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Prevalence of Dermatophytosis in Canine in and Around Kolkata Metropolitan Area

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ABSTRACT

The aim of the present study was to isolate and identify the pathogenic fungi of canine dermatophytosis in and around Kolkata Metropolitan area, also study the clinical signs of dermatophytosis from January 2015 to December 2015. Out of 186 dogs 41 cases showed an overall incidence of 22.04% in dogs in the present study. Dermatophytes were isolated from 24 (19.35%) adult dogs and 17 (27.42%) puppies. Clinically 15 dogs and 12 bitches showed skin lesion resembling ringworm. The laboratory examination by culturing materials from the lesions revealed that 13 dogs and 11 bitches were infected by dermatophytes resulted the incidence of this disease in dogs and bitches as 20.0% and 18.64% respectively. Clinically examination indicated that 11 male and 7 female puppies were having lesions resembling dermatophytes. Cultural examination of the samples in the laboratory confirms the presence of dermatophytes in 10 males and all the female puppies resulted and incidence of dermatophytosis of 24.39% in male and 33.33% in female puppies. Occurrence of dermatophytosis was found comparatively high during summer and rainy season. The most common species of dermatophytes isolated from dogs was *M. canis* (58.55%o) followed by *M. gypseum* (24.39%), *T. Mentagrophytes* (12.18%) and *T. rubrum* (4.88%).

HIGHLIGHTS

- Isolation and identification of most prevalent dermatophytosis causing fungi.
- Detection of prevalence as well as age, sex and seasonal variation of dermatophytosis in canines.

Keywords: Prevalence, dermatophytosis, canine, fungus, Kolkata

Dog a lovable creature of our almighty. Dog is famous for its friendly nature, obedience, affiliation and sacrifice towards man. It has been proved to be the best and faithful companion among all animals on earth. It is opined that dog has appeared on this earth about 20 million years ago and it is believed that association of the man and dog has started since the civilization begins. This association has gradually been transformed to domestication. Dog stands first amongst all domesticated pet animals. The man felt necessity of a faithful and loyal companion who could be associated with hunting and guarding the livestock's at firm and will also pull the sledge cart in snow bound areas (Debnath *et al.,* 2016). Slowly with advancement of time, man started recognizing the extra sensory powers in dogs gifted by God and thought to convert this natural instinct

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into a right way for serving mankind. Later, by means of good selection, some special breeds of dogs were develop to meet the various requirement of man like hunting, detecting crime, veterinary and medical research works, amusement in circus and shows.

These dogs are prone to several systemic and specific diseases. Out of them, skin diseases are very problematic and obstinate. Skin is the mirror of the body. The healthy condition of the body reflected by its body coat i.e. both skin and hair. Skin is the largest visible organ of body and it is the anatomical and physiological barrier between body and environment. Skin helps in thermoregulatory system, acid-base balance, synthesis of vitamin-D, immunity and keeps the inner systems and tissues out of reach from external pathogens. Skin is consisting of two primary layers; epidermis (outer layer) and dermis (inner layer). Epidermis again composed of four layers; Stratum corneum, stratum granulosum, stratum spinosum and basal cell layer including hair follicles, apocrine glands and sebaceous glands. Dermis is located between the epidermis and subcutis and consists of a loosely assemblage of connective tissues and contains blood vessels, nerves, lymphatic glands and muscles.

Dermatophytes are the members of the genera Trichophyton, Microsporum and Epidermophyton. They are closely related filamentous fungi that regularly invade the nails, hair, and stratum corneum, the outermost layer of the skin. The term "dermato-phyte" literally means "skin plant," and although the documented use of the word did not appear until 1882, the infections they cause have existed for hundreds of thousands of years.

Despite increasing reports of dermatophytosis in different tropical and sub-tropical regions of India, there is scanty data on this issue from Kolkata Metropolitan area which is situated in the Eastern part of India. There is scanty information on application of keratin utilization test to study the pathogenesis of the dermatophytes in animals; particularly dermatophyte species prevalent among canine population.

