



A rare case of *Candida famata* associated endometritis in cross bred Jersey cow

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Abstract

Endometritis, an inflammation of endometrium, is one of the major causes of reduced reproductive efficiency in bovines. Among microbial etiologies of endometritis, fungal causes are rarely reported. A 4-year-old, cross bred Jersey cow with high fever history and whitish mucopurulent discharge from vulvar opening for 1.5 years was treated with long acting oxytetracycline (intramuscularly) for 3 days with failure to cure. The uterine discharge was collected from the cow and processed for isolation of causative agent and further antimicrobial susceptibility testing. Pure yeast like colonies were observed on SDA after 48 hours of incubation at 37°C. Which was purified and confirmed as *Candida famata* based on phenotypic characteristics. Antifungal susceptibility testing following disk diffusion method revealed sensitivity of isolate for Fluconazole, Ketoconazole, "Amphotericin-B, Nystatin, Itraconazole and Miconazole but was resistant to Clotrimazole. In conclusion, *C. famata* was detected as causal agent of endometritis in cow and thus identification of fungal etiologies in antibiotic treatment of unresponsive cases of endometritis is important.

Key words: *Candida famata*, endometritis, cattle, antifungal

Endometritis in cattle is characterized by inflammation of uterine mucosal membrane with mucopurulent or purulent discharge from the vulvar opening (Sheldom *et al.* 2008). Bacterial pathogens are usually associated with endometritis in cow which include *Escherichia coli*, *Trueperella pyogenes*, *Klebsiella* spp, *Proteus* spp, *Pseudomonas* spp, *Clostridium* spp, *Staphylococcus* spp etc. (Udhayavel *et al.* 2013; Bicalho *et al.* 2017; Pascottini *et al.* 2021). Fungal pathogens are rarely reported from the endometritis cases and more likely occur under immunosuppressive conditions. Among fungal infections, frequency of *Candida* spp. associated reproductive tract infections has been on rise (Pohlman and Chengappa 2013). *Candida famata* (formerly known as *Debaryomyces hansenii* and *Torulopsis candida*) is an opportunistic pathogen, found in dairy products and the environment (Kam and Xu 2002). In previous reports the organism has been detected in teats of dairy cows (Wawron *et al.* 2011), wild birds (Mendes *et al.* 2014) and many other species. The study was aimed at isolation of fungal

pathogens from uterine discharge of cow diagnosed with endometritis and secondly, to evaluate antifungal sensitivity of isolated fungi against commonly used antifungal drugs.

Materials and methods

Case history: A 4-year-old, cross bred Jersey cow suffering from endometritis for 1.5 years was presented at Veterinary Clinic with high fever along with whitish mucopurulent discharge from vulvar opening (Fig. 1). The discharge was collected in sterile sheath and transferred to the Department of Veterinary Microbiology, DGCN COVAS, CSKHPKV, Palampur for identification of etiological agent.

Microbial isolation: The uterine discharge was inoculated on to two plates of Sabouraud Dextrose Agar (SDA) and incubated at 37°C and 28±2°C for 24 hours and 1 week for the isolation of yeast and molds, respectively (Kumar *et al.* 2011).

Gram's staining: A loopful of isolated colony was mixed with a drop of normal saline and heat fixed for

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Fig.1 Whitish mucopurulent vaginal discharge from a crossbred cow suffering from endometritis

performing Gram's staining. The morphology of organism was observed under 100x using microscope (Boyanova 2018).

Sugar fermentation test: Phenol red broth base was prepared comprising protease peptone, sodium chloride with pH indicator phenol red. The medium was poured into test tubes and sterilized by autoclaving. Further, the sugar discs (galactose, glucose, maltose, lactose, trehalose and sucrose) were added to the tubes followed by inoculation of organism. The tubes were incubated at 37°C up to one week and examined at every 24 hour interval for the production of acid that will change the color of phenol red to yellow (Giri and Kindo 2012).

Antifungal susceptibility testing: Susceptibility test for the fungal isolate was performed by the disk diffusion method on Mueller Hinton agar (MHA) using seven antifungal drugs i.e. Clotrimazole, Nystatin, Fluconazole, Amphotericin-B, Ketoconazole, Itraconazole and Miconazole. The plates were incubated for 24 hours to 5 days at 37°C (CLSI 2018).

Results and Discussions

Animal candidiasis is considered as opportunistic

mycoses which affect wide range of domestic animal species. Presently, gradual rise in *Candida* spp. infections prompts the need of early diagnosis and accurate antifungal therapy for the treatment of animals. Although *C. famata* is not considered as most virulent *Candida* species, but its role as pathogenic organism can't be ignored. In this case report, the uterine discharge was inoculated onto SDA and single type of colonies were observed after 24 hours of incubation at 37°C. Based on the colonial morphology on SDA, creamish small sized colonies with soft consistency, entire border and convex elevation were consistent with the typical morphology of yeasts (Fig. 2a). Owing to slow growth, the colonies were further incubated for two weeks. After two weeks, the colonies grew well and larger in size (Fig. 2b). Gram's staining has been widely used for the presumptive identification of yeasts (Haw *et al.* 2012). In the Gram's staining, the yeasts appeared purple, round to oval and few budding cells (Fig. 2c). It is well documented that yeast cell wall is made up of thick peptidoglycan layer and retains crystal violet on Gram's staining (Haw *et al.* 2012).

