Journal of Veterinary Behavior 8 (2013) 362–366

Contents lists available at SciVerse ScienceDirect

Journal of Veterinary Behavior

journal homepage: www.journalvetbehavior.com

Research

ROC analysis of prepartum feeding time can accurately predict postpartum metritis development in HF crossbred cows

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ARTICLE INFO

Article history:
Received 25 June 2012
Received in revised form
14 April 2013
Accepted 1 May 2013
Available online 17 June 2013

Keywords:
ROC analysis
feeding time
metritis
threshold value
relative risk

ABSTRACT

Receiver operating characteristic (ROC) analysis is a useful statistical tool to visualize, organize, and select diagnostic variables. Accuracy of the test is represented by the area under the ROC curve (AUC), sensitivity (Se), and specificity (Sp). In the present study, ROC analysis of prepartum feeding time (day −11 to day −2) of 20 pluriparous Holstein-Friesian crossbred cows were recorded, and the cows were followed up for detecting postpartum metritis development if any. Prepartum daily feeding time data during whole study was grouped into 2 periods (P1 [day −11 to day −7] and P2 [day −6 to day −2]). The AUC of feeding time for the P1 and P2 periods was 0.64 ($P = 0.29$) and 0.86 ($P = 0.006$), respectively. Cows with daily feeding times below the optimum threshold value of 284.5 min/d during P2 (Se = 75% and Sp = 91.67%) had more chance of developing postpartum metritis compared with those with daily feeding time above the threshold value (positive likelihood ratio = 9.0). Furthermore, relative risk (RR) analysis revealed that the risk of development of postpartum metritis increased as feeding time decreased below the optimum threshold value during P2 (RR = 5.571, $P = 0.004$). Thus, the optimum threshold value of feeding time during P2 may be used to predict risk of postpartum metritis, allowing for preventive measures to be implemented in the early postpartum period to reduce the likelihood of postpartum complications.

Introduction

Metritis is an important postpartum health disorder, which affects reproductive performance and productivity of high-yielding dairy cows and buffaloes. Heavy productivity loss because of increased days open, days dry, and calving interval along with significant reduction in lactation length has been reported in crossbred cows at National Dairy Research Institute, Karnal (Balasundaram et al., 2011; Sharma et al., 2011). Earlier studies on blood metabolites and feeding behavior revealed that these parameters can be effectively used for early prediction of metritis (Urton et al., 2005; Huzzey et al., 2007; Ospina et al., 2010). Prepartum feeding time below an optimum threshold value (75 min/d) was found to be highly correlated with increased incidence of postpartum metritis (Urton et al., 2005). The optimum threshold value is defined as the threshold value that has maximum combined sensitivity (Se) and specificity (Sp) for predicting diseases (Urton et al., 2005; Ospina et al., 2010).

The common method used to find the optimum threshold value is through receiver operating characteristic (ROC) analysis. It is a simple statistical tool used to characterize a variable in terms of area under the ROC curve (AUC) and provides optimum threshold values along with the corresponding Se, Sp, and likelihood ratio (LR). The Se measures the proportion of sick and animals that are identified by the diagnostic test as sick (true positives [TPs]), whereas Sp measures the proportion of healthy cows correctly identified by the test as healthy (true negatives [TNs]). The LR refers to the number of times a cow is more likely to develop a disease when they are below a particular threshold value relative to if they were above this threshold value. The assumption behind ROC

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http://dx.doi.org/10.1016/j.jveb.2013.05.001

analysis is that a diagnostic variable (continuous classifier, i.e., feeding time) has binary outcomes (metritis or normal).

The optimum threshold value for daily feeding time to predict ensuing metritis in medium- to high-yielding crossbred cows is not available. Hence, the present study was conducted in Holstein-Friesian (HF) crossbred cows to find out a suitable optimum threshold value for prepartum feeding time by ROC analysis for early prediction of metritis along with its Se, Sp, and LR+. Using these optimum threshold values, the relative risk (RR) of development of postpartum metritis was also estimated.

Materials and methods

Experimental animals and management

The present experiment was conducted at Cattle Yard, National Dairy Research Institute, Karnal. Karnal is located on 29° 42′ N latitude and 72° 02′ E longitude at an altitude of 250 m above the mean sea level. Twenty pluriparous dry pregnant HF crossbred cows (HF × Tharparkar) were based on normal body condition score (3.5-4.5 in 6-point scale; Prasad, 1994); lactation yield (3800-4500 L during 305 days) and body weight (450-550 kg) were selected. Experimental cows were maintained in a loose housing system of management during December to March (2010-2011). The temperature during study period ranged from 2°C (minimum) to 30°C (maximum) and relative humidity from 35% (minimum) to 96% (maximum). The cows were fed measured amount of concentrate (20% crude protein and 70% total digestible nutrients) as per National Research Council (2001) standards. The concentrate mixture comprised of maize (33%), groundnut cake (21%), mustard cake (12%), wheat bran (20%), deoiled rice bran (11%), mineral mixture (2%), and common salt (1%). The concentrate mixture fed was 1.5 kg/d/cow for body maintenance and extra concentrate of 1.5 kg/cow 3 weeks before expected date of calving. In addition to concentrate, ad libitum seasonal green fodder (berseem and oat) was provided 2-3 times a day. The dry matter (DM) percent of concentrate, berseem, and oat was approximately 90, 12, and 14%, respectively. Daily ration was given in the common manger located at one side of the loose house with adequate manger space (0.6 m per animal).

