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Determination of optimal diagnostic criteria for purulent vaginal discharge and cytological endometritis in dairy cows

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ABSTRACT

The objectives of this observational study were to identify the optimal diagnostic criteria for purulent vaginal discharge (PVD) and cytological endometritis (ENDO) using vaginal discharge, endometrial cytology, and leukocyte esterase (LE) tests, and to quantify their effect on subsequent reproductive performance. Data generated from 1,099 untreated Holstein cows (28 herds) enrolled in a randomized clinical trial were used in this study. Cows were examined at 35 (±7) d in milk for PVD using vaginal discharge scoring and for ENDO using endometrial cytology and LE testing. Optimal combinations of diagnostic criteria were determined based on the lowest Akaike information criterion (AIC) to predict pregnancy status at first service. Once identified, these criteria were used to quantify the effect of PVD and ENDO on pregnancy risk at first service and on pregnancy hazard until 200 d in milk (survival analysis). Predicting ability of these diagnostic criteria was determined using area under the curve (AUC) values. The prevalence of PVD and ENDO was calculated as well as the agreement between endometrial cytology and LE. The optimal diagnostic criteria (lowest AIC) identified in this study were purulent vaginal discharge or worse (≥4), ≥6% polymorphonuclear leukocytes (PMNL) by endometrial cytology, and small amounts of leukocytes or worse (≥1) by LE testing. When using the combination of vaginal discharge and PMNL percentage as diagnostic tools (n = 1,099), the prevalences of PVD and ENDO were 17.1 and 36.2%, respectively. When using the combination of vaginal discharge and LE (n = 915), the prevalences of PVD and ENDO were 17.1 and 48.4%. The optimal strategies for predicting pregnancy status at first service were the use of LE only (AUC = 0.578) and PMNL percentage only (AUC = 0.575). Cows affected by PVD and ENDO had 0.36 and 0.32 times the odds, respectively, of being pregnant at first service when using PMNL percentage compared with that of unaffected cows; odds ratios were 0.33 and 0.69 for PVD and ENDO, respectively, when LE was used. Kappa value (agreement) of the pairwise comparison for the PMNL percentage and LE was 0.43. Vaginal discharge, endometrial cytology, and LE can be used to diagnose PVD and ENDO, and to predict pregnancy status at first service. The use of LE could be a good alternative to endometrial cytology for on-farm testing.

Key words: dairy cow, uterine disease, endometritis, diagnosis

INTRODUCTION

Reproductive performance is a major concern in the dairy industry because of its important economic impact (LeBlanc, 2007). Reproductive tract diseases such as purulent vaginal discharge (**PVD**) and cytological endometritis (**ENDO**) are associated with detrimental effects on subsequent reproductive performance (Dubuc et al., 2010a). To implement an on-farm disease surveillance program and an immediate treatment strategy for PVD and ENDO, practical and easy-to-use diagnostic tools are needed. Multiple diagnostic tools have been validated for PVD and ENDO. For example, a vaginoscope or a Metricheck device (Simcro, Hamilton, New Zealand) can be used to diagnose PVD, and both tools provide relatively similar results (McDougall et al., 2007). The diagnosis of ENDO using endometrial biopsy or cytology techniques has been validated (Kasimanickam et al., 2004; Gilbert et al., 2005; Chapwanya et al., 2010) but does not provide on-farm results. Leukocyte esterase (**LE**) colorimetric testing from uterine lavage or cytobrush sample was shown to provide interesting results and it was developed to provide immediate on-farm results (Cheong et al., 2012; Couto et al., 2013).

Diagnosis of PVD and ENDO requires the use of thresholds to classify the animals as diseased or not because the techniques used provide quantitative or semiquantitative results (de Boer et al., 2014). Al-

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though many studies have reported different diagnostic criteria, most of them were relatively similar (Barlund et al., 2008; Runciman et al., 2009; Dubuc et al., 2010a). However, few studies have considered PVD and ENDO diagnosis simultaneously (de Boer et al., 2014). It is unclear at this point what the best combination of diagnostic criteria for PVD and ENDO is when taken together and whether this combination would provide better accuracy to diagnose reproductive tract disease globally. Therefore, the first objective of this study was to define PVD and ENDO diagnostic criteria using vaginal discharge and endometrial cytology in postpartum dairy cows considering both conditions simultaneously and to quantify the effect of these conditions on subsequent reproductive performance. The second objective was to validate the use of LE colorimetric testing as a diagnostic tool for ENDO and its effect on reproduction. The third objective was to evaluate the accuracy of these diagnostic tests for predicting the subsequent reproductive performance of dairy cows.

