

Mycoflora of cervicovaginal fluids in dairy cows with or without reproductive disorders

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Abstract Fungal infection of reproduction system of dairy cattle have not been received much more attention. The aim of this study was to determine the fungal infection of Holstein dairy cows with reproductive disorders or healthy. Fungal isolates of cervicovaginal fluids of 176 Holstein dairy cows were collected by using the double rod swabs for cervix and the sterilized cotton swabs for discharges of vagina. They were evaluated for fungal infections. The treatment group included 70 dairy cows with reproductive diseases, such as abortion, repeat breeder, endometritis, metritis, retention of fetal membrane, dystocia, cervicitis, and vaginitis. The control groups were included 42 healthy non-pregnant cows and 64 pregnant cows. Isolates of fungi were obtained from cervix and vagina of 27.1% and 28.6% of treatment group, 26.7% and 31.2% of

pregnant cows, and 33.3% and 21.4% of healthy non-pregnant cows, respectively, indicating no significant differences. It is showed that the cervix and vagina of the treatment group have been infected by six different mycotic isolates. However, the cervix and vagina of pregnant and non-pregnant healthy cows in control group were infected with 5, 6 and 5, 4 different fungal agents, respectively. *Penicillium* and *yeast* were the most common isolated agents. Regarding to the result of this study, it is concluded that fungal infections can occur in cervicovaginal cavity of Holstein dairy cows with or without reproductive diseases.

Keywords Mycoflora · Cervicovaginal · Dairy cows · Reproductive disorders · Healthy reproduction system

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Introduction

Fungal infections of the female reproductive system of domestic animals have not received much attention in the past. However, with use of antibiotics and hormonal therapy, for treatment of genital system, mycobiotic infections are becoming more common. Vaginitis and cervicitis are important diseases that may influence on reproductive performance, and knowledge of continuous or occasional fungal isolates living in the animals' cervicovaginal environment is relevant to a better understanding of these

pathological processes. However, few data on the impact of fungal cervicovaginal infections have been described in dairy cattle.

Fungal microorganisms have been isolated from the genital area of various animal species. Different fungi could cause genital disorder by *Aspergillus* spp., *Penicillium* spp., *Acremonium* spp., *Aureobasidium pullulans*, *Cladosporium* spp., *Mortierella* spp., *Candida*, *Zygomycetes* and yeast has been observed in cows and buffalos [1, 2]. Endometritis and cervicitis, due to fungal infection in mares has been described [3–5]. Fungal contamination of the normal vagina are found in female dogs and vary according to the stage of the estrous cycle which is known to constitute an endogenous source of infection [6]. Different fungi are isolated and identified from vaginal flora in lions [6]. In women, the prevalence of vulvovaginites (mainly with source of *Candida*) is described [7]. It has been estimated that approximately 75% of women experience at least one episode of vulvovaginitis during their lifetime [8].

The recent study was undertaken in order to determine the occurrence of fungi in the cervicovaginal fluids of dairy cows with reproductive diseases and healthy.

Materials and methods

Samples were obtained from 176 Holstein dairy cows. The treatment group included 70 dairy cows with reproductive diseases such as abortion (No: 4, 5.71%), repeat breeder (animals that had a regular estrous cycle but failed to conceive after three artificial inseminations) (No: 8, 11.42%), endometritis (No: 14, 19.44%), metritis (No: 13, 18.57%), retention of fetal membrane (No: 5, 7.14%), dystocia (No: 9, 12.85%), cervicitis (No: 9, 12.85%) and vaginitis (No: 8, 11.42%). The control groups were involved 42 healthy non-pregnant cows and 64 pregnant cows. The external genitalia were thoroughly washed with 5% povidine Iodine solution and lips of the vulva were dried with sterilized tampon prior to sample collection. The vaginal walls were manually spread in order to allow a deep insertion of a sterile cotton swab and avoid external contaminations. Discharges of vagina of both groups were obtained by using the sterilized cotton swabs [1]. Discharge swabs from the cervix of the treatment and

control groups were collected by using the double rod swab (Equi. Vet. Denmark). The samples were transferred to sterilized Stuart transport media (Difco Laboratories, USA) and were brought on ice to the laboratory. They were inoculated in Sabouraud Dextrose Agar 4% at 28°C for 3 weeks. Chloramphenicol was used in the agar media for initial fungal isolation. Duplicate culture was used for every sample. The yeast and molds were identified on the basis of colony characteristics, microscopic morphology, as sugar fermentation tests, germ tube formation test. The data were analyzed by using the χ^2 statistic method.

Results

Fungal isolates were isolated from cervix and vagina of 27.14% and 28.57% of treatment group, 26.56% and 31.25% of pregnant cows and 33.33% and 21.42% of healthy non-pregnant cows, respectively. The different fungi isolated in treatment and control groups are shown in Table 1. There were no significant differences among the treatment and control groups ($P \geq 0.05$). The cows with reproductive diseases were infected by six different kinds of fungi isolates. However, the cervix and vagina of pregnant and non-pregnant of control groups were infected by 4–6 different fungi isolates. The present study showed that some cows in treatment and control groups were infected by one kind of fungi isolate. However, some cows were infected by 2–3 kind of fungi isolates. Since, some fungi isolates were uncommon, so there were impossible to identify the species of whole fungi isolates.

