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Etiology, Transmission, Epidemiology, Clinical Spectrum, Diagnosis and Management of Fungal Mastitis in Dairy Animals: A Mini Review

Mahendra Pal

Narayan Consultancy on Veterinary Public Health and Microbiology, B-103, Sapphire Lifestyle, Gujarat, India.

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***Corresponding author:** Mahendra Pal, Narayan Consultancy on Veterinary Public Health and Microbiology, B-103, Sapphire Lifestyle, Gujarat, India.

Abstract

Mastitis is a major production disease of dairy animals, which is caused by more than 136 microorganisms. The disease is highly significant due to huge economic losses to the dairy farmers. It is reported in both developing and developed nations of the world. The infection of the mammary gland is more commonly encountered in exotic and cross-bred animals. The incidence of subclinical mastitis seems to be higher than clinical mastitis. There are many species of fungi that are associated with the etiology of mastitis in dairy animals, such as cows, buffaloes, goats, camels, and sheep. Among fungal agents, Candida species are the most frequently isolated from mastitis cases. The extensive, prolonged, and indiscriminate use of antibacterial antibiotics and hydrocortisone in the conventional treatment of mastitis has predisposed dairy animals to mycotic mastitis. Fungal pathogens have the potential to produce both clinical and subclinical mastitis in milch animals. Fungi are widely prevalent in the environment, and hence, unhygienic sheds of animals may provide a source of fungal infection of the mammary gland. The infection may spread through the milker's hand and milking machine. Clinical signs and symptoms of fungal mastitis resemble bacterial mastitis. Laboratory techniques are required to confirm the diagnosis of the disease. The fungi are easily isolated from the mastitic milk on Sabouraud dextrose agar and Pal sunflower seed medium. Microscopic morphology of fungal isolates can be studied in Narayan stain. Nystatin therapy has shown encouraging results in the management of mastitis cases caused by Candida species. The treatment of mycotic mastitis, mainly due to Cryptococcus neoformans is challenging. The implementation of good hygienic practices during milking, proper sanitation measures in the cow sheds, and immediate cessation of antibacterial antibiotics may help to reduce the prevalence and incidence of fungal mastitis.

Keywords

Dairy animals, Fungi, Mastitis, Narayan stain, Pal sunflower seed medium

1. Introduction

Despite substantial research work in veterinary medicine, mastitis still remains a persistent enigmatic problem in dairy animals worldwide. Mastitis, a disease complex of multiple etiologies, results in considerable financial losses to dairy farmers [1]. It is a sequel to the interplay between animal husbandry practices and the organism. The disease can manifest in acute or chronic form and occur in sporadic as well as epidemic forms. The infection may be a single quarter or may involve multiple quarters. Mastitis in dairy animals is caused by several types of organisms, which include bacteria, fungi, *Mycoplasma* and *Prototheca* [2, 3]. Among bacterial agents, *Staphylococcus aureus* is recognized as a significant pathogen of clinical and sub-clinical mastitis in India and other countries of the world [1]. Most of the organisms are prevalent in the environment and can gain access to the teat canal during the act of milking [1, 4]. Mastitis is of great economic

importance as it results in substantial economic losses to the dairy industry worldwide [1, 4].

Mastitis a disease of multifactorial etiology, is essential from an economic as well as public health point of view [1]. The disease is primarily observed in lactating animals about 4 to 8 weeks after parturition. In the majority of cases, only one-quarter of the mammary gland is affected. It is mentioned that 5 to 10% of cows in dairy herd are affected during their lactation period [1]. In Australia, economic losses due to mastitis are estimated at more than UD Dollar 120 million each year [1]. The financial losses incurred due to mastitis in India were reported to be about US dollars 98,228 million per lactation by Sharma and co-workers [5]. The infection of the mammary gland is most commonly encountered in exotic and cross-bred cows [1]. The incidence of subclinical mastitis is much higher as compared to clinical mastitis in dairy animals. Laboratory help is imperative for the confirmation of mastitis [6]. Due to the high specificity and sensitivity, PCR-based tools have been employed to diagnose mastitis in dairy animals [7].

Fungi have been reported to be associated with subclinical and clinical mastitis in dairy cattle [6, 8, 9]. It is mentioned that *Candida* species are most frequently isolated from infected glands [10]. Several factors, such as unsanitary milking practices, hot and humid climate, the excessive and misuse of antibiotic therapy, feeding of fungal contaminate fodder, poor hygienic conditions of the byre, and incorrect diagnosis may predispose the dairy animals to fungal mastitis [6, 11]. There is a paucity of information on the fungal mastitis in dairy animals. Therefore, the present communication is an attempt to describe the etiology, transmission, epidemiology, diagnosis, and management of mycotic mastitis in milch animals.

