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B Lahari Reddy
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha, India

R Mishra
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha, India

PK Rath
Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha, India

BK Patra
Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha, India

BP Mishra
Department of Livestock Products Technology, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha, India

N Soren
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha, India

Chandana Sree
Department of Animal Breeding and Genetics, College of Veterinary Science and animal Husbandry, OUAT, Bhubaneswar, Odisha, India

Corresponding Author:
B Lahari Reddy
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha, India

Microbial prevalence of dermatitis in dogs in Odisha

B Lahari Reddy, R Mishra, PK Rath, BK Patra, BP Mishra, N Soren and Chandana Sree

Abstract
Due to increasing awareness with respect to 'one health 'programme, in the present study an attempt has been made to find out the prevalence of microbial isolates in pet dogs. Dogs with skin infections represents the main reason of presentation for treatment in the Teaching Veterinary Clinical Complex (TVCC) of Odisha Veterinary college, Bhubaneswar India. In the present study 45 dogs presented with skin infections from February, 2021 to December, 2022 were subjected to microbial analysis and their antimicrobial susceptibility pattern were depicted. Skin infections like pyoderma in dogs, (n=22) itching and allergic inflammation (n=5) and alopecia (n=28) in dogs were observed in the study. The most common isolates from the dermatophytes-infected dog cases were *Microsporum* spp (49.3%) followed by the *Trichophyton* spp (12.2%). Detailed antifungal sensitivity profile revealed Miconazole (80.95%) was most effective. *Staphylococcus* spp was the most common bacteria (82.35%, n=42) while gentamicin was found to be having good in-vitro sensitivity (76.1%).

Keywords: Dermatitis, dog, Odisha, one health, *Staphylococcus*

Introduction
One health is the integrative effort of multiple disciplines working locally, nationally, globally to attain optimal health for people, animals and environment (WHO guidelines, 2020). The irrelevant use of antimicrobials in both human beings and animals is leading to antimicrobial resistance expansion, one of the main threats of demography and is responsible for pandemic and infections globally threatening public health care system (Brinkac *et al*., 2017) [3] Pets also acts as the reservoir for transmission of antimicrobial resistant zoonotic bacteria. The pet associated bacterial zoonoses represent a relatively neglected area. So, in the present study an emphasize will be given to the potential antibiotic resistance pattern of the pets living in common environment. In this study antimicrobial and antifungal susceptibility of dog's skin infection will reveal the potential microbial isolates that are present in dogs. The breed wise microbial isolates will reveal the prevalence of microbes the particular breed. The data obtained in this study could be utilized to guide empirical antibiotics selection treatment in pets.

Materials and Methods
The 45 samples were collected from German Shepherd (n=20, Fig-1) and Spitz breeds (n=25, Fig. 2) of dogs (Table-1) belonging to various ages and sexes, visiting to TVCC, College of Veterinary Science and Animal Husbandry, Bhubaneswar, Odisha. All the samples underwent primary isolation for bacterial and fungal isolates. The samples pertaining to fungal isolates went through Potassium hydroxide (KOH) mount, culturing on Sabouraud Dextrose Agar (Fig. 3, SDA) and lactophenol cotton blue staining (Fig. 4). The isolates were identified by the macroconidia, septa. For isolation of bacteria, the samples were inoculated in to Brain-Heart infusion (BHI) broth for enrichment, followed by culturing on to specific media. Susceptibility tests were carried by agar-based disk diffusion method using Mueller-Hinton Agar (MHA).

