

Comparison of clinical signs and bacterial isolates of postpartum endometritis in holstein dairy cows in Iran

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Abstract

The aim of this study was to determine the uterine bacteria in cows with endometritis and to compare other characteristics in cases of endometritis without bacterial growth, with *Trueperella pyogenes* (*Arcanobacterium pyogenes*) or other bacteria. In total, 86 Holstein cows with postpartum endometritis from 13 commercial dairy herds were sampled once between 21-35 days postpartum. We used several diagnostic techniques for endometritis such as external observation, vaginal exam, rectal palpation, ultrasonography, and cervical and uterine cytological examination. Clear mucus with flakes of pus (E1), mucopurulent discharge (E2), and purulent discharge (E3) are three groups of endometritis. A transcervical double-guarded swab was used for bacterial sampling. The samples were cultured aerobic and anaerobically and biochemical tests were used for differentiation. Measurements were compared between groups: A, no growth (n=47); B, positive bacterial growth without *T. pyogenes* (n=21); and C, positive bacterial growth with *T. pyogenes* (n=18). There were no differences (P>0.05) in uterine wall thickness, body condition score (BCS) and milk yield between the groups. The uterine horn diameter was largest in groups A (4.30±0.88) in comparison with groups B (4.81±1.17) and C (5.53±2.17) (P<0.05). The percentage of neutrophils in smears of the uterine discharge (45.7±33.4) in group C was higher (P<0.05) than in groups A (14.5±22.7) and B (23.5±24.0). Eighty-three percent of group C have shown purulent discharge. In conclusion, the facultative anaerobe *T. pyogenes* may be the most common bacterial agent of postpartum endometritis in Holstein dairy cows in Iran.

Introduction

A recent approach classifies endometritis into two categories, clinical and subclinical.^{1,2} Clinical endometritis is defined as the presence of purulent or mucopurulent uterine discharge detectable externally or in the anterior vagina and/or a cervical diameter greater than 7.5 cm after 26 days in milk (DIM).¹ Subclinical endometritis is defined as the presence of polymorphonuclear leukocytes (PMN) in uterine cytology samples and/or ultrasonographic imaging of fluid in the uterine lumen after 21 days postpartum in the absence of clinical endometritis.²

The varying degrees of endometritis can cause a significant proportion of reproductive disorders. Endometritis is a common reproductive disorder in female domestic animals with a wide range of symptoms including no effect on reproductive performance to permanent infertility. Endometritis affects animals general health and strongly effects their reproductive performance. The lactational incidence of endometritis has been reported from 7.5 to 61.6%.³ Transrectal uterine palpation, vaginoscopic test, uterine fluids cultivation, uteri biopsy and uteri cytology, and also various sampling after birth that have a role in definition of disorder and founding treatment option, are some diagnostic techniques which are used in endometritis cases.¹ According to uterine cytological test the subclinical endometritis is common in dairy cows and has a profound negative impact on reproductive performance.⁴ Subclinical endometritis may occur in many postpartum cows that were previously presumed to be normal. Subclinical endometritis leads to poor reproductive performance in cows.²

Inflammation, histological lesions of the endometrium and delayed uterine involution are some symptoms of the presence of bacteria in uterus.⁵ Furthermore, uterine bacterial infection or bacterial products suppress pituitary LH secretion and disturb growth of postpartum ovarian follicle and its function, which disrupts ovulation in cattle.⁶ Therefore, endometritis is associated with lower mating rates, increased intervals from calving to first service or conception, and more culls for lack of conception.^{1,7}

The aim of this study was to determine the uterine bacterial type in cows with postpartum endometritis and to compare other clinical characteristics in cases of postpartum endometritis without bacterial growth, with *Trueperella pyogenes* (*Arcanobacterium pyogenes*) or other bacteria in Holstein dairy cows in Iran.

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Key words: Clinical endometritis; Bacteria; *Trueperellapyogenes*; Vaginal discharge.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 23 June 2015.

Revision received: 25 August 2015.

Accepted for publication: 1 September 2015.

