Research Report

Diagnosis of clinical cervicitis and vaginitis in dairy cows in relation to various postpartum uterine disorders

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Abstract

There is poor agreement between clinical findings and results of diagnostic tests in postpartum uterine diseases that may be explained at least in part by the presence of inflammation and infection at sites other than the endometrium. It was hypothesized that clinical cervicitis and vaginitis are components of reproductive tract inflammatory disorders in postpartum dairy cows. A total of 61 postpartum dairy cows were enrolled in a nested case-control design. Periparturient disease occurrence in weeks 1 (1w), 3 (3w), and 5 (5w) postpartum were recorded. Clinical cervicitis was diagnosed in 36.0, 40.1, and 31.1% of cows in 1w, 3w, and 5w postpartum, respectively. Approximately 64% of cows with clinical endometritis in 5w postpartum also had clinical cervicitis ($p \leq 0.05$). Prevalence of clinical and cytological vaginitis was 0% in 5w postpartum. Average days open: 110 for healthy cows and 117 for cows with clinical cervicitis ($p \leq 0.001$), 145 for cows with clinical cervicitis and clinical endometritis ($p < 0.005$), and 199 for cows with clinical cervicitis and cytological endometritis ($p < 0.001$). Contrary to vaginitis, high prevalence of clinical cervicitis and its association with clinical endometritis and longer average days open suggested a substantial role of the condition in postpartum uterine diseases.

Keywords: Cervicitis, vaginitis, postpartum uterine diseases, neutrophils, inflammation

Introduction

Postparturient cows sustain substantial damage to endometrium along with uterine bacterial contamination, triggering an active inflammatory process that clears cellular debris and bacteria, as well as repairs the endometrium, as part of normal uterine involution. In healthy cows, uterine inflammation eventually subsides and bacteria are eliminated by 4 - 6 weeks postpartum.[1] In scenario where pathogenic bacteria persist in uterus, reproductive tract inflammation is prolonged that has a substantial role in postpartum uterine diseases' pathogenesis.[2,3] In dairy cows, occurrence of postpartum uterine diseases (PUDs) is high (up to 40%) and include clinical conditions like retained fetal membranes, metritis, clinical endometritis, and pyometra, as well as nonclinically evident conditions (e.g. subclinical endometritis). These conditions have negative effects on fertility, causing decreases in pregnancy rates and increases in the number of services per pregnancy and days open.[4] Infertility is directly linked to changes in the uterine environment and ovarian function. Uterine inflammatory response and the presence of bacterial toxins like lipopolysaccharides (*Escherichia coli [E. coil]*) and pyolysin (*Trueperella pyogenes*) have negative effects on ovarian function, resulting in the establishment of anovulatory conditions and postpartum anestrus.[2,5] Distinction between physiological and pathological uterine involution depends on the severity of the disorder, stage postpartum, duration of inflammatory process and, most importantly, whether it impairs cow's fertility at the end of the voluntary waiting period.

Diagnosing PUDs is complicated by a lack of clarity in case definition, use of various gold standard tests, reduced sensitivity and specificity of diagnostic tests, and simply the difficulty

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inherent in exploring bovine reproductive tract.[6,7] Several techniques used for diagnostic purposes are not suitable for widespread use in clinical practice because they are not rapid, neither easy to use nor cost-effective. Sometimes there is poor agreement between clinical findings and diagnostic test results due to an inflammatory process at a site other than endometrium, such as cervix, vagina, or uterus.

Cervix is a self-contained organ of the reproductive tract that serves as an anatomical and functional barrier between vagina and uterus. However, our understanding of cervical inflammation and bacterial contamination, and its subsequent influence on reproductive efficiency in dairy cows, is incomplete. Research has suggested that cervicitis is a distinct condition that has both a separate and additive effect on reproductive performance of dairy cows.[8] Cervicitis has a prevalence of 11 - 30% in dairy cows, and ~ 75% of cows with cervicitis also exhibit clinical or subclinical endometritis.[9] In women of reproductive age, diagnosing cervicitis is difficult, partly because it is frequently asymptomatic and so remains undiagnosed.[10,11] In 20 - 25% women cervicitis is associated with an increased risk of pelvic inflammatory disease and adverse pregnancy outcomes.[10]