Table 1: History of the German shepherd and spitz from TVCC

Sl. No	Breed	Age	Sex	History
1	German shepherd	2.6 yrs	m	Pruritis, erythematous lesions on belly, nose bridge, mouth
2	spitz	6 months	f	Alopecia, intense pruritis
3	German shepherd	5 yrs	f	Patchy alopecia
4	German shepherd	3.6 yrs	m	Alopecia, pruritis
5	Spitz	10 m	m	Red coloured patchy lesions all over the body
6	German shepherd	2 yrs	m	Pododermatitis
7	Spitz	6 m	f	Pruritis, lesions in interdigital space, alopecia
8	Spitz	1.4 y	f	Intense pruritis, bad odour, blackish discoloration on thigh
9	German shepherd	4 y	f	Pruritis, alopecia
10	German shepherd	2.8 y	m	Alopecia, pustule
11	Spitz	10 m	f	Alopecia in forelimbs and hind limbs
12	German shepherd	1.3 y	f	Intense pruritis, erythematous patches around elbow, back, shoulders
13	Spitz	5 m	m	Alopecia, pruritis
14	German shepherd	1.4 yrs	f	Alopecia, reddened lesions around groin and thighs
15	Spitz	2 y	m	Alopecia, erythematous lesions in some parts of the body
16	German shepherd	1 y	m	Patchy alopecia in abdominal region, hardening of skin in hairless part
17	Spitz	2.5 yrs	f	Pruritis, redness in tail region
18	Spitz	2 y	m	Hyperpigmentation
19	Spitz	3 months	m	Lesions over the axillary region
20	Spitz	5 y	m	Pruritis, erythematous lesions all over body
21	German shepherd	2.5 y	f	Pruritis, erythematous lesions at ventral abdomen, pododermatitis
22	German shepherd	1 y	f	Alopecia, red skin lesions in hind &forelimbs
23	Spitz	6 months	m	Alopecia, pruritis, all over the body
24	Spitz	3 m	f	Lesions over groin region, right elbow, pruritis,
25	German shepherd	1.5 y	M	Erythematous lesions all over body &interdigital space, alopecia
26	Spitz	2.7 y	f	Lesions around eye, erythematous lesions all over the body
27	German shepherd	4 m	f	Alopecia, redness in ventral portion of abdomen &in medial side of thigh
28	Spitz	5 y	m	Pruritis & redness over belly
29	German shepherd	6 m	m	Alopecia around eyes, pruritis
30	Spitz	2 y	m	Reddened lesions on legs, nose, belly
31	German shepherd	2.7 y	f	Alopecia, Severe skin infection
32	Spitz	1.5 y	f	Alopecia, erythematous lesions on abdomen
33	German shepherd	4.5 y	m	Patchy dry skin
34	Spitz	5 m	m	Patchy dermatitis
35	Spitz	1 y	f	Alopecia, pruritis
36	Spitz	6 m	f	Redness over limbs, pruritis
37	German shepherd	45 d	f	Pruritis, scab around ear, mouth
38	Spitz	4 y	f	Alopecia, allergic conjunctivitis
39	German shepherd	1.2 y	m	Pruritis, alopecia on the dorsal part
40	German shepherd	3 y	m	Patchy alopecia, lesions over the limbs
41	Spitz	1 y	m	Pruritis, erythematous lesions around extremities
42	Spitz	1 y	f	Erythematous lesions all over body, pruritis
43	Spitz	4 y	f	Alopecia
44	Spitz	5 Y	M	Alopecia, pruritis
45	German shepherd	3.5 y	M	Patchy alopecia

Fig 1: German shepherd infected with fungi in the paw region and pyoderma

Fig 2: Spitz showing dermatological lesions

Results

In the present study, particularly in German shepherds, spitz a total number of 45 samples were collected from the 45 dogs of various ages, sex, and characteristic lesions like alopecia, scaly patches, erythema presented to the college of veterinary science and animal husbandry, OUAT, Bhubaneswar in the duration of February 2021 to December 2022. All these samples have undergone routine fungal identification. out of 45 samples, 21 samples have shown positive for fungal growth.

Fig 3: Growth of fungi on SDA

Fig 4: Lactophenol Cotton Blue staining

Prevalence of dermatophytes breed wise

All 45 dog skin samples underwent thorough isolation, characterization, direct microscopic examination, and cultural examination which revealed that 21 cases yielded dermatophytes with an overall incidence rate of 46.66% (21/45). Out of the two breeds, German shepherd and spitz screened for fungal infection, it was observed that the highest fungal infection was found in the in the German shepherd 61.9% (13/21) and in spitz accounting for 38.1% (8/21). The breed-wise prevalence of canine dermatomycosis was depicted in (Table-2). This highest prevalence in German Shepherd might be due to their long hair coats and higher humid conditions playing a major role in faster multiplication and propagation of fungal elements as per Bhardwaj *et al*., (2012) [2].

Table 2: Breed-wise occurrence of dermatomycosis in canine species

Breed Type	Total no of cases	No of positive cases	Percent from total no of positive cases (N=21)
German shepherd	20	13	61.9%
Spitz	25	8	38.1%
Total	45	21	100

Age-related prevalence of dermatophytes in dogs

Present study concluded that the younger population aged between 6 months to 1.5 years was relatively more susceptible to dermatomycosis infection with a higher rate of occurrence 47.6% than the 1.5-3-year-old age group (28.6%) with least occurrence among the age group above 3years (23.8%). The age-wise occurrence of dermatomycosis in dogs was given in the Table -3

Table 3: Age-wise occurrence of dermatomycosis in dogs

Age group of canines	No of positive cases	Percent from total no of positive cases	*Microsporum* spp (%)	*Trichophyton* (%)
6-1.5 yrs	10	47.6	26.62	6.2
1.5-3 yrs.	6	28.6	13.48	3.9
Above 3yrs	5	23.8	9.2	2.1
Total	21	100	49.3	12.2

Sex-related prevalence of dermatophytosis

Table-4 depicts the sex wise distribution of fungal infections among dogs with highest among male dogs (66.67%, n=14) and in females (33.33%, n=7).

