RESEARCH ARTICLE

Haemato-Biochemical Changes in Dermatitis Associated with *Penicillium* spp. in Dogs

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Abstract

Skin infection in dogs is classified as primary infection, caused by infectious organisms directly and secondary infections, which are augmented by underlying conditions that act as catalysts for infections such as bacterial or fungal infections. Among fungal infections, *Penicillium* is one of the largest genera. *Penicillium* species can cause allergies and both superficial and invasive infections. Keeping the above facts in view, the present research was undertaken to study the haemato-biochemical alterations in clinically affected dogs with *Penicillium* spp. associated dermatitis. A total of 200 dogs were selected for the study. Skin scraping samples were collected for culture and microscopic identification. Out of 200 dogs screened, 23 (11.5%) dogs were found positive for *Penicillium*-associated dermatitis based on skin scraping, *Penicillium* culture and identification. *Penicillium*-associated dermatitis in dogs revealed an increase in TLC, lymphocytes, eosinophils and total protein, and a decrease in serum zinc and copper concentration. On the basis of heamato-biochemical profile group T_1 showed better recovery. Twelve positive dogs, 6 each, treated with Tab. Terbinafine @ 30 mg/kg b.wt. (T1), and Tab. Itraconazole @ 10 mg/kg b.wt. (T2), PO, OD, for 21 days, with supportive therapy, resulted in normalization of these altered profiles by day 28 of treatment, at par with healthy control group, (T3, n=6). Basis of clinical recovery and haemato-biochemical analysis treatment Tab. Terbinafine showed better results from *Penicillium*-associated canine dermatitis.

Key words: Dermatitis, Dogs, Haemato-biochemical profile, *Penicillium* spp, Therapy.

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Introduction

There are more than 300 species of *Penicillium*, a common fungus in the environment (Langlois *et al*., 2014). Most *Penicillium* species are believed to be opportunistic and nonpathogenic to both humans and animals (Miyakawa *et al*., 2011). Dermatological problems are common in dogs, and their frequency varies according to the season, the local climate, and the management methods employed (Sharma *et al*., 2008). Dermatological issues are among the most frequently reported (about 20-70%) and challenging issues that veterinarians face in small animal medicine (Scott *et al*., 2001). Sindha *et al*. (2015) reported significantly greater TLC values in all cases of non-parasitic dermatoses as compared with the control, although the average values of Hb, PCV and TEC were significantly lower in bacterial, fungal, and other non-parasitic dermatological diseases. In acariasis, there was a drop in Hb, PCV and TEC and an increase in eosinophils (Narang *et al.,* 2015). Mange and pyoderma are associated with lymphopenia and neutrophilia. With the exception of bacterial dermatitis, all etiological groups show a substantial rise in total proteins and globulins and a decrease in albumin and the A:G ratio (Narang *et al*., 2015). El-Motaily *et al*. (2022) reported that serum copper and zinc concentrations decrease in dogs with dermatitis. Zinc is an essential trace element found in all body tissues and body fluids. Around 85% of body zinc is present in muscles and bones, 11% in skin and liver, and 2% to 3% in other organ tissues. Zinc also has a particular role in keratinization of the epidermis (Pond *et al*., 2004). The current study was aimed to evaluate haemato-biochemical changes in canine dermatitis associated with *Penicillium* spp.

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Materials and Methods

The present study was conducted on clinical cases presented at the outpatient ward of the Department of Veterinary Medicine, Veterinary Clinical Complex, Bihar Veterinary College, Patna (India), to determine the prevalence of *Penicillium*-associated dermatitis in dogs in and around Patna.

A thorough medical history including documentation of

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any prior treatments was recorded. The dogs showing lesions of suspected mycotic infection, such as alopecia (patchy or generalized), erythema, papules, pustules, scabs, scales, scars and varied degrees of inflammation, irrespective of their age, sex or breed were selected for the dermatological study. Two hundred dogs were screened for this study.

Collection of Skin Scraping

The lesion of selected cases suspected for *Penicillium*-associated dermatitis was taken for scraping of the epidermal layer together with hair along with healthy tissue along the margin of the lesions after cleaning with normal saline to prevent the growth of other saprophytic organisms. The collected samples were stored in an airtight, sterile container and labeled for further investigation.

Inoculation and Identification of *Penicillium* spp.

