

Uterine Mycotic Flora in Healthy Buffaloes and Buffaloes with Reproductive Disorders

Salma Ismail¹, Mehdi chavoshi^{2*}, Jamal Hussaini², Shahrbanoo Malekshaabani²

¹Faculty of Science, University of Malaya (UM), Malaysia

²Faculty of Medicine, University Technology MARA (UiTM), Malaysia

Abstract: Buffalo's uterine mycotic flora has not been studied too much yet. The aim of current study is to investigate fungi in uterine of healthy buffaloes with the history of uterine disorders like metritis. Uterine lavage was done in 100 buffaloes (50 healthy and 50 problematic). Samples were cultured in Subro Glucose agar medium and Chloramphenicol in 28°C for 2 weeks. For more identification of fungi, Corn Meal Tween 80 agar medium and Chapex dox agar were used. The data were analyzed by Chi-square test in SPSS software. Fungi colonies were isolated from 20% (10) and 26% (13) of healthy and problematic buffalo cows, respectively and the difference was significant ($p < 0.05$). The most prevalent fungi in buffaloes with uterine disorders were *Aspergillus fumigatus* and *Penicillium* spp., respectively. *Mucor* was dominant in healthy uterine. Uterine disorders may predispose uterine mycotic infections in buffaloes and lead to subfertility.

[Salma Ismail, Mehdi chavoshi, Jamal Hussaini, Shahrbanoo Malekshaabani. **Uterine Mycotic Flora in Healthy Buffaloes and Buffaloes with Reproductive Disorders.** *Life Sci J* 2014;11(5s):400-402] (ISSN:1097-8135). <http://www.lifesciencesite.com>.80

Keywords: Buffalo, Uterine, Fungi, Infection, Mycotic flora

1. Introduction

Smith (1920) reported the first fungal contamination of bovine uterine, and then it is reported from all parts of reproductive tracts of other animals [3]. Mycotic abortion caused by *Aspergillus*, *Candida*, *Zygomycetes* and yeasts is reported in cattle and buffalo. Mares' endometritis and cervicitis caused by fungal contamination are reported [1, 2, 7]. Fungi are the normal vaginal flora in human and some animals like dogs, and can be a source of infection in some cases [3]. In all countries the most reported fungal infection of bovine uterine was associated with *Aspergillus fumigatus* and *Zygomycetes* [4, 5]. Mycotic placentitis may have haemoral source [12]. Reproductive disorders due to fungi in bovine may be result of misdiagnosis of causative agent, and that in these cases detection of fungi and toxins from contaminated foods may be effected [13]. Ergot alkaloids secreted by *Claviceps* genus result in many reproductive disorders. Intake of food like barley, wheat and oat contaminated with this fungus is the only entrance to the body. Natural Ergot alkaloids cause severe stimulation of uterine muscles [13]. Small amount of it is uterotonic. Uterine response to Ergot alkaloids depends on gestation period and fetal growth stages. Ergotamine group of this alkaloid causes vasocontraction and results in gangrene in extremities [13]. The aim of current study is to determine mycotic flora in healthy buffaloes and buffaloes with the history of uterine problems and the infection rate in different seasons.

2. Materials and methods

100 Azeri ecotype buffaloes in Northwest of Iran were randomly selected by using statistical

sampling method (50 healthy, 50 problematic) and were divided in 2 groups. They were bred in traditional system by natural mating. Their diet was composed of alfalfa forage, barley, wheat, soybean meal and other supplements. Hand milking was done twice a day.

Control group

The 50 selected buffaloes were clinically healthy. They were in different stages of postpartum period and at least 150 days were past from their parturition but they did not become pregnant after several mating. They did not have received any antibiotic until the trial has begun.

Treatment group

These 50 buffaloes were in different stages of post partum period with at least 150 days passed after parturition and had mating history of no pregnancies as the same as control group. Although all buffaloes have had a history of endometritis, metritis, dystocia, infertility, abortion, retained placenta or repeat breeder and some of them had received antiseptic and antimicrobial therapy, they were clinically healthy when sampling.