2. Etiology

Many fungi, such as *Aspergillus flavus, A. fumigatus, Aspergillus nidulans, Aspergillus niger, A. terreus, Candida albicans, C. tropicalis, C. guillermondii, C. krusei, C. parapsilosis, C. rugosa, Candida etchellsii Cryptococcus neoformans, Debarryomyces hansenlii, Geotrichum candidum, Hansenula anomala, Pichia farinosa, Rhodotorula glutinis, Rhodosporidium diobovatum* (2.3%), *Galactomyces geotrichum* (3.6%), *Geotrichum terreste Saccharomyces fragilis, S. marxianus* and *Trichosporon ashehi [beigelii], Yarrowia lipolytica* etc. are isolated from mastitic milk of dairy animals throughout the world [3, 6, 12-15]. The etiologic role of zoo-pathogenic basidiomycetic yeast *Cryptococcus neoformans*, with mastitis in goats and buffaloes, was established for the first time by Pal in 1975 and 1980, respectively [6].

The isolation of *Candida albicans* for the first time from mastitic milk of she-camel in Ethiopia was described by Pal. Among the fungal pathogens, *Candida* species has been identified as the primary fungus, which is found to be associated with mycotic mastitis in dairy animals in India and elsewhere [3, 11, 15, 16-18]. The majority of fungi, which are implicated in the etiology of mycotic mastitis, occur as saprophytes in diverse types of natural substrates and can serve as a source of infection to susceptible animals [6].

3. Transmission

The fungi exist as saprobe in nature and may enter the udder through the injured teat canal. The fungi can also be introduced through yeast-contaminated antibacterial preparations given by intramammary route to dairy cows [6]. The unsanitary surroundings of the animals and contaminated teat cups may also act as a source of infection [6].

4. Epidemiology

Mycotic mastitis is a widely prevalent disease in dairy animals, which includes cows, buffaloes, goats, camels, and sheep. The disease has been reported from many countries of the world, such as Israel, USA, Brazil, Egypt, Ethiopia, Poland, Mexico, India [3, 9, 15, 16, 18-24]. Fungal mastitis can affect a single animal in a sporadic form or may involve several animals in the form of an outbreak [6, 25, 26]. One study conducted by Pachuri and co-investigators [9] reported the prevalence of *Candida albicans* in clinical and sub-clinical mastitis of animals in and around Mathura, India, as 30 % and 25%, respectively.

A severe mastitis problem due to *Cr. neoformans* in a large dairy herd that affected 106 out of 235 cows was investigated by Ponden and others in 1952 in the USA [27]. Similarly, another outbreak of cryptococcal mastitis in cows was recorded in 1953 by Simon and co-investigators [25]. These authors suggested that the administration of antibacterial antibiotics to milch cows was the source of fungal mastitis. Cryptococcal mastitis is often recognized severe and profound form of the disease, which usually does not respond to treatment, and hence, the affected animals have to be culled and disposed [6].

Fungal infections may account for up to 2 to 13% of all cases of mastitis in cows [21]. Lassa and Smulski [28] reported that the prevalence of fungal mastitis in Europe in cows may range from 1 to 10%. Elad and co-investigators [20] reported that contamination of animal feed, such as silage and wheat, was the most probable source of fungal mastitis in cows with *Candida krusei* in Israel. It is pertinent to mention that the unhygienic conditions of cow sheds support the growth of fungi in the immediate environment of dairy animals. This may provide an opportunity for the fungi to enter the

parenchyma of the mammary tissue and initiate the infection [6, 20, 29].

5. Clinical spectrum

The first symptoms of yeast-induced mastitis may appear 5 to 8 days following the intramammary administration of yeast-contaminated antibacterial antibiotics. In a few cases, mastitis develops after 2 days, and in others, it may take about 10 to 12 days. The signs and symptoms, such as subcutaneous oedema of the udder, enlargement, swelling, induration of the udder (Fig. 1) and supra-mammary lymph nodes, anorexia, temperature, and decreased milk yield have been observed in the affected dairy animals [6, 12, 14, 22, 27]. On clinical examination, the udder appears hot, tender, and painful. Mammary secretions from an affected gland with mycotic mastitis may appear watery or clear yellow and gelatinous with strands of cloudy material. Greyish-white, stringy, and mucilaginous fluid is observed in cryptococcal mastitis [6].

Fig. 1. A goat affected with cryptococcal mastitis showed enlargement, swelling, and induration of the mammary gland. *Cryptococcus neoformans* were quickly isolated from the milk of the affected goat by observing brown pigmented colonies on Pal sunflower seed medium. Source: [6].