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Veterinary Science Development 2017; 7:6083
doi:10.4081/bsd.2016.6083

Materials and Methods

Animals

This study was conducted on 13 big commercial dairy herds of Iran. Four hundred and two postpartum dairy cows were examined once between 21 and 35 days after birth. Totally, 86 cows that had clinical endometritis were selected. Cattle were calved in calving boxes hygienically and kept in single boxes for at least 10 days after delivery. The ration that was used for cows nutrition was a combination of corn silage, alfalfa hay, and concentrates. In this research, we did not use any intrauterine or reproductive hormonal therapy at least 10 days before sampling in cattle.

Clinical examination

At first, during the test cattle were investigated in order to find fresh discharge on the vulva, perineum, or tail. If no discharge was visible externally, cows were investigated vaginally. The cow's vulva was cleaned completely using dry paper towel and then a clean, lubricated, gloved hand was inserted via the vulva. Lateral, dorsal and ventral walls of the vagina of each cow were examined and then the mucus contents withdrawn manually for test.⁸ The color and pus ratio of vaginal mucus were examined. Clear mucus with flakes of pus (E1), mucopurulent discharge or fluctuating contents in uteri (E2), and purulent discharge with or without palpable contents in uterus (E3) are three groups of endometritis.⁹

Following the investigation, transrectal palpation of the reproductive tract was done. Some features such as cervical diameter, location of the uterus, symmetry of the uterine horns, diameter of the (larger) uterine horn, texture of uterine wall, palpable uterine lumen, dominant palpable ovarian structure including corpus luteum (CL), follicle or no palpable structures were recorded.¹

Ultrasonographic evaluation of uterus and ovaries was performed using a 5MHz rectal linear probe (AMI Company, Canada). Some parameters were measured in cows, such as Diameter of the uterus, echotexture and thickness of the uterine wall and intraluminal fluid accumulation. The overall shape of ovaries was scanned.¹⁰

Collecting uterine swab and bacteriological culture

For each cow, a transcervical protected swab was collected from body of uterus. The swab was comprised of a long copper wire bearing a cotton wool tip sheathed in a metal guard tube (8 mm external diameter; 58 cm long), and was wrapped and sterilized at 121°C for 15 min by autoclaving. The guard tube was covered with sterile plastic sheath in order to prevent contamination of the swab during the cervix insertion.

The cow was restrained and its tail secured and then the perineal region was washed and cleaned. Firstly, the cervix was differentiated from rectum and then a sterilized catheter was passed via the cervix into body of uteri. For sampling, the inner rod of catheter was pushed forward in order to expose the swab to endometrium, the swab was rotated opposite the uterine wall and finally withdrawn within the catheter. The catheter was cleaned with alcohol to avoid contamination. Swabs were cultured on sheep blood agar and MacConkey agar (MERCK) immediately, and incubated at 37°C for 48 h. The same cultivation on sheep blood agar (MERCK) was incubated anaerobically for up to 7 days. Samples were isolated and identified using standard biochemical tests.¹¹ Results of bacteriological cultivation were categorized in three groups: A, no growth; B, positive bacterial growth without *T.pyogenes*; and C, positive bacterial growth with *T.pyogenes*.

Cervical and uterine cytology

Cytological samples were kept from discharge of cervical mucus and uterine fluid. Samples could be collected by two methods: i) gentle suction from the cervical external and ii) with a plastic pipette, then samples were aspirated by suction with a 50 mL syringe.¹² Each uterine swab was used for bacteriological culture and cytological examination. After the samples had been taken the swabs were rolled

on glass slides.

Thin smears were prepared for cytological test by smearing a drop of cervical mucus on a clean slide and then left to dry at room temperature for 30-35 minutes. Slides were moved to laboratory and examined within two hours after collection. Giemsa-stained slides were used for differential cell count of each smear in each of the 20 microscopic fields (900); 100 to 200 cells were counted. Types of recorded cells were epithelial, large vacuolated epithelial, neutrophils, lymphocytes and macrophages.¹³

Blood sampling and P4 assays

Blood samples were collected from the artery or coccygeal vein into evacuated tubes and moved on ice to the laboratory. Blood serum was separated by centrifugation at 2500 × g for 10 minutes and then stored at -20°C until required. Radioimmuno assay was used to measure plasma progesterone concentration (Spectria® Progesterone RIA, Espoo, Finland) with a sensitivity of 0.1 ng/mL and intra- and inter-assay coefficients of variation of 10.2 and 6.5 % respectively.

