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DIMORPHIC FUNGI ISOLATED FROM CAMEL DERMAL MYCOSES

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ABSTRACT

Out of 207 fungal isolates from sporadic cases of camel dermal mycoses, 8 isolates were diagnosed as dimorphic fungi. These fungi included *Sporothrix schenckii, Coccidioides immitis/posadasii* and *Penicillium marneffei.*

Key words: Camel, dermal mycosis, dimorphic fungi, skin

Camel is the most suitable mammal for uses in extreme climatic conditions (Wilson, 1984; Yagil, 1985). The skin infections causing contagious skin necrosis, dermatitis, wounds, abscesses or similar lesions are a constant problem in camel. In practice, antibiotic therapy also does not work satisfactorily. During 5 year-period (from July 2007 to June 2012), information concerning the occurrence of bacterial and fungal diseases along with managemental practices adopted by the farmers for the treatment and control of bacterial and fungal diseases of camel were recorded. Various dimorphic fungi isolated from camels, skin lesions are being presented.

Materials and Methods

Information concerning the occurrence of bacterial and fungal diseases along with managemental practices adopted by the farmers for the treatment and control of camel diseases were recorded during 5 year-period (from July 2007 to June 2012). It was done at camel rearing villages of Bikaner, Nagaur, Barmer, Hanumangarh, Jodhpur, Pali, Udaipur, Jaisalmer, Churu and Jhunjhunu districts with the help of local veterinary officers and heads of the camel rearing Raika community.

Collection of samples: In affected camels with skin lesions, ointments or other local applications present were first removed with an alcohol wipe. Scrapings were collected from lesions using a blunt scalpel in cases of multiple lesions the most recent were chosen for scrapings. The tops of any fresh lesions were removed as the fungus is often plentiful in the roof of the lesions. Swab samples were collected from lesions with abscess formation in sterile vials. These were transferred to the laboratory in thermocol box packed with brine packs.

Isolation and identification of fungi

1. Direct microscopic examination: Scrapings were placed on a glass slide with 2 or 3 drops of 20 per cent potassium hydroxide and placing a cover slip over it and was warmed for 5 minutes over a flame; then carefully examined microscopically for the presence of hyphae and/or arthroconidia.

2. Cultural examination: Samples were first mixed with Sabouraud's dextrose chloramphenicol broth and were than incubated for up to 24 hours. These samples were inoculated on to Sabouraud's dextrose chloramphenicol agar (SDCA) plate and were incubated at 28°C for 3-4 weeks. In case the growth appeared to be of dimorphic fungi, another plate was subcultured and incubated at 37°C for up to 2 weeks for confirming the yeast stage of the isolate. In cases where secondary bacterial infection was suspected and separate swabs for routine bacteriology were not collected, the swabs were directly inoculated first on a blood agar plate, followed by the SDCA plate. These plates were examined daily for the growth of the fungi. The resultant growth was examined for the colony morphology. Microscopic examination was carried out using either lactophenol cotton blue or calcofluor white stains using wet mount method (Halley and Standard, 1973). Fungal species were identified (de Hoog *et al*, 2000; Sutton *et al*, 1998; Weitzman and Summerbell, 1995).

Results and Discussion

Type of lesions: Cases of camel dermal mycoses which were advanced complicated (Fig 1-2), from such lesions dimorphic fungi were isolated mostly in association with either dermatophytes or other filamentous fungi. Except in case of *Sporothrix*

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schenckii which was isolated as pure culture, from the moist, multicentric type of lesions (Fig 3-4).

Species of dimorphic fungi identified: Out of the total 207 fungal isolates from camel skin infections only 8 isolates were dimorphic fungi. These included *Coccidioides immitis/posadasii* (4), *Penicillium marneffei* (3) and *Sporothrix schenckii* (1).

Coccidioides are thermally dimorphic fungi found in soil particularly at warm and dry areas with low rain fall, high summer temperatures and low altitude. The 2 species *Coccidioides immitis* and *C. posadasii* are morphologically identical but genetically and epidemiologically distinct (Fisher *et al*, 2001; Fisher *et al*, 2002). The 2 species can be distinguished by genetic analysis and different rates of growth in the presence of high salt concentrations (*C. posadasii* grows more slowly). *C. immitis* is geographically limited to California's San Joaquin valley region, whereas, *C. posadasii* is found in the desert southwest of the United States, Mexico, and South America. The 2 species appear to co-exist in the desert southwest and Mexico specifically inhabits alkaline soil. *C. immitis/posadasii* is a pathogenic fungus and is among the causative agents of true systemic (endemic) mycoses. Imported cases may occur following travel to endemic areas (Cairns *et al*, 2000).