Vagina is the female copulatory organ that extends from cervical caudal segment of cervix to vestibular border at the external urethral orifice. One important function of vagina (similar to cervix) is to serve as a line of defence against bacterial invasion by secreting fluids that inhibit undesirable bacterial growth. Very limited information is available on vaginitis in postpartum dairy cows. Objective was to investigate whether cervicitis and vaginitis are potentially a substantial problem in postpartum dairy cows. We examined the occurrence of these 2 disorders during the postpartum period and their associations with other PUDs.

Materials and methods

Research was conducted in compliance with the experimental practices and standards approved by the animal care committee of the University of Montréal (Protocol No. 211-03), and efforts were made to minimize animal suffering in compliance with the Canadian Council on Animal Care guidelines.

Study population and experimental design

This prospective observational longitudinal cohort study was carried out in postpartum Holstein dairy cows (n = 61) between first and third lactation from 3 convenient commercial dairy herds. Nested case-control design was used, cows were enrolled systematically based on calving date. Cows were housed in tie-stall barns and milked twice daily. Rolling herd average for milk production was 9,000 kg. Cows were fed a total mixed ration of corn and hay silage to meet the nutrient requirements of dairy cows as recommended by the National Research Council (NRC, 2011). Farms were visited weekly by the same veterinarian. Cows were vaccinated intramuscularly twice against *E. coli* before calving (2 ml on day 40 and on day 26 before parturition; J-VAC®, Boehringer Ingelheim, Burlington, Ontario, Canada) and once against IBR, BVD (type 1 and 2), PI3, and BRSV after calving (2 ml between days 10 and 30 postpartum; Bovi-Shield GOLD5® FPTM 5 L5, Zoetis, Kirkland, Quebec, Canada). In addition, pregnant cows were injected with selenium 5.0 ml on day 60 before calving (MU-SE, Intervet, Merk, Kirkland, Quebec, Canada).

Cows were examined 3 times: week 1 postpartum (1w), week 3 postpartum (3w), and week 5 postpartum (5w) postpartum. At each examination, a complete clinical and reproductive examination was performed. Reproductive assessment comprised: 1. vaginoscopy to assess cervical and vaginal appearance as well as the presence of purulent vaginal discharge and 2. transrectal ultrasonographic examination (7.5 mHz probe; Ibex, E.I. Medical Imaging, CO, USA) to assess uterine horn symmetry (2.5 cm beyond the external bifurcation).

Cytological sampling

To diagnose subclinical conditions, cytobrush technique[7] was used to collect samples of mucosa from vaginal fornix (1w, 3w, 5w), cervix (5w), and uterus (1w, 3w, 5w), Cytobrush samples were collected using a modified Casou cannula. Double-protected Casou containing cytobrush was inserted into vaginal fornix and uterus. At each location, cytobrush was exposed and rolled over the mucosa before being recovered in the double-protected Casou. New brush and protective sheet were used for each sampling location. Once withdrawn from the reproductive tract, cytobrush was rolled over a sterile slide, fixed, and stained. One smear was used per location and examined under the microscope (400 x magnification) by a reviewer without knowledge of the identity of the cows. Total of 200 cells were counted and characterized.[12] Percentage of polymorphonuclear neutrophils (PMNs) was determined for each 3 sampling sites (vagina, cervix, uterus).