MATERIALS AND METHODS

The study was carried out for one year from, January 2015 to December, 2015 (Winter, Summer and Rainy) from Belgachia dog Ward and several other private clinics in and around Kolkata where dogs were brought for treatment of skin disorders. A number of 186 domesticated dogs with dermatomycosis lesions sent to laboratory of the Department of Veterinary Pathology and the Department of Veterinary Public Health in the Faculty of Veterinary and Animal Sciences of West Bengal University of Animal and Fishery Sciences, Kolkata were evaluated for the presence of dermatophytes. The results were correlated to the breed, age, coat, habit, seasonal effect and clinical conditions in order to understand the distribution of these fungi among pet dogs. To determine the skin lesions, which were alopecia, crust, scales, nodules, pustules and inflammation, all infected domestic dogs had their skin examined clinically by inspection and palpation. The shape, size, position, distribution and time of the appearance of skin lesions as well as the age, breed, sex of the dogs were also recorded. After a cleansing with 70% ethyl alcohol in sterile falcon, skin scrapings and hair samples were taken from the periphery of the lesions. Infective animals that suffered from lesions indicating ringworm infection were collected for samples.

Cultural examination

The identification of fungal species was based upon its colony characteristics and conidial cell it produced. The factors in the identification of conidia were the size, shape, presence of septae, number of septae, thickness of conidial wall and arrangement of conidial cells around the hyphae. For some dermatophytes it was important to select an appropriate substrate for bacterial isolation and identification, since they are known to be poorly produced of conidia (Moretti *et al.,* 2013). The other important variables concerning optimization of growth were the inoculums size, temperature and duration of incubation. The medium containing Chloramphenicol and Cycloheximide were more suited for primary isolation since they suppressed bacteria and saprophytic fungal growth, respectively. The specimens were inoculated onto three sets of test tubes, one containing sabouraud's dextrose agar with 0.05% chloramphenicol, second containing sabouraud's dextrose agar with 0.05% chloramphenicol Plus 0.5% Cycloheximide and third onto dermatophyte test medium. Sabouraud's dextrose agar (SDA) with 0.05% chloramphenicol and sabouraud's dextrose agar with 0.05% Chloramphenicol plus 0.5% Cycloheximide were incubated at 28ºC for up to 4 weeks, and was observed

periodically for growth. If no growth has been observed after four weeks, it is regarded as having a negative effect on fungal growth. Dermatophyte test medium was also incubated at 28ºC for up to 10 days and was observed for colour change (Fig. 1).

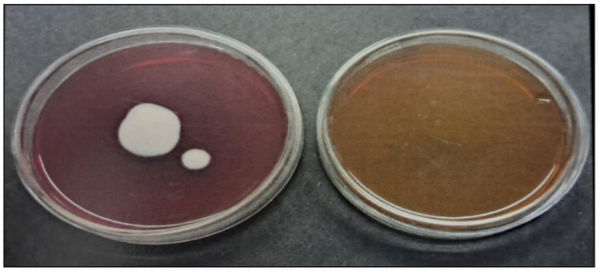

Fig. 1: Growth of dermatophytes on DTM agar medium (Right sided plate showing un-inoculated control plate and the left sided plate showing development of red colour characteristics for the growth of dermatophytes)

Microscopic Examination of Culture

Tease Mount or Needle mount: Cultures were examined microscopically by removing a portion of aerial mycelium with a spud and placed on a glass slide in to a drop of lactophenol cotton Blue and matted mycelium mass was gently teased with a pair of Teasing needles and the cover slip was placed on it. The needle mount was observed under low and high power objective of microscope, tor the presence of hyphae, macroconidia, macroconidia and other accessory structures of vegetative hyphae and the characters of each was noted (Fig. 2, 3, 4 and 5).

Biochemical and physiological tests

Following tests are performed to differentiate different species of Dermatophytes.

