Prevalence of opportunistic fungi and their possible role in postpartum endometritis in dairy cows

Abdollah Derakhshandeh,[1]
Seyed Morteza Aghamiri,[2,3]
Mohammad Rahim Ahmadi,[2]
Abdollah Mirzaei[2]

[1]Department of Pathobiology, and[2]Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University; [3]Department of Clinical Sciences, School of Veterinary Medicine, University of Shahid Bahonar Kerman, Iran

Abstract

The aim of this study was to identify fungal infections by culture from uterine lavages of 172 Holstein dairy cows between 25 and 35 days postpartum and two weeks later. In the first examination, 61/172 (35.5%) cows were suffering from clinical endometritis. The positive rate of fungal growth was fifteen (8.7 %) swabs and the remaining 157 (91.3%) showed no fungal growth. The most frequently isolated fungi were *Aspergillus* spp. (60%) followed by *Penicillium* spp. (26%) and Yeast (13%). In the second examination, 20/128 (16%) cows showed endometritis. Nine (5.5%) swabs were fungal positive. No significant differences between cows with positive and negative fungal cultures in the percentage of polymorphonuclear leukocytes of cytological samples were seen. In conclusion, treatment of cows affected with postpartum endometritis with intrauterine infusion of oxytetracycline, hygiene of bed, number of cows in one yard, age and parity of cows may cause increase in incidence of mycotic endometritis.

Introduction

Endometritis is an inflammation limited to the endometrium without clinical signs.[1] Physical damages to birth canal during parturition could result in an upsurge of microbial infections in the cow.[2] During the first weeks after parturition, immune responses of cows eliminate the microbes. But up to 40% of animals still have a bacterial infection three weeks after calving.[2,3] Also, the fungi are capable of infection in the uterus in cows.[4,5] Increased time of pregnancy and lower conception rates occur after uterine infections.[1,6] There have been many studies regarding pathogenic bacteria that showed their important role in occurrence of endometritis. But fewer surveys have been done on fungal infections of the postpartum uterus in dairy cows. Fungal infections of genital tracts are becoming more common because of indiscriminate use of antibiotics and hormonal therapy.[4] The aim of this study was to identify fungal infections by culture of uterine lavages in Holstein dairy cows between 25 and 35 days postpartum and two weeks later.

Materials and Methods

Animals

The study was carried out in a large commercial dairy farm near Shiraz, Fars province, in the south of Iran (29°58 34 N, 52°40 45 E). One hundred and seventy two postpartum dairy cows (1st and 2nd calving) were examined twice, between 25 and 35 days postpartum and two weeks later. The farm milked 1900 Holstein cows three times daily. Cows were housed in freestall barns with mat bedding for primiparous and sand bedding for multiparous cows. Cows calved throughout the last year and the herd had annual average milk yields of 8800 liters per cow. The cows received corn silage, alfalfa hay and concentrates (containing corn meal, soybean meal, vitamins and minerals). The cows were maintained in close-up dry group for three weeks before calving. The cows calved in an open shed barn. Fresh cows were kept in a transition group for one month. None of the cows received any intrauterine or reproductive hormonal therapy for at least 14 days before sampling. All cows were examined once between 25 and 35 days postpartum and reexamined in the next 14 days. In the first examination, samples were obtained from 172 cows and in the second examination samples were obtained from 128 cows in 2011 and 2012 during the winter season. After examinations, Prostaglandin F2 and Oxytetracycline (OTC) were administered to all cows with clinical endometritis.

Clinical examination

During examination, the cow's vulva was thoroughly cleaned and disinfected using a Savlon (chlorhexidine and cetrimide) solution, also lubricated, gloved hand was inserted through the vulva and the mucus contents of the cranial vagina were withdrawn manually for examination. The vaginal mucus was assessed regarding its color and proportion of pus. Endometritis was classified into three categories: clear mucus with flakes of pus (E1), mucopurulent discharge or fluctuating contents in the uterus (E2), and purulent discharge with or without palpable contents in the uterus (E3) (7). Then, cows were classified into 2 groups: healthy (vaginal discharges score ≤E1) and endometritis affected (vaginal discharges score ≥E2).[8]

Uterine samples collection

Uterine secretion samples were collected as follows: cows were restrained and the perineum area was cleansed and disinfected using a Savlon (chlorhexidine and cetrimide) solution. Sterile covered plastic infusion pipettes (pipettes were first autoclaved and then put on inside plastic sheaths, UV light was used to sterilize covers) were inserted into the cranial vagina and passed through the caudal cervix. The sheath was subsequently ruptured and the sterile pipette tip was manipulated through the cranial cervix into the uterus. A total of 60 mL of sterile saline solution was injected into the uterus, agitated gently, and a sample of the fluid was aspirated. The volume of recovered fluid ranged from 2 to 5 mL. Samples were maintained in ice prior to laboratory processing.[9]