Uterine bacterial culture and identification

Uterine cytobrush samples for bacteriological examination that were only collected in 3w after calving, were subjected to aerobic and anaerobic culturing using standard bacteriology testing methods (API system, Biomérieux, Marcy Étoile, France). Samples for bacteriology were stored in a culture tube on ice (Starplex Scientific Inc, Etobicoke, Ontario, Canada) and transported at room temperature to the Faculty of Veterinary Medicine's diagnostic laboratory within 3 hours. For microbiological analysis, brushes were plated onto sheep blood agar (soy agar with 5% sheep blood; Becton, Dickinson and Co, Sparks, Maryland, USA) using a sterile disposable inoculating loop within 6 hours after arrival. Plates were incubated for 48 hours at 35°C under aerobic conditions and examined. When growth was observed, colony type was identified based on morphology, pigmentation and hemolytic pattern. Very small beta-hemolytic, catalase-negative colonies consisting of gram-positive coryneform rods were identified as *Trueperella pyogenes*. Swabs were quickly plated on Brucella agar containing neomycin (100 g/ml) and incubated anaerobically at 35°C for 5 days. When gram-negative rods were observed, colonies were examined using Analytical Profile Index 20 A gallery for identification of *Fusobacterium necrophorum* and *Prevotella melaninogenica*. For *E. coli* isolation, swabs were plated on blood agar and MacConkey agar (Oxoid, Ottawa, Ontario, Canada) at 37°C at the WOAH Reference Laboratory for *E. coli* (Faculty of Veterinary Medicine, University of Montréal). For confirmation of *E. coli*, isolates were submitted to 3 biochemical tests: indole spot, Simmons citrate, and motility.

Disease definitions and selection of cases and controls

Dairy cows were examined for 7 PUDs, defined as follows: 1. Clinical metritis (MET): an abnormally enlarged uterus and purulent uterine discharge in week 1 postpartum (1w)[13]; 2.

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Clinical endometritis (CE): purulent vaginal discharge (PVD) of uterine origin in weeks 3 postpartum (3w) with normal-sized uterus and no fluid[14]; 3. Cytological endometritis (CYTOe); absence of PVD (3w and 5w) plus PMNs exceeding 5% on cytobrush examination, a normal-sized uterus and no fluid in the uterus[15]; 4. Clinical cervicitis (CC): cervical folds on vaginoscopy in 5w were assessed, where Grade 0 = normal cervical fold, Grade 1 = second cervical fold was swollen without redness and protruding through first cervical fold, and Grade 2 = second fold swollen with redness and protruding through first cervical fold[9]; 5. Cytological cervicitis (CYTOc): PMNs exceeding 5% on cytobrush sampling between first and second fold in 5w; 6. Cytological vaginitis (CYTOv): PMNs exceeding 5% on cytobrush sampling of the vaginal fornix at 5w without purulent discharge; and 7. Clinical vaginitis: red and edematous mucosa on vaginoscopy without purulent discharge. Note that these postpartum reproductive tract diseases were not associated with systemic clinical signs. At each reproductive tract examination, body condition score was recorded.

Variable definitions

Following variables were included in the analysis: 1. herd variables; average parity and herd effect; 2. disease variables: metritis, clinical endometritis, cytological endometritis, clinical and cytological cervicitis (5w postpartum), and clinical and cytological vaginitis (1w, 3w, 5w); and 3. clinical variables: body condition score; cervical, vaginal fornix and uterine PMN counts, vaginal discharge Grade, and uterine asymmetry Grade.

Data analyses

Descriptive analysis was performed using frequency tables to determine the percentage of each variable category in the study population. Percentage of cows affected by various postpartum conditions (MET, CE, CYTOe, CC, CYTOc, and CYTOv) were calculated by dividing the number of cows with the condition by the number of cows studied. Co-occurrence of CYTOv and CYTOc, CYTOv and CYTOe, and CYTOc and CYTOe were assessed using Kappa coefficients. Associations between clinical cervicitis and other postpartum diseases were assessed using contingency tables and Chi-square tests. Logistic regression model was used to explore the association between risk factors and occurrence of CC. Univariate analysis was conducted first for each independent variable (herd, clinical, disease variables), with CC or CYTOv as the outcome variable (separate models for each outcome variable). Variables with a p value < 0.15 were considered for the multivariate analysis that was conducted using backward selection with a p value of 0.05. Confounding and interaction effects were examined via noncausal exposure-outcome associations.[16] Variable parity and body condition score were considered potential confounding variables. Random herd effect in a mixed-effects logistic regression model was explored. Hosmer–Lemeshow test was used to determine the goodness of fit of the logistic regression models. Analyses were conducted using Stata® Statistical Software (Release 15, StataCorp LLC, College Station, Texas, USA).

