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Use of reagent test strips for diagnosis of endometritis in dairy cows

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Abstract

The use of leukocyte esterase (LE), protein, and pH tests were evaluated on widely available urinary test strips (Multistix 10 SG; Bayer Corporation, Elkart, IN, USA) on uterine lavage samples as a potential cow-side test for the diagnosis of cytologic endometritis. Uterine lavage samples of 563 lactating Holstein cows between 40 and 60 days postpartum from 28 herds were evaluated. Endometrial cytology was used as the reference for endometritis, with a cutoff point of $\geq 10\%$ neutrophils. All three (LE, protein, and pH) were increased in cows with cytologic endometritis and the associations were highly significant. Optimal cutoff points determined by receiver operating characteristic analysis for LE, protein, and pH were $\geq ++$, ≥ 300 mg/dL, and ≥ 7.0 , respectively. Combining the results for LE and pH improved the performance of the test strip, but this resulted in a group of cows (20.6% of cows) which were approximately equally likely (46% with endometritis and 54% without endometritis) to have cytologic endometritis or not, and therefore could not be accurately classified. The direct relationship between reagent strip test and reproductive performance was also evaluated. Reproductive impairment due to endometritis was restricted to multiparous cows; significantly decreased reproductive performance was observed for multiparous cows with lavage fluid LE $\geq +++$ (154 vs. 115 median days not-pregnant), as well as cows with pH ≥ 7.0 (150.5 vs. 111.5 median days not-pregnant), but not in cows with high protein, even at the highest cutoff point. In conclusion, reagent strip test results were strongly associated with cytologic endometritis and reproductive impairment; however, in comparison with conventional cytology, the performance of reagent strip as an alternative test was relatively poor and may require further refinement.

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1. Introduction

Endometritis is an inflammatory uterine disease that persists beyond normal uterine involution and impairs

reproductive performance [1–4]. Affected cows frequently have no external symptoms [5,6]. Diagnostic methods, such as ultrasonographic evaluation of the reproductive tract and uterine content are inferior to cytologic examination of uterine content [1,3], which lead to the proposed disease definition based on cytology as the presence of $>18\%$ neutrophils in uterine samples collected between 21 and 33 days postpartum, or $>10\%$ neutrophils between 34 and 47 days postpartum, in the absence of purulent vaginal discharge [5]. Cytologic evaluation of uterine samples is currently the best method to diagnose inflammatory disease of the

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uterus. In a farm setting, however, this method is inconvenient, as it involves collection of the sample, preparation of the slides and staining, followed by microscopic examination and identification and enumeration of cells. Uterine samples collected using the cyto-brush method [1,7] allow easier slide preparation compared with samples collected using low-volume uterine lavage, but still require the time-consuming cell evaluation step. The lack of a practical cow-side test is a major reason endometritis is not monitored or managed in commercial herds.

A candidate cow-side test for the diagnosis of endometritis from uterine lavage fluid is leukocyte esterase (LE), for example on a reagent strip intended for urinalysis, such as Multistix 10 SG (Bayer Corporation, Elkhart, IN, USA). In a smaller study, Santos et al. [8] reported high sensitivity (83%) and specificity (94%) when using the LE strip to diagnose endometritis. Multistix 10 SG (Bayer Corporation) is a reagent strip of 10 tests, namely: LE, nitrite, urobilinogen, protein, pH, blood, specific gravity, ketone (acetic acid), bilirubin, and glucose. The LE compound is present in neutrophils; therefore, a positive result of this test is the most direct indicator of inflammatory cells in urine using reagent strips. In addition, protein and pH reagent tests may be useful in the diagnosis of endometritis, as well as providing insight into the pathogenesis of the condition. Fluid accumulation in the uterine lumen is used as an indicator of inflammation [1,3] which, if present, could elevate the protein content of the recovered fluid of low volume uterine lavage, making protein concentration a potential diagnostic test. Furthermore, inflammation of the udder or vesicular glands elevates the pH of milk [9] and seminal fluid [10], respectively, but it is unknown if inflammation of the uterus is associated with an elevation of pH in uterine fluid.

The objectives of this study were to: (1) determine if LE, protein, pH, or a combination of reagent strip tests were associated with cytologic endometritis; (2) identify cutoff points for associated reagent tests based on cytology and reproductive outcome; and (3) identify other factors associated with LE, protein, and pH in uterine lavage samples.

2. Materials and methods

2.1. Sample collection

Uterine lavage samples used in this experiment were part of a larger study [4]. The present study was initiated after 10 herds had already been sampled. All samples collected from that point on were included in

the present study. Selection of herds for the study was from a convenience sample of herds that were willing to participate in the study. The inclusion criteria for herds sampled were: located in New York State, large herd size (minimum of 400 milking cows), and used DairyComp 305 (Valley Ag Software, Tulare, CA, USA) for maintaining herd records. The inclusion criteria for cows sampled were: between 40 and 60 days postpartum, apparently healthy (by cursory visual examination), no external vaginal discharge observed by visual examination, not inseminated, and at least 2 days before the end of the voluntary waiting period for that specific farm (average 59 days; range 50 to 70). Herd records were obtained at the time of sampling and reproductive outcomes were obtained by follow-up herd records collected 4 and 6 mo after sampling.

Animal procedures were approved by the Cornell University Institutional Animal Care and Use Committee. Uterine lavage samples were obtained as previously described [2]. Briefly, paper towels were used to cleanse the perineum of the cow, then a 63.5 cm sterile flex tip infusion pipette (Exodus Breeders Corporation, York, PA, USA) was introduced into the uterus through the cervix, and 20 mL sterile saline solution (0.9% Sodium Chloride Injection USP; Baxter Healthcare Corp., Deerfield, IL, USA) was infused into the uterus. Approximately 5 to 8 mL of fluid was recovered by aspiration. The samples were put on ice and transported to the laboratory for analysis. One drop of uterine lavage sample was added to each test on the Multistix 10 SG (Bayer Corporation) reagent strip. Protein and pH results were evaluated after 1 min and the LE result evaluated after 2 min, as per manufacturer instructions. Protein results were recorded in six categories which were: negative, trace, + (30 mg/dL), ++ (100 mg/dL), +++ (300 mg/dL), and ++++ (>2000 mg/dL); pH results were recorded in seven categories: 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5; and LE results were recorded in five categories: negative, trace, + (small), ++ (moderate), and +++ (large). Cytologic evaluation of the uterine lavage samples was performed after cytocentrifugation ($105 \times g$ for 3 min) and staining using Camco stain Pak stain (Cambridge Diagnostic Products, Inc., Fort Lauderdale, FL, USA) by counting 200 cells (neutrophils, lymphocytes, macrophages, and uterine epithelial cells, excluding erythrocytes) and results were expressed as the percentage of total cells. Cows were considered positive for endometritis if neutrophils were >10% of total cells [5].

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