Correspondence: Mohammad Rahim Ahmadi, Departments of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Postal Code: 7144169155, Shiraz, Iran.
Tel.: +98.917.7001074 - Fax: +98.322.86940
E-mail: rahmadi@shirazu.ac.ir

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Cervical sample cytology

Cytological samples were obtained from the discharge of cervical mucus. Cervical aspirated samples were collected by gentle suction from the cervical external os with a plastic uterine pipette and aspirated by suction with a 50 mL syringe. Once the samples had been taken, the swabs were rolled on glass slides. Thin smears were prepared for cytological examination by smearing a drop of cervical mucus on a clean slide. The smears were then allowed to dry at room temperature for 30-35 minutes. Slides were transported to the laboratory and examined within two hours of collection. A differential cell count of each smear was done on Giemsa-stained slides. One hundred to 200 cells were counted in each of 20 microscopic fields (×900). Recorded cell types were epithelial, large vacuolated epithelial, neutrophils, lymphocytes and macrophages.[10]

Fungal culture

Fungal isolation Sabouraud's Dextrose agar (SDA) spot inoculation technique was employed. The samples were inoculated against SDA and incubated at 25°C for 2 weeks. Chloramphenicol was used in the agar media for initial fungal isolation. Duplicate culture was used for each sample. The cultures were examined daily for any mycobiotic growth during the incubation period. Visual examinations of the fungal colonies were made, and their colonial morphology or characteristics, such as texture, pigment and rate of growth on media, were recorded. For microscopic examination, the fungal culture was stained as wet monut with lactophenol cotton blue stain. Identification of fungal agents was made on the basis of colony characteristics and staining reaction was observed under the microscope.

Statistical analysis

In the first and second examinations, percentage of cows with and without endometritis for fungal culture results was statistically analyzed with the Chi-square test using SPSS (SPSS for Windows, version 11.5, SPSS Inc, Chicago, IL, USA). Comparison of the neutrophil percentages between different groups of studied cows for fungal culture was done by Independent-Samples T-test at the first and second examinations. Data presented as the number (percentage) and probability values of $P \leq 0.05$ were considered statistically significant.

Results

In total, 172 Holstein cows were selected and sampled at 25-35 days postpartum. Of these, 128 cows were sampled again, two weeks later. In the first examination (25 to 35 days postpartum), assessment of vaginal mucus showed 61 out of 172 (35.5%) cows were suffering from clinical endometritis (vaginal score ≥E2). Fifteen (8.7%) swabs were found fungal positive and the remaining 157 (91.3%) showed no fungal growth (Table 1). In the first examination, the most frequently isolated fungi were *Aspergillus* spp. 9 (60%) *Penicillium* spp. 4 (26%) and Yeast 2 (13%) respectively (Table 2).

In the second examination (39 to 49 days postpartum) 20 out of 128 (16%) cows showed endometritis. Among them, 9 (5.5%) swabs were fungal positive (Table 1). All 9 swabs were collected from clear mucus in the second examination.

In the first examination, 10 cases were affected by endometritis and treated by intrauterine Oxytetracycline. But 5 cases were clean and did not receive any intrauterine infusion. In the first examination, 10 out of 15 positive fungal were in the first parity cows and just one case was positive, yet in the second examination all fungal agents were isolated from healthy cows (Table 1).

In the first examination, corpus luteum (CL) was presented in five cases of positive fungal culture. In the second examination, four positive fungal culture cases had corpus luteum.

During 100 days after calving, 45.2% (71/157) of negative fungal culture cows became pregnant. Among positive fungal culture cows, 40% (6/15) of cases became pregnant during 100 days postpartum (P>0.05) and the other 9 cases were open in this period.

In the second examination, five out of nine positive fungal culture cows received intrauterine OTC in the first examination.

The number (percentage) of the negative and positive fungal cultures at the first and second examinations of healthy and endometritic cows is shown in Table 1. In the first examination, endometritic cows had a significantly higher rate of infection to fungi compared to healthy cows (P<0.05).

The results showed that 39.1% of cows with positive fungal cultures needed ≥3 inseminations and 25.4% of cows with negative fungal

Table 1. Number (%) of the fungal negative and positive culture at the first and second examination for healthy and endometritic cows.

	Healthy cows (%)	Endometritic cows (%)
First exam		
Negative	105 (94.6)	52 (85.2)
Positive	6 (5.4)[a]	9 (14.8)[b]
Total	111 (100)	61 (100)
Second exam		
Negative	99 (91.7)	20 (100)
Positive	9 (8.3)	0 (0)
Total	108 (100)	20 (100)

[a,b]Values within row having different superscripts differ significantly (P<0.05).

Table 2. Number of isolated fungi in the uterine lavage at the first and second examination.

