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Accuracy of a New Milk Strip Cow-Side Test for Diagnosis of Hyperketonemia

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Abstract

The objectives of this study were to determine the accuracy of the PortaBHB™ milk strip for detection of hyperketonemia in early lactation cows, and to compare the agreement of results from quarter and composite samples. A total of 577 Holstein cows of all parities, from 88 commercial herds, were sampled once during this study. Cows were sampled simultaneously for blood and milk between one and 60 days-in-milk. Blood samples were collected from coccygeal vessels, and were analyzed on-farm using an electronic β-hydroxybutyrate (BHBA) hand-held meter. Milk samples were collected from one quarter (n=577), as well as from four quarters (composite; n=299). All milk samples were tested using PortaBHB™ milk strips (0, 50, 100, 200, and 500 µmol/L). Blood BHBA concentration was considered to be the gold standard, with hyperketonemia defined as a blood BHBA concentration ≥ 1400 µmol/L. With this cut-point, the true prevalence of hyperketonemia in the study population was 24.6%. Using a threshold of 100 µmol/L, sensitivity and specificity of PortaBHB™ milk strips was 89.2% and 79.6%, respectively. Using a threshold of 200 µmol/L, sensitivity and specificity of PortaBHB™ milk strips was 40.3% and 99.5%, respectively. The kappa coefficient for agreement between milk results from quarter and composite samples, using a threshold of 100 µmol/L, was 0.95. Study results suggest that PortaBHB™ has good accuracy, and that there is no benefit to collect milk samples from four quarters compared with sampling one quarter.

Keywords: dairy cow, hyperketonemia, ketosis, diagnostic test

Résumé

Cette étude avait pour but de connaître la précision des bandelettes réactives PortaBHB^{MC} pour le dépistage de l'hyperacétonémie dans le lait chez des vaches en début de lactation, et de vérifier la concordance des résultats de ce test effectué sur le lait d'un seul quartier et d'un échantillon composé. Dans cette étude, nous avons échantillonné une seule fois 577 vaches (de tous niveaux de parité) provenant de 88 élevages commerciaux. Nous avons prélevé en même temps sur les vaches des échantillons de sang et de lait entre le premier jour et le 60^{e} jour en lactation. Les échantillons de sang ont été prélevés du vaisseau coccygien puis analysés à la ferme au moyen d'un doseur électronique portatif de β-hydroxybutyrate (BHBA). Quant aux échantillons de lait, ils provenaient d'un seul quartier (n=577) ainsi que des quatre quartiers (n=299). Tous les échantillons ont été testés au moyen des languettes réactives PortaBHB^{MC} (0, 50, 100, 200 et 500 µmol/L). Nous avons considéré les concentrations sanguines de BHBA comme étant nos valeurs étalons, la teneur déterminant l'hyperacétonémie étant ≥ 1400 µmol de BHBA par litre de sang. Avec cette valeur de référence, la vraie prévalence d'hyperacétonémie dans la population étudiée était de 24,6 %. Avec un seuil de 100 µmol/L, la sensibilité et la spécificité des languettes réactives PortaBHB^{MC} étaient de 89,2 % et 79,6 %, respectivement. Avec un seuil de 200 µmol/L, la sensibilité et la spécificité de ces languettes étaient de 40,3 % et de 99,5 %, respectivement. Les résultats obtenus à partir d'un seul quartier du pis ou de l'ensemble des quartiers, avec un seuil de 100 µmol/L, affichaient un coefficient de Kappa de concordance de 0,95. Cette étude nous permet de dire que pour le dépistage de l'hyperacétonémie dans le lait, les bandelettes réactives PortaBHB^{MC} ont une bonne précision et qu'il n'y a pas d'avantages à prélever un échantillon dans les quatre quartiers plutôt que dans un seul de ceux-ci.

Introduction

Hyperketonemia is defined as an abnormally high concentration of circulating ketone bodies during the postpartum period.[1] Cows affected by hyperketonemia include both cows with and without clinical signs of ketosis. Hyperketonemia is prevalent in dairy herds[5] and

