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Therapeutic management of fungal dermatitis in captive wild animal

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Abstract

The study was conducted to determine fungal dermatitis in captive wild animals of Assam State Zoo cum Botanical Garden, Guwahati, Eastern Assam Wildlife Division, Bokakhat and Centre for Wildlife Rehabilitation and Conservation, Bokakhat for a period of 1 year (Aug 2013- July 2014). In the study a total of 105 cases belonging to 21 different species were studied. Fungal isolates were found in 16 animals out of 62 affected with skin diseases. *Trichophyton tonsurans*, *Trichophyton concentricum*, *Epidermophyton floccosum*, *Microsporum nanum*, *Aspergillus niger* and *Penicillium sp* were isolated on the basis of cultural and microscopic characteristic.

Keywords: Fungal dermatitis, captive wild animal, *Trichophyton tonsurans*, *Trichophyton concentricum*

Introduction

Fungal dermatitis is one of the major dermatological disorders observed in captive wildlife facilities of Assam. The Assam State Zoo cum Botanical Garden (popularly known as Guwahati Zoo) which is a largest of its kind in the North East region of India has the prevalence of fungal dermatitis, which is recorded in less captives, but the recovery does not happen in time and reoccurrence is noticed significantly. Although the cause of occurrence of the disease is not known but injury, age, immune deficiency and poor health condition may be possible factors according to the caretakers of the facility. Similar, clinical manifestations have been noticed by the forest veterinarian and caretakers in captive elephants of Eastern Assam Wild life division. Pododermatitis in Asian elephant is another problem, which requires intensive care and management. Another captive facility, Centre for wildlife Rehabilitation and Conservation, Bokakhat that rehabilitates the rescued animals as soon as they recovered from the ailments. This facility is also facing similar problem but in less number. Considering the above factors, objective of present study was undertaken for isolation and identification of causative dermatophytes and to derive an effective therapeutic regimen of fungal dermatitis in captive wild animals.

Materials and Methods

Prior to collection of samples, history of each affected animals was recorded. The affected animals were immobilized by applying following three methods viz: Chemical immobilization, physical restraining and squeeze cage. Royal Bengal tiger, White Tiger, Leopard, Himalayan Black bear and Stump tailed macaque were tranquilized by Ketamine hydrochloride @ 5-10 mg/kg and Xylazine hydrochloride 0.1-0.5 mg/kg and in Hog deer Ketamine hydrochloride @ 1-2 mg/kg and Xylazine hydrochloride 0.4-0.5 mg/kg was used. Reversal Yohimbine hydrochloride was used at the dose rate equal to the Xylazine in each cases. The materials from the skin lesions were collected by maintaining possible aseptic precautions for investigation of mycotic infection according to the procedure described by AL-Doory (1980)^[1]. The area showing visible skin lesions was washed with 70 % alcohol or sterile water and allowed to dry. Materials like scales, scraps and a part of defoliating skin were collected with the help of a sterile scalpel and forceps from the margin as well as from the centre of the lesion and transferred immediately into sterile glass vial/test tubes and brought to the laboratory for detection of dermatophytes by culture. Direct microscopic examination of the specimen, was done by placing the collected material on a clean glass slide with 1-2 drops of 10 % Potassium hydroxide (KOH).

A cover-slip was placed over the mixture and the slide was heated gently over a flame and then removed just before the mixture reaches the boiling point. The slide was examined under a microscope to detect hyphal segments, spores, budding yeast and mycotic granules. Further primary isolation of the fungus was done in Sabouraud's Dextrose Agar with Chloramphenicol (SDA) medium. Scraps, scales defoliating skin materials collected were inoculated onto the SDA medium plate as per Scott (1988) [10]. The inoculated plates were sealed by parafilm and kept in a dark place, away from sunlight under the room temperature for 30 days to develop. The plates were checked every 2-3 days for the growth of fungi. Observation of gross appearance, nature of growth and growth rate was one of the criteria for colony identification on culture media. This observation also includes size, shape, colour, undersurface and texture. The identification of fungi was done as per the method described by Al-Doory (1980) [11] and Ellis *et al.* (2007) [12]. A tuft of the fungal growth on SDA was picked up with the help of a sterile platinum loop and placed into a drop of lacto phenol cotton blue on a clean grease free slide. The material on the slide was then teased several times by sterile teasing needle and a cover slip was placed on it. The wet mount thus prepared was observed under microscope to study the morphology and for the identification of macro conidia and micro conidia. The presence of hyphae and spores were also recorded.

