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FUNGAL ISOLATION FROM SALIVA AND SKIN–SWAB SAMPLES OF CAPTIVE ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*)

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Abstract: Microorganisms are found in the environment; any unhygienic condition may have source of pathogens, which cause infections in animals. Most of the harmful organisms enter into host through air, food and water. Therefore managing hygienic conditions has been emphasized to avoid such pathogenic effect. Asian elephants (*Elephas maximus*) managed in captivity by the Hindu temples, private owners, zoos and circuses are prone to several diseases due to unhygienic conditions of the enclosure, food and water. The present study was carried out to identify fungal species from skin and saliva samples of six captive elephants managed by Hindu temples in Tamil Nadu. Through microbial culture methods form skin-swab and saliva, 15 species including four *Candida* species of fungi were identified. Of these, few species like *Aspergillus* and *Candida* species have pathogenic potentials depending on the physiological condition of the host. The significance of the presence of the pathogens to the health and hygiene of elephants in temples has been discussed.

Keywords: captive Asian elephant, microbes, fungal diseases

INTRODUCTION

The captive Asian elephants constitute about 22–30% of the remaining Asian elephants (Lair 1997) and India harbours about 3400–3600 captive elephants. In India, captive elephants are managed by the state forest departments, Hindu temples and private owners (MoEF 2004). Most of the temple elephants are chained and confined in small enclosures (Vanitha 2007) unlike those in the wild that move extensively with an average of 4–6 km on day-to-day basis for various activities (Baskaran 1998). Confinement in small enclosures or houses may result in behavioural changes like stereotypic body movements (Schmid 1995, Gruber *et al.* 2000, Vanitha 2007) and enhanced susceptibilities to disease due the damp unhygienic conditions that result from build up of urine and faeces in enclosures which the elephant cannot escape from (Galloway 1991, Chandrasekharan *et al.* 1995).

Microorganisms such as bacteria, viruses, fungi, and protozoans are found in the environment. Any unhygienic condition may have source of pathogens causing infections to animals (Ananthanarayan and Paniker 2002).

Main bacterial pathogens are *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Serratia* and *Pseudomonas*. Viral diarrhoea, chickenpox, cytomegalo virus, herpes virus, influenza, enteroviruses, arena viruses may also cause infection. The range of environmental pathogens also includes yeasts (*Candida*), moulds (*Aspergillus, Mucor*) and protozoa (*Entamoeba histolytica, Plasmodium, Toxoplasma gondii*). The main types of nosocomial infections are urinary, wound, respiratory tract, intravascular and gastrointestinal infection (Ananthanarayan and Paniker 2002).

Candida is a polyphyletic genus of ascomycete yeast (Thompson 2001). There are 1.5 million species within the genus (Hawksworth 1992). Although the majorities of them are known to cause disease in animals, *Candida albicans, C. krusei, C. glabrota, C. pseudotropicalis, C. guilliermondii, C. parapsilosis, C. lusitanial* and *C. rugosa* are known as animal pathogens. Conditions such as unhygienic surroundings, wounds and injuries favor the development of candidiasis (Wilson and Sande 2001). *Candida spp.* are among the infectious agents commonly associated with dermatitis in elephants (Mc Culler 1994, Sikes 1971, Redrobe 2000). A *Candida sp.* yeast was isolated together with bacterial infection from the eye of an Asian elephant with ocular lesions (Kodikara *et al.* 1999). The present study examined the fungal species from saliva and skin-swab samples of six captive Asian elephants managed at Hindu temples in Nagapattinam and Tanjore districts of Tamil Nadu, south India between June 2007 and March 2008.

MATERIALS AND METHODS

A total of three to four skin-swabs collected from sunburns, bedsores and injuries of captive elephants were immediately immersed in the saline solution. The saliva samples collected from trunk of the elephant were transferred into a sterile leak proof, screw top container. From each saliva and skin sample, through serial dilution method 10^{-3} and 10^{-4} dilutions were obtained and from these diluted samples, using Sabouraud's dextrose agar medium through plate culture method common fungal species and yeast cells were identified. Later the yeast cells were isolated from the culture plate and sub-cultured for isolation of *Candida* species using Gram's staining method and carbohydrate fermentation tests using standard procedures of Bergey's manual of determinative bacteriology (1993).

RESULTS AND DISCUSSIONS

In total, 12 species of fungi were isolated together from the cultured skin-swab and saliva samples of captive elephants (Table 1). The genus *Aspergillus* was found in all the six elephants and *A. niger* was isolated five out of six elephants tested, revealing common occurrence of these species among the captive elephants. While the genus *Mucor, Penecillium and Trichoderma* were found each in one elephant. The elephant Boomadevi had the maximum number of fungal species among the six elephants studied (Table 1).

Of the six skin-swab and saliva samples analyzed, two elephants (40%) *viz.* Avayambal and Periyanayaki tested positive results for *Candida* sp. indicating a moderate occurrence of *Candida* among the captive elephants. Four different Candidial species were isolated from these samples using germ tube test and carbohydrate fermentation tests and these include *C. tropicalis, C. guilliermondii, C. krusei* and *C. parapsilosis* (Table 2). All the four *Candida* species showed positive results in skin-swab, while in the saliva samples, except the *C. parapsilosis,* remaining three species showed positive results. Among forty-six isolates, *C. tropicalis* was found most commonly (67%) both in skin-swab and in saliva samples, followed by *C. parapsilosis* (15%), and *C. guilliermondii* (13%) and *C. krusei* was the least found species (4%).