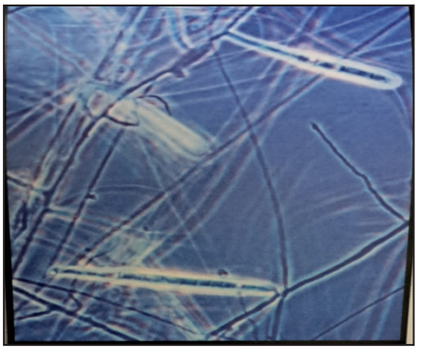

Fig. 2: *M. gypseum*

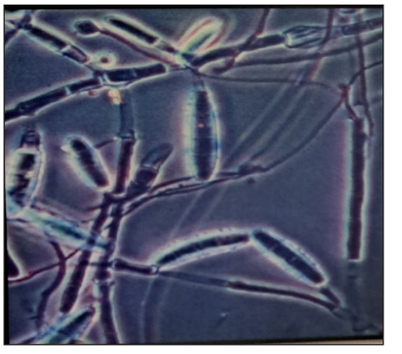

Fig. 3: *M. canis*

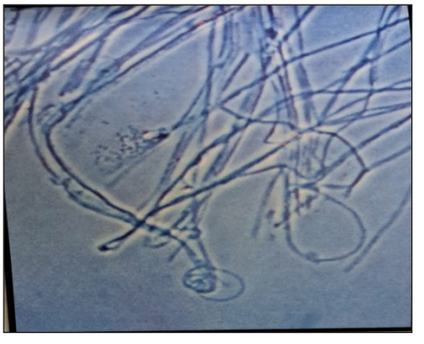

Fig. 4: *T. mentagrophytes*

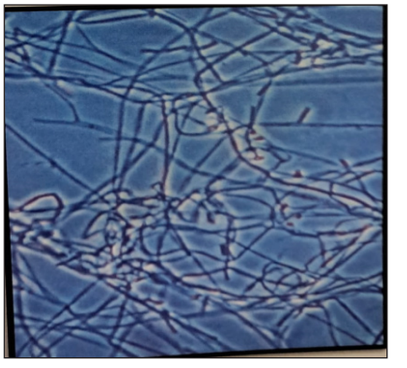

Fig. 5: *T. rubrum*

Fig. 2-5: LPCB mount under the phase Contrast Microscope

Urease Activity

This test was performed to differentiate between *T. mentagrophytes* and *T. rubrum*. Christensen's urea agar slant was inoculated with the test fungus and incubated at 28°C for 7 days. *T. mentagrophytes* demonstrated the urease activity usually within 7 days changing the colour of the medium to pink. *T. rubrum* isolates were negative for urease test (Fig. 6).

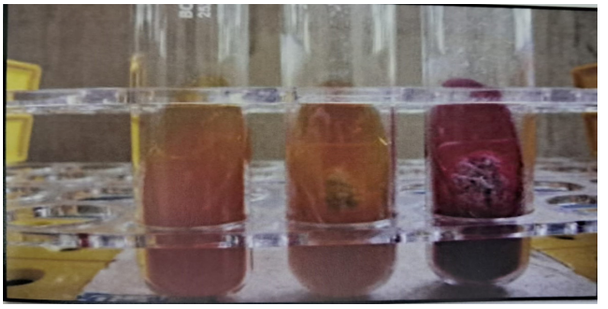

Fig. 6: Urease test (Left side tube un-inoculated control and the development of pink coloration on right side by dermatophytes indicating urease positive test)

In-vitro hair perforation test

Child hair were cut into small bits; cleaned, washed and placed in sterilized McCartney bottle, lightly plugged with cotton wool, and autoclaved at 121ºC for 10 minutes at 15 Ibs. Pressure per square inch. In sterile 100 mm petridish about 25 ml of sterile distilled water was taken and 3-4 drops of 10% yeast extract were added. Several bits of sterile child hair were placed in it.

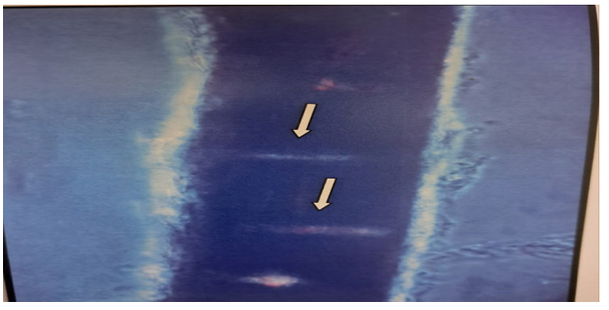

Fig. 7: Hair perforation test for the *T. mentagrophytes*, arrows are indicating wedge shaped erosion of hair shaft

The contents were inoculated with a few fragments of test culture from Sabouraud medium. Then the dish was incubated at 28°C for 4 weeks. After 7-10 days of incubation, 3-4 hair fragments were aseptically removed from the dish, mounted on a clean glass slide with Lactophenol cotton blue stain and examined under the microscope for perforation. Perforations appeared as wedge shaped erosions occurring at irregular intervals along the hair shaft (Fig. 7).