Results

Study population consisted of 85 cows, but an initial exclusion resulted in the removal of some cows due to culling (n = 5), use of antimicrobials (n = 4), metabolic disease (n = 5) or missing data (n = 10). A total of 61 cows were without symptoms related to systemic illness. Clinical cervicitis was diagnosed in 36.7, 40.1, and 31.1% of cows in 1w, 3w, and 5w postpartum, respectively. Many cases of cervicitis occurred concurrently with another postpartum condition. In total, 47% of cases of clinical cervicitis (CC) were associated with purulent vaginal discharge (PVD), and 21% with cytological endometritis (CYTOe). Only 32% of cases of CC did not co-occur with another clinical condition. Association between cervicitis in 5w and other postpartum diseases in 1w, 3w and 5w is provided (Table 1). Sixty percent (n = 15) of cows with CC and 37.5% (n = 12) of cows with clinical endometritis in 3w postpartum had clinical cervicitis in 5w postpartum (Table 1). However, the association was not significant. Cows with clinical endometritis in 5w postpartum (64.2%, n=9) had clinical cervicitis (p = 0.002). CYTOv, CYTOc and CYTOe (3w and 5w) were not associated with CC in 5w (p > 0.05). Average days open were: 110 for healthy cows, 117 for cows with CC, 145 for cows with cervicitis plus purulent vaginal discharge, and 199 for cows with clinical cervicitis plus CYTOe. There were low to very low levels of co-occurrence between CC and CYTOv (Kappa = 0.024), CYTOc (Kappa = 0.077) and CYTOe (Kappa = 0.132). The results were similar to the co-occurrence of CYTOe and CYTOv (Kappa = 0.041) and CYTOc (Kappa = 0.253). There was slight agreement between vaginal and cervical findings and the results of the cytological evaluation (Kappa = 0.211).

There was no significant herd effect when herd was used as a random effect in a mixed-effects logistic regression model. Univariate logistic regression analysis concluded that the variable parity, body condition score in 1w postpartum, diagnosis of subclinical ketosis (BHB > 1.2 mmol/l) in 1w postpartum, high uterine PMN count in 1w postpartum (> 5%), and positive culture for *T. pyogenes* (15/61) in 3w postpartum were associated (p < 0.15) with clinical cervicitis on 5w postpartum. Cows with clinical ketosis were removed from the study and no other bacteria were present in substantial amounts. Univariate analysis did not detect any associations between independent variables and vaginitis (p < 0.15) and hence vaginitis was not included as an outcome variable in the multivariate analysis.

Results of the multivariate logistic regression model for CC are provided (Table 2). Using a backward elimination process in the final model, body condition score in 1w postpartum was a confounder and was forced in the model. In the model, subclinical ketosis in 1w postpartum increased the odds of CC compared to cervicitis-free cows (OR: 5.2, 95% IC: 1.3 – 19.8), and a positive culture for *T. pyogenes* in 3w postpartum increased the odds of cervicitis compared to cows with a negative culture (OR: 4.2, 95% IC: 1.0 – 16.7).

Discussion

Bovine cervix is a thick-walled cylindrical structure composed of 3 or 4 annular folds that separate the uterus from the vagina. It forms a recess in the vagina known as the fornix.[17] Because of its strategic position, it is exposed to changes that occur in the vagina or uterus, such as infection/inflammation. For that reason, fornix and cervical status can be a good indicator of PUDs. However, in this study, there were low levels of co-occurrence between CC and CYTOv and CYTOe, and between CYTOe and CYTOv that indicated a substantial compartmentalization of the reproductive tract in cows. Based on the assumption that cervical involution is slower than the remainder of the reproductive tract, cervical status in 5w may

Table 1. Contingency table and Chi-square test results: clinical cervicitis diagnosed in 5 weeks postpartum in relation to postpartum uterine disorders diagnosed in 1, 3 and 5 weeks postpartum in dairy cows