Statistical analysis

SAS software, version 9.1 was used for data analysis. T test and One Way ANOVA were used for comparison of cervical and uterine cells of endometritic cows with different discharges. To determine significant difference between groups, Duncan multiple range test was used as a *post hoc* test. Chi-square and Fisher exact tests were used to determine association of ovarian structure, P4 level of serum and discharge status with bacterial culture results in postpartum endometritic cows. P<0.05 was considered as a level of significance.

Results

The uterine horn diameter was higher in group C (5.53±2.17) compared to A (4.30±0.88) and B (4.81±1.17) groups. The percentage of neutrophils in smears of the cervical mucosa and uterine discharge in group A (14.5±20.6, 14.5±22.7) and B (12.7±17.4, 23.5±24.0) was lower than group C (26.7±22.4, 45.7±33.4). There were no differences in uterine wall thickness, body condition score (BCS) and milk yield between the groups (Table 1).

There was a significant difference between the uterine horn diameter and the size difference between the left and right uterine horns in cows with endometritis category E3 (5.47±2.15) compared to category E1 (4.34±0.91) and E2 (4.81±1.17). The percentage of neutrophils in smears of the cervical mucosa (28.05±26.81) and uterine discharge (42.70±30.93) in group E3 was higher

(P<0.05) than group E1 (12.65±18.37, 16.27±23.86) and group E2 (14.92±10.92, 27.36±31.91) respectively. There were no differences in uterine wall thickness, palmar P4 concentration, BCS, parity and milk yield between the groups (Table 2).

Eightythree percent of group C showed purulent discharge. There was no significant difference between the cervical and uterine cytology of the endometritic cows. There was a significant difference between the same cells in different status of uterine discharge (Tables 1-3). Ovarian characters and P4 level of serum were independent from the bacterial culture results (Table 3). Vaginal mucus characters were compared with bacterial culture results (Table 4).

Discussion and Conclusions

The objective of this study was to compare bacterial growth and clinical characters, in cases of affected, to postpartum endometritis. Higher uterine horn diameter and percentage of neutrophils in cervical and uterine mucosa in cows with endometritis grade 3 were shown in our study. Similarly, Westermann and colleagues¹⁴ found a significant positive correlation between findings of *T.pyogenes* and PMN, but not between other bacteria and PMN. The results of one sample may not give an accurate picture of the infection status.¹⁵ However, the intrauterine bacterial infection does not necessarily mean a clinical manifestation of disease; this depends on the immune status of the host.¹⁶ The uterine bacterium in cattle with postpartum endometritis was evaluated. The clinical severity and other characteristics in cases of postpartum endometritis without bacterial growth, and with *Trueperella pyogenes* or other bacteria in Holstein dairy cows in Iran were compared.

Postpartum endometritis has a negative effect on the reproductive performance, such as increased services per mating, the calving to first service interval and the calving to conception interval,¹⁷ reducing the pregnancy ratio,¹ and decreasing the conception rate.¹⁸ It is believed that *T.pyogenes* is the most severe pathogen of the genital tract, and is rarely isolated alone in the postpartum period.^{1,19} In the first few days after calving, *E.coli* is dominant in the uterus and *T.pyogenes* is found later in animals with severe clinical endometritis.⁷ Various research have confirmed that most of the clinical and reproductive aftermath are related to the presence of certain and non-specific pathogens: mainly to *T.pyogenes*, either singly or along with other bacteria such as *E.coli* and gram negative obligate anaerobes.¹⁶ Separation of *T.pyogenes* at the late involution period (28-35 days after calving) is related to

significant reduction in re-conception rate.⁷ *Trueperella pyogenes*, *E. coli*, and non-differentiated *streptococci*, *staphylococci* and *bacilli* were the most commonly isolated bacteria in endometritis.²⁰ Gram-negative anaerobes and other facultative pathogens including *T. pyoge-*

nesare important pathogens that cause severe uterine inflammation.²¹ There is general agreement that *T. pyogenes* and *E. coli* are relevant uterine pathogens, *T. pyogenes* being the most frequently isolated species {LeBlanc, 2002 #18692}.¹⁶ Uterine pathogens, however,

were also isolated from the uteri of cows that did not show clinical signs of endometritis.⁷ In accordance with previous reports *T. pyogenes* was the most prevalent bacteria in our study and positively related with vaginal discharge score,^{7,9} although *E. coli* is thought to play a