The collected samples were inoculated on the Mycobiotic Agar culture (SDA) plates with the help of an inoculation loop, labeled and incubated at 25°C (Simpanya and Baxter, 1996). The samples were observed twice a week for the presence of any growth. It was kept in an incubator for one month. If no growth was detected in one month, then the sample was considered negative.

The positive *Penicillium* culture was identified based on colony characteristics, shape, size and microscopic examination of conidial cell structure produced and the presence of septate with number, and the arrangement of conidial cells around the hyphae following lactophenol cotton blue staining (Seker and Dogan, 2011).

Therapeutic Study

Twelve positive cases of *Penicillium* associated dermatitis were taken for therapeutic study and were randomly divided into two groups T1, and T2 consisting of 6 dogs each to assess the efficacy of two therapeutic regimens, *viz.,* Tab. Terbinafine @ 30 mg/kg b.wt., and Tab. Itraconazole @ 10 mg/kg b.wt., PO, OD, for 21 days, respectively, along with Herbal supplement orally containing *Phyllanthus emblica* (Amla), *Cynarascolymus* (Artichoke) and Bitter melon @ 5, 10 and 15 gm/day for small breeds below 10 kg, 11-25 kg body weight and 26 kg & above body weight, respectively, and other need based symptomatic treatment in both the groups till day 28. Six healthy dogs were kept as healthy control group T3.

Blood Collection and Haemato-Biochemical Analyses

Approximately 5 mL of blood was collected aseptically from the cephalic or saphenous vein of all selected dogs for treatment, including control, on day 0 and day 28th. Three millilitres of blood in EDTA vials were analyzed for haematological parameters using an automated haematology analyzer (Celltac α VET, Nihon Kohden, India). The remaining 2 mL was collected in plain vacutainers, centrifuged at 1500 x g for 10 min to obtain serum, and stored at −20°C until biochemical analysis. Serum total protein, sodium, and potassium were estimated using a Fujifilm Dry Chemistry Analyzer (Fuji Photo Film Co., Ltd., Japan), while serum zinc and copper concentrations were determined using commercial colorimetric assay kits (Thermo Fisher Scientific).

Statistical Analysis

Data were statistically analyzed using SPSS software version 29, with Mean ± SE determined by the descriptive statistical method. One-way analysis of variance and Duncan's multiple range tests were applied to compare means among different groups with their respective base values as described by Snedecor and Cochran (1994). P-values <0.05 were considered statistically significant.

Results and Discussion

A total of 2975 numbers of canine cases were presented at TVCC, Patna, during July 2024 to December 2024, in which 592 cases were suspected for dermatitis. Out of 592 suspected cases, 200 cases were screened for the study of *Penicillium*-associated dermatitis. Out of them, 23 dogs were found positive for *Penicillium*-associated dermatitis with an incidence of 11.5%. A similar result (9.8%) of *Penicillium* spp was found by Pandey (2024) in Patna. The confirmatory diagnosis was made using lactophenol cotton blue stained fungal growth from the culture media and examining it microscopically to observe the characteristic morphological features of the isolate (Fig. 1 & 2)

Haematological Changes

The mean values of haematological parameters are illustrated in Tables 1 and 2. The values of Hb, TEC and PCV were non-significantly lower in T_1 and T_2 groups as compared to control group of dogs. However, a significant ($p<0.05$) increase in TLC was observed in Group T_1 and T_2 as compared to the control group (Table 1). These findings aligned with the work of El-Motaily *et al*. (2022) and Remi *et al.* (2012), who documented transient leukocytosis in dogs with dermatophytosis. Findings of this study were consistent with Sindha *et al.* (2015), who also reported leukocytosis in dogs suffering from mycotic dermatitis. The increase in leukocyte count may be attributed to toxins released as a result of tissue damage or necrosis caused by inflammation, or it may stem from a secondary bacterial infection. Prior studies by Stockham *et al.* (2003) and Nair and Nauriyal (2007) have found non-significant decreases in the TLC in dermatophytosis-infected dogs.