Rice grain test

This medium was used for growing *Microsporum spp.* It differentiated *M. audouinii* from other *Microsporum spp*. Rice grain medium was inoculated with the test culture and incubated at 28ºC for 7-10 days. All species of Microsporum group grew very luxuriantly on this medium indicating the non-isolation of *M. audouinii* in the present study (Fig. 8).

Fig. 8: Rice grain test for *Microsporum canis* (development of yellow colour suggests the growth *M. canis*)

Trichophyton Agar Medium

This medium was used for Trichophyton growth. For each fungal isolate tested on this medium, one set of tubes from each of the seven media were inoculated at 28°C for 7-10 days, and examined for the presence of any growth.

RESULTS AND DISCUSSION

Genus Microsporum

Microsporum is a zoophilic dermatophyte and the type species is *Microsporum canis* and in case of anthropophilic varieties *M. audouinii* is regarded as type species. Cultures of Microsporum on SDA were cottony, wooly, matted or powdery, aerial mycelium varied in colour from white to

buff to deeper shades of brown coloration. A bright golden reverse pigment was noted, but non pigmented strains were also common. Macroconidia were typically echinulate and were characterized by the presence of rough walls which were asperulate, or verrucose. Macroconidia had thin, moderately thick to thick walls and one to two septa (small) to 6-12 septa (large spindles). Microconidia were single celled, small, sessile or stalked and clavate and usually arranged singly along the hyphae. Identification of individual species was made on the basis of the following characteristics:

Microsporum canis

Cultures of *M. canis*, on SDA, developed quickly a cottony to wooly aerial mycelium that became powderyand buff to light brown in the center. The reverse of the colonywas brilliant reddish-brown to orange in colour (Fig. 9&10). The species developed a luxuriant growth on Rice medium. The culture developed numerous multi septate spindle shaped, rough, thick-walled macroconidia. A few single celled clavate microconidia borne laterally along the hyphae were also noticed in some cases.

Microsporumgypseum

Cultures of *M. gypseum*, on SDA, grew quickly, becoming powdery and buff to light brown in the center. Some strains developed a white wooly aerial mycelium that later became powdery and light brown in the centre with the radiating furrows. The reverse of the colony was reddish-brown to orange in colour (Fig. 11 & 12). The macroconidia were large, four to six septate, ellipsoid, thin-walled, and echinulate.

Genus Trichophyton

The growth on SDA appeared as cottony, granular, or powdery or glabrous, smooth and waxy. Pigmentation varied greatly and the cultures were white, pink, red, purple, violet, orange, yellow or brown in colour. The pigmentations were either lost on transfer of cultures, or varied in intensity and appeared only on reverse of colony, sometimes involving the aerial mycelium, otherwise remained diffused through the medium. Microconidia were usually more abundant than macroconidia, small, single-celled, thin-walled, Globose, pyriform or clavate, sessile or stalked, and were borne singly along the sides

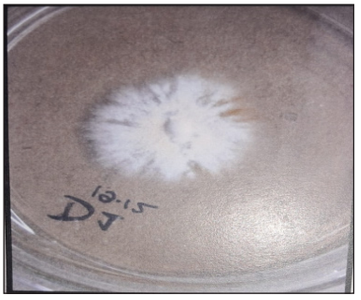

Fig. 9

Fig .10

Fig. 9 & 10: *Microsporum canis* growth on Sabouraud Dextrose Agar (SDA) Obverse (Fig. 9) and Reverse (Fig. 10)

Fig. 11

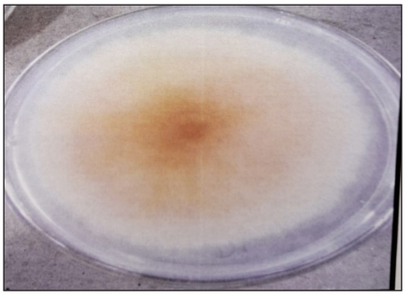

Fig. 12

Fig. 11&12: *Microsporum gypseum* growth on Sabouraud Dextrose Agar (SDA) Obverse (Fig. 11) and Reverse (Fig. 12)

of the hyphae or in grape-like clusters. Macroconidia were comparatively rare, appeared as large, multi-celled (one to 12 septa), smooth, thin-walled or thick-walled hyaline, clavate to fusiform.