Table 1. Comparison of bacterial culture results and clinical characteristics of endometritis cows at 21-35 days in milk.

Clinical characters	Bacterial culture		
	No growth (n=47)	Mixed growth (n=21)	<i>Trueperella</i> (n=18)
BCS, 1-5	2.82±0.31	2.63±0.35	2.69±0.28
Larger uterine horn, cm	4.30±0.88 ^a	4.81±1.17 ^{ab}	5.53±2.17 ^b
Difference of 2 horns, cm	1.16±0.69 ^a	1.52±0.80 ^a	2.33±1.67 ^b
Uterine wall thickness, mm	4.93±2.17	5.26±1.76	5.94±2.04
Parity	2.52±1.39	2.94±1.92	2.19±1.38
Milk yield, kg	29.84±10.90	32.74±11.57	32.31±9.55
P4, ng/mL	1.22±1.63	1.15±2.12	1.39±2.23
Cervical PMN, %	14.51±20.58 ^a	12.71±17.40 ^a	26.67±22.38 ^b
Uterine PMN, %	14.54±22.70 ^a	23.55±24.04 ^a	45.73±33.41 ^b

Data are mean ± standard deviation. Values with different superscript in the same row are significantly different.

Table 2. Comparison of endometritis classification and clinical characters of endometritis cows at 21-35 days in milk.

Characters	Endometritis classification		
	E1 (n=58)	E2 (n=12)	E3 (n=19)
BCS, 1-5	2.75±0.32	2.79±0.38	2.68±0.27
Larger uterine horn, cm	4.34±0.91 ^a	4.83±1.23 ^{ab}	5.47±2.15 ^b
Difference of 2 horns, cm	1.22±0.71 ^a	1.50±0.77 ^a	2.21±1.72 ^b
Uterine wall thickness, mm	5.12±2.30	5.37±1.43	5.60±1.83
Parity	2.57±1.50	2.54±1.86	2.50±1.38
Milk yield, kg	29.45±10.45	31.10±10.13	34.90±11.82
P4, ng/mL	1.12±1.79	1.53±1.69	1.40±2.25
Cervical PMN, %	12.65±18.37 ^a	14.92±10.92 ^a	28.05±26.81 ^b
Uterine PMN, %	16.27±23.86 ^a	27.36±31.91 ^{ab}	42.70±30.93 ^b

Data are mean ± standard deviation. Values with different superscript in the same row are significantly different (P<0.05).

Table 3. Comparison of ovarian characters and clinical characters of endometritis cows at 21-35 days in milk.

Characters	Ovarian characters		
	No structures (n=5)	Follicle ≥8 mm (n=57)	Follicle + corpus luteum (n=27)
BCS, 1-5	2.75±0.25	2.66±0.30	2.90±0.32
Larger uterine horn, cm	4.60±0.82	4.71±1.50	4.54±1.18
Difference of 2 horns, cm	1.80±0.57	1.53±1.18	1.28±0.90
Uterine wall thickness, mm	7.00±1.87 ^a	5.46±2.28 ^{ab}	4.52±1.37 ^b
Parity	3.67±2.52	2.24±1.45	3.00±1.38
Milk yield, kg	26.97±14.25	30.01±10.76	33.34±10.23
P4, ng/mL	0.43±0.38 ^a	0.51±1.19 ^a	2.94±2.12 ^b
Cervical PMN, %	14.60±19.33	18.91±22.78	10.92±14.09
Uterine PMN, %	45.20±42.27	20.96±25.29	23.20±30.55

Data are mean ± standard deviation. Values with different superscript in the same row are significantly different (P<0.05).

