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Concurrent infections of *M. audouinii* and Mucormycosis in buffaloes and attributed risk factors for fungal infections

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Abstract
Studies on the prevalence of fungal diseases carried along with their clinical signs in an organized livestock farm in Jamdoli, Jaipur, having 19 cows, 18 Murrah buffaloes,6 Murrah calves, with varying age groups. Few Murrah milch animals and buffalo calves revealed skin lesions. Hence all the buffaloes and their calves were clinically examined and the skin scrapings were screened for their etiological agents' involved in the causation of the cutaneous lesions. In this study the adult buffaloes suffered with hair loss with white spots, alopecia, muco-cutaneous ulceration, scaling, plaques, necrotizing fasciitis and eschar. Affected animals develop discrete scaling batches of hair loss with grey-white crust that later become a thickly suppurated crust in variable locations. The lesions observed mostly on the neck, chest, face, dewlap, lower abdomen and inter-maxillary skin. Skin scrapings from this buffalo and their calves revealed concurrent fungal infections of microsporum and Mucormycosis. The risk factors attributed for the development of cutaneous lesions in these bovine populations discussed in this study.

Keywords: Buffaloes, microsporum, mucormycosis, risk factors

Introduction
Dermatophytosis is a common contagious disease caused by fungi known as dermatophytes. Most of the fungi reside in the soil and in living tissues. Zoophilic species are adapted to animals. Geophilic species live in the environment but occasionally act as parasites. In living tissues dermatophytes remain in superficial tissues such as epidermis, hair and nails. The illness causes disfiguring and uncomfortable, especially when the lesions are widespread. Economic effects, such as damage to the hides are important in livestock. In immune compromised animals the dermatophytes invade subcutaneous tissues and other sites. The pathogenic dermatophytes causing keratin digestion are trichophyton, microsporum and epidermophyton species. Most of the dermatophytes becomes adapted to people or animals and are maintained in these reservoirs. Most or all zoophilic dermatophytes are thought to be zoonotic, although some are transferred to people more often than others. There are numerous species of anthropophylic dermatophytes in the 3genera i.e trycophyton, microsporum and epidermophyton. The predominant species of dermatophytes involved in human cases vary with climate and location and other factors such as exposure to livestock, pets or exotic species. Zoophilic dermatophyte *M. canis* infects ruminants also. Anthropophylic dermatophytes reported infrequently in animals. Some species documented are *M. audouinii, T. rubrum, T. tonsurans, T. violaceum, T. schoenleinii,* and *E. floccosum.* Potential predisposing factors such as tumors or treatment with immunosuppressive drugs were reported in some cases. (The Centre for food security and public health, Institute for international Cooperation in Animal Biologics: Iowa state university, OIE and USDA 2013: Papini *et al* 2009; Nweze,2011) [19, 20]. Fungal infections are relatively common in healthy and immuno-competent animals compared with bacterial and viral, since fungal infections are present in the environment in great numbers. (Kohler *et al*.2015; Gnat *et al*. 2020a) [15, 13]. The prevalence of fungal infections are low and recurrent caused by true and opportunistic pathogens, noticed worldwide in animals and human beings. (Fisher *et al*. 2012; Bishnoi *et al*. 2018; Shenoy and Jayaraman 2019; Gnat *et al*. 2020c) [14, 9, 2, 27].

succumbed. Gasping immediately after making a small abdominal incision might be suggestive of communication between thoracic and abdominal cavities.

Mucormycosis is an opportunistic invasive fungal infection belongs to the genus rhizopus, mucor, rhizomucor etc. (Hoffmann *et al*. 2013; Upadhyay *et al*. 2019; Walther *et al*. 2019) [14, 32, 34]. Ali R, *et al.* (2006) reported that the mucor pathogens found in soil, decaying debris and cow feed, such as straw. The natural ecological niche of mucorales fungi are soil and decaying material. Ravise *et al.* 1978 [22]; Schonmann *et al*. 1977 [24]; Connolly *et al.* 2005 [6]; Thirion-Delalande *et al*. (2005) [31] reported cutaneous mycoses due to mucor species in cattle, horses and cat.

Results and Discussion

Fig 1a &1b: Buffaloes: Combined cutaneous infection of *M. audouinii* & Mucormycosis

Fig 2: Buffaloe- *M. audouinii* Mucormycosis in SDA culture

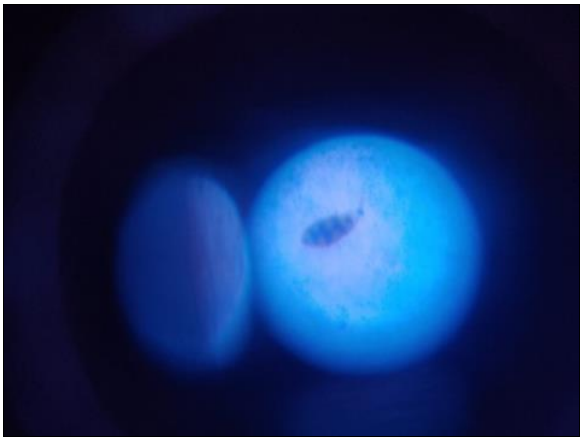

Fig 3a: Buffaloe- *M. audouinii* stained in LPCB

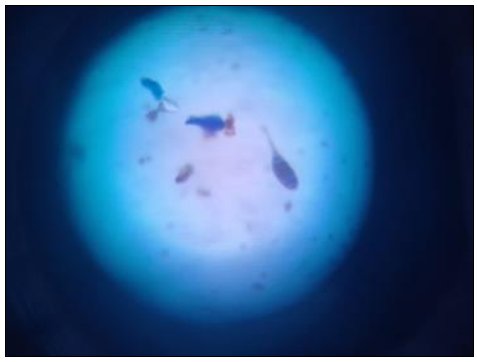

Fig 3b: *M. audouinii* stained in LPCB.

