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Latent class evaluation of a milk test, a urine test, and the fat-to-protein percentage ratio in milk to diagnose ketosis in dairy cows

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ABSTRACT

In this study, 3 commonly used tests to diagnose ketosis were evaluated with a latent class model to avoid the assumption of an available perfect test. The 3 tests were the KetoLac BHB (Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya, Japan) test strip that tests milk for β-hydroxybutyrate, the KetoStix (Bayer Diagnostics Europe Ltd., Dublin, Ireland) test strip that tests urine for acetoacetate, and the fat-to-protein percentage ratio (FPR) in milk. A total of 8,902 cows were included in the analysis. The cows were considered to be a random sample from the population of Danish dairy cattle under intensive management, thus representing a natural spectrum of ketosis as a disease. All cows had a recorded FPR between 7 and 21 d postpartum. The KetoLac BHB recordings were available from 2,257 cows and 6,645 cows had a KetoStix recording. The recordings were analyzed with a modified Hui-Walter model, in a Bayesian framework. The specificity of the KetoLac BHB test and the KetoStix test were both high [0.99 (0.97–0.99)], whereas the specificity of FPR was somewhat lower [0.79 (0.77–0.81)]. The best sensitivity was for the KetoStix test [0.78 (0.55–0.98)], followed by the FPR [0.63 (0.58–0.71)] and KetoLac BHB test [0.58 (0.35–0.93)].

Key words: ketosis, diagnostic test evaluation, latent class model, gold standard

INTRODUCTION

Ketosis is a common disease in adult cattle. It typically occurs in dairy cows in early lactation and is clinically characterized by nonspecific signs such as anorexia, milk loss, central nervous symptoms, and loss of body fat. The pathogenesis of ketosis involves a complex set of manifestations of excessive mobilization of body reserves of stored energy, mostly fat, due to a lack of balance between the cow's energy demand for milk production and her energy intake. The cause of low energy intake can be related to poor quality of feedstuff, including ketogenic substances in the feed, other diseases like milk fever (post-parturient hypocalcaemia), or displaced abomasums. However, increased body condition score at calving may also in itself decrease feed intake (Broster and Broster, 1998).

A range of biochemical tests are available to diagnose ketosis. Measurements of BHBA in serum or milk and acetoacetate (**AcAc**) in urine probably are the most common. Several studies have used serum concentrations of BHBA of 1400 μmol/L as the suggested cut-off or even gold standard to define ketosis, but several cut-offs for the available tests have been suggested (Geishauser et al. 1998; Carrier et al., 2004; Iwersen et al. 2009). Previous studies on the accuracy (sensitivity and specificity) of available tests for ketosis that can be used in the herd (cow-side test) have primarily used serum levels of BHBA as the gold standard. However, BHBA and AcAc measurements vary for several reasons. For example, diurnal variation (Nielsen et al., 2003), high levels of somatic cells in the milk that will give false-positive results of a BHBA test on milk (Jeppesen et al., 2006), temperature (Geishauser et al., 2000), time of reading of urine sticks that influences color changes (Jeppesen et al., 2006), and pure measurement error. Fat-to-protein percentage ratio (**FPR**) in the milk has also been suggested as an indication of ketosis (Duffield et al., 1997; Čejna and Chlàdek, 2005). Excessive mobilization of fat will be reflected in an elevation of fat percentage. Because protein percentage is rather stable and cow-specific, FPR should be an indicator of ketosis that is adjusted for a cow effect. Although neither of the above tests can be considered perfect, they are all relevant tools in the dairy herd from a practical point of view and evaluation of their performance under field conditions is needed. Also, neither level of BHBA in serum, nor any other tests are appropriate to define a gold standard to describe the dynamic level of metabolic stress the cow is exposed to. The term gold standard should only be applied to diagnostic tests that have a sensitivity and specificity of 1. However, as an alternative, the term criterion standard is adopted

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by the American Medical Association and defined as a method having an established or widely accepted accuracy for determining a diagnosis, providing a standard to which a new screening or diagnostic test can be compared. Thus, previously obtained estimates of test accuracy for ketosis tests may be biased, due to the misclassification bias, which occurs from using a less than perfect test in a testing scheme to define cases and non-cases (Nielsen and Toft, 2002). Cow-side ketosis tests are ideally tools to detect ketosis at a stage where the possibly diffuse clinical symptoms are not yet present and not just to verify a clinical suspicion. This provides the practitioner the possibility to intervene efficiently. Thus, a practically relevant disease definition relates to cows in all stages of ketosis, rather than one subject to the selection bias imposed by a classification scheme using (e.g., serum as a gold standard or clinical symptoms).