Identification of individual species was made on the basis of the following characteristics:

Trichophyton mentagrophytes

The cultures were powdery to granular, light buff to rose-tan in colour, the growth varied from fluffy, cottony type to velvety and pure white. The reverse of the growth was wine coloured to brownish (Fig. 13 & 14). Growth on Corn meal dextrose agar was consistently yellow pigmented. The species was urease (+) positive in 5-7 days. The organism was hair perforation (+) positive. The powdery and granular cultures developed, both macroconidia and microconidia and the growth showed coils, nodular bodies and chlamydospores. The cottony cultures rarely developed such structures. This species grew slowly at 37°C and the species grew luxuriantly in Trichophyton agar medium.

Trichophyton rubrum

The cultures on SDA were cottony to velvety and sometimes powdery. The culture developed reddish to purple pigmentation on the reverse that spread to the hyphae (Fig. 15 & 16). The organism resembled *T. mentagrophytes* very closely. The differentiating features were: growth of *T. rubrum* on Corn meal Dextrose agar was consistently red-pigmented, *T. rubrum* was Urease (-) negative in 7 days. The species was hair perforation (-) negative on hair culture. This species also grew abundantly on Trichophyton agar media but the growth was bright red in colour. The species failed to grow at 37 ^{0}C microconidia were more numerous.

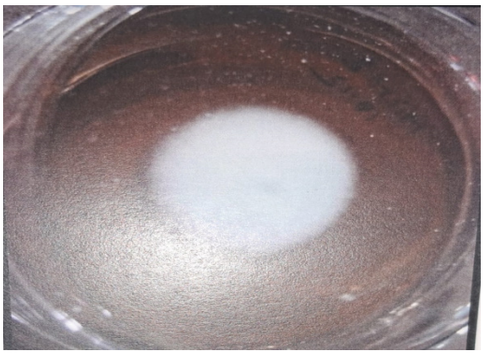

Fig. 13

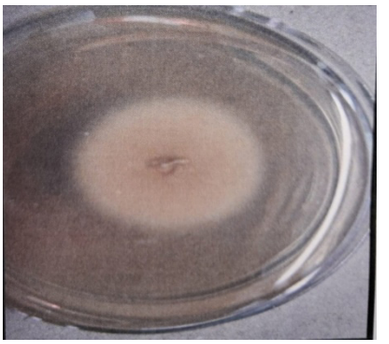

Fig. 14

Fig. 13 & 14: *Trichophyton mentagrophytes* growth on Sabouraud Dextrose Agar (SDA) Obverse (Fig. 13) and Reverse (Fig. 14)

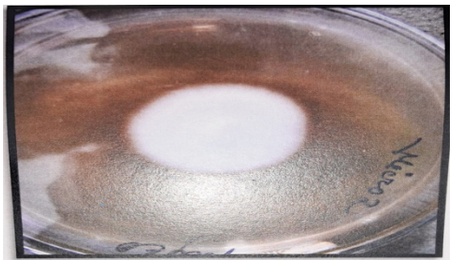

Fig. 15

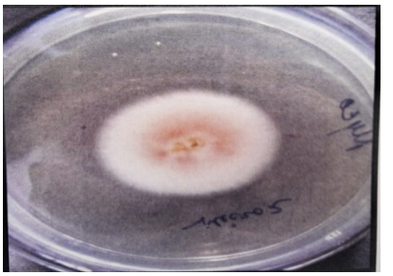

Fig. 16

Fig. 15&16: *Trichophyton rubrum* growth on Sabouraud Dextrose Agar (SDA) Obverse (Fig. 15) and Reverse (Fig. 16)

