

Accuracy of diagnosis of clinical endometritis with Metrichheck™ in postpartum dairy cows

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Summary

Clinical endometritis, an important condition during the postpartum period in dairy cows, is diagnosed by different methods. A recently-developed vaginal device (Metrichheck™, Simcro Tech Limited, Hamilton, NZ) utilizes information on the nature of vaginal exudate in diagnosis. In the cytobrush method, information about endometrial inflammation as determined by endometrial cytology is used in diagnosis. In this clinical investigation, the accuracy of Metrichheck™ for diagnosis was compared to that of the cytobrush method. Specifically, this study tested the hypothesis that vaginal exudate is not always accompanied by endometrial inflammation. One hundred twenty two cows were chosen at random from a population of postpartum dairy cows that were between 28 and 41 days postpartum. The prevalence of clinical endometritis as diagnosed by Metrichheck™ and cytobrush was 15.6% and 13.1%, respectively. The percent positive agreement was 36.8% and the percent negative agreement was 91.3% between these tests. The sensitivity and specificity measures of Metrichheck™ were 43.8% and 88.7%, respectively. These findings support the hypothesis that a vaginal exudate is not always accompanied by endometrial inflammation. Only 37% of cows diagnosed positive for clinical endometritis by the Metrichheck™ method had evidence of endometrial inflammation.

Keywords: Dairy cow, postpartum, clinical endometritis, Metrichheck™, cytobrush

Background

Early resumption of continuous cyclic ovarian activity in postpartum cows is desirable since it distinctly enhances reproductive efficiency. One of the major impediments to the early resumption of cyclic ovarian activity is postpartum uterine health that is less than ideal.¹⁻⁶ Postpartum endometritis is an important condition amongst postpartum uterine health issues and is influenced by peripartum nutrition and other factors.⁶ Evidence of inflammation in the absence of systemic clinical signs is defined as endometritis.⁷ For clarity, endometritis is further classified as clinical or subclinical. Clinical endometritis is characterized by the presence of a purulent or mucopurulent uterine discharge after 21 or 26 days postpartum⁷⁻⁹ while subclinical endometritis is characterized by the presence of >18% polymorphonuclear inflammatory cells (PMN) in uterine (endometrial) cytology samples collected 21 to 33 days postpartum, or >10% PMN in samples collected at days 34 to 47.^{7,10} Cows with subclinical endometritis do not have a uterine discharge; however, it is very important to recognize that the severity of the disease is still considered sufficient to impair reproductive performance.⁷

The prevalence of endometritis varies depending on the management of the farm, the time the diagnosis is made during the postpartum period, and the method employed for diagnosis. For example, a study conducted in southern Ontario involving 228 cows in two tie-stall dairy farms and using the endometrial cytology technique as a diagnostic procedure, found that the prevalence of endometritis was 41.4% at 34 to 47 days postpartum.¹¹ Another study, conducted in central New York involving 141 Holstein cows in five commercial dairy herds that utilized samples obtained by uterine lavage for diagnosis concluded that at 40 to 60 days postpartum the prevalence of endometritis was 53% and among herds the range was 37 to 74%.¹² These percentages are influenced by the cut-off points adopted for number of PMN. The former study calculated different cut-off points for different days postpartum using receiver operating characteristic (ROC curves) while the latter study arbitrarily selected a cut-off point of 5%. A subsequent study¹³ selected a cut-off point based upon the use of sequential model to detect the least level of PMNs between 5 and 15% that produced a significant decrease in pregnancy rate. Recent studies^{14,15} followed the first approach.¹¹ Endometritis how ever defined or diagnosed has been shown to have a significant detrimental effect on subsequent reproductive performance. Affected cows are at a

higher risk of not being detected in estrus before the breeding season, have a lower first service conception rate and a lower pregnancy rate, and are more likely to be culled.^{9,11-13,16}

Historically, clinical endometritis has been diagnosed and scored by various methods and observations, namely, manual vaginal examination, vaginoscopy, transrectal palpation, transrectal ultrasound examination of the uterus, cytological evidence of inflammation, bacterial culture, and detection of cervical and vulvar discharges.^{9,11-13,17-20} Recently an intravaginal device* been used in a few studies.^{16,21} Other studies^{11,13,22} have used a cytobrush technique to document the cytological evidence of endometrial inflammation.

