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Fungal endometritis in bovines

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Abstract
Fungus is well capable of producing reproductive failures in bovines. As fungus is an opportunistic pathogen, there are more chances for fungus to cause infection in the uterus when certain predisposing factors like prolonged intra-uterine antibiotic therapy, persistent endometritis, presence of necrotic foci, immunosuppression, and pneumovagina are available. Various authors have reported the incidence of fungal endometritis in repeat breeder endometritic cows and buffaloes. Uterine culture along with the cytological examination of the uterine secretions considered being best way to diagnose this condition; however, endometrial biopsy can be used to access the efficacy of treatment to invasive yeast. Intra-uterine treatment with antiseptic solutions and Lugol's iodine had been found effective in bovines..
Keywords: Bovines, Fungal endometritis, Opportunistic.

Introduction

Fungus is an opportunistic pathogen that can establish in a long-term disturbed uterine or vaginal environment (Stout, 2008). Fungi are capable of producing reproductive failure in animals either by directly establishing infection in the reproductive system or by *in vitro* production of toxic metabolites which is later on taken up by the animal (Laing *et al*., 1988; Garoussi *et al*., 2007). Getting one calf per year from cattle provide maximum benefit to farmers, but when animal fails to conceive after three to four repeated inseminations in the absence of any apparent detectable abnormality, then it becomes a costly problem for dairy producers (Wodaje and Mekuria, 2016). Over 100 species of fungi are involved with serious human and animal infections (Cvetnic and Pepeljnjak, 1997). There are various predisposing factors which help fungus to cause infection in uterus like pneumovagina, persistent endometritis, and repeated intra-uterine antibiotic therapy; however, the exact conditions which make the fungus capable to colonize the uterus are still not known (Stout, 2008). Yeast are widely distributed in the soil, animal excreta, and even in the vegetative parts of the plants (Hensyl and Oldham, 1982) and through these, they may gain entry into the reproductive tract of animals, but the most common source is thought to be skin or feces (Stout, 2008). Infact fungal isolates have also been cultured from urethra and semen of stallion (Malmgren *et al.*, 1998), but there is no report of occurrence of fungal endometritis through mating with such infected stallions. Fungi have even been reported in other pathological conditions of genital tract like cervicitis, vulvo-vaginitis, abortions, and metritis (Laing *et al*., 1988; Verma *et al*., 1999a; Garoussi *et al*., 2007). There have been many studies regarding pathogenic bacteria causing endometritis in bovines, but there are very few survey reports available on role of fungus in endometritis so there is a need to understand and explore this field.

Incidence in cows and buffaloes

Various studies reported the prevalence of fungal endometritis in repeat breeder cows to range between 10.5% and 33.3% (Verma *et al*., 1999b; Sharma and Singh, 2012; Ramsingh *et al*., 2013). In another study, the prevalence of pathogenic fungi in repeat breeding cattle and buffaloes was reported to be 17.98% and the most common isolated fungi were *Aspergillus fumigatus* and *Penicillium* spp. in cattle and buffalo, respectively (Ahmed and Bhattacharyya, 2015). Derakhshandeh *et al.* (2015) reported 8.7% (25–35 d post-partum) and 5.5% (39–49 d post-partum) prevalence of mycotic endometritis in Holstein cows and the most frequently isolated fungi were *Aspergillus* spp. (60.0%), *Penicillium* spp. (26.0%), and Yeast (13.0%), respectively.

The occurrence of fungi (mainly *Penicillium* and Yeast) in cervico-vaginal fluids of Holstein dairy cows has been observed from cervix and vagina as 27.14% and 28.57% from infertile/repeat breeder cows (Garoussi *et al*., 2007). Ahmadi *et al*. (2015) reported that microbiological cultures from reproductive tract of repeat breeder dairy heifers did not show any fungal growth. Vlcek *et al*. (1989) found pathogenic and potentially pathogenic fungi to be 22% and 17% cows, at first and second months after parturition, respectively. Only *Aspergillus* species was found in abnormal cervical mucus discharge of Holstein-Friesian cows and heifers and incidence was reported to be 4%

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(Ata *et al*., 2010). There has been a report of 27.80% mycotic isolates from endometritic buffaloes (Verma *et al*., 1999b). Along with many bacterial species, uterine culture showed 12.96% and 16.67% *Candida* spp. in healthy and inflamed uteri, respectively, of Anatolian Water Buffaloes (Yilmaz *et al*., 2012).

Candida albicans, an important opportunistic yeast, was identified as the cause of endometritis in water buffalo (Pal, 2002). Mycological examination of the uterine discharge samples from unbred buffalo heifers and repeat breeder buffaloes revealed the presence of mycoflora in 60% and 58.33%, respectively (Walia, 1996).