Table 2 shows the different fungi found in the cervicovaginal of female cows with reproductive-disorders. There were no significant differences among the different kinds of reproductive disorders for fungal infection.

Discussion

Fungi are able to grow saprobiologically, since their nutritional requirements are less exacting. These fungi are ubiquitous in nature and are usually saprophytic, although occasionally they produce serious disease.

Table 1 Different fungi isolated from cervicovaginal fluids of Holstein dairy cows with or without reproductive disorders

Mycotic agents	Experimental groups					
	Treatment		Controls			
	C	V	Pregnant		Non-pregnant	
			C	V	C	V
<i>Penicillium</i>	4	7	3	3	–	2
<i>Aspergillus fumigatus</i>	5	1	1	1	1	–
<i>Mucor & yeast</i>	–	–	–	1	–	–
<i>Geotrichum</i>	3	3	1	3	–	2
<i>Candida albicans</i>	1	1	3	3	6	3
<i>Mucor</i>	1	1	–	–	4	–
<i>Penicillium & Aspergillus fumigatus</i>	1	–	1	1	–	–
<i>Yeast</i>	4	6	3	5	2	1
<i>Mucor & Penicillium</i>	–	1	3	–	–	1
<i>Aspergillus niger</i>	–	–	–	1	–	–
<i>Aspergillus niger & penicillium</i>	–	–	1	–	–	–
<i>Penicillium, mucor & yeast</i>	–	–	–	1	–	–
<i>Penicillium, mucor & Aspergillus fumigatus</i>	–	–	1	1	–	–
<i>Cryptococcus neoformans</i>	–	–	–	–	1	–
Total	19	20	17	20	14	9

C: Cervix; V: Vagina

Table 2 Fungi isolated in the cervicovaginal fluids region of the cows with reproductive diseases

Reproductive disorder	No. of cows tested	Fungi isolated	
		Cervix	Vagina
Abortion	4	1	2
RFM ^a	5	3	2
Endometritis	14	2	2
Metritis	13	3	3
Repeat breeder	8	2	2
Dystocia	9	2	2
Cervicitis	9	3	3
Vaginitis	8	3	4

^a RFM: Retention of fetal membrane

Many species of fungi have been generally identified as pathogens of animals [1]. These mycotic agents can usually be pathogen under unusual circumstances, such as abnormal susceptibility, prolonged antibiotic treatment, especially intrauterine therapy, local treatment of vagina and cervix, immunosuppressants, traumatic implantation, vaginal wind sucker (pneumovagina), and bedding. The

mycotic isolates are then capable of causing clinical conditions, because they can invade tissue under such conditions.

In the present study, the overall prevalence of cervix and vaginal fungal isolates from cows with reproductive disorders, healthy pregnant and healthy non-pregnant were established (Table 1). Eight different fungal genera were isolated from bovine uterine by Singh et al. [9]. Verma et al. could isolate eight different fungi from genital system of cows with reproductive disorders in India [1]. In this study, we observed that the cervix and vagina of the treatment group have been infected by six different mycotic isolates. However, the cervix and vagina of pregnant and non-pregnant healthy cows in control group were infected with 5, 6 and 5, 4 different fungal isolates (Table 1). The lower prevalence of mycotic isolates in this study may be due to the topographic condition and lower humid in suburb of Tehran-Iran. *Penicillium* and *yeast* were the most common isolated isolates. Yeasts are found in the environment, often on plants or plants materials. However, bedding may be the source of yeast contamination. They may also occur as commensals on the skin or mucosal

membranes of animals. They cause opportunistic infections, which are categorized as exogenous when derived from the environment, or endogenous when resulting from overgrowth of commensals. In most instances, yeast is replaced after respond to initial therapy. Yeasts do not invade tissue but reside on the mucosal lining [10].

Fungi can produce reproductive failure in animals either as a direct result of establishing infection in the genital system or by producing toxin metabolites (mycotoxins) in vitro, which are subsequently ingested and absorbed. Mycotic abortion is the most important consequence of fungal infection of the genital tract, although fungi have been implicated occasionally in other syndromes such as vulvovaginitis or endometritis. The genera of *Aspergillus* and *Penicillium* can grow in a suitable substrate under appropriate conditions. They can produce toxins which are accounted the majority of abortion cases and can occur metritis in cows [11, 12].

Candida species occur worldwide on plant materials and, as commensals, in the digestive and urovaginal tracts of animals and human. *Candida albicans* is isolated from environmental sources less frequently than other *Candida* species, suggesting adoption toward a parasitic rather than a saprophytic existence. [13].

In conclusion, we observed that the occurrence of fungi (mainly *Penicillium* and *Yeast*) in cervicovaginal fluids of Holstein dairy cows with or without reproduction diseases. We suggest that those microorganisms are members of the resident or transitory of cervicovaginal flora of Holstein dairy cows and further investigations should be done to understand the role of opportunistic fungal pathogens in various therio-genicological processes.

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