Results and Discussion

In the present study fungal isolates were found in 16 animals out of 62 affected with skin diseases. A total of 26 fungal isolates was recorded from 10 positive cases of fungal and 6 cases of mixed infection of fungal and bacterial dermatitis. Out of 26 fungal isolates dermatophytes detected were *Trichophyton tonsurans* 6 (23.07 %) (Fig. 1) and *Mucor* sp. 2 (7.69 %) from Brown fish owl, *Trichophyton concentricum* 5 (19.24 %) (Fig. 2) and *Penicillium* sp. 3 (11.54 %) from elephant, *Microsporum nanum* 3 (11.54 %) and *Aspergillus niger* (11.54 %) from Leopard and *Epidermophyton floccosum* 2 (7.69 %) and *Aspergillus niger* 2 (7.69 %) from White tiger as shown in table no. 1. Chakraborty (2003) [5] reported *Aspergillus niger*, *Candida albicans*, *Zygomycosis*, *Trichophyton terrestre* and *Microsporum* sp. from foot and body lesion of captive elephants in Assam. Present finding of *Trichophyton concentricum* is contrary to his findings. While, Fowler (2006) [6] mentioned *Penicillium* sp. as the causative agent of pododermatitis in elephant species which is similar to the present findings. Bacterial isolates *S. intermedius* from 2 white tiger and *S. aureus* from 2 leopard and 2 elephants was isolated from mixed infection by bacteria and fungi respectively. Similar studies conducted by Vodika (2007) [12] isolated staphylococcus species from the skin lesion of trunk pyoderma in a male Ceylon Elephant (*Elphus maximus maximus*) while Fowler (2006) [6], described *S. aureus* as a pathogenic organism isolated from the elephant feet. Their findings of staphylococcus species from the skin lesion is found similar with the present study.

Ten positive cases of fungal dermatitis were recorded in three male elephants (2 sub-adults and 1 adult), one adult male leopard and six unsexed adult Brown fish owl. The isolation, identification and drug sensitivity of the fungal isolate was a long process, that delays rendering treatment to the affected ones and also due to time bound research period, keen clinical observation of the skin lesion and morphology and microscopy of the cultural study of isolates was given priority for prompt treatment. Although *in vitro* drug sensitivity test

for bacterial isolates was done and treatment was given based on the result and easy availability of the concerned drugs in the market. All the species affected with fungal dermatitis were observed for clinical recovery after treatment at weekly interval up to 60 days in Asian elephant, while in Leopard and brown fish owl 42 days.

In Asian Elephant, the skin lesions were mainly noticed in the abdomen, neck, leg and foot regions. The animal remains apparently normal with sound appetite, but signs of dullness and rubbing the body in the surrounding trees were observed. All the three elephants were treated with clotrimazole powder (Candid powder, Glanmark Pvt. Ltd.) after proper dressing of the lesion with povidone iodine solution (Bactocept Solution, Ranbaxy Pvt. Ltd.) for two weeks and continued for another week on alternate days. They were supplemented with vitamin (Vimeral, Virbac Animal Health India) and mineral Supercare liquid (Exceller Pvt. Ltd.) orally at the rate of 40 ml and 100 ml respectively once daily for 4 weeks. On day 7th slight improvement was seen, on day 21st clinical improvement was observed in affected area, on day 35th high recovery and on day 42nd two of the elephants showed complete clinical recovery. The recovered elephants showed disappearance of clinical lesions. However, one of the elephants could not showed marked recovery and on day 35th slight disappearance of the skin lesion was noticed. No significant recovery was observed with this elephant till the day 60th.