On SDCA, *C. immitis/posadasii* colonies grew rapidly. At 25 or 37°C the colonies are moist, glabrous, membranous, and greyish initially, later producing white and cottony aerial mycelium. The colonies become tan to brown in colour with age (Fig 5).

Microscopic morphology varies depending upon the incubation temperature at 25°C, hyphae and arthroconidia are produced (Fig 6). Arthroconidia are thick-walled, barrel-shaped. These arthroconidia alternate with empty disjuncture cells. At 37°C, large, round, thick-walled spherules filled with endospores are observed (Fig 7) The definitive identification of an isolated *C. immitis/posadasii* strain requires demonstration of spherule production *in vitro*, use of DNA probes, application of exoantigen tests, or demonstration of spherule production *in vivo* by animal experiments (Larone, 1995; Lindsley *et al*, 2001).

C. immitis/posadasii is the causative agent of coccidioidomycosis in humans. Coccidioidomycosis is one of the true systemic mycoses (Galgiani, 1999). Inhalation of the dry arthroconidia of *C. immitis/ posadasii,* which are carried by dust storms, initiates the infection. The infection remains as an acute and self-limited respiratory infection in most exposed hosts, Spontaneous healing is observed in as high as 95% of the otherwise healthy hosts. It may progresses to a chronic and sometimes fatal disease in others. Airway coccidioidomycosis involving the endotracheal and endobronchial tissues may develop (Polesky *et al*, 1999). Haematogenous spread of the organism results in infection of skin, bones, joints, lymph nodes, adrenal glands and central nervous system (Ampel *et al*, 1986; Bayer and Guze, 1979; Blair and Logan, 2001). Dissemination may occur particularly during pregnany and carries a high risk of mortality (Powell *et al*, 1983). Although, coccidioidomycosis basically effects; otherwise healthy immuno-competent hosts due to the true pathogenic nature of the fungus, it may also develop in immuno-compromised patients, such as patients with AIDS and organ transplant recipients (Blair and Logan, 2001; Medoff *et al*, 1992). Coccidioidomycosis has also been described in warm-blooded water animals such as bottlenose dolphins (Reidarson *et al*, 1998) and horses (Ziemer *et al*, 1992).

Amphotericin B, itraconazole (Li *et al*, 2000) and voriconazole (Kappe, 1999; Li *et al*, 2000) are active *in vitro*. Nikkomycins are additive to synergistic *in vitro* with fluconazole or itraconazole against this fungus (Li and Rinaldi, 1999). Amphotericin-B (Drutz, 1983) and azoles, such as fluconazole, itraconazole, and ketoconazole are used for treatment of coccidioidomycosis (Blair and Logan, 2001; Galgiani *et al*, 2000; Medoff *et al*, 1992). Animal experiments suggest that caspofungin (Gonzalez *et al*, 2001), sordarins (Aviles *et al*, 2001; Clemons and Stevens, 2000; Odds, 2001) and nikkomycins (Hector *et al*, 1990) are also promising in treatment of coccidioidomycosis.

Vaccine development for prevention of coccidioidomycosis are in progress (Dixon *et al*, 1998; Jiang *et al*, 1999; Peng *et al*, 1999; Zimmermann *et al*, 1998).

Amongst various species of Penicillium, only one species *P. marneffei* is thermally dimorphic and produces filamentous, flat, radially sulcate colonies at 25°C. These colonies are bluish-gray-green at centre and white at the periphery. The red, rapidly diffusing, soluble pigment observed from the reverse is very typical (Fig 8-9), at 37°C colonies are cream to slightly pink in colour and glabrous to convolute. Microscopically, the yeast phase is visualised as globose to elongated sausage-shaped cells (3 to 5 µm) that multiply by fission. Microscopically, the filamentous stage of *P. marneffei* is similar to other species.

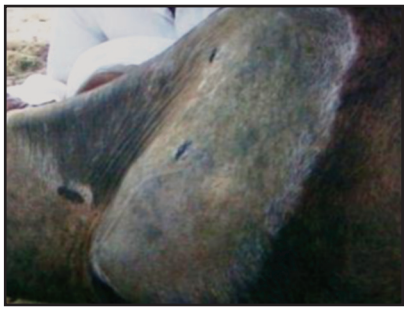

Fig 1. Acid burn like lesion dry and fast spreading.

