



Mycotic endometritis in repeat breeder cows and buffaloes of Himachal Pradesh

PINKI SAINI¹, MADHUMEET SINGH², PRAVESH KUMAR³ and B M SHARMA⁴

CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh 176 062 India

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Among all the reproductive problems, endometritis is one of the most dramatic diseases that causes interference with the functioning of the reproductive tract of cows and hence decreases the livestock profitability (Adnane *et al.* 2017). Several infectious agents like bacteria, virus, protozoan and fungus are known to have direct impact on reproductive health of cows (Yoo 2010). Fungus is an opportunistic pathogen which can establish itself in disturbed environment of vagina and uterus (Stout 2008). It can cause infection under unusual circumstances such as prolonged intra-uterine antibiotics treatment, local treatment of vagina and cervix, traumatic implantation, pneumovagina (Garoussi *et al.* 2007). Several workers had reported different species of fungi from reproductive tract of the repeat breeder endometritic cows (Sharma and Singh 2012, Ahmed and Bhattacharyya 2015) and buffaloes (Pal 2002). The purpose of this report was to document the fungal isolations identified from uterus of endometritic cows and buffaloes of Himachal Pradesh.

Repeat breeding is defined as condition when cow/buffalo is cycling normally, with no clinical abnormalities but has failed to conceive after two or more successive inseminations. Animals selected for this study were endometritic repeat breeder cows and buffaloes of different agro-climatic zones (Dry-temperate, Wet-temperate, Sub-tropical and Sub-humid) of Himachal Pradesh. The animals selected for this study were maintained under variable managemental conditions in different zones. Uterine discharge samples were collected from 327 cows and 54 buffaloes from different districts of Himachal Pradesh. Collected uterine discharge samples were inoculated on Sabouraud's Dextrose agar plates by spot inoculation technique and the inoculation point was marked outside the plates to rule out the growth of the contaminants. Inoculated plates were incubated at 25–30°C in an upright position. Plates were checked each day for presence of any growth. Fungal isolates were identified by studying gross

morphological characters and microscopic characters after staining with Lactophenol Cotton Blue stain (Larone 1993). Gross morphological characters included growth rate, colony topography, colony texture, colony pigmentation and microscopic characters included hyphae, spores, vesicle, yeast like cell and any other identifying special feature. Yeast isolates were tested for their sensitivity to various antifungals by disc diffusion method for yeast (according to CLSI procedure) using Muller Hinton agar supplemented with 2% glucose and methylene blue (Himedia Laboratories Pvt. Ltd.) against 8 antifungal diffusion discs (Hi-media) namely itraconazole (10 mcg/disc), clotrimazole (10 mcg/disc), ketoconazole (10 mcg/disc), amphotericin B (100 units/disc), nystatin (50 mcg/disc), variconazole (1 mcg/disc), fluconazole (10 mcg/disc) and miconazole (10 mcg/disc). Different fungal isolates recovered from endometritic cows and buffaloes have been shown in Table 1.

Different geographical location, climate and meteorological factors like temperature, wind speed, relative humidity and rainfall are responsible for level of fungal spores and their dispersion in the atmosphere (Tomas 2003,

Table 1. Fungal isolates obtained

Isolate	N (%)
<i>Cows</i>	
<i>Rhizopus</i> spp.	6 (17.65)
<i>Aspergillus niger</i>	4 (11.76)
<i>Blastomyces</i> spp.	3 (8.82)
<i>Mucor</i> spp.	3 (8.82)
<i>Aspergillus</i> spp.	3 (8.82)
<i>Rhodotorula</i> spp.	3 (8.82)
<i>Penicillium</i> spp.	3 (8.82)
<i>Candida</i> spp.	2 (5.88)
<i>Penicillium vermiculatum</i>	1 (2.94)
<i>Aspergillus versicolor</i>	1 (2.94)
<i>Murogenella</i> spp.	1 (2.94)
<i>Carvularia</i> spp.	1 (2.94)
<i>Nigrospora</i> spp.	1 (2.94)
<i>Alternaria</i> spp.	1 (2.94)
<i>Pithomyces chartrum</i>	1 (2.94)
<i>Buffaloes</i>	
<i>Rhodotorula</i> spp.	1 (33.33)
<i>Mucor</i> spp.	1 (33.33)
<i>Fusarium</i> spp.	1 (33.33)

Present address: ¹Veterinary Officer (sainipinky142@gmail.com), Veterinary Hospital Kataula, District Mandi, Himachal Pradesh. ²Professor and Head (madhumeetsingh2004@gmail.com), ³Assistant Professor (pk9919@gmail.com), Department of Veterinary Gynaecology and Obstetrics; ⁴Professor (drbrijsharma@gmail.com), Department of Plant Pathology, College of Veterinary and Animal Sciences.