In Leopard, skin lesion was seen over the body forming scab and alopecia on the neck, back, lumbar and caudal aspect of the tail. Rubbing the body surfaces against surrounding objects was a prominent sign. It appeared, dull, depressed and off-fed. The affected area was treated with povidone iodine and then Miconazole (Kiskin ointment, Intas Pharm. Ltd.) was applied. It was supplemented with oral vitamin (Viselum, Brihans Pvt. Ltd.) at the rate of 20 ml once daily for 3 weeks. The animal showed clinical improvement from the day 7th and 14th. Marked recovery on day 21st and complete recovery were observed on day 28th. (Fig.3 & 4).

In Brown fish Owl, Clinical lesion was seen in each eyelid of the three birds. Granular type lesion was observed on distance examination. On close examination the crusts are felt with defoliating scales and loss of feather from the eyelid. Scabs are also observed in the legs of two birds. Decreased flight with inappetence was one of the prominent sign. Gradually, similar symptom was observed in rest of the three birds kept in the same enclosure. First three affected birds were given an oral dose of Fluconazole (Zocon 5 mg, FDC Ltd) 1/3rd of 5 mg tab, once daily for one week and then continued on alternate days for another one week. Other, three birds were treated with topical application of Ketoconazole ointment (Nizral, Johnson & Johnson Ltd.) once daily for one week and then continued alternate days for another one week. Both the groups were supplemented with 1 ml of Zyncovit Syrup (Apex Lab. Pvt. Ltd.) once daily for three weeks (Fig. 5 & 6). On day 7th birds treated with Ketoconazole ointment showed slight recovery, on day 14th clinical recovery and by the day 21st to 28th complete clinical recovery was observed. It was characterized by decrease in thickness of the inflamed eyelids, loss of scabs and granulation. The birds became gradually active with normal appetite. On the other hand, owls treated with Fluconazole, started showing slight clinical improvement from the day 14th and by the day 21st to 28th complete clinical recovery was observed. But, one bird could not show any clinical improvement till day 42nd.

Mixed infection of fungal and bacterial dermatitis was recorded in two sub adult male Asian elephant, two male and female sub- adult white tiger and two adult male leopards. Elephants were found apparently normal with sound appetite. The lesion was mainly observed in the foot pad with pyogenic exudates in the inter-digital space. They showed frequent lifting of the affected limb and reluctant in walking.

The lesion was first washed with strong solution of KMnO_4 and then povidone iodine is applied topically. Then a paste comprises of clotrimazole, povidone iodine ointment and boric acid is applied. The elephants were supplemented with vitamins (Vimeral) and Mineral (Supercare) once daily for 4 weeks. Till day 7th no improvements were observed in the lesions. On day 14th slight healing was observed in one of the elephant, from day 21st clinical recovery was observed as the animals did not showed sign of pain during walking. From day 35th and 42nd improvement in clinical recovery was observed, while from 49th to 60th day, both the elephants showed complete disappearance of the skin lesions. The animals were kept in rest until complete recovery.

In White Tiger and Leopard, superficial to deep pyodermatitis was seen in neck, back, lumbar, and in the inter-digital space. Licking of the affected area and rubbing the body against the surrounding object was noticed. The area remains wet and rough body coat with alopecia was another clinical manifestation.

Both the species were administered with Ceftriaxone/Sulbactam @ 2.5 g i/mly for seven days along with application of povidone iodine (Bectosept) and miconazole (Kiskin) for 14 days once daily topically. Supportive therapy of multivitamin (Multistar pet, Mankind Pvt. Ltd.) was administered @ 20 ml. daily for two weeks. Clinical improvement was observed from day 7th and complete recovery was observed from the day 28th onwards. However, one leopard did not show any sign of recovery with this treatment schedule.