Monitoring of feeding time and metritis diagnosis

The cows were moved to an experimental paddock to monitor and record behavior 3 weeks before the expected date of calving. When a cow started showing physical signs of imminent calving (udder enlargement, milk letdown, and relaxation of sacrosciatic ligament), she was shifted to the calving pen. Cows were marked with hair dye on their back and sides for easy identification. Feeding behavior (daily feeding time) was monitored continuously by means of 3 video cameras with infrared lighting arrangement and recorded by an 8-channel digital video recorder (MPEG4/H.264; TS MicroTech, Inc, California, USA). A cow was considered to be feeding when its head was in the manger with active ingestion. Daily feeding time data were analyzed by replay of recorded data with 2× to 32× speed depending on feeding activity pattern. Start and end points of feeding were noted to calculate duration of feeding time (difference between start and end points).

Cows were regularly monitored (3-4 days of interval) for health problems up to 21 days postpartum, and uterine discharge was collected through rectovaginal method for metritis diagnosis. The uterine discharge was aspirated from the uterus using sterile blue sheath (IMV Technologies, L'Aigle, France) and universal artificial insemination gun and transferred to a clean test tube. Cows that showed fetid uterine discharge (purulent or red–brown) along with high body temperature (>39.5°C) within 3 weeks postpartum were diagnosed as metritic (Sheldon et al., 2006).

Statistical analysis

In the present study, owing to variation between expected and actual date of calving, we recorded complete daily feeding time data from day −11 to day −2 during prepartum period. The prepartum daily feeding time data during the whole study were grouped into 2 periods, that is, P1 (day −11 to day −7) and P2 (day −6 to day −2), and the cows were divided into 2 groups as normal and metritic after diagnosis of metritis. Descriptive analysis was conducted to find the mean, standard error, and range of daily feeding time. Feeding time, dependent variable, was analyzed by 2-way analysis of variance to evaluate the effect of health (metritis and normal) and periods (P1 and P2) on this measure. All pairwise differences in mean were compared by Tukey post hoc test. Different optimum threshold values of feeding time were analyzed by ROC analysis using SigmaPlot 11 software package (Systat software, Inc, California, USA). Power analysis was conducted using MedCalc, version 12.3 software package (MedCalc Software, Ostend, Belgium). The ROC analysis produces AUC, a 2-dimensional graph (Se and 1 − Sp plotted in Y-axis and X-axis, respectively, for the range of potential optimum threshold values), which gives the accuracy. Accuracy of diagnostic test was interpreted based on the AUC and said to be noninformative if AUC is 0.5, accurate if AUC is 0.5-0.7, very accurate if AUC is 0.7-0.9, highly accurate if AUC is 0.9-1.0, and finally perfect if AUC is 1 (Swets, 1988).

The Se values depend on the number of TP and false negative (FN) cases, whereas Sp values depend on the number of TN and false positive (FP) cases. Furthermore, the Se and Sp together define the positive LR (LR+). The TP indicates the number of metritic cows having prepartum feeding time below the threshold value, and FP indicates the number of metritic cows having prepartum feeding time above the threshold value. The TN indicates the number of normal cows having prepartum feeding time above the threshold value, and FN indicates the number of normal cows having prepartum feeding time below the threshold value (Lalkhen and McCluskey, 2008). The Se was defined as the proportion of cows with metritis that had feeding time below the threshold value ([TP]/[TP + FN]). The Sp was the proportion of normal cows that had feeding time above the threshold value ([TN]/[TN + FP]). The LR+ was the number of times a cow was more likely to develop metritis below a particular threshold value (LR+ = Se/[1 − Sp]) (Greiner et al., 2000; Akobeng, 2007; Fawcett, 2006; Faustini et al., 2007; Lalkhen and McCluskey, 2008).

The optimum threshold value of feeding time was used to dichotomize cattle into 2 health categories (feeding time above each threshold value indicated "0" value [healthy] and below each threshold value indicated "1" [metritic]), and a simple 2 × 2 contingency table was constructed to analyze RR of metritis (Simon, 2001; Cummings, 2009). The RR is the ratio of proportion of cows that had feeding time below the threshold value developed metritis to the proportion of cows that had feeding time above the threshold value developed metritis ({TP/[TP+ FN]}/{FP/[FP+TN]}).

Results

Feeding time

Among the 20 experimental cows, 8 cows developed metritis during postpartum period. There was a significant difference ($P < 0.001$) in prepartum feeding time between normal and metritic cows during both the periods studied (Table 1). Similarly, the feeding time of cows irrespective of the health status differed