Sampling

Uterine sampling was done by lavage after washing, disinfecting and drying of the perineal region. A sterile double cover catheter was directed to both uterine horns. 20 ml normal saline was injected then the fluid was aspirated and collected in a sterile tube contained Stuart transport medium (Difco laboratories, USA). The samples were labeled and transferred to the laboratory.

Fungal culture

1 ml of each sample was surface cultured on 4% Subro Glucose agar medium + Chloramphenicol

and was incubated in 28 °C for 3 weeks. For determining yeast types, Germ tube test in Corn Meal Tween 80 agar was used. Macroscopic and microscopic methods along with Chapex dox agar culture was used for identifying *Aspergillus* species. Data were analyzed by Chi square test in SPSS software.

3. Results

From total 100 buffalo cows, 23% had mycotic infection. Fungi colonies were isolated from 20% (10) and 26% (13) of healthy and problematic buffalo cows, respectively and the difference was significant ($p < 0.05$) (Table 1).

Table 1: Prevalence of uterine mycotic infection in both healthy and problematic groups

Fungi	Groups	
	Treatment (%)	Healthy (%)
<i>Penicillium</i>	2 (15.38)	1 (10)
<i>Aspergillus fumigatus</i>	3 (23.07)	1 (10)
<i>Geotrichum</i>	1 (7.69)	-
<i>Candida albicans</i>	1 (7.69)	2 (20)
<i>Mucur</i>	1 (7.69)	4 (40)
<i>Penicillium</i> + <i>Aspergillus f.</i>	3 (23.07)	-
Yeast	1 (7.69)	-
<i>Aspergillus f.</i> + <i>Mucur</i>	1 (7.69)	-
<i>Aspergillus niger</i>	-	1 (10)
<i>Penicillium</i> + yeast	-	1 (10)
Total isolated	13 (26)*	10 (20)*
Total no isolated	37 (74)	40 (80)

*Difference is significant ($p < 0.05$)

Table 2: Frequency of reproductive disorders in treatment group

Reproductive disorders	Mycotic infection		Total (%)
	+ (%)	- (%)	
Endometritis	11 (8.8)	21 (16.8)	32 (25.6)
Metritis	9 (7.2)	26 (20.8)	35 (28)
Dystocia	8 (6.4)	6 (4.8)	14 (11.2)
Repeat breeder	6 (4.8)	15 (12)	21 (16.8)
Infertility	8 (6.4)	8 (6.4)	16 (12.8)
Retained placenta	4 (3.2)	3 (2.4)	7 (5.6)
Total	46 (36.8)	79 (63.2)	125 (100)

No significant difference ($p > 0.05$)

Table 3: Prevalence of uterine mycotic infection in both groups in different seasons of the year

Group	Season of the year				Total
	Spring (%)	Summer (%)	Fall (%)	Winter (%)	
Treatment	5 (38.46)	1 (7.7)	4 (30.77)	3 (23.08)	13
Control	4 (40)	2 (20)	1 (10)	3 (30)	10
Total (%)	9 (39.13)	3 (13.04)	5 (21.73)	6 (26.09)	23

No significant difference ($p > 0.05$)

It was revealed mycotic infection isolated from both groups. The most prevalent fungi isolated from treatment group were *Aspergillus fumigatus* (23.07%), *Aspergillus Fumigatus*+ *Penicillium* (23.07%) and *Penicillium* (15.38%), respectively. In control group *Mucur* (40%) and *Candida albicans* (20%) were the most isolated agents (Table 1).

On the basis of historical records for buffalo cows, reproductive disorders were analyzed for both positive and negative results of mycotic infections in treatment group. Some of cows had

more than one disorders. Endometritis and metritis were the most disorders related to mycotic infections but no significant difference observed (Table 2).

It was shown spring is the season with the most mycotic infection occurrence and conversely, infection in summer is minimum (Table 3).