Table 1: Erythrogram of dogs with dermatitis associated with *Penicillium* spp. compared with that of apparently healthy dogs on day 0 or 28th of treatment (Mean ± SE)

Group	Day	Hb (g/dL)	PCV (%)	TEC (x10^6/µL)	TLC (x10^3/ µL)
Group T_1	0	13.67±0.26	39.68±1.09	5.93±0.18	14.32±0.44^{Aa}
	28	14.75±0.44	42.91±1.04	6.38±0.26	10.48±0.70^{b}
Group T_2	0	13.53±0.37	38.90±1.83	5.93±0.23	14.37±0.77^{Aa}
	28	14.21±0.23	40.50±0.94	6.00±0.15	10.55±0.43^{b}
Control	0	14.93±0.85	43.50±2.95	6.58±0.48	11.08±1.00^{B}
	28	14.72±0.68	42.83±2.71	6.37±0.45	10.87±1.02^{b}

The different uppercase superscripts between groups and lower case superscripts within the group (between days) differ significantly (p<0.05).

Table 2: Leukogram of dogs with dermatitis associated with *Penicillium* spp. compared with that of apparently healthy dogs (Mean ± SE)

Group	Day	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)	Basophils (%)
Group T_1	0	62.50±2.17	30.83±1.66^{Aa}	3.33±0.21^{Aa}	4.83±0.48	0
	28	67.00±0.83	24.16±0.30^{b}	2.50±0.22^{b}	5.33±0.33	0
Group T_2	0	62.33±2.92	28.67±1.96^{Aa}	3.33±0.42^{Aa}	4.67±0.56	0
	28	69.17±0.60	22.33±0.88^{b}	2.00±0.26^{b}	4.67±0.33	0
Control	0	68.83±0.30	23.67±0.42^{B}	1.83±0.30^{B}	5.33±0.33	0
	28	68.83±0.48	22.67±0.67	2.00±0.26	5.83±0.48	0

The different uppercase superscripts between groups and lower case superscripts within the group (between days) differ significantly (p<0.05).

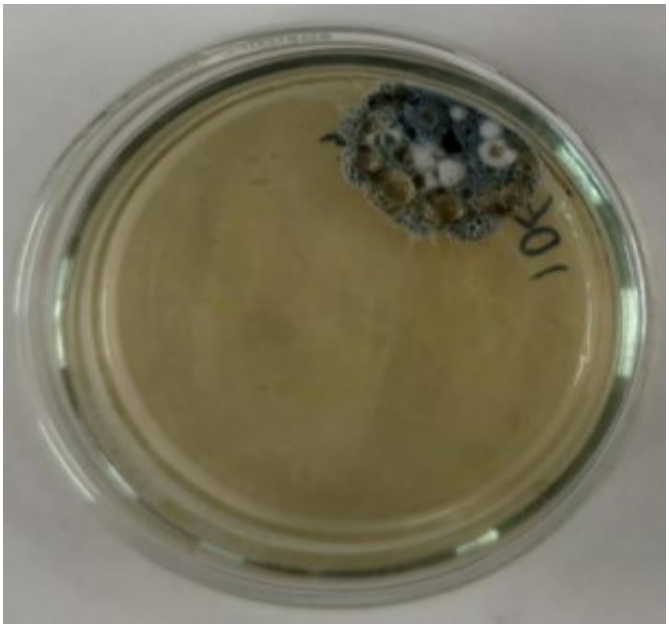


Fig. 1: Culture growth of *Penicillium* Spp. on Mycobiotic SDA Agar plate



Fig. 2: Microscopic view of *Penicillium* Spp. on 100X

The mean ± SE values of lymphocytes and eosinophils in dogs were observed to be significantly (p<0.05) increased in Group T_1 and T_2 compared to the control group, although neutrophils and monocytes did not vary between three groups (Table 2). These findings were consistent with the observations of Ambily *et al (*2022) and Sharma *et al.* (2022), who also reported lymphocytosis in allergic fungal dermatitis attributed to continuous antigen exposure from chronic infection or the prolonged inflammatory response triggered by fungal pathogens. Their study highlighted that lymphocyte levels tend to increase during fungal infections as part of the adaptive immune response. However, Würsch *et al*. (2006), Brar *et al*. (2017) and Soad *et al*. (2021) found non-significant increase in eosinophils in fungal dermatitis in dogs. As stated by García (2020), fungal pathogens like *Penicillium* can trigger eosinophilic responses through cytokine stimulation, particularly IL-5. Additionally, eosinophil persistence in fungal infections has been documented as part of chronic inflammatory pathways (Meason-Smith *et al*., 2020). A hypersensitivity reaction occurs due to an increase in histamine levels, which triggers the release of eosinophils into the bloodstream. It was also observed that atopic dogs exhibited elevated eosinophil levels due to mast cell stimulation through IgE