Under certain conditions, latent class analysis (**LCA**) can be used to estimate the sensitivity (**Se**) and specificity (**Sp**) of diagnostic tests without the assumption of one being a gold standard (Toft et al., 2007a). The basic LCA relies on what is generally referred to as the Hui-Walter paradigm (Hui and Walter, 1980): 2 or more tests must be evaluated in 2 or more subpopulations with different prevalence of the disease, the tests must have constant Se and Sp across the populations, and the tests must be conditionally independent, given disease status. The LCA methods have gained increased acceptance as a means of evaluating diagnostic tests for infectious diseases and are now endorsed by the OIE (World Organization for Animal Health) as an alternative to classic test evaluations in the OIE fitness for purpose concept (OIE, 2010).

The objective of this study was to compare the test performance of a milk-based cow-side BHBA test, a urine-based cow-side AcAc test, and the FPR in milk as tools to diagnose ketosis without the assumption of an available gold standard.

MATERIALS AND METHODS

Diagnostic Tests

The KetoLac BHB (Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya, Japan; marketed as KetoTest in the United States) test is a dip-stick that measures the milk contents of BHBA on a semiquantitative scale. In this study we used the cut-off $\geq$200 μmol/L of BHBA in milk, recommended by the manufacturer, to define a positive test. Tests were performed as described by the manufacturer. However, variation in the procedure between veterinarians might exist (such as preceding testing for mastitis).

The KetoStix (Bayer Diagnostics Europe Ltd., Dublin, Ireland) is a dip-stick that measures the AcAc content in the urine on a semiquantitative scale. A concentration of 4 mmol/L (moderate) or above of AcAc in urine was chosen as the cut-off for a positive KetoStix test (Carrier et al., 2004). Tests were performed as described by the manufacturer on urine collected as catheter sampling, spontaneous urination, or urination induced by manual manipulation the distal urethra in the vagina.

The FPR was calculated based on 1 of the 11 annual milk recordings from the Danish milk control program (RYK, 2010). Fat-to-protein percentage ratio values above 1.5 were defined as test positive for ketosis, as suggested by Čejna and Chlàdek (2005).

Data Collection

Data were extracted from the Veterinary Production and Consultancy (**VPR**) database, which is a subset of the National Danish Cattle Database (Krogh and Enevoldsen, 2006), on June 7, 2009. The VPR database consists of herds, where the veterinarians perform systematic clinical recordings on well-defined groups of cows. At least 12% of the Danish dairy herds have their cows routinely tested in early lactation for ketosis by a veterinarian. The veterinarians voluntarily submit cow-level registrations to the VPR database. In most Danish dairy herds, milk yield, fat percentage, and protein percentage are measured at up to 11 annual test days. The primary inclusion criteria for our study were that cows had been tested for ketosis with either the KetoLac BHB or the KetoStix between 7 and 21 d postpartum and had a milk test-date recording 1 d before the day of the ketosis test. The restriction with a minimum of 7 d postpartum was imposed because some cows might still have some colostrum in the milk up to this time point and colostrum will affect the measurement of fat content in the milk. Milk recordings at or after the day of testing for ketone bodies were also excluded because test results might have induced treatments of ketosis (usually glucocorticoids parentally or propylene glycol given orally; Radostits et al., 2000), which might affect FPR. Initial analyses of the data suggested that treatments could affect the milk composition very quickly. Records of ketosis treatment were available but not used, except for the initial data analyses. Out of 141,133 ketosis tests available from individual cows, a total of 8,902 cows were selected based on the milk test date. Of these, 2,257 cows were examined with the KetoLac BHB test and 6,645 cows with the KetoStix test. The KetoLac BHB tests were recorded from February 2004 to April 2009. The KetoStix tests were recorded between February