The objective of the present study was to evaluate the efficacy of Metriceck™ in the diagnosis of clinical endometritis. Information on the vaginal exudate and its nature is used in the Metriceck™ method to conclude whether or not the cow has clinical endometritis. However, diagnosis based on evidence of endometrial inflammation as determined by endometrial cytology^{11,13,22} appears to be sensitive provided the right cut-off value for PMNs is adopted. It has also been suggested that cytobrush is a good reference diagnostic criterion and reliable test for clinical endometritis because of its greater intra-observer repeatability as evidenced by its higher concordance correlation coefficient.¹³ Hence in the present study, observations made by the Metriceck™ technique were evaluated in comparison to those of the of cytobrush method findings. The study tested the hypothesis that vaginal exudate is not always accompanied by endometrial inflammation.

Case presentation

Animals

This clinical investigation was conducted in September of 2008 and involved 122 cows from 11 seasonally calving dairy herds in the Matamata region of New Zealand. Herd size ranged from 300 to 1500 lactating cows maintained on pasture and the percentage of cows chosen for the study from these herds ranged from 1.5 to 3.3 %. As part of reproductive health management, each cow was tested for the presence of vaginal exudate by Metriceck™. Every fourth cow was moved into the chute for the cytobrush procedure. The cows were within 28 to 41 days postpartum with a median of 34 days and their ages ranged from three to seven years with a median of five years. The project was conducted in accordance with the New Zealand Animal Welfare Act, National Animal Welfare Advisory Committee 1999 stipulations.

Vaginal fluid samples

Samples of vaginal fluid were collected by Metriceck™ and evaluated immediately as described previously.¹⁶ This intravaginal device consists of a 4 cm silicon hemisphere with its concave surface attached (facing) the tip of a 50 cm stainless steel rod. Briefly, the Metriceck™ was inserted through the cleaned vulvar lips, advanced to the cranial extent of the vaginal fornix, and with the handle slightly elevated the device was retracted caudally. Material adhering to the surfaces of the silicon hemisphere was assessed visually. This procedure was performed by four veterinarians from Matamata Veterinary Services Ltd.

Endometrial samples

After the collection of vaginal fluid samples, the selected animals were moved into a chute to obtain an endometrial sample. The procedure was conducted solely by the senior author. An endometrial sample for cytological examination was collected using a non-sterile brush (Cytobrush® Plus, GT Cooper Surgical Company, Trumbull, CT) modified for use in cattle as described previously.¹¹ Before threading the brush into the stainless steel rod (65 cm long, 4 mm outside diameter) and its stainless steel sheath (50 cm long, 6 mm outside diameter) both were cleaned with alcohol. The handle of the brush (17 cm) was shortened to 3 cm and was threaded into the hole at the tip of the stainless steel rod. The stainless steel

* http://www.simcro.com/products/other_accessories/metricheck.html

rod was retracted into the stainless steel sheath until the brush was hidden completely inside the sheath. The vulva and perineum were cleaned with a paper towel. The assembled device was covered by a plastic sanitary chemise (IMV Technologies, Maple Grove, MN), and introduced into the vagina. Next, a sleeved arm was introduced into the rectum to facilitate passage of the instrument through the cranial vagina and cervix. At the external os of the cervix, the plastic sleeve was perforated and the metal sheath was manipulated through the cervix into the body of the uterus and the brush was exposed by pushing the metal rod forward and the rod was turned clockwise approximately one quarter turn for the brush to gather cellular material from the adjacent endometrium. The rod was withdrawn into the sheath to prevent the brush from collecting cellular material from the cervical and vaginal area. A slide was prepared immediately and the slides collected at each visit were stained within two h of collection.