Fig 4a & 4b: *M audouinii* in rice culture-brown color

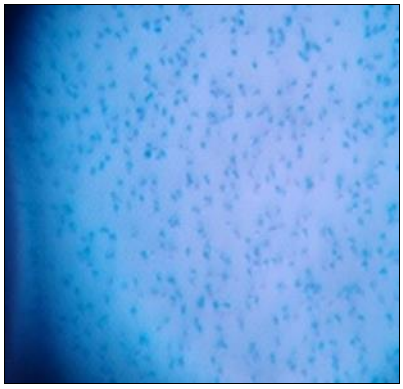

Fig 5: Mucormycosis Sporangiospores stained by LPCB

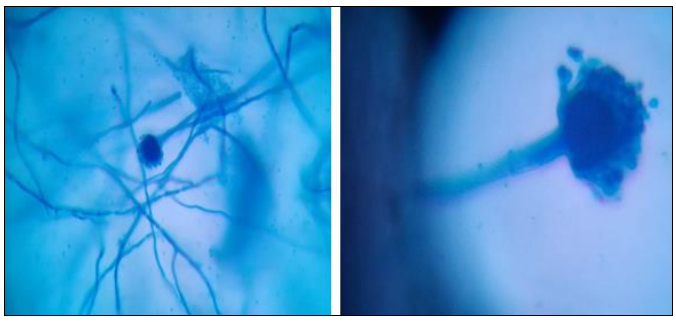

Fig 6a **Fig 6b**

Fig (6a & 6b): Mucormycosis- Mycelium with sporongia & sporesby LPCB staining

Clinical examination of the buffaloes

In this study the buffaloes and their calves suffered with hair loss, white spots, alopecia, muco-cutaneous ulceration, scaling, plaques, necrotizing fasciitis and eschar. (Fig.1)

In cattle, affected animals develop discrete scaling batches of hair loss with grey-white crust that later become a thickly

suppurated crust in variable location. (Mignon and Monod 2011; Swai and Sanka, 2012) [18, 29]. Castrejon-Perez *et al*, 2017; Lin *et al*. 2017; Sahota *et al*. (2017) [5, 23, 16] reported subcutaneous tissues and skin with subcutaneous mycoses. Ram-maert *et al*. 2014; Shields *et al*. 2019; McMahon *et al*. (2020) [21, 26, 17] reported the clinical manifestations of ecthyma-gangrenosum—like lesions, muco-cutaneous ulceration, eschar, necrotic papulo nodules, cellulytic plaques and necrotizing fasciitis or any combination of these lesions in cattle population due to Mucormycosis. In this study the buffaloes and their calves suffered with hair loss, white spots, alopecia, muco-cutaneous ulceration, scaling, plaques, necrotizing fasciitis and eschar. (Fig.1a& 1b).

Culture studies of the skin samples

The skin scrapings collected from the ailing animals subjected to culture studies using SDA revealed both microsporum species and mucormyses species (Fig2). The morphology of the culture and tests with lacto phenol cotton blue and rice grain color pigmentation studies confirmed the presence of the above fungi as *M. audouinii*. (Fig.3a&3b) Further studies for species wise identification of *M. audouinii* carried out by urease enzyme analysis and by rice culture exposure method. (Fig.4a &4b) as per the method of Ellis *et al.* (2013) [8] who differentiated *M. canis* non –sporulating strain, and *M. audouinii* using rice grain for its brown color pigment. Wray *et al.* (2008) [35] identified the mucor species by its morphophological characteristics of the mycelium, filamentous growth of a yellowish/brown spreading colony, sporangiophores and spores in SDA culture. In this study also the mucor species was identified as per this procedure by its morphological structures. (Fig.5,6a & 6b).

The skin scrapings revealed the presence of *M. audouinii* and Mucormycosis by culture in Sabroud dextrose agar media (SDA) and by lactophenol cotton blue staining. Sharma *et al.* (2010) [25] reported the infection of cattle population with *Microsporum gypseum*, *Trichophyton mentagrophytes* and mucor species as a contaminant species. Sravani and Ganesan (2023) [28] reported the prevalence of *M. audouinii* in buffaloe calves. Vipparti 2014 [33]; Gawaz and Weisel (2018) [11] reported co-existence of yeast-like fungi and dermatophytes or mould in the same lesion.

de Crecy *et al*. 2009 [7]; Casadevall *et al*. (2019; Casadevall *et al*. (2020) [3-4] opined that the climatic changes associated with anthropogenic pressure led to adaptation of harmless fungal species to become infectious. Friedman and Schwartz (2019) [10] stated that the expansion of the currently known fungal pathogens happens in the areas with higher average temperatures and wetter environment. The present study was carryout in Jaipur where the city experiences both extreme weather conditions which favor the adaptations of fungal species to become infectious. These observations coincide with the outcome of the present studies.

Conclusions

Studies on the clinical examinations followed by cutaneous lesions examination were carried out in buffaloes. The buffalo population confirmed the prevalence of both microsporum and mucormyces fungal infections with cutaneous lesions. The reasons attributed for the fungal infections in cattle population are; thermal bearing capacity of the animals, anthropogenic pressure on the fungal agents to become infectious, expansion of more pathogens due to higher temperature and wetter environment, more possibilities of transmission of fungal infections from human to animals, and the undefined source of asymptomatic & carrier animals for further infection. Studies needed to characterize the pathogens and the host - dependent disposing factors to the disease state.

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