Predisposing factors for fungal endometritis

One important factor is prolonged local intra-uterine antibiotic therapy. The exact mechanism how intra-uterine antibiotics let the fungus to cause infection in the uterus is not yet clear, but it is presumed that it might disturb functioning of normal uterine defense mechanism which prevents fungus or yeast to colonize (Zafracas, 1975; Chengappa *et al*., 1984). Even it is also believed that the leakage of antibiotics from uterus to vagina at the time of intra-uterine therapy support the fungal growth as it disturbs commensal flora and reduce the competition for fungus by killing bacteria and changes pH of caudal reproductive tract. Although the primary reservoir of fungal pathogen is caudal reproductive tract, but sometime contamination form feces may also occur due to pneumovagina (Dascanio, 2000). It is presumed to cause ascending reproductive tract infection (Zafracas, 1975). Due to the opportunistic nature of fungal pathogen, disease results when it accidentally penetrate host barriers and during immunologic deficiency or debilitating conditions which help fungus to enter and grow inside the host (Hogan *et al*., 1996). The negative balance of energy, vitamins, and minerals may lead to immunosuppression (Cai *et al*., 1994; Galvao, 2013), which could be another factor for mycotic endometritis.

The retention of placenta also provide favorable medium in the form of residual tissue for growth of microbes in uterus. Presence of any necrotic foci due to the retention of placenta is also considered as an important predisposing factor for fungal endometritis (Hurtgen and Cummings, 1982). Abnormal calving and dystocia can induce trauma of the endometrium and calving assistance favors the introduction of pathogen into the uterus, thus, increases the potential for clinical and subclinical endometritis to develop (Bruun *et al*., 2002; Prunner *et al*., 2014). Feed and fodder materials like low-quality alfalfa forage and bedding are the major sources of contamination. Mycotoxins are secreted from *Aspergillus* and *Penicillium* that grew on foods in humid weather (85%) and temperature between 12°C and 25°C. Fungal spores are found in good quality fodder material at 106/g but more in low quality fooder (Laing *et al*., 1988), so such contaminated food material can act as a direct or indirect source of fungal pathogen to the bovines.

Diagnosis

Confirmatory diagnosis of fungal endometritis can be done by a combination of aerobic culture and cytological examination of uterine secretions (Stout, 2008).

Uterine culture

Mycological culture should be incubated for at least 5 d before declaring a sample negative because fungal growth can be very slow (Freeman *et al*., 1986). Visual characteristics of the fungal colonies include texture, pigment, and rate of growth on medium and microscopic examination under a light microscope to determine the morphological structures of the fungus species on slide mounted in Lactophenol-cotton blue (Carter and Chengappa, 1991) and are helpful in the identification of the fungal isolates.

Cytological examination

It is usually done in conjunction with uterine culture for diagnosis of the condition. Modified Wright's stain is used for staining purpose and subsequent cytological examination reveals signs of an ongoing inflammatory reaction, i.e., presence of neutrophils in most of the cases, and may also help in detecting obvious yeasts or, less commonly, elongated fungal hyphae (Stout, 2008).

Endometrial biopsy

If a fungal infection is suspected, but culture results are negative or inconclusive, a diagnosis can be made by staining a histopathologic specimen from the uterine biopsy with a silver stain such as Gomori's methenamine silver stain (Dascanio and McCue, 2014). This technique may be, especially, useful for indicating whether treatment is likely to result in a satisfactory outcome or a predisposing cause and resulting fungal infection are associated with poor subsequent fertility (Hurtgen and Cummings, 1982).

Antifungal sensitivity test

Selection of anti-fungals to treat fungal endometritis should be based on *in vitro* susceptibility of specific fungal isolates to available drugs. But, this may be complicated because fungi has special culture media requirement and need prolonged incubation period (Beltaire *et al*., 2012). Anti-fungal susceptibility testing is still not offered by many veterinary laboratories and thus, the methods are currently being standardized between laboratories by the Clinical and Laboratory Standards Institute. No reports regarding use of antifungal in case of bovines were found. Ferris (2016) performed antifungal sensitivity test in mares suffering from fungal endometritis using Amphotericin-B, Natamycin, Nystatin, Clotrimazole, Ketoconazole, Miconazole, Itraconazole, and Fluconazole and reported sensitivity of 96%, 100%, 100%, 80%, 81%, 43%, 62%, and 44%, respectively. Beltaire *et al*. (2012) recorded 100%, 100%, 94%, 100%, 74%, 97%, and 75% sensitivity of yeasts isolates against Amphotericin-B, Nystatin, Clotrimazole, Ketoconazole, Miconazole,

Itraconazole, and Fluconazole and 75%, 75%, 100%, 38%, 86%, 50%, and 0% sensitivity of Amphotericin-B, Nystatin, Clotrimazole, Ketoconazole, Miconazole, Itraconazole, and Fluconazole against mold isolates from cases of fungal endometritis in mare.