MATERIALS AND METHODS

A prospective cohort study was conducted from October 2011 until December 2013. A convenience sample of 28 herds was recruited in a 250-km radius around St-Hyacinthe, Québec, Canada. Participating herds were required to use a computerized record system and to use an ovulation synchronization protocol to synchronize the first breeding of all cows at around 70 DIM. All herds used exclusively AI for breeding. Each cow could only be enrolled once in the study.

A total of 1,168 enrolled cows were targeted in the study. Sample size was calculated to identify a difference of 10% in pregnancy risk at first service between diseased (20%) and nondiseased (30%) cows with 95% confidence and 80% power (Dohoo et al., 2009) when expecting an overall disease prevalence of 25%. The present study was part of a larger research project involving a randomized clinical trial of intrauterine cephapirin treatment for endometritis in postpartum dairy cows. All cows enrolled in the present study were control animals and did not receive any intrauterine treatment that could influence their reproductive performance. Cows that were not bred before 100 DIM were excluded from the project.

Farms were visited every other week by an animal health technician and a veterinarian. All participating cows were examined for PVD and ENDO at 35 (±7) DIM. First, vaginal discharge was evaluated with the Metricheck device technique (McDougall et al., 2007) and scoring (0 = no discharge, 1 = clear mucus, 2 = mucus with flecks of pus, 3 = mucopurulent discharge, 4 = purulent discharge or 5 = foul-smelling discharge). A cytological sample was then collected from the uterus using a cytobrush (VWR CanLab, Mississauga, Canada) adapted for use in cattle (Kasimanickam et al., 2004; Dubuc et al., 2010a). After collection, the cytobrush was rolled on a clean glass microscope slide and plunged into an individual vial (3 mL) containing 1 mL of 0.9% saline (NaCl 0.9% Irrigation, Baxter Corp., Mississauga, ON, Canada). Within 12 h of collection, endometrial cytology slides were stained with a modified Wright-Giemsa stain (Hema3, Biochemical Sciences, Swedesboro, NJ) and coverslips were applied when dry (Barlund et al., 2008). Microscopic evaluation of cytology slides was done separately at 400× magnification by 2 experienced observers (animal health technician and veterinarian; Kasimanickam et al., 2005). Percentage of PMNL was determined in 2 different regions of each slide based on a differential count of 200 cells (polymorphonuclear and endometrial cells). Slide readers were blinded to on-farm clinical findings.

Within 12 h of collection, LE testing was performed by shaking the individual vial for 10 s and putting a drop of the solution on an LE commercial test strip (Multistix 10 SG, Bayer Corporation, Elkart, IN) with a pipette. Test results for every cow were read according to the manufacturer's colorimetric chart after 2 min, and the score was recorded as follows: 0 = negative, 0.5 = trace of leukocytes, 1 = small amount of leukocytes, 2 = moderate amount of leukocytes, and 3 = large amount of leukocytes (Couto et al., 2013). Test readers were blinded to the on-farm disease clinical findings.

Statistical Analyses

All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC). The cow was considered as the unit of interest in this study. Individual cow data such as parity (first, second, and third or greater), season of calving (winter: January to March; spring: April to June; summer: July to September; fall: October to December), and reproductive and culling events were exported from a DSA computerized record system (DSAHR Inc., Saint-Hyacinthe, QC, Canada) into Microsoft Excel (Microsoft Corp., Redmond, WA).

Diagnostic Criteria. To determine to optimal diagnostic criteria for PVD and ENDO, a dichotomous dummy variable was created for each disease (PVD and ENDO). When the score of vaginal discharge was at or above the vaginal discharge diagnostic criterion, the variable PVD was considered positive, regardless of the cytology result. If the percentage of PMNL was at or above the PMNL diagnostic criterion, the variable ENDO was considered positive. All possible