Fig 5. *C. immitis;* colony at 37°C (7 days front).

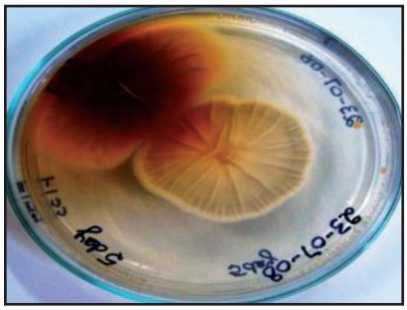

Fig 9. *P. marneffei;* colony producing red diffusible pigment (5 day reverse).

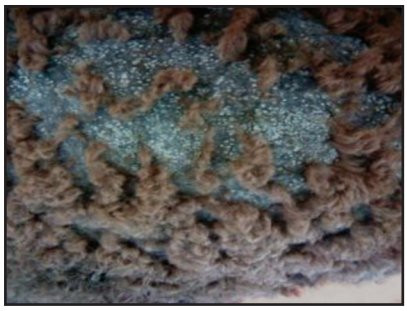

Fig 2. Moist lesion on the flank with lot of granular debris deposit.

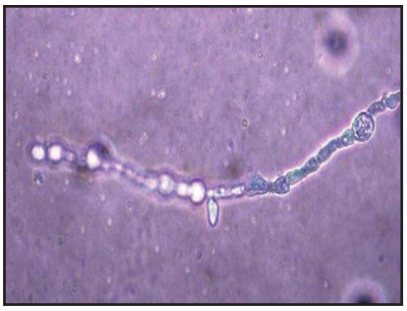

Fig 6. *C. immitis;* arthroconidia at 25°C.

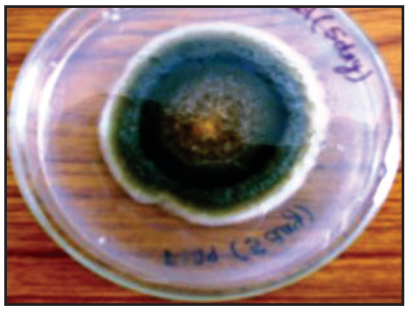

Fig 10. *S. schenckii;* colony at 28°C (5 days front).

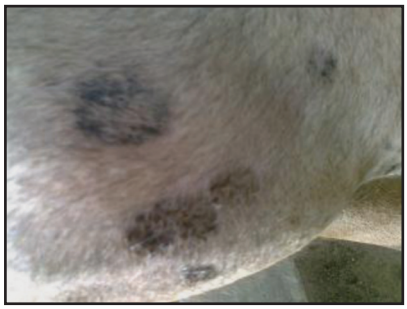

Fig 3. *S. schenckii* moist, multicentric lesions on the belly.

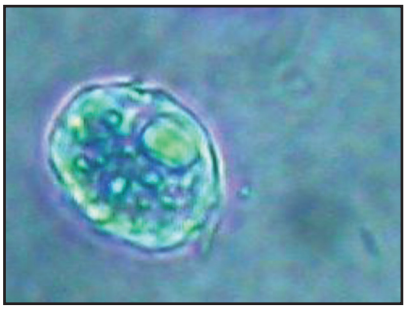

Fig 7. *C. immitis;* spherule with endospores.

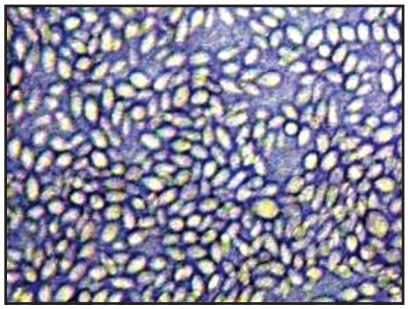

Fig 11. *S. schenckii*; yeast cells at 37°C.

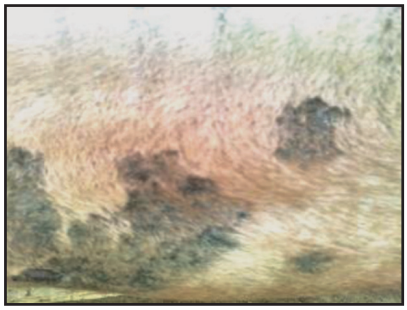

Fig 4. *S. schenckii,* fast spreading moist lesions on the belly.