is associated with reduced milk production,[4] decreased probability of pregnancy at first service,[5,9] and increased risks of health problems such as displaced abomasum, clinical ketosis, and metritis.[4] The gold standard diagnostic test for hyperketonemia is the measurement of β-hydroxybutyric acid (BHBA) in serum or plasma by a laboratory process.[5] Using this test, a threshold of 1400 μmol/L was reported to be the most accurate for diagnosing hyperketonemia.[4] However, serum or plasma testing is inconvenient because results are not immediate and provided cow-side. An alternative to the laboratory method is blood BHBA measurement[6] that can be used as an on-farm cow-side test for hyperketonemia with a near perfect accuracy (Precision Xtra[a]; sensitivity 96%, specificity 97%). The Keto-Test[b] milk strip was also developed and validated as a cow-side test for hyperketonemia.[2,7] Its accuracy was variable between studies, but was reported to be good.[2,7] A new semi-quantitative cow-side milk strip test (PortaBHB™[-c]) is available for dairy producers and veterinarians to quantify BHBA concentration in milk. However, its accuracy remains unknown, and it is unclear if sampling milk from a single quarter provides a representative sample for BHBA concentration compared to a composite sample from all four quarters. Therefore, the objectives of this study were to determine the accuracy of the PortaBHB™ milk strip for detection of hyperketonemia in early lactation cows and to compare the agreement between results of individual quarter and composite milk samples.

Materials and Methods

A total of 577 Holstein cows, from 88 herds in southwestern Québec (Canada), were used in this study. Herd selection was based on convenience sampling (location). Participating herds were visited regularly between June and December 2010. During farm visits, blood and milk samples were collected simultaneously from fresh cows (between one and 90 days-in-milk (DIM)). Cows were sampled once during the postpartum period. Blood (1 mL) was drawn from coccygeal vessels and immediately analyzed with a BHBA hand-held meter (Precision Xtra). A single quarter (front left) milk sample was collected from all cows enrolled on the study (n=577). In addition, a composite sample (from all four quarters) was taken from a subpopulation of cows (n=299). Milk samples were transported on ice to the bovine ambulatory clinic laboratory of the Université de Montréal (Saint-Hyacinthe, Québec) and then kept in a refrigerator (39°F; 4°C). Samples were pulled out of the refrigerator (room temperature 68°F; 20°C) one hour before being analyzed. Each milk sample was analyzed within six hours of sampling by dipping one PortaBHB™ milk strip into the sample. The semi-quantitative test result was read one minute after dipping using the color chart provided on the test bottle (0, 50, 100, 200, or 500 μmol/L). All samples were read by the same technician, who was blinded to BHBA blood results and to cow identification (unable to differentiate between samples from the same cow). If the technician evaluated that a test result fell between two categories, it was decided a priori that the lowest value would be chosen. Statistical analyses were performed using SAS.[d] Blood BHBA concentration measured with the Precision Xtra device was used as a reference test. Hyperketonemia was defined as a blood BHBA concentration ≥1.4 mmol/L, and data were dichotomized using this cut-point. PortaBHB™ test results from one- and four-quarter samples were dichotomized using the threshold values of ≥100 and ≥200 μmol/L. Sensitivity, specificity, positive predictive values, negative predictive values, and overall accuracy were calculated for both thresholds (FREQ procedure in SAS). The agreement between results from quarter and composite samples was tested using the Kappa statistic for agreement beyond chance (FREQ procedure in SAS).[3]

Results and Discussion

A total of 577 cows were used in this study. These cows had an average parity of 2.8 (SD=1.6; median=2.0; minimum=1; maximum=9), and were sampled on average at 18.7 DIM (SD=14.0; median=16.0; minimum=1; maximum=90). The average blood BHBA value was 1.2 mmol/L (SD=1.0; median=0.8; minimum=0.2; maximum=6.3), and the true prevalence of hyperketonemia, as defined by the reference test, was 24.6%. This value of true prevalence is similar to other reports.[5,8] In cows sampled for milk from one quarter (n=577), using a threshold of ≥100 and ≥200 μmol/L provided an apparent prevalence of 37.4 and 10.3%, respectively. In cows sampled for milk from four quarters (n=299), using a threshold of ≥100 and ≥200 μmol/L provided an apparent prevalence of 39.5 and 13.7%, respectively. Measures of accuracy for quarter and composite samples are presented in Tables 1 and 2, respectively. These values are similar to pooled sensitivity (100 μmol/L: 83%; 200 μmol/L: 54%) and specificity (100 μmol/L: 82%; 200 μmol/L: 94%) values summarized by Oetzel for the Keto-Test milk strip.[7] The kappa coefficients for agreement between quarter and composite samples, when using thresholds of ≥100 and ≥200 μmol/L, were 0.95 (95% CI=0.93-0.97) and 0.91 (95% CI=0.89-0.93), respectively. These kappa values indicate that there was almost perfect agreement between milk groups.[3] Overall, these data suggest that the PortaBHB™ milk strip is a good test for the detection of hyperketonemia, and that when collecting milk for BHBA testing there is little benefit of using a composite sample compared with sampling just one quarter. Discussion of practical strategies for monitoring hyperketonemia in dairy herds was not the

Table 1. Accuracy of using PortaBHB™ milk strip tests on milk samples collected from one quarter for diagnosis of hyperketonemia in dairy cows (n=577) when compared with blood β-hydroxybutyric acid concentration (threshold ≥1.4 mmol/L).