Weeks postpartum	Disease		Clinical cervicitis (week 5)	
			No	Yes
1	Metritis	No	26 (70.3%)	11 (29.7%)
		Yes	15 (62.5%)	9 (37.5%)
	Grade 2 cervix	No	25 (65.8%)	13 /34.2%)
		Yes	16 (69.6%)	7 (30.4%)
3	Clinical endometritis	No	22 (75.9%)	7 (24.1%)
		Yes	20 (62.5%)	12 (37.5%)
	Grade 2 cervix	No	32 (88.9%)	4 (11.1%)
		Yes	10 (40%)	15 (60%)
	CYTOv	No	35 (68.6%)	16 (31.4%)
		Yes	7 (70%)	3 (30%)
	CYTOe	No	21 (72.4%)	8 (27.6%)
		Yes	21 (65.6%)	11 (31.4%)
5	Clinical endometritis*	No	37 (78.7%)	10 (21.3%)
		Yes	5 (35.7%)	9 (64.3%)
	CYTOc	No	26 (66.7%)	13 (33.3%)
		Yes	8 (36.4%)	14 (63.6%)
	CYTOe	No	39 (68.4%)	18 (31.6%)
		Yes	3 (75%)	1 (25%)

(%) = Represent the percentage of cows in a row
CYTOv = Cytological vaginitis
CYTOe = Cytological endometritis
CYTOc = Cytological cervicitis
*Pearson Chi-square ≤ 0.05

be an important element in attempts to develop more sensitive, specific and easier to use diagnostic tools for postpartum uterine diseases (e.g. CC and CYTOe). Weak associations between purulent vaginal discharge (resulting in colonization by putative bacteria), presence of endometrial inflammation (as measured by PMNs in uterus), and absence of visible inflammation of vaginal mucosa (clinical vaginitis) in postpartum cows (unpublished data), coupled with the strong reproductive tract compartmentalization, point to the need for a better understanding of reproductive tract involution.

This study examined cervicitis and vaginitis in postpartum dairy cows as diagnosed by visual and cytological examination, respectively, in established cases of CE and CYTOe in the absence of exclusion criteria. Occurrence of CC in postpartum dairy cows at 5w was 31.2% (35-39 DIM) similar to a previous report (26.1% 42–50 DIM).[9]There was a decrease of ~ 22% in the percentage of cows with CC between 3w and 5w (Table 1) but not as important as the decrease of CE (56%). Similar decreases of CE in the same postpartum period were measured in other studies.[12,18]

Our study is the first to report changes in the percentage of cows diagnosed with CC over the postpartum period: 36.7 in 1w, 40.1 in 3w, and 31.1% in 5w. Due to the phenomenon of spontaneous clinical cure during physiological uterine involution, prevalence of postpartum reproductive tract diseases is expected to decrease with the number of days in milk (DIM). However, as cervical involution is slower than uterine involution[19] one would also expect the rate of spontaneous clinical recovery of the cervix to be lower than that of the uterine horn in postpartum cows. Prevalence of CYTOc in 5w in the present study was 6.6%, 42% lower than that reported[8] for a similar postpartum period (< 35 DIM). In the latter study, higher prevalence of CYTOc may have been associated with undiagnosed CE given that no assessment of vaginal purulent discharge was performed. This could also explain the presence of bacteria on their endometrial cytology samples. Similar to that study, we used a threshold of 5% PMNs to diagnose CYTOc. Note that we performed cervical cytology only in 5w.

Clinical cervicitis occurred in conjunction with various PUDs and our final model revealed that CC cases increased (OR = 3.8 p = 0.05) when cows had a positive culture for *T. pyogenes.* There was concomitant CE and CYTOe in 47 and 21% of CC cases, respectively. In a previous study, ~ 12% of cows with CC between 42 and 50 days postpartum also had cytological endometritis.[9] In that study, cows with CE and abnormal uterine content were excluded. In another study where no exclusion criteria were applied, 75% of cows with CYTOe also had CYTOc, and ~ 50% of cows with CE had CYTOc.[8]

Numerous risk factors have been documented for postpartum uterine diseases like MET, CE and CYTOe. However, because studies often have limited statistical power, as is true of this study, it is difficult to rank possible risk factors. In the case of