Treatment

Intra-uterine antiseptic treatment

Intra-uterine treatment has been found effective with 0.05% povidone-iodine solution and 2% acetic acid (Zafracas, 1975). However, intra-uterine povidone-iodine infusion should be used with caution as there are reports of it resulting in severe endometrial damage, including fibrosis and adhesion formation (Perkins, 1999). Intra-uterine infusion of 0.1% Lugol's iodine was found clinically efficacious in the treatment of fungal endometritis (Sharma and Singh, 2012; Ramsingh *et al*., 2013). Iodine compounds have been successfully used by Kremlev and Banakova (1979) to treat fungal endometritis. Lugol's iodine infusion can be used as the first choice in all the cases without a clear cut diagnosis of the uterine infection (Morrow, 1980).

Intra-uterine antifungals

Before administering any antifungal agents, a drug sensitivity screen should be done to aid in the selection of appropriate and specific anti-fungal agents (Dascanio *et al*., 2001).

Usually, the duration of intra-uterine therapy should be 7–10 d though longer duration may be needed for resistant infections. However, for resistant infections, intra-uterine therapy should be used in conjunction with oral anti-fungals (Dascanio, 2000). Although the choice of anti-fungal agent for treatment is decided by culture sensitivity test, many a times *in vitro* sensitivity patterns may not correlate with *in vivo* effectiveness (Dascanio, 2011). Some try to avoid intra-uterine antifungal treatments with the concern that repeated intra-uterine treatment may make the mare more susceptible to re-infection or prolonged inflammation. An alternative would be oral antifungals, which may be expensive (Dascanio, 2011). Up until now, there are no reports of antifungal agent use in bovines. Dascanio (2011) treated fungal endometritis in mares with 100–200 mg of Amphotericin-B, 400–700 mg of Clotrimazole, 100 mg of Fluconazole, 500–700 mg of Miconazole, and 0.5–2.5 million IU of Nystatin through intra-uterine route.

Systemic anti-fungals

Anti-fungals may be administered systemically. Their use has been recorded in mare (Dascanio, 2011). In mare, oral route of administration of the antifungal agents has also been used. Oral fluconazole has been recommended for the treatment of *Candida* spp., whereas oral itraconazole has been suggested for the treatment of *Aspergillus* spp. Oral anti-fungals have also been used in conjunction with intra-uterine therapy for resistant infections (Dascanio, 2000). Dascanio (2011) treated fungal endometritis in mare with systemic dosage of antifungal agents like Amphotericn-B at 0.3–0.9 mg/kg q 24 to 48 h IV, Fluconazole at 14 mg/kg loading, then 5 mg/kg q 24 h IV/Per os, Itraconazole at 2 g q 24 h IV or per os Azole Itraconazole 5 mg/kg q 12 to 24 h, and Ketoconazole at 20 mg/kg q 12 h in 0.2 N HCl through naso-gastric intubation.

Lufenuron

Lufenuron inhibits chitin synthesis, since fungi also have chitin-rich cell walls. Hess *et al*. (2002) proposed that lufenuron might have activity of inhibiting fungal growth, but, Lufenuron may not be effective in all cases as not all fungal organisms have chitin in their cell walls (Ferris, 2017). The effectiveness of lufenuron still remains in question (Hector *et al*., 2005; Mancianti *et al*., 2009).

Dimethyl sulfa-ixide infusion

Biofilms are aggregates of bacteria or fungi encased in an adherent polymeric matrix, which may inhibit antibiotic penetration (Donlan and Costerton, 2002) and dimethyl sulfoxide (DMSO) lavage may be useful in augmenting tissue penetration followed by disruption of microbial biofilms (Leblanc, 2008). During an *in vitro* study, DMSO at 10%–20% concentration decreased the growth of *C. albicans*, whereas at concentration greater than 30%, it inhibited growth (Pottz *et al*., 1967).

In conclusion, mycotic endometritis is one of the causes of infertility in cows and buffaloes. Prolonged intra-uterine therapy, pneumovagina, environment, dystocia, retention of placenta, and immunosuppression are various predisposing factors which help fungus to colonize the uterus. Diagnosis can be done with help of uterine culture and cytological examination if needed endometrial biopsy can also be used. Up until now, there are no reports of antifungal intra-uterine and systemic use of antifungals to treat fungal endometritis in bovines, but their dosage has been reported in mare. As there is very less understanding of reproductive disturbances caused by this opportunistic fungal pathogen in cows, there is further need of investigation in this field/area.

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