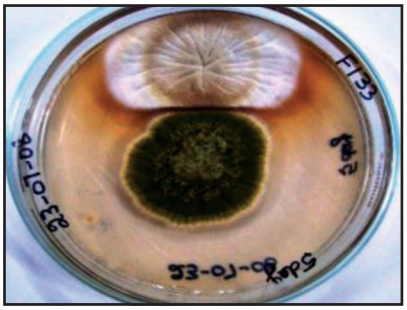

Fig 8. *P. marneffei;* colony with green saprophytic spp. (5 day front).

P. marneffei is pathogenic and endemic specifically in Southeast Asia where it infects bamboo rats which serve as epidemiological markers and reservoirs for human infections. *P. marneffei* is pathogenic particularly in patients with AIDS and its isolation from blood is considered as an HIV marker in endemic areas (Deng *et al*, 1988; Singh *et al*, 1999; Supparatpinyuo *et al*, 1992). *P. marneffei* infections have also been reported in patients with haematological malignancies and those receiving immunosuppressive therapy (Wong *et al*, 2001). Infection is acquired via inhalation and results in initial pulmonary infection followed by fungemia and dissemination of the infection (Rimek *et al*, 1999; Singh *et al*, 1999; Garbino *et al*, 2001). The lymphatic system, liver, spleen and bones are usually involved. Acne-like skin papules on face, trunk, and extremities are observed during

the course of the disease. *P. marneffei* infection is often fatal.

Amphotericin B, oral itraconazole and oral fluconazole have so far been used in treatment of *P. marneffei* (Cheng *et al*, 1998; Lortholary *et al*, 1999; Rimek *et al*, 1999). Oral itraconazole was found to be efficient when used prophylactically against *P. marneffei* in patients with HIV infection (Chariyalertsak *et al*, 2001).

Sporothrix schenckii is a thermally dimorphic fungus. At 28°C, colonies are slow growing, moist and glabrous with a wrinkled and folded surface. Some strains may produce short aerial hyphae and pigmentation may vary from white to cream to black (Fig 10). Conidiophores arise at right angles from the thin septate hyphae and are usually solitary, erect and tapered towards the apex. Conidia are formed in clusters on tiny denticles by sympodial proliferation of the conidiophore, their arrangement often suggestive of a flower. As the culture ages, conidia are subsequently formed singly along the sides of both conidiophores and undifferentiated hyphae. Conidia are ovoid or elongated, hyaline, one-celled and smooth-walled. In some isolates, solitary, darkly pigmented, thick-walled, one-celled, obovate to angular conidia may also be observed along the hyphae. At 37°C, colonies are glabrous, white to greyish yellow and yeast-like, consisting of spherical or oval budding yeast cells (Fig 11).

As shown in Figs 3 and 4, the type of moist, multicentric lesions *S. schenckii* was isolated from such lesions *S. schenckii*, has 2 important mechanisms through which its potential to infect the mammalian host is maximised. First, *S. schenckii* has the ability to change phases to an ascomycete telemorph that survives on living or decaying plant material. This fungus has been isolated from decaying vegetation such as thorns, straw, hay, wood, moss and soil. Second, after entering the skin via puncture, bite, or scratch, the fungus converts to a yeast phase, thereby causing lesions locally and possibly systemically in the mammalian host. *S. schenckii* can be found worldwide. Sporotrichosis is particularly common in the tropics where high humidity and temperatures promote fungal growth.

S. schenckii is the causative agent of sporotrichosis or rose handler's disease (Rex and Okhuysen, 2000). Sporotrichosis is a subcutaneous infection with a common chronic and a rare progressive course. Following entry of the infecting fungus through the skin with a minor trauma infection may spread via the lymphatic route and nodular lymphangitis may develop (Kostman and DiNubile, 1993; Tomimori-Yamashita *et al*, 1998). Patients infected with *S. schenckii* may be misdiagnosed as pyoderma gangrenosum due to the large ulcerations observed during the course of sporotrichosis (Byrd *et al*, 2001).

Itraconazole is generally used for the treatment of lymphocutaneous infection (Conti *et al*, 1992; Lortholary *et al*, 1999; Sharkey-Mathis *et al*, 1993), while amphotericin B is indicated for severe infections or when itraconazole therapy fails (Kauffman *et al*, 2000). Potassium iodide is one of the oldest therapeutic modalities used for treatment of sporotrichosis (Tomimori-Yamashita *et al*, 1998)

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