	First examination		Second examination		Total
	Affected cows	Healthy cows	Affected cows	Healthy cows	
Aspergillus spp.	2	7	0	1	10
Penicillium spp.	3	1	0	0	4
Cladosporium spp.	0	0	0	3	0
Scopulariopsis spp.	0	0	0	2	2
Rhizopus spp.	0	0	0	1	1
Yeast	1	1	0	2	4
Total	6	9	0	9	24

cultures were conceived after three or more inseminations (Table 3; P=0.17), however, this difference was not significant.

The results of cytological change percentages of PMNs (mean ± SD) of the negative and positive fungal cultures at the first and second examination are shown in Table 4. There was no significant difference between cows with positive and negative fungal cultures in the first and second examination (P>0.05).

Discussion

The uterine lumen was sterile before parturition. After parturition, the microorganisms inflow from the animal's environment, skin, and feces to the uterine lumen.[11] The fungi can invade tissues and cause clinical infections.[4] About 100 species of fungi are generally identified as pathogens of humans and animals.[4] In the present study, six different genera, *Aspergillus*, *Penicillium*, *Cladosporium*, *Scopulariopsis*, *Rhizopus* and Yeast were isolated from healthy and endometritic cows (Table 2). The isolation of fungi in the first examination was higher than the second (8.7% *vs* 7%); however, this difference was not significant (P>0.05). In the first examination the growth of fungi in endometritis affected cows was higher than healthy cows. But in the second examination there was no isolation of fungi in the endometritic cows and there were nine isolations in the healthy cows. Verma *et al*. reported mycobiotic agents from 27.8% and 33.3% of endometritic buffaloes and cows, respectively.[4] Contamination of fungi in endometritic and healthy cows was 39.34% and 28.57% respectively.[12] Sharma and Singh found 15.5% mycotic isolation in repeated breeder cows.[13] Ramsingh *et al*. have reported that out of 168 uterine discharge samples from repeated breeder endometrtic cows, a total of 168 (10.5%) mycotic isolates were recorded and identified.[14] In our study, there was a lower prevalence of mycobiotic agents. This may be because of the hot and dry climate of Shiraz. The cows in our farm were housed in mat or sand bed. However, the examined cows in the present study were young and have greater ability to clean their uteri from contaminations. Other effective factors are the density of animals and topographic variations.[4]

Aspergillus spp. were the most important fungi isolated from all samples (10 from 24) which means from samples in first examination (9 from 15) and from endometritis affected cows (7 from 9). According to the survey of Verma *et al., Aspergillus* spp. was reported as the most important mycobiotic agent, 43.7% (7/16), among endometritic cases in cows and buffaloes.[4] Also, *Aspergillus* spp., *Penicillium* spp. and *Cladosporium* spp. were the highest isolated fungi from vaginal mucus of cows with complicated puerperium.[15] In another survey, *Penicillium* spp. and *Candida albicans* were the most common isolated fungi. *Penicillium* spp. was more commonly isolated from uterus of cows with reproductive diseases and *Candida albicans* was more frequently isolated from healthy uterus.[12]

Cows with lower rate of infected uteri to fungi get pregnant during a 100 day period and a higher percentage of them require three or more inseminations to become pregnant, although it was not significant. Vlcek *et al*. found increase of the insemination interval, service-period and insemination index in cows that yielded pathogenic and potentially pathogenic micromycetes.[15]

No significant difference between cows with positive and negative fungal cultures in the first and second examinations (P>0.05) confirmed that the immune system did not respond to the exposure of uterus to fungi. This issue is reported for the first time and to the best of the researchers' knowledge there have been no reports regarding this subject. No significant difference between conception in positive and negative fungal cultures may indicate that the immune system in reproductive tract of cow with positive fungal culture was unaffected. In the second examination, there were five cows with contaminated uteri to fungi that received intrauterine OTC in the first examination. It is suggested that use of intrauterine antibiotics can cause fungal infections.[4,14] Antibiotic therapy, especially from intrauterine route, or corticosteroid therapy, might eliminate bacterial agents from the reproductive tract but produce immunosuppression and trigger fungal infections.[4]

Table 3. Number (%) of cows that had fungal negative and positive culture in both exams based on the service per conception.

Service per conception	Fungal culture	
	Negative (%)	Positive (%)
≤2	103 (74.6)	14 (60.9)
≥3	35 (25.4)	9 (39.1)
Total	138 (100)	23 (100)

Table 4. Comparison of cytological change of neutrophils percentages (mean ± SD) of the fungal negative and positive culture at the first and second exam.

Fungal culture	Examination	
	First exam	Second exam
Negative	11.3±14.3 (142)	8.2±13.6 (135)
Positive	11.6±9.3 (13)	4.1±3.2 (9)

Conclusions

In conclusion, treatment of cows affected by postpartum endometritis with intrauterine infusion of antibiotic such as OTC, hygiene of bed, number of cows in one yard, age and parity of cows may increase incidence of mycotic endometritis. The clinical signs of endometritis may be not found in cows that were affected by mycotic endometritis.

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