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Short Communication

Isolation and Prevalence of *Malassezia* Species from Ear Canals of Healthy and Otitic Buffaloes (*Bubalus bubalis*)

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ABSTRACT

Prevalence of *Malassezia* in the ear canals of healthy and otitic buffaloes was studied. Ear swabs were collected from 121 otitic (uni or bilateral) buffaloes (166 ears) and 20 (40 ears) apparently healthy buffaloes. Mycological analysis was carried out by roll swab for cytology and fungal culture on Sabouraud's dextrose agar. Secretion or, cerumen were collected with two sterile swabs Cytological examination by roll swab technique was done by gently rolling one swab on to a clean glass slide and stained with New Methylene Blue and examined under oil immersion. The second swab was inoculated into Sabouraud's Dextrose broth. The broth culture was streaked onto Sabouraud's Dextrose agar, supplemented with olive oil, and incubated at 32°C for one week**.** On cytological examination, 47.5 per cent of healthy and 50.6 per cent of otitic ears showed typical Malassezial cells. The density of Malassezial organisms was greater than 30 organisms per field in majority of otitic ears. Cultural examination revealed that 59.0 per cent of the otitic ears were positive for *Malassezia* species, indicating higher sensitivity. *Malassezia* from otitic ears was isolated either in combination with bacteria (18.7%), parasites (21.1%) or both (19.3%). Though *Malassezia* was prevalent in both healthy and otitic ears, the presence of higher density of organisms in otitic ears might suggest their probable contribution to the pathogenesis of otitis.

Key words: Incidence, otitis, *Malassezia*, buffaloes, Malassezial otitis

INTRODUCTION

Malassezia species are lipophilic yeasts that are normal mycobiota of the human skin and inhabit the skin of various animal species. However, these yeasts are associated with dermatological disorders of the human skin, such as atopic dermatitis, dandruff, folliculitis, pityriasis versicolor or seborrheic dermatitis and intravascular catheter acquired infections. They have been reported from different skin disorders of animals, mainly otitis externa and dermatitis (Cabanes *et al*., 2005).

Few studies revealed *Malassezia* in frequencies ranging from 16 to 33 per cent in the ear and skin samples from healthy cattle (Guillot *et al*., 1994 and Duarte *et al*., 1999). The role of yeasts and fungal mycelia was reported in cattle with parasitic otitis caused by nematodes (Rhabditiform) and Railletia mites (Duarte *et al*., 2001b). However, the role of *Malassezia* in parasitic otitis has not been completely elucidated (Duarte and Hamdan, 2006). Perusal of literature revealed no systematic studies on the occurrence of yeast in buffaloes.

The objective of the present study was to isolate *Malassezia* and determine its prevalence from healthy and otitic buffalo ears.

MATERIALS AND METHODS

Cerumen, secretions or inspissated pus from the external ear close to the external acoustic meatus were collected with the help of two sterile swabs from 20 (40 ears) healthy and 121 (166 ears) otitic buffaloes (*Bubalus bubalis*). The samples were collected from buffaloes aged above 3 years old and of either sex located in different regions in the state of Andhra Pradesh, India. Majority of these buffaloes were Graded Murrah and few were non-descript. Mycological analysis was carried out by roll

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swab for cytology and fungal culture on Sabouraud's dextrose agar. Secretion or cerumen were collected with two sterile swabs from each ear. **C**ytological examination by roll swab technique was done by gently rolling one swab on to a clean glass slide stained with New Methylene Blue as per standard procedure and examined under oil immersion (1000 X)**,** average of the 10 random microscopic fields was taken to record the number of typical Malassezial cells per field (Mactaggart, 2008). Isolation of *Malassezia* was performed by inoculating the material from the 2nd swab in to Sabouraud's Dextrose broth supplemented with 1% v/v of pure olive oil and incubating at 32^{o}C for 48-72 hours (Lee and Lee, 2010). Broth containing the growth was streaked on to 9 cm petri dishes containing Sabouraud's Dextrose agar added with chloramphenicol 150 mg/l. Two drops of olive oil was swabbed on the surface the Sabouraud's Dextrose agar after specimen seeding (Duarte *et al*., 1999). In the present study SDA with olive oil was used for the isolation of lipid dependent *Malassezia* species as suggested by Duarte *et al*., (2003) who opined that olive oil with SDA was superior to Dixion agar for the isolation of *Malassezia* from cattle of tropical region. The plates then were incubated at 32°C for 7 days. The plates were checked every day for development of colonies and their characters. Yeasts were identified according to morphological characteristics and growth in Sabouraud's Dextrose agar. Smears were made out of the colonies**,** stained with New Methylene Blue and observed under oil immersion for *Malassezia***.** Further confirmation was also done by catalase reaction, determined by addition of hydrogen peroxide onto a portion of the colony taken on a glass slide.