Prevalence of dermatophytes in dogs

Clinical examination of 186 dogs indicated that 45 dogs had ringworm like lesions on different part of their bodies (Fig. 17 & 18). Direct microscopic examination (Fig. 19) of the skin scrapings collected from these animals revealed the presences of fungal elements in 43 samples (Table 1). On cultural examination of all the clinically suspected animals yield dermatophytes from 41 cases resulting an overall incidence of 22.04% in dogs in the present study (Table 2). Dermatophytes in dogs have been studied by many workers all over the world. The incidence of dermatophytes in dogs, as reported by different workers are 83.33% (Mohammed, 2013); 49.5% (Nweze, 2011); 32.5% (Beraldo *et al.*, 2011); 18.18% (Menelaos, 2006); 9.8% (Chermette *et al*., 2008). Many of the above mentioned reporters' are in line of this study. The variation in incidence may be due to the differences in various epidemiological factors as well as size of the sample, sampling procedure and many other related factors.

Age related prevalence of dermatophytes in dogs

The dogs above 6 months of age were considered as adult while those below 6 months of age were regarded as young. Of the 124 adult dogs 27 animals and of the 62 young animals 18 pups showed evidence of skin infection. Dermatophytes were isolated from 24 (19.35%) adult dogs and 17(27.42%) puppies (Table 3). Menelaos (2006) were also found highest prevalence of dermatophytosis in animals up to one year old. Devi and Vijayakumar (2013) observed highest per cent of infection in dogs between 1 and 6 months of age. Observations of present study are in agreement with those reported by Devi and Vijayakumar (2013). Boehm and Mueller (2019) reported that the younger animals had poor cell mediated immunity as they may suffer from nutritional deficiencies. Moretti *et al.* (2013) stated that delay in the development of host immunity predisposed the young animals to dermatophyte infections. Possibly acquired resistance of epidermal tissues through repeated exposure to lower grade

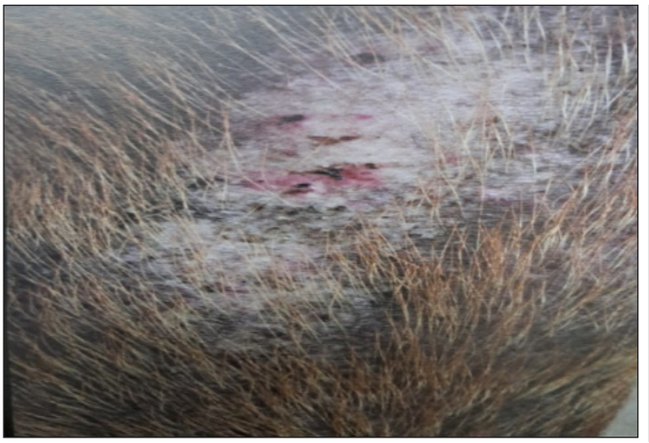

Fig. 17: Showing skin lesions; alopecia, desquamation

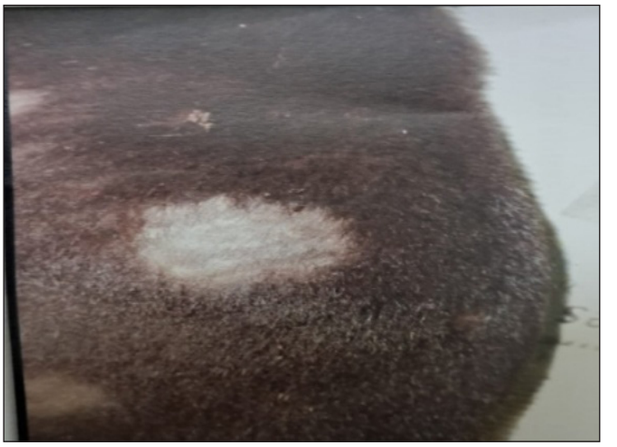

Fig. 18: Clinical cases showing characteristic ring-like lesions

Table 1: Number of animal suspected for dermatophytosis

Sl. No.	Species of animal	Number of animals screened	Suspected for dermatophytosis	Percentage (%)
1	Dog	186	43	23.11

Table 2: Prevalence of Dermatophytes in dog

Total number examined	Suspected Clinically	Positive by microscopy	Culturally Positive cases	Overall incidence (%)
186	45	43	41	22.04

infestation with mycotic elements may have played a role in reduced prevalence rate in aged dogs. Similar finding has been reported by Copetti *et al*. (2006).