Table 4: Sex-wise occurrence of dermatomycosis in dogs

Sex	No. of the animals positive	Percent
Females	7	33.33
Males	14	66.67
Total	21	100

Isolation of various species of dermatophytes from dogs

The two predominant causative agents in dermatophytes: *Microsporum* and *Trichophyton* spp. were isolated from the dogs. These isolates were identified by the presence of well-developed macroconidia with septa and small microconidia with stalked appearance.

Antifungal susceptibility test of dermatophytes

All the fungal isolates isolated from 21 dogs are subjected to an antifungal susceptibility test by agar-based disk diffusion method. This test resulted in the susceptibility of the antifungal drugs in the following way ketoconazole (15 µg): 16(76.2%) sensitive, 3(14.28%) intermediate, 2(9.52%) resistance. Miconazole (10 µg):17(80.95%) sensitive, 1(4.77%) intermediate, 3(14.28%) resistance. Fluconazole (25 µg): 18(85.72%) intermediate, 3(14.28%) resistance, clotrimazole (10 µg): 15(71.42%) sensitive, 6(28.58%) resistance. In this method of antifungal susceptibility test it was found that miconazole (10 µg) was found to be highly sensitive followed ketoconazole (15 µg) Clotrimazole (10 µg) and against dermatophyte (Table-5).

Table 5: Antifungal susceptibility test of dermatophytes in dogs

Result	Antifungal drugs			
	Ketoconazole (15 µg)	Miconazole (10 µg)	Fluconazole (25 µg)	Clotrimazole (10 µg)
Sensitive	16(76.2%)	17(80.95%)	-	15(71.42%)
Intermediate	3(14.28%)	1(4.77%)	18(85.72%)	-
Resistance	2(9.52%)	3(14.28%)	3(14.28%)	6(28.58%)

Isolation of bacteria

In this study out of 45 samples collected 51 bacterial isolates were identified through culturing onto the specific media and through gram staining. The most common bacterial spp. was *Staphylococcus* accounting for 82.35% (n=42), followed by *Pseudomonas* spp 11.7% (n=6) and *E. coli* 3.9% (n=2). *Staphylococcus spp* were identified through gram staining (Fig. 5) after culturing on specific growth media like Mannitol Salt Agar (Fig. 6). On antimicrobial susceptibility testing by disc diffusion method, it was found that *Staphylococcus* spp isolates showed sensitivity to Gentamicin (76.19%), Amoxacillin+clavulanic acid (AMX20 µg+10 µg, 59.52%), followed by cephalexin (LEX, 30 µg), Co-trimoxazole (SXT, 23.75 µg of sulphamethaxazole+1.25 µg of trimethoprim, 50%), and moreover, it was observed that Ciprofloxacin (CIP, 5 µg), Clindamycin (CLI, 2 µg), Doxycycline (DOX, 30 µg) were less sensitive against *Staphylococcus* spp and Gentamicin (GEN, 10 µg) showed good efficacy against *Staphylococcus* spp. Table-6 depicted the details of antimicrobial sensitivity test results against *Staphylococcus* Spp. our study.

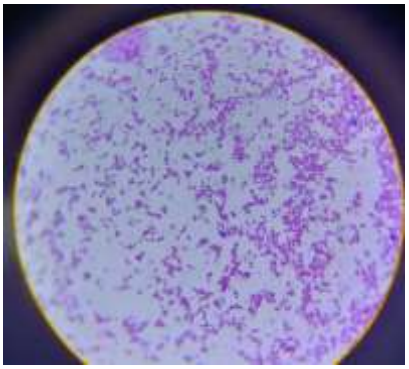

Fig 5: *Staphylococcus* spp under grams staining (100X)

Fig 6: Growth of *Staphylococci* in Mannitol Salt Agar

Table 6: Antimicrobial susceptibility test for *Staphylococcus* spp

Antibiotics	Sensitive	Intermediate	Resistance
Ciprofloxacin (CIP, 5 µg)	19 (45.24%)	15 (35.71%)	8 (19.05%)
Clindamycin (CLI, 2 µg)	16 (38.1%)	19 (45.24%)	7 (16.66%)
Doxycycline (DOX, 30 µg)	18 (42.86%)	17 (40.47%)	7 (16.67%)
Gentamicin (GEN, 10 µg)	32 (76.19%)	6 (14.29%)	4 (9.52%)
Co-trimoxazole (SXT, 23.75 µg of sulphamethaxazole+1.25 µg of trimethoprim)	21 (50%)	11 (26.19%)	10 (23.81%)
Amoxicillin+ Clavulanic acid (AMX 20 µg+10 µg)	25 (59.52%)	4 (9.52%)	13 (30.95%)
Cephalexin (LEX, 30 µg)	23 (54.76%)	13 (30.95%)	6 (14.29%)