Table 3: Biochemical changes of dogs with dermatitis associated with *Penicillium* spp. compared with that of apparently healthy dogs (Mean ± SE)

Group	Day	Total protein (g/dL)	Sodium (mmol/L)	Potassium (mmol/L)	Zinc (µg/dL)	Copper (µg/dL)
Group T_1	0	7.72 ± 0.19^{Aa}	140.83±1.01	5.18±0.22	89.33 ± 4.56^{Bb}	94.32 ± 3.69^{Bb}
	28	6.41 ± 0.14^{b}	141.17±1.27	4.53±0.19	101.85 ± 1.29^{a}	110.38 ± 1.92^{a}
Group T_2	0	7.27 ± 0.20^{Aa}	141.67±0.76	5.52±0.23	89.52 ± 4.2^{Bb}	94.24 ± 3.65^{Bb}
	28	6.55 ± 0.16^{b}	141.5±0.88	4.43±0.32	100.28 ± 0.99^{a}	108.25 ± 2.049^{a}
Control	0	6.40 ± 0.19^{B}	141.00±1.24	4.68±0.28	101.85 ± 0.92^{A}	110.38 ± 1.92^{A}
	28	6.47±0.21	140.67±1.28	4.58±0.25	101.35±0.92	110.00±2.98

The different uppercase superscripts between groups and lower case superscripts within the group (between days) differ significantly ($p<0.05$).

receptors (Würsch *et al*., 2006). By day 28 of treatments, all these altered haematological parameters came to normal levels at par with healthy control group, proving the efficacy of both the treatments.

Biochemical Changes

Biochemical changes in dogs from different groups presented in Table 3 revealed a significant ($p<0.05$) increase in serum total protein, and drop in serum zinc and copper concentrations in Group T_1 and T_2 compared to the control group on day 0, which returned to normal levels by day 28 of treatment at par with healthy control group. However, no such change was noted in serum sodium and potassium levels. Sindha *et al*. (2015) and Joshi (2019) suggested that increased production of immunoglobulins results in a rise in the globulin fraction of the blood. Since globulins are a major component of total protein, their increased value leads to higher overall protein levels due to fungal infection. According to Aujla *et al.* (1999), these elevated values may result from an increase in immunoglobulin production and enhanced systemic immune responses. The findings of significant ($p<0.05$) drop in serum zinc and copper concentrations aligned with El-Motaily *et al*. (2022) and Gong *et al*. (2023), who suggested that chronic inflammation linked to dermatological disorders can disrupt trace mineral metabolism, potentially resulting in lower serum zinc and copper levels.

Zinc plays a crucial role in synthesizing antioxidant enzymes, such as Zn/SOD1, which shield cells from reactive oxygen species (ROS) and counteract the catalytic effects of metals like iron that promote the formation of oxidative radicals. According to Hefnawy and El-Khaiat (2015), copper is another antioxidant element that is necessary for the activity of antioxidant enzymes such as ceruloplasmin and superoxide dismutase. It is a necessary trace element that plays a role in the activation of over 20 metalloenzymes and metalloproteinases that are involved in the production of connective tissue, myelin development, pigmentation and the elimination of free radicals. Fungal dermatitis infection imposes stress on dogs by disrupting their antioxidant defense system, leading to a reduction in antioxidant trace element levels. Beigh *et al.* (2014) and Sharma *et al.* (2017) also found a significant decrease in serum zinc and copper concentration in dogs associated with dermatitis. However, in contrast to our study, several studies have shown no significant variation in the Zn level of diseased dogs (Ural *et al*., 2009; Dina, 2021).



Fig. 3



Fig. 4: Dogs infected with *penicillium* spp. associated dermatitis

Conclusion

The present study demonstrated that *Penicillium*-associated dermatitis accounted for 11.5% of suspected canine dermatitis cases screened. Affected dogs exhibited significant haematological alterations including leukocytosis, lymphocytosis, and eosinophilia, along with biochemical changes characterized by increased serum total protein and decreased serum zinc and copper concentrations. These findings indicate that *Penicillium* infection induces systemic inflammatory and immune responses associated with altered trace mineral metabolism. These altered profiles returned to normal one by day 28 of treatment with Tab. Terbinafine @ 30 mg/kg b.wt., and Tab. Itraconazole @ 10 mg/kg b.wt., PO, OD, for 21 days, with supportive therapy. On the basis of clinical recovery and haemato-biochemical analysis treatment Terbinafine showed better results.

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