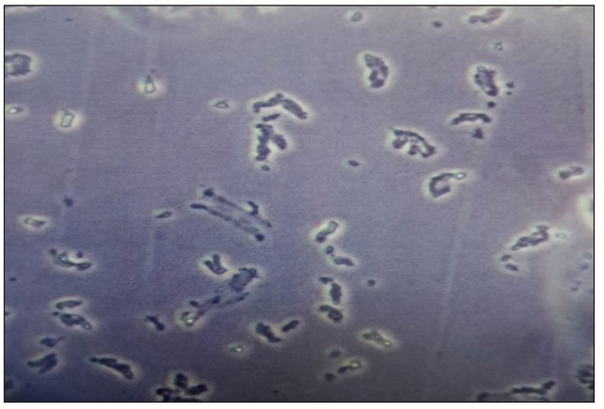

Fig. 19: Fungal elements in skin scraping (KOH method X 100)

Sex related prevalence of dermatophytosis in adult dogs

The adult dog population included in the present study consisted of 65 dogs and 59 bitches. Clinically 15 dogs and 12 bitches showed skin lesions resembling ringworm. The laboratory examination by culturing materials from the lesions revealed that 13 dogs and 11 bitches were infected in dermatophytes resulting the incidence of this disease in dogs and bitches as 20.00% and 18.64% respectively (Table 4). Similar kinds of observation have been reported by Singathia *et al.* (2014). However Ivaskiene *et al*. (2009) and Menelaos (2006) suggested that sex had no significant effect on the prevalence of Dermatophytosis.

Sex related prevalence of dermatophytosis in puppies

In total 41 males and 21 females puppies were examined during the present study. Clinical examination indicated that 11 male and 7 female puppies were having lesions resembling dermatophytes. Cultural examination of the samples in the laboratory confirms the presence of dermatophytes in 10 males and all the female puppies resulting an incidence of dermatophytosis of 24.39% in male and 33.33% in female puppies in this study (Table 5). Seker and Dogan (2011) also reported similar finding from their studies about the incidence of dermatophytes and suggested that there is no any significant differences between sexes of animals and prevalence of the disease.

Table 3: Age related prevalence of dermatophytes in dog

Total number examined		Suspected clinically		Culturally positive cases		Overall incidence (%)	
Adult	**Young**	**Adult**	**Young**	**Adult**	**Young**	**Adult**	**Young**
124	62	27	18	24	17	19.35	27.42

Table 4: Sex related prevalence of dermatophytes in adult dog

Total number examined		Suspected clinically		Culturally positive cases		Percentage (%)	
Dog	**Bitch**	**Dog**	**Bitch**	**Dog**	**Bitch**	**Dog**	**Bitch**
65	59	15	12	13	11	20	18.64

Table 5: Sex related prevalence of dermatophytes in puppies

Total number examined		Suspected clinically		Culturally Positive cases		Positive percentage (%)	
Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
41	21	11	7	10	7	24.39	33.33

Table 6: Prevalence of different species of dermatophytes in Dog

Type of animal	Total number examined	Number of positive	Species isolated	Number of isolations	Incidence (%)
Dog	65	13	*M. canis*	7	53.85
			M. gypseum	4	30.77
			T. mentagrophytes	2	15.38
			T. rubrum	0	0
Bitch	59	11	*M. canis*	6	54.55
			M. gypseum	2	18.18
			T. mentagrophytes	1	9.09
			T. rubrum	2	18.18
Puppies (male)	41	10	*M. canis*	7	70
			M. gypseum	2	20
			T. mentagrophytes	1	10
			T. rubrum	0	0
Puppies (female)	21	7	*M. canis*	4	57.14
			M. gypseum	2	28.57
			T. mentagrophytes	1	14.29
			T. rubrum	0	0

Table 7: Seasonal distribution of dermatophytes

Seasons	Number of animals examined	Culturally positive	Percentage (%)
Winter	62	8	12.9
Summer	57	15	26.32
Rainy	67	18	26.86
Overall	186	41	22.04

Seasonal distribution of dermatophytes

From the (Table 7) it can be realized that the occurrence of dermatophytosis was comparatively high during summer and rainy season. Similar observations were also reported by various authors Singathia *et al.* (2014) and Murmu *et al.* (2015). It may be due to high average rainfall, humidity and temperature in the area of study during the summer and rainy season. Higher humidity is congenial for faster multiplication and propagation of fungal elements as reported earlier by Bhardwaj *et al*. (2012). There are conflicting reports regarding influence of season on the prevalence of the disease. Cafarchia *et al.* (2006) observed an increased incidence of dermatophytosis in autumn to winter months.