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Table 2. Results for multivariate logistic regression model of clinical findings associated with clinical cervicitis to 35 days in milk in dairy cows

Disease	Variable	OR	Std. Err.	p	95% CI
Clinical cervicitis	Intercept	0.18	0.03	<0.001	(0.12 – 0.28)
	Body Condition Score				
	1w				
	< 3.0 / 5.0	Ref.	-	-	-
	3.0 – 3.25 / 5.0	0.51	0.46	0.468	(0.08 – 3.04)
	3.5 – 3.75 / 5.0	0.19	0.16	0.062	(0.03 – 1.08)
	≥ 4.0 / 5.0	0.08	0.12	0.079	(0.005 – 1.33)
	Subclinical ketosis				
	1w				
	No	Ref	-	-	-
	Yes	5.22	3.55	0.015	(1.37 – 19.85)
	Trueperella pyogenes				
	3w				
	Negative culture	Ref	-	-	-
	Positive culture	4.25	2.96	0.038	(1.08 – 16.70)

CE, dystocia, twin calves, stillbirth, male calves, retained fetal membranes, and puerperal metritis have been reported as important risk factors.[20] Taking into account independent variables like herds, clinical variables, and disease variables, CC in 5w was significantly associated with CE in 5w, implicating the involution process. If CC is associated with trauma during calving, the condition should be visible earlier in the postpartum period (e.g. in 3w). Additionally, PMNs of > 5% in the uterus in 1w was associated ($p < 0.001$) with CC in 5w. A threshold of 18% may have been more appropriate in 1w and could have altered the results. Similarly, *T. pyogenes* in 3w was associated ($p < 0.001$) with CC. However, presence of PMNs of > 5% in the vagina in 3w was not associated ($p < 0.05$) with CC in 5w.

In the present study, cows with CC and CYTOe had 89 additional days open compared to cows with only CC. Effect of CC on reproductive efficiency is amplified ($p < 0.001$) when associated with other postpartum uterine diseases, thus supporting previous reports for various PUDs.[20] This amplified effect may also be associated with the fact that involution is slower in the cervix than it is in the uterus. Cows affected by CE were 1.7 times more likely to be culled compared to cows without endometritis.[20] Cows with postpartum clinical metritis exhibited reduced conception rates, and time to first insemination was extended by 7.2 days, ultimately leading to subfertility.[21] In addition, postpartum dairy cows with more than 1 disease have poorer reproductive performance. Cows with CE and CYTOe had worse reproductive performance than those with only 1 pathological condition.[20] Similarly, CC reduced reproductive performance,[9] and its negative effect is amplified when the disease is combined with other uterine diseases.

It is believed that postpartum uterine infection/inflammation is caused by bacteria ascending from the vagina or travelling through the vagina from the outside environment when the cervix dilates during parturition,[22] and that this state continues until the uterus returns to normal functioning. However, this concept is not necessarily associated with infection of the vagina as demonstrated (very low prevalence of CYTOv (3%) and absence of clinical vaginitis in the cows) in our study. Vaginal microbiota in dairy cows harbour main uterine pathogens.[23] In addition, it has been hypothesized that the vagina is responsible for a portion of the purulent vaginal discharge observed in postpartum endometritis in dairy cows; however, there was no visual evidence of this in the present study.

Conclusion

Prevalence of CC and CYTOc in 5 weeks postpartum dairy cows was 31.1 and 6.6%, respectively. Clinical cervicitis occurred concomitantly with other common uterine conditions like endometritis and metritis, with 32% of cows exhibiting only CC. High prevalence of CC with its association with clinical endometritis and longer average days open suggested that it would be advantageous to conduct a visual vaginal examination during 5 weeks postpartum as part of a complete reproductive examination. Absence of clinical vaginitis and low prevalence of CYTOv in cows indicated that these conditions do not have an important role in PUDs in high-producing dairy cows. Better understanding of the interaction between CC and other PUDs should be possible with larger studies.

Conflict of interest

Authors declare that they have no conflict of interest that would prejudice their impartiality in conducting this research or in publishing the results.

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