Discussion

A detailed study was carried out by analysing the prevalence of dermatomycosis and bacterial infection in dogs particularly in German shepherd and spitz as these are commonly found canine species in Bhubaneswar. Out of the two breeds screened for fungal infection German shepherd has the highest prevalence rate followed by the spitz which is in agreement with Devi and Vijayakumar (2013) [4].

In this study, it was found that the younger dog population aged between 6 months to 1.5 years age was relatively more susceptible to dermatomycosis infection with a higher rate of incidence (47.6%), followed by the age group between 1.5 years -3 years with an incidence rate of (28.6%). Singathia *et al*. (2014) [13] also reported that a higher incidence rate was found between the age group of 6 months-1. 5 years. Devi and Vijayakumar (2013) [4] observed that the highest rate of incidence was found in the age group between 1-6months.this might be due to a poorly developed immune system and deficiency of the fungistatic linoleic acid. The observations of the present study are in correlation with studies reported by Singathia *et al*. (2014) [13], Mancianti *et al*. (2002) [6], and Devi and Vijayakumar (2013) [4]. In the present study, it was also revealed that the prevalence of fungal infection was more in males compared to females which is in agreement with the reports of Bhardwaj *et al*. (2012) [2], Murmu *et al*. (2017) [8]. However, Brilhante *et al*. (2003) [17] suggested that there are no significant differences between the sexes of animals and the prevalence of the disease

The most common isolates from the dermatophytes-infected dog cases were *Microsporum* spp. (49.3%) followed by the *Trichophyton* spp. (12.2%). There are many reports from India and abroad that correlates with our findings regarding the isolation of *Microsporum* spp. as the predominant species of dermatophytes in dogs as per, Beigh *et al*. (2014) [1], Serkan and Dogan (2011) [11], Mohammed (2013) [7] and Murmu *et al*. (2017) [8]. *Trichophyton* spp was the second most prevalent species with a rate of occurrence of 12.2% of cases of dermatophytes in dogs.

In this study to evaluate the effectiveness of the antifungal drugs against dermatophytes a total of 21 clinically diagnosed dermatophytes samples were subjected to a susceptibility test by agar-based disk diffusion method. The method is a practical, agar-based method that enables the determination of the activity of various antifungal drugs against various fungal genera and spp. Broth micro and macro dilution assays can be used to determine the antifungal susceptibility of

dermatophytes, but these methods are expensive and require specific media. Some suggest that disk diffusion is a reproducible method which in general shows a good Correlation with the reference method for microdilution antifungal susceptibility Barry *et al*. (2002) [18]. Detailed sensitivity profile revealed that Miconazole (80.95%) was the effective/highest sensitive antifungal against dermatophytes. Pakshir *et al*. (2009) [11], Shalaby *et al*. (2016) [12] found that clotrimazole is effective followed by the miconozole

Among the 45 samples collected 51 bacterial isolates were obtained, among which the most common species is *Staphylococcus* spp which constitutes 82.35% (n=42) of the total bacteria isolates isolated. In this, study all 42 isolated staphylococci were subjected to an antimicrobial sensitivity test, and gentamicin was the only antibiotic showing good invitro activity against this pathogen. Present study revealed only 11.9% of isolates were resistant to gentamicin but in the study conducted by Thaysa Souza Silva *et al*., (2022) [14] only 14% showed resistance to Gentamicin. The next sensitive antibiotic is cephalexin, followed by amoxicillin+clavulanic acid, and co-trimaxozole this is in agreement with Vanni *et al*., (2009) [15], Khinchi *et al*., (2022) [5] but only 30.2%isolates showed sensitive to Gentamicin, cephalexin (100%), amoxicillin+clavulanic acid (95.35%) It was observed that *Staphylococcus* was less sensitive to Ciprofloxacin (CIP), clindamycin (CLI), doxycycline (DOX). This resistance of antibiotics in *Staphylococcus* spp is due to many mechanisms such as enzymatic inactivation, efflux, impermeability, low permeability of its cell wall and mutants as well as the combination of these different mechanisms. The significant difference in the prevalence of multidrug resistance and multi-drug sensitive isolates highlights the importance of preventing the further spread of multidrug resistance clones among the dog and human population.

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