Isolation of various species of dermatophytes from dogs

The present study revealed that of the 13 diseased male dogs, 7(53.85%) were infected with *M. canis*, 04 (30.77%) with *M. gypseum* and 02 (15.38%) were infected with *T. mentagrophytes*. Of the 11 diseased Bitches, 06 (54.55%) were infected with *M. canis*, 02 (18.18%) with *M. gypseum,* 01(9.09%) were infected with *T. mentagrophytes* and *T. rubrum* 02(18.18%). In case of 10 diseased male puppies, 07 (70.00%) were infected with *M. canis,* 02 (20.00%) with *M. gypseum* and 01(10.00%) with *T. mentagrophytes*. Of the 07 infected female puppies, 04(57.14%) were infected with *M. canis*, 02 (28.57%) with *M. gypseum* and 01 (14.29%) were infected with *T. mentagrophytes*.

In the present study dermatophytes belonging to two genera: Microsporum and Trichophyton were isolated from dogs, which were of four different species *M. canis*, *M. gypseum, T. mentagrophytes* and *T. rubrum*. In the decreasing order of prevalence of these were *M. canis* from 24(58.55%) animals including, 7 (53.85%) from dogs, 6 (54.55%) from bitch,7 (70.00%) from male puppies and 04 (57. 14%) from female puppies; *M. gypseum* from 10 (24.39%) animals including, 04 (30.77%) from dogs, 02(18.18%) from bitch, 02 (20.00%) from male puppies and 02 (28.57%) from female puppies; *T. mentagrophytes* from 05 (12.18%) of animals including,02 (15.38%) dogs, 01 (9.09%) bitch, 01 (10.00%) male puppies and 01(14.29%) female puppies; *T. rubrum* from 02 (4.88%) animals including 02(18.18%) bitches (Table 6).

The most common species of dermatophytes isolated from dogs was *M. canis* (58.55%) (Table 1). These dermatophytes have also been reported by Copetti *et al.* (2006), Prado *et al.* (2008), Ivaskiene *et al.* (2009). Nichita and Marcu (2010), Beraldo *et al* (2011), Nweze (2011), Seker and Dogan (2011), Mohammed (2013) and Murmu *et al.* (2015). The work of above mentioned workers Support the findings of the present work. Cafarchia *et al.* (2006), Ates *et al*. (2008) and Gangil *et al.* (2012) could not isolated this species from dogs.

M. gypseum was the 2nd most prevalent dermatophyte isolated from 24.39% of dogs suffering from dermatophytes in the present study. This finding is supported by the work of Chermette *et al*. (2008), Beraldo *et al.* (2011), Mohammed (2013) and Murmu *et al.* (2015), while Nweze (2011) observed *M. gypseum* at number three in order of prevalence and isolation. Again, Gangil *et al.* (2012) found highest percentage of *M. gypseum* infection in dogs.

T. mentagrophytes was the third in order of prevalence and was isolated from 12.18% of cases of dermatophytes in dogs. This finding are in line of those reported by Murmu *et al.* (2015). However workers like Nweze (2011) and Gangil *et al.* (2012) placed *T. mentagrophytes* in second most prevalent dermatophytes causing infection in dogs.

T. rubrum was isolated only in two cases making it 4th and least prevalent dermatophyte species. Some authors also reported occasional isolation of dermatophytic species as etiological agents of dermatophytosis by Begum and Kumar (2020).

CONCLUSION

This study concludes that direct microscopy and *in vitro* culture are equally efficient in diagnosis of dermatophytosis. The prevalence of dermatophytosis in the canines of this study ranged between 4.88 and 58.55%, and *M. canis* was most abundant (58.55 %) followed by *M. gypseum* (24.39%), *T. Mentagrophytes* (12.18%) and *T. rubrum* (4.88%). Furthermore, the prevalence of canine dermatophytosis is affected by some factors represented by age, breed, coat, habitat and season of years.

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