Endometrial cytology

Slides were stained using a Romanowsky stain (Diff-Quick; Fisher Diagnostics, Middletown, VA). After drying, each slide was examined using 400 X magnification by the senior author, and a differential count using a minimum of 100 cells (endometrial cells, PMN, and squamous cells) was obtained to provide a quantitative assessment of endometrial inflammation.

Analysis

Material gathered by the Metricheck™ was scored as follows: 0 = no discharge, 1 = clear mucus, 2 = flecks of purulent material within otherwise clear mucus, 3 = mucopurulent but <50% purulent material, 4 = mucopurulent with >50% purulent material and 5 = mucopurulent with >50% purulent material and an odor. For the purpose of data analysis, scores of 0 and 1 were recorded as negative and the rest (2 and greater) were recorded as positive. For the cytobrush, the presence of 8% or more PMN in the endometrial sample was recorded as positive. This PMN cut-off point was chosen based on the findings of a recent study in which endometritis was diagnosed based on the lowest percentage of PMN that was significantly associated with the time to pregnancy.¹³ The McNemar's test was applied to the data to determine whether differences existed between expected results between the two tests. To assess the agreement (percent positive and percent negative agreement) between these tests and to determine the sensitivity and the specificity measures using the Bayesian theory,²³ a specific analysis developed for this purpose was used (SAS Version 9.1 for Windows, SAS Institute, Cary, NC).

Outcome

The prevalence of clinical endometritis as diagnosed by Metricheck™ and cytobrush was 15.6% and 13.1%, respectively. The McNemar's test showed that the different categories which defined the contingency table did not significantly differ from what was expected, an observation necessary to proceed further with subsequent analysis of data. There was a fair agreement between the two tests ($\kappa = 0.30$). In 94 negative cases and seven positive cases Metricheck™ and cytobrush agreed. Twelve cases that were positive by Metricheck™ were negative by cytobrush and nine cases that were positive by cytobrush were negative by Metricheck™. The findings are summarized in the table. The percent positive agreement was 36.8% and the percent negative agreement was 91.3%. In other words, only 37% of the cows that had a vaginal exudate showed evidence of endometrial inflammation. The sensitivity and specificity measures of Metricheck™ were 43.8% and 88.7%, respectively compared to cytobrush.

Discussion

The tests used in the present study may result in over-diagnosis of endometritis because all cows have some degree of endometritis associated with normal uterine involution. Hence the timing when these tests are performed during the postpartum period is very critical. It has been established very clearly that a test conducted after day 28 postpartum has merit in accurately diagnosing the condition^{9,11,13} and furthermore cows that were found positive at this time had lower reproductive performance in the ensuing period compared to negative cases.¹³ Hence, it is suggested that besides using the PMN cut-off points in

defining metritis the effect of metritis in lowering the reproductive performance such as pregnancy rate should also be considered.

The findings of this study support the hypothesis that cows can have a vaginal exudate without evidence of endometrial inflammation. Presence of vaginal exudate in the absence of endometrial inflammation suggests that these cows may have had any of the following conditions during the postpartum period: vaginitis, cervicitis, and possibly resolving endometritis. Thus it is possible that some of the cows that had a purulent exudate in the vagina could have had a healthy endometrium. It is important to note that only 37% of the cows that had a vaginal exudate showed evidence of endometrial inflammation which demonstrated the test's reduced specificity in diagnosing subclinical endometritis. Further studies are required to investigate the economic relevance of this finding. These studies should focus on the impact of treatment on subsequent fertility.

Learning points

Findings of this study suggest that a vaginal exudate as diagnosed by an intravaginal device is not always accompanied by endometrial inflammation. This may result in the over-diagnosis of endometritis when using this technique.

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Table: Prevalence of clinical endometritis as diagnosed by Metricheck™ and cytobrush in 122 cows 28-41 days postpartum.

Cytobrush	Metricheck™		
	Negative	Positive	Total
Negative	94	12	106
Positive	9	7	16
Total	103	19	122