There are approximately 1.5 million described species of fungi and a little more than 400 of these species are known to cause disease in animals with few of them pathogenic to mammals (Hawksworth 1992). Among the fifteen species of fungi recorded from the saliva and skin-swab samples of captive elephants in the present study, *Candida* sp. are highly infectious pathogens and could cause skin diseases (Mc Culler

1994). Further, *Aspergillus* sp. and *Mucor* sp. are highly infectious pathogens and cause lung infection, allergy, bronchopulmonary disease and keratitis (inflammation of the cornea) (Inglis 1996). Various health problems have been reported due to *Candida* sp. among the African as well as Asian elephants. *Candida* spp. are among the infection agents commonly found associated with dermatitis in elephants (Mc Culler 1994). Dermatitis has been reported commonly in captive elephants as a result of an inadequate daily skin care (Schmidt 1978, 1986). It may occur in association with sunburn, urine scalding, trauma or infectious agents, or may be of unknown cause; infectious agents associated with dermatitis in elephants include most commonly *Candida* sp., *Staphylococcus* sp. and *Streptococcus* sp. (Mc Culler 1994). Prolonged irritation of the skin of the foot, especially at the skin–sole junction, may cause hyperkeratosis or thickening of the epidermis. During winter, these lesions crack and develop cracked heels (Fowler 1993). *Candida albicans* infection was first reported in a wild African elephant by Sikes (1971) and later was detected in a captive Asian elephant, and is associated with hyperkeratosis of the skin (Redrobe 2000). However, no detailed data are available on the occurrence of fungal pathogens and their impact on wild elephants.

The present study identified the presence of *Candida* sp. in two out of six elephants surveyed and skin diseases, viz. dermatitis and hyperkeratosis, have also been recorded in them. The temple elephants in the study area are chained most of the time in small indoor enclosures with poor hygienic conditions such as unclean shelter, infrequent use of disinfectants, improper disposal of waste and inadequate bathing facility (Vanitha 2007). Further these two elephants were found to have injuries or bedsores and these could also favor in the development of candidiasis as reported elsewhere (Wilson and Sande 2001). The bedsores or pressure sores develop mostly in the cheek and hip regions of the elephant owing to hard surface in which they lie down (AZA 2001). Over 90% of the elephants of temples in Tamil Nadu have enclosures with granite (64%) and cement (32%) floors (Vanitha, 2007). The American Zoo and Aquarium Association (AZA 2001) recommends the flooring of the enclosure to be impervious to water, quick to dry and sloped to drain. Therefore, the hard cement/granite flooring used in elephant households should be modified into smooth surfaces in order to prevent bedsores. Similarly, managing hygienic conditions such as daily bathing, application of disinfectants, clean food and water supply and regular veterinary care should be ensured to reduce the occurrence of the fungal diseases and for upkeep of the temple elephants. Detailed studies need to be conducted to document the occurrence of microbes on captive elephants, and their environment and their impact including on human beings who associate with the captive elephants.

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Table 1: Fungal species recorded from the skin-swab and saliva of the temple elephants studied (using fungal culture method)

S. No.	Elephant age/sex (name)	Place	Fungi species isolated	
			Skin-swab	Saliva
1.	SAF/10 yrs. (Boomadevi)	Kumbakonam	*Aspergillus fumigatus*, *A. sulfurius*, *A. flavus*, *Cladosporium* sp., and *Penicillium* sp.	*Alterneria* sp., *Curvularia* sp., and *A. niger*
2.	AF/21 yrs. (Abirami)	Thirukadaiyur	*A. niger*, *A. flavus*, and *A. fumigatus*	*Curvularia* sp. and *A. niger*
=\3.	AF/43 yrs. (Gomathi)	Thiruvidaimaruthur	*A. niger*, *A. flavus*, and *Cladosporium* sp.	*A. flavus*, and *A. fumigatus*
4.	AF/33 yrs. (Mangalam)	Kumbakonam	*A. fumigatus*, *A. niger*, *A. sulfurius*, and *Curvularia* sp.	*A. niger*, and *Alterneria* sp.
5.	AF/42 yrs. (Avayambal)	Mayiladuthurai	*Rhizopus* sp., *Trichoderma* sp., *A. niger*, and *Candida* sp.	*A. niger*, *A. flavus*, and *Candida* sp.
6.	AF/ 59 yrs. (Periyanayaki)	Vaitheeshwarankoil	*Mucor* sp., *Rhizopus* sp., *A. niger*, *A. flavus*, and *Candida* sp.	*A. niger*, *A. flavus*, *A. fumigatus*, and *Candida* sp.

SAF = Sub-Adult Female, AF = Adult Female.

Table 2. Results obtained for various *Candida* species using of different tests

Candida sp.	Germ tube test	Surface growth	Carbohydrate fermentation test				Urease
			Glucose	Sucrose	Lactose	Maltose	
C. tropicalis	–	+	+	+	–	+	–
C. guilliermondii	–	–	+	+	–	–	–
C. kruset	–	–	+	–	–	–	+
C. parapsilosis	–	–	+	–	–	–	+

+ = Positive and – = Negative

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