4. Discussion

Fungi are able to induce disorders in reproductive performance directly or indirectly by means of their toxins [6]. Mostly, 100 fungi strains

have been diagnosed to cause human and animal diseases. These fungi can cause infection after some predispositional factors such as uterine antimicrobial and antiseptic therapies leading to intrauterine trauma^[10].

Buffalo uterine infections have not studied well yet. Nowadays due to more use of antibiotics and hormone therapy, fungal infections have become more prevalent. Fungi may enter systemic blood circulation by mating, inhalation or orally^[9]. Because of less prevalence of uterine mycotic infections and difficulties in diagnosis, veterinarians often do not diagnose these cases. They always will diagnose when clinical symptoms start. Use of antibiotics and corticosteroids are the major cause of mycotic infections^[11]. Limitation of bacterial flora by long term use of microbial makes enough opportunity to colonization of fungi^[9]. So, more infection rate in treatment group may be due to antimicrobial use in cases of reproductive disease (table 1) and more likely due to introducing contaminated intrauterine catheter for injection of antibiotics.

Food supply like low quality alfalfa forage or bedding are the major source of contamination. Fungal spore rate in good quality food is 10^6 / g but more in low quality foods^[6].

Mycotoxins are secreted from *Aspergillus* and *Penicillium* that grew on foods in humid weather (85%) and temperature between 12 to 25 °C^[6]. Significant relation is reported between rain and abortion rate resulted by *Aspergillus*^[6]. Table 3 shows the most uterine infection is in spring in both groups, while the least infection was in summer. Probably it is due to suitable humidity and temperature in spring.

5. Conclusion

The results showed the uterine of healthy and problematic buffalo causes are contaminated with many fungi and yeast types, and endometritis and metritis were the most prevalent diseases (table 1,2)

and *Aspergillus* is a fungus more isolated from these cases. It is concluded that buffaloes with reproductive disorders are more at risk than healthy buffaloes in case of uterine mycotic infection and it may be because of antimicrobial use and more uterine handling.

References

- [1] Blue, M. G. "Mycotic invasion of the mare's uterus." *Veterinary Record* 113.6 (1983): 131-132.
- [2] Collins, S. M. "A study of the incidence of cervical and uterine infection in Thoroughbred mares in Ireland." *Vet Rec* 66 (1964): 673-6.
- [3] A Moraes Ismar; SP Jussara; Stussi; WaltervLilenbaum; Alcides Pissinatti; P Fabio; Luz. And Maria R Ferreira; *Am. J. Primatol.*, 2004, 64: 334-343.
- [4] HE Jensen; HV Krogh; H Schonheyder; J. Vet. Med. Series B.; 1991, 38: 33-40.
- [5] Krogh, H. V. "Bovine mycotic abortion in Denmark." *Nordisk veterinaermedicin* 37.1 (1984): 27-33.
- [6] JA Laing; WJ Bringley Morgan; WC Wagner; *Fertility and infertility in veterinary practice*. 4thEd. Bailliere tindall. 1988, 228-229.
- [7] DG Pugh; MT Martin; JW Shull; JM Bowen; J. *Equine Vet. Sci.*, 1986, 6: 40-43.
- [8] SJ Roberts; *Veterinary obstetrics and genital diseases (Theriogenology)*. Woodstock publication. 1991, 145.
- [9] SD Semrad; *JAVMA*, 1993, 203, 10: 1404-1410.
- [10] S Verma; RC Katoch; SK Jand; BM Sharma; P Nigam; *Vet. Res. Communi.* 1999, 23: 337-341.
- [11] BJ Waurzyniak; JP Hoover; KD Clinkenbeard; *Vet. Path.*, 1992, 29: 566-569.
- [12] CE Whiteman; MM Benjamin; L Ball; MWM Hill; *Vet. Path.*, 1972, 9: 408-425.
- [13] RS Youngquist; *Current therapy in large animal theriogenology*. USA. W. B. Saunders Company. 1997, 389-391.