RESULTS

Upon microscopic examination of roll swab smears, *Malassezia* appeared as small ovoid or peanut shaped bodies and it was noted that 47.5 % (19/40) of healthy and 50.6% (84/166) of otitic ears were positive for *Malassezia* organisms (Fig. 1). The number of *Malassezia* organisms per field ranged from 2 to 22 in healthy ears and 4 to 43 in otitic ears. It was observed that the organisms were <10 in majority (52.6%) of healthy and >10-30 and above 30 in 42.9% and 44.1% of otitic ears respectively. However**,** it was revealed that presence of more than 30 organisms per field in majority of otitic ears (Table 1).

During isolation of *Malassezia*, chloramphenicol was added to Sabouraud's Dextrose agar to check the bacterial growth, while olive oil was added, as different species of *Malassezia* noticed in buffaloes are lipid dependent. The cultural studies revealed development of cream colored, smooth, convex colonies with pasty texture in 3 to 4 days. They were catalase positive with production of characteristic gas bubbles. Cultural isolation revealed *Malassezia* in 47.5% (19/40) and 59.0% (98/166) of healthy and otitic ears respectively (Fig. 2). Using cytological examination, fungal culture showed good relative specificity (83%) and sensitivity (98%). The results of this study highlight the good specificity of fungal culture compared with the cytological examination because only 1 of 166 samples examined by both techniques was positive by cytological examination but negative by fungal culture. However 14 negative cytological samples were positive on fungal culture. These findings suggest that cultural examination is a more sensitive method of diagnosis than roll swab cytology (Table 2) as the presence of bacteria, inflammatory debris and colored artefacts might complicate the direct microscopic observation of roll swab smears (Duarte *et al*., 2004a). Further, it was observed that one sample positive for *Malassezia* by roll swab cytology was negative by cultural examination.

Cultural studies in healthy ears revealed presence of Malassezia alone and *Malassezia* along with bacteria in 25% (10/40) and 22.5% (9/40) of healthy ears respectively. Similarly *Malassezia* in combination with parasites and bacteria were isolated from 21.1 and 18.7 per cent of otitic ears respectively, while in 19.3 per cent of otitic ears, all the three pathogens were present as mixed infection. Further, yeast always occurred in combination with other pathogens and never recorded as a single entity in otitic buffaloes. PCR was performed in 28 samples (5 healthy and 23 otitic) for the identification of *Malassezia* species and all the samples were positive for *M. sympodialis* yielding a product of approximately 580 bp size specific for 26 S rDNA gene sequence.

DISCUSSION

The prevalence of *Malassezia* species identified by either method was 47.5% in healthy ears that was in accordance with the findings of Gustafson (1960); and Duarte *et al*. (2003) who reported the occurrence of *Malassezia* species from healthy ears of cattle by means of cultural studies as 48.0, and 39.6 per cent respectively. *Malassezia* species in the present study contributed to a prevalence of 59.0% (through cultural examination) in otitis of buffaloes. These findings were in agreement with Duarte *et al*. (1999) who reported the cultural prevalence of *Malassezia* species as 54.7% and the same in another study was reported as 68.9% in cattle with parasitic otitis (Duarte *et al*., 2001a). Though *Malassezia* are normal inhabitants of healthy ears, the number of organisms observed per field in the present study through roll swab cytology in healthy and otitic ears differed greatly. It was observed that the number of Malassezia organisms were less in healthy ears when compared to otitic ears. Similar observation was made by Thomas (1994) who reported that yeasts are generally found in greater numbers in diseased ears rather than apparently healthy ears. The observation of occurrence of *Malassezia* either along with parasites, or bacteria, or both in this study gained support by the findings of Duarte *et al*. (1999; 2001b and

Table 1: Cytological enumeration (per field) of Malassezia in healthy and otitis affected ears (1000x)

S. No	Ear status	Total Ears	Positive	No of *Malassezia* per field			
				< 10	10 to 20	>20 and up to 30	>30
1	Healthy	40 (100)	19 (47.5)	10 (52.6)	5 (26.3)	4 (21.1)	0
2	Otitis	166 (100)	84 (50.6)	11 (13.1)	17 (20.2)	19 (22.6)	37 (44.1)

Figures in parenthesis indicate percentage of occurrence.