Table 4. The relationship between microbial growth and vaginal discharge.

Bacteria	Vaginal mucus character			Total
	Mucus with flecks of pus	Mucopurulent	Purulent	
No growth, (%)	40 (46.51)	6 (6.97)	1 (1.16)	47 (54.65)
Mixed growth (%)	13 (15.11)	5 (5.81)	3 (3.49)	21 (24.41)
Arcano. (%)	2 (2.32)	1 (1.16)	15 (17.44)	18 (20.93)
Total (%)	55 (63.95)	12 (13.95)	19 (22.09)	86 (100)

There was significant difference (P<0.0001) between vaginal mucus character groups.

key role in the metritis-endometritis complex.^{9,22} To determine the origin of exudates (which may stem from the uterus, cervix, or vagina) the vaginal speculum test can help.⁹ Some researchers reported that the most useful tool for the diagnosis of endometritis is vaginoscopy for detection of pus and cervical contents,^{1,3} while Sheldon and Dobson²³ confirmed that the most useful method for diagnosis of endometritis is the examination of the contents of the vagina for the presence of pus. In the present study, we compared bacterial isolation and the vaginal mucus characters. Positive results from bacterial culture were more commonly associated with mucopurulent or purulent vaginal mucus. These observations are in agreement with Williams and colleagues.⁹ They reported that the bacterial growth densities for recognized and potential pathogens were associated with purulent vaginal mucus, and higher growth density for recognized pathogens was associated with mucopurulent mucus character. Pus is a combination of bacterial infection and a mixture of viable and dead neutrophil leucocytes, necrotic tissue and tissue fluid, so it is not surprising that bacterial growth density of pathogenic bacteria is associated with purulent vaginal mucus.⁹ The associations found between a vaginoscopy examination performed 28-37 days prior to Mating start date and subsequent reproductive performance indicate that vaginoscopy is an effective tool for predicting cows with reduced odds of pregnancy rate.²⁴

There were also significant differences in neutrophils percentages between different groups of bacterial culture results and between different grades of endometritis severity, however, the results of this study support that the increase in neutrophils percentage in uterine fluid was in accordance with the increase of neutrophils percentage in cervical mucosa. The results of this study also revealed that the contamination of uterus with *T. pyogenes* causes increased neutrophils to migrate to the uterus, and the severity of endometritis. The color of normal discharge ranged from dark brown to red or white and usually should not be considered abnormal unless there are other aberrant clinical signs or if the discharge is malodorous.¹ Presence of a white or whitish-yellow mucopurulent vaginal discharge is one of the symptoms of endometritis.⁸ The volume of the discharge is variable, but increases at estrus stage when the cervix dilates and there is great vaginal mucus. The uterine PMN% was a better predictor of reproductive performance than either intra-uterine bacteriology or gross vaginal inflammation score.²⁵ Cows in the highest quartile for PMN% at both days 28 and 42 had lower pregnancy rates, took longer to conceive, and had a lower milk yield than those in the lower PMN% categories.

It is believed that the uterus is less sensitive

to invasion by pathogens or experimental bacterial challenge after delivery in animals until the first ovulation, and during the follicular phase of the next cycles. In a model study²⁶ progesterone treatment increased the susceptibility of animals to intrauterine inoculation with *T. pyogenes* and *E. coli*, and also reduced the circulating PGF₂, and elevated the plasma PGE₂ for a few days after treatment. Using concanavalin (A-stimulated lymphocyte proliferation) measurement we found that ovariectomy increased and progesterone treatment decreased the cellular immune response.^{26,27} Progesterone makes the uterus susceptible to infection after calving, ovariectomy allows the dam to maintain resistant, and prostaglandins of uterus may mediate the process.^{26,27} In conclusion, the present study provides evidence that *T. pyogenes* may be the most common bacterial agent of postpartum endometritis in Iranian dairy farms. Choice treatment of postpartum cows affected by *T. pyogenes* can reduce the severity of endometritis, prevent chronic endometritis and repeat breeder cows. Consequently, more study needs to be done to detect the relationship between *T. pyogenes* and infertility in dairy cows.

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