Table 2. Incidence of mycotic endometritis in cows and buffaloes in different agro-climatic zones of Himachal Pradesh

Agro-climatic zone	Cows			Buffaloes		
	Total samples	Positive for fungal growth	Incidence (%)	Total samples	Positive for fungal growth	Incidence (%)
Dry temperate (2200 m AMSL)	68	10	14.70	—	—	—
Wet temperate (1801-2200 m AMSL)	93	10	10.75	—	—	—
Sub-humid (651-1800 m AMSL)	59	5	8.47	15	1	6.66
Sub-tropical (350-650 m AMSL)	107	9	8.41	39	2	5.12
Overall	327	34	10.39	54	3	5.55

AMSL, above mean sea level.

Okten *et al.* 2005). Sharma and Singh (2012) corroborated our findings as they also reported 15.50 and 3.70% incidence of mycotic endometritis in cows and buffaloes, respectively. Ahmed and Bhattacharyya (2015) reported a comparative high prevalence (17.98%) of pathogenic fungi in repeat breeder cattle and buffaloes. Fungi are common in indoor and outdoor environment. Higher incidence (14.70%) of mycotic endometritis in cows was found in dry temperate zone (Table 2). This may be due to indoor favourable environment of cow sheds for fungal growth, as the cows in these areas are mostly confined in their sheds for almost throughout the year. The negative balance of energy, vitamin and minerals may lead to immunosuppression (Galvao 2013), which could be another reason for high incidence of mycotic endometritis in cows of these districts, as they are just maintained on available fodder crops only. The comparatively low incidence (8.47 and 8.41%) was found in sub-humid and sub-tropical zone (Table 2) than dry temperate zone which may be attributed to immuno-competence of the animals. Incidence of mycotic endometritis in buffaloes was reported from sub-tropical and sub-humid zones which may be due to favourable temperature and humidity conditions for fungal growth.

In this study, yeast isolates were found 100, 75, 75 and 37.5% sensitive to itraconazole, miconazole, clotrimazole and ketoconazole. Nearly similar high sensitivity of antifungals had been recorded in mares where itraconazole, miconazole, clotrimazole and ketoconazole were 97, 74, 94 and 100% sensitive, respectively (Beltaire *et al.* 2012). However, a comparatively low susceptibility of itraconazole and miconazole with high sensitivity against clotrimazole and ketoconazole had also been reported (Ferris 2017). Others antifungals namely fluconazole, variconazole, nystatin and amphotericin-B were 100% resistant. There is no known specific reason for their resistance, but it may be due to intrinsic/ natural resistance of these yeast isolates owing to less use of antifungals in field conditions.

This study was aimed to explore the fungal etiology of endometritis in repeat breeder cows and buffaloes of different agro-climatic zones of Himachal Pradesh. Uterine discharge samples were collected from 327 endometritic cows and 54 buffaloes and were subjected to fungal isolation by culturing on Sabouraud's Dextrose agar. Overall incidence of mycotic endometritis was 10.39 and 5.55% in cows and buffaloes, respectively. The mycotic isolates from cows included

Rhizopus spp., *Aspergillus niger*, *Blastomyces* spp., *Mucor* spp., *Aspergillus* spp., *Rhodotorula* spp., *Penicillium* spp., *Candida* spp., *Penicillium vermiculatum*, *Aspergillus versicolor*, *Mucor* spp., *Carvularia* spp., *Nigrospora* spp., *Alternaria* spp. and *Pithomyces chartrum*. *Fusarium* spp., *Mucor* spp. and *Rhodotorula* spp. were isolated from buffaloes. Yeast isolates were subjected to *in vitro* antifungal sensitivity testing and itraconazole antifungal was found to be most sensitive.

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