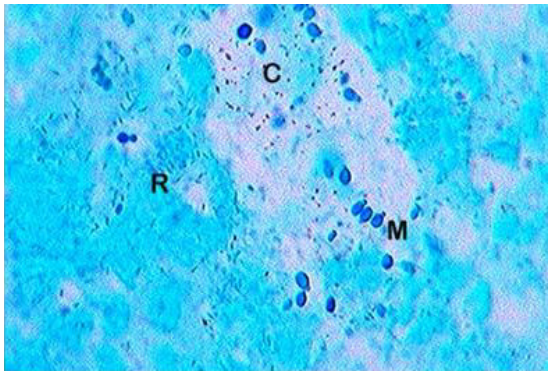

Fig. 1: Photomicrograph of ear cytology revealing cocci (C), rods (R) and Malassezia (M) organisms

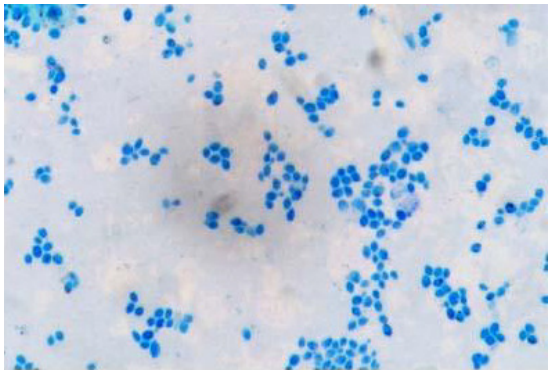

Fig. 2: Photomicrograph showing Malassezia organisms from pure culture.

Table 2: Agreement between results of fungal culture and cytological examination; relative sensitivity (Se) and specificity (Sp) of fungal culture compared with cytological examination

	Cytology positive	Cytology negative	Total
Culture positive	83	14	97
Culture negative	1	68	69
Total	84	82	166

K Value = 0.82(PA); Sensitivity = 98%; Specificity = 83%; Accuracy = 90%; PA = Perfect agreement; Adopting cytological examination as gold standard, fungal culture showed good relative specificity and sensitivity.

2003) who opined that a growth stimulation phenomenon for *Malassezia* occurred by the presence of compounds secreted during the inflammatory process in parasitic otitis. Similarly the presence of *Staphylococci* favored the growth of *Malassezia* and probably the two organisms are mutually benefited through utilization of end products formed by bacterial and yeast lipases (Matausek and Campbell, 2002).

Though previous studies (Huang, 1995) reported that cytological examination should be relied upon for diagnosis of yeast infections. It was observed in the present study, that fungal culture was necessary for more accurate identification, although cytology aids in immediate diagnosis as also reported by Duarte *et al*. (2004a) and Girao *et al*. (2006). One sample positive for *Malassezia* by roll swab cytology was negative by cultural examination might be due to the presence of yeast cells that have lost culturability or due to inhibition of growth due to presence of contaminants (Duarte *et al*., 2004a).

A relative high prevalence of *Malassezia* in buffaloes with or without otitis might be due to anatomical shape of the external ear with accumulation of cerumen favoring growth and maintenance of these yeasts. Cerumen/wax is a mixture of keratin and fat rich compounds. Certain studies revealed that fat percentage in the cerumen of female cattle might be used as an indicator of fat percentage in the milk and opined that increased accumulation of fat rich cerumen in dairy cattle might favor the multiplication of parasites and yeast (Quintavalla *et al*., 2004).

It was concluded that *Malassezia* was prevalent in both healthy and otitic ears, though the rate of prevalence and number of organisms were more in otitic ears. Presence of higher density of organisms in otitic ears might suggest their probable contribution to the pathogenesis of otitis.

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