6. Diagnosis

Clinical signs are not very characteristic to confirm the diagnosis of mycotic mastitis. Therefore, several techniques like mycological, histopathological, and molecular have been used by researchers to establish an unequivocal diagnosis of fungal mastitis in dairy animals [6, 7, 13, 24, 30]. About 5 to 10 ml of mid-stream milk collected aseptically from the teats of dairy animals should be centrifuged at 10,000 rpm at 5^0C for 10 minutes for direct microscopy as well as for cultural isolation of the fungi. The diagnosis should be confirmed by the detection of fungal elements in the smear prepared from the milk sediment by staining with 0.5% crystal violet [6]. The fungal agents can be easily isolated from the milk of affected quarter/s on Sabouraud dextrose agar with chloramphenicol and Pal sunflower seed medium (pulverized sunflower seed 45 gm, agar powder 20 gm, distilled water 1,000 ml and chloramphenicol 500 g [6, 31]. The later medium is highly selective and thus helps in the early identification of *Cryptococcus neoformans* by imparting brown pigment to the colony of this opportunistic pathogen. *Candid*a species failed to produce brown color on Pal sunflower seed agar (Fig. 2) [31]. The microscopic morphology of the fungal agents can be studied in a newly developed stain, designated as "Narayan" (4.0 ml of glycerin, 0.5 ml of 3% solution of methylene blue, and 7.0 ml of dimethyl sulfoxide [6]. The detailed study of the cultural, morphological, physiological, and biochemical characteristics of the isolates helps in the identification of the fungi involved in mastitis [6]. Histopathological examination of biopsy tissues from the affected mammary gland after staining periodic acid Schiff (PAS) and Gomori methenamine silver (GMS) can demonstrate the fungal elements. However, the isolation of fungi from clinical specimens is imperative to identify the species involved in causing mastitis [6]. It is suggested that routine cultural examination of milk for fungi may reveal more cases of mycotic mastitis in dairy animals.

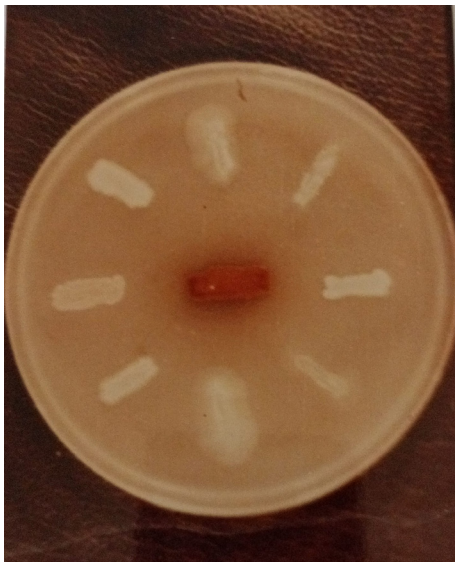

Fig. 2. Colony of *Cryptococcus neoformans* in the centre of Pal sunflower seed medium exhibited brown pigmented growth whereas isolates of *Candida* species inoculated at the periphery of medium failed to reveal a brown colour effect. All species of *Candida* at the periphery showed a white colour on Pal sunflower seed medium. Source: [31].

Pengov [15] used API 20 G and API 20 CAUX (BioMerieux) systems to confirm the diagnosis of *Candida* species, which were isolated from mastitic milk. Molecular tool like multiplex PCR has been employed to diagnose fungal mastitis due to *Cr.neoformans* in dairy goats [24]. The drug sensitivity with nystatin, miconazole, amphotericin can be performed against the fungal isolates by in vitro disc diffusion test [6]. A drug that shows high sensitivity should be used to treat mastitis cases [6].

7. Management

Nystatin, an antifungal drug, was used as an intramammary infusion in the affected udder to treat the animals suffering from *Candida* mastitis [6, 12]. It is essential to mention that the animal should be first milked out, and then intramammary infusion of the drug should be administered.

Cryptococcal mastitis usually presents a therapeutic problem as no effective drugs are available. Intramammary administration of miconazole and clotrimazole was not found effective in treating clinical cases of cryptococcal mastitis in cows [M. Pal Personal communication].

The literature scan revealed that the efficacy of fluconazole and itraconazole therapy for fungal mastitis has not been established, and therefore, the use of both broad-spectrum antifungal antibiotics needs to be investigated in clinical cases of mycotic infections of the udder in dairy animals. It is hoped this may prove a milestone in the treatment of mycotic mastitis it still remains a challenge to the field veterinarians.

Specific measures like immediate cessation of antibacterial antibiotics and hydrocortisone, proper sterilization of dairy utensils, use of sterile distilled water to dissolve the drug by intramammary administration, hygienic conditions of the animal sheds, personal hygiene of milk attendants, and avoidance of moldy feed and treatment with antifungal drug may help to reduce the prevalence and incidence of mycotic mastitis in dairy animals [6]. In this context, Eland and c0investigators [30] reported that the outbreak of fungal mastitis in dairy cows stopped after the replacement of the silage and improvement in milking hygiene. It is recommended that the rules of dairy hygiene should be followed strictly by the farmers to prevent the occurrence of mastitis.

8. Conclusion

Mastitis is a multifactorial disease of serious concern for dairy farmers both in the developing as well as developed nations of the world. A number of fungi are implicated in the etiology of mycotic mastitis in dairy animals. However, maximum cases of fungal infections of the mammary gland are due to *Candida* species. The routine application of Pal sunflower seed medium and Narayan stain will undoubtedly help the microbiologist in the study of mycotic mastitis of dairy animals. It is recommended that an animal unresponsive to antibacterial antibiotic therapy must be suspected of mycotic mastitis, and a detailed mycological investigation should be conducted to establish the role of fungi in mastitis. As fungal mastitis is a global problem, comprehensive and systemic studies on the development of cheap, safe, and effective antifungal drugs should be undertaken.

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