In the present study treatment of fungal dermatitis with povidone iodine was found effective in case of elephants and leopard. Efficacy of povidone iodine in fungal infection was described by Kondo *et al.* (2011) [7]. The result of the treatment with Miconazole was found to be 100 % effective in Leopard. While in Brown Fish owl Ketoconazole was found to be 83.34 % effective. Efficacy of Ketoconazole in canine dermatophytosis was studied by Bhardwaj *et al.* (2012) [4]. The affected dogs were treated with Tab. Nizral 10 mg/kg twice daily orally. The present finding was contrary to the species affected, and the route of administration. Though the route of administration was different but the result of efficacy is similar.

Application of clotrimazole, povidone iodine and boric acid in the affected areas along with administration of oral multi vitamin resulted in the resolution of mixed fungal dermatitis in captive elephants. Further study is suggested for the efficacy of combine effects of clotrimazole, povidone iodine and boric acid. Two White tiger and one Leopard were clinically recovered from the day 14th onwards with the treatment of ceftriaxone/sulbactam injection along with oral multi vitamin and mineral administration. Described the efficacy of this combination of drugs in dogs. Though the present study was conducted in White Tiger and Leopard, the efficacy of the drug showed similar results.

Vitamin A is essential for wide range cell and tissue function, its cutaneous role is particularly directed towards keratinocyte proliferation and differentiation by regulating expression of keratin. Considering the fact a supportive therapy of

multivitamin was administered orally to the affected animals. Use of vitamin A in canine dermatitis was described by Beigh *et al.* (2013) [3] and Baviskar *et al.* (2013) [2]. Reference regarding dermatitis in different species of wild and captive wild animals is very scarce and the author had taken the reference of domesticated animals. As, it is worth to mention that many species of wild animals are zoologically related to domestic animals and the diseases of wild animals are almost the same as of their domestic counterpart except for differences in the severity and other epidemiological features. However, the wild animals are generally considered more resistant to the disease as compared to the domestic animals, although they serve as reservoirs of some epizootics. Treatment of diseases of wild animals is also based on the same fundamental principles as in domestic animals. Administration of medicines, however, remains a problem which may largely depend on the skill and presence of mind of the veterinarian but at the same times may become difficult.

Very little information is available on the wild animal pharmacology (drug action, dose, frequency of administration, blood concentration, drug metabolism, idiosyncrasies, toxicity, contraindication etc.) and generally the information available in veterinary pharmacology is applied, Sharma (2003) [11] thus there is need for some more studies.



Fig.1: Photograph showing microscopic morphology of macroconidia of *Trichophyton tonsurans*



Fig. 2: Photograph showing macroscopic colony morphology of *Trichophyton concentricum* on SDA



Fig.3: Photograph showing fungal dermatitis in dorsum, before treatment of a Leopard (*Panthera pardus*)



Fig 5: Photograph showing fungal dermatitis in eyelids, before treatment in a Brown Fish Owl (*Ketupa zeylonensis*)



Fig.4: Photograph showing disappearance of fungal dermatitis in dorsum, after treatment of a Leopard (*Panthera pardus*)



Fig 6: After treatment.

Table 1: Isolation of fungus from skin lesions of captive wild animals

Species	Isolates	No. of isolates	Percentage (%)
Elephant	<i>Trichophyton concentricum</i>	5	19.24
	<i>Penicillium sp.</i>	3	11.54
White tiger	<i>Epidermophyton floccosum</i>	2	7.69
	<i>Aspergillus niger</i>	2	7.69
Leopard	<i>Microsporium nanum</i>	3	11.54
	<i>Aspergillus niger</i>	3	11.54
Brown fish owl	<i>Trichophyton tonsurans</i>	6	23.07
	<i>Mucor sp.</i>	2	7.69
Total		26	100.00

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