	PortaBHB milk strip threshold	
	≥100 μmol/L	**≥200 μmol/L**
True prevalence (%)	24.6	24.6
Apparent prevalence (%)	37.4	10.3
Sensitivity (%; (95% CI[1]))	89.2 (85.4-93.0)	40.3 (36.1-44.5)
Specificity (%; (95% CI[1]))	79.6 (76.2-83.0)	99.5 (96.4-100)
Positive predictive value (%; (95% CI[1]))	58.8 (56.0-61.6)	96.6 (92.1-100)
Negative predictive value (%; (95% CI[1]))	95.8 (93.4-98.2)	83.6 (80.1-87.1)
Overall accuracy (%)	81.9	85.0

[1] 95% CI: 95% confidence interval

Table 2. Accuracy of using PortaBHB™ milk strip tests on milk samples collected from four quarters (composite samples) for diagnosis of hyperketonemia in dairy cows (n=299) when compared with blood β-hydroxybutyric acid concentration (threshold ≥1.4 mmol/L).

	PortaBHB milk strip threshold	
	≥100 μmol/L	**≥200 μmol/L**
True prevalence (%)	24.6	24.6
Apparent prevalence (%)	39.5	13.7
Sensitivity (%; (95% CI[1]))	92.0 (88.4-95.6)	50.7 (46.9-54.5)
Specificity (%; (95% CI[1]))	78.1 (74.6-81.6)	99.7 (96.9-100)
Positive predictive value (%; (95% CI[1]))	58.5 (55.6-61.4)	92.7 (88.6-96.8)
Negative predictive value (%; (95% CI[1]))	97.7 (95.2-100)	85.7 (82.5-88.9)
Overall accuracy (%)	81.6	86.6

[1] 95% CI: 95% confidence interval

objective of this paper, but excellent information on this topic can be found elsewhere.[7]

For logistical reasons, milk samples were not analyzed on farms. It is unclear if cooling and warming milk samples could have influenced results of this study compared with the cow-side use of PortaBHB™ test. This information should be considered when interpreting the study results. Furthermore, these results were obtained by comparing milk samples to Precision Xtra test results, which has a near perfect accuracy, but is not the gold standard test for hyperketonemia.

Conclusions

This study was conducted to evaluate the accuracy of a new milk strip test for diagnosis of hyperketonemia. Study results suggest that this cow-side test has good accuracy, and that there is no benefit of collecting a composite milk sample compared to sampling a single quarter. Thus, the on-farm application of this milk strip may be useful to identify cows with hyperketonemia, or to quantify herd-level incidence of hyperketonemia.

Endnotes

[a]Precision Xtra, Abbott, Mississauga, ON, Canada
[b]Keto-Test, Sanwa Kagaku Kenkyusho Co., Nagoya, Japan
[c]PortaBHB™, PortaCheck Inc., Moorestown, NJ
[d]SAS, version 9.2, SAS Institute, Cary, NC

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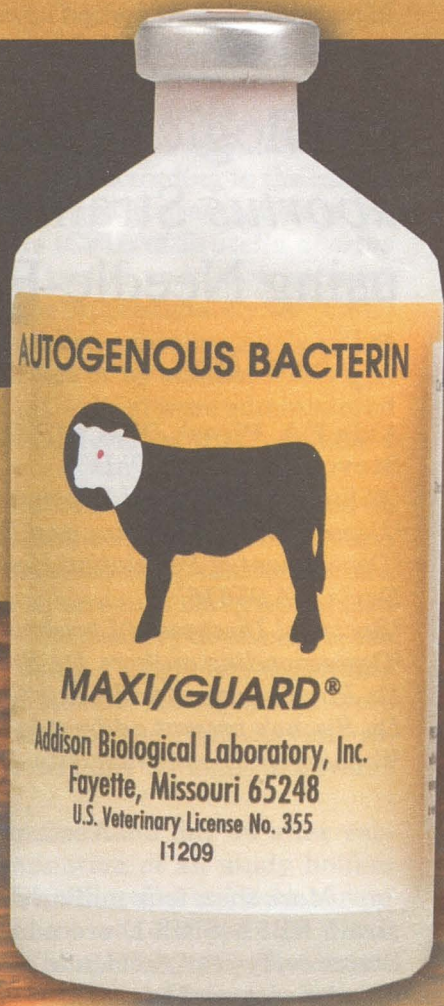

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