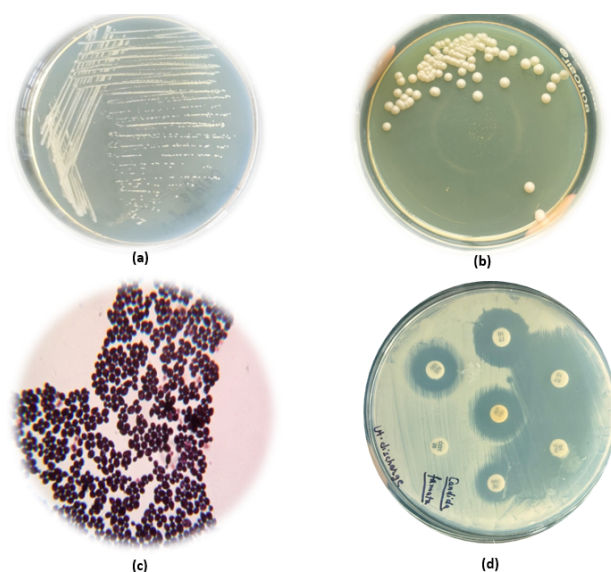


Fig. 2: (a) Identification of *Candida famata* depicting colonies after 24 hours, (b) after 2 weeks of incubation at 37°C on SDA, (c) Gram staining showing budding, (d) Isolate showing high susceptibility to Nystatin, Fluconazole, Amphotericin B, Ketoconazole, Itraconazole; moderate susceptibility to Miconazole and resistance to Clotrimazole on MHA

For further confirmation, sugar fermentation test of isolated *Candida* spp. was performed for species

confirmation. The results showed *Candida* spp. had ability to ferment glucose (colour change from red to yellow) only among all the sugars and displayed positive result (acid production) (Fig. 3). This finding is in accordance with another study where *C. famata* has given similar results (Imran and Abuad 2015). Sugar fermentation is the conventional method for the identification of *Candida* species and reported to with varying degrees of sensitivity and specificity (Jones 1990). According to the previous studies, *Candida tropicalis* gave positive reactions to glucose and sucrose along with negative reaction to lactose whereas *C. albicans* is not able to ferment sucrose and lactose (Maikan *et al.* 2022). Also, *C. glabrata* ferments only glucose and trehalose (Odds 1988) whereas *C. parapsilosis* is unable to ferment maltose (Trofa *et al.* 2008). On the basis of above findings, *Candida* spp. was confirmed as *Candida famata*.

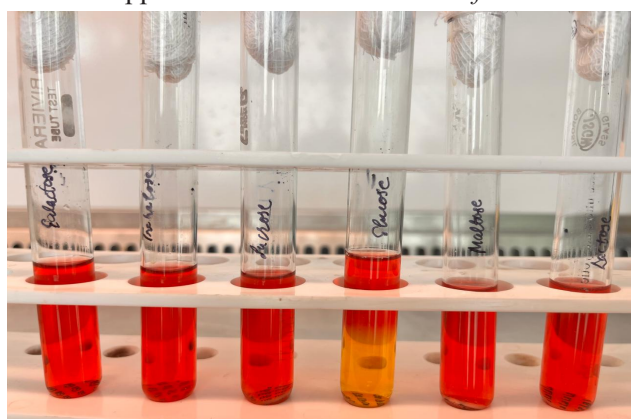


Fig. 3 Sugar fermentation test for identification of *Candida famata* showing fermentation of glucose

The isolate of *C. famata* was tested for antifungal susceptibility and it was found that Nystatin,

Fluconazole, Amphotericin-B, Ketoconazole and Itraconazole were more effective against *Candida famata*. The isolate was moderately sensitive and resistant to Miconazole and Clotrimazole, Antphotericin-B (Fig. 2d). Previous studies reported Nystatin and Ketoconazole were found highly effective against yeast from cases of fungal endometritis followed by Clotrimazole, Miconazole, Itraconazole and Fluconazole (Beltaire *et al.* 2012). The report highlights the clinical significance of mycotic pathogens in bovine endometritis. Further, it is suggested that genome characterization of the yeast isolates to identify various *Candida* spp. should be carried out with special emphasis on the involvement of *Candida famata* in bovine endometritis.

Conclusions

In the present report, *Candida famata* was isolated and identified in the uterine discharge of a cow showing endometritis signs, which indicated the importance of fungal infections in the endometritis cases along with bacterial pathogens. So, the association of fungal pathogens in endometritis should be studied systematically, so that effective treatment can be provided to the animals.

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Conflict of interest: The authors declare that there is no conflict of interest among the authors in this research paper.

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