Isolation and Identification of Dermatophytes in Apparently Healthy Skin and Skin with Clinical Lesions in Different Breeds of Dogs

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

PUSA (SAMASTIPUR) BIHAR

(FACULTY OF POST - GRADUATE STUDIES)

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

IN

MICROBIOLOGY

By

Dr. Shashi Bhushan Sudhakar

Registration No. - M/V. Micro/29/2002-2003

Department of Veterinary Microbiology

BIHAR VETERINARY COLLEGE

PATNA (BIHAR)

2004

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DEPARTMENT OF VETERINARY MICROBIOLOGY

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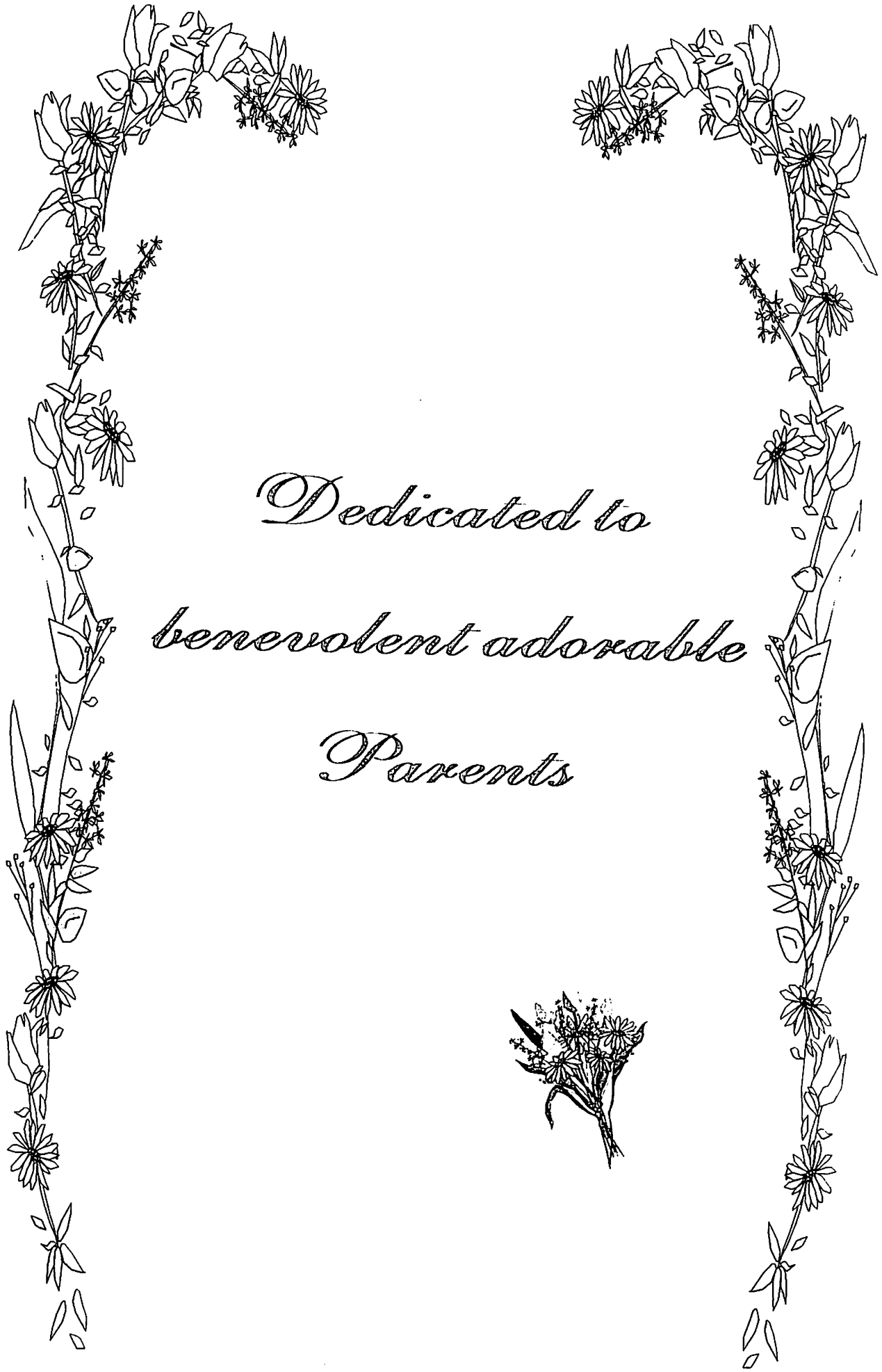

Dedicated to

benevolent adorable

Parents

DEPARTMENT OF VETERINARY MICROBIOLOGY
BIHAR VETERINARY COLLEGE, PATNA-14
RAJENDRA AGRICULTURAL UNIVERSITY
PUSA (SAMASTIPUR) , BIHAR.

Dr. S.D. Singh, M.V.Sc., Ph. D
Chairman, Deptt. of Vety. Microbiology &
Assoc. Dean-cum-Principal
Bihar Veterinary College, Patna 14

CERTIFICATE-I

This is to certify that thesis entitled "***Isolation and identification of dermatophytes in apparently healthy skin and skin with clinical lesions in different breeds of dogs***" submitted in partial fulfilment of the requirements for the Degree of **Master of Veterinary Science (Veterinary Microbiology)** of the Faculty of post-graduate studies, Rajendra Agricultural University, Pusa, Samastipur, Bihar is the record of bonafide research work carried out by ***Dr. Shashi Bhushan Sudhakar*** Registration no. M/V.Micro/29/2002-2003, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received during the course of this investigation and preparation of the thesis have been fully acknowledged.

12/04

(*S.D. Singh*)

Major Advisor

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PUSA (SAMASTIPUR) , BIHAR.

CERTIFICATE - II

We, the undersigned members of the Advisory Committee of ***Dr. Shashi Bhushan Sudhakar***, Registration No. M/V.Micro/29/2002-2003, a candidate for the Degree of Master of Veterinary Science with Major in **Veterinary Microbiology** have gone through the manuscript of the thesis and agree that the thesis entitled "***Isolation and identification of dermatophytes in apparently healthy skin and skin with clinical lesions in different breeds of dogs***" may be submitted by ***Dr. Shashi Bhushan Sudhakar*** in partial fulfilment of the requirements for the degree.

(***S.D. Singh***)

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3. Dr. J.N. Singh
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CERTIFICATE - III

This is to certify that the thesis entitled "*Isolation and identification of dermatophytes in apparently healthy skin and skin with clinical lesions in different breeds of dogs*" submitted by *Dr. Shashi Bhushan Sudhakar,* Registration No. M/V.Micro/29/2002-2003, in partial fulfilment of the requirements for the Degree of Master of Veterinary Science (Veterinary Microbiology) of the Faculty of Post-Graduate studies, Rajendra Agricultural University, Pusa, Samastipur, Bihar, was examined and approved on 18.1..... 2006

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DRI-cum-Dean, P.G. Studies

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I am flooded with emotions and bow my head, expressing profound sense of gratitude to my beloved mother Smt. Renu Das and father Sri B.K. Das for their psychological support, blessings, love, gracious sacrifice and inspiration to pursue higher education and to achieve the goals in my life.

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Place : Patna

Date : 22.12.04

S. B. Sudhakar

(Shashi Bhushan Sudhakar)

CONTENTS

LIST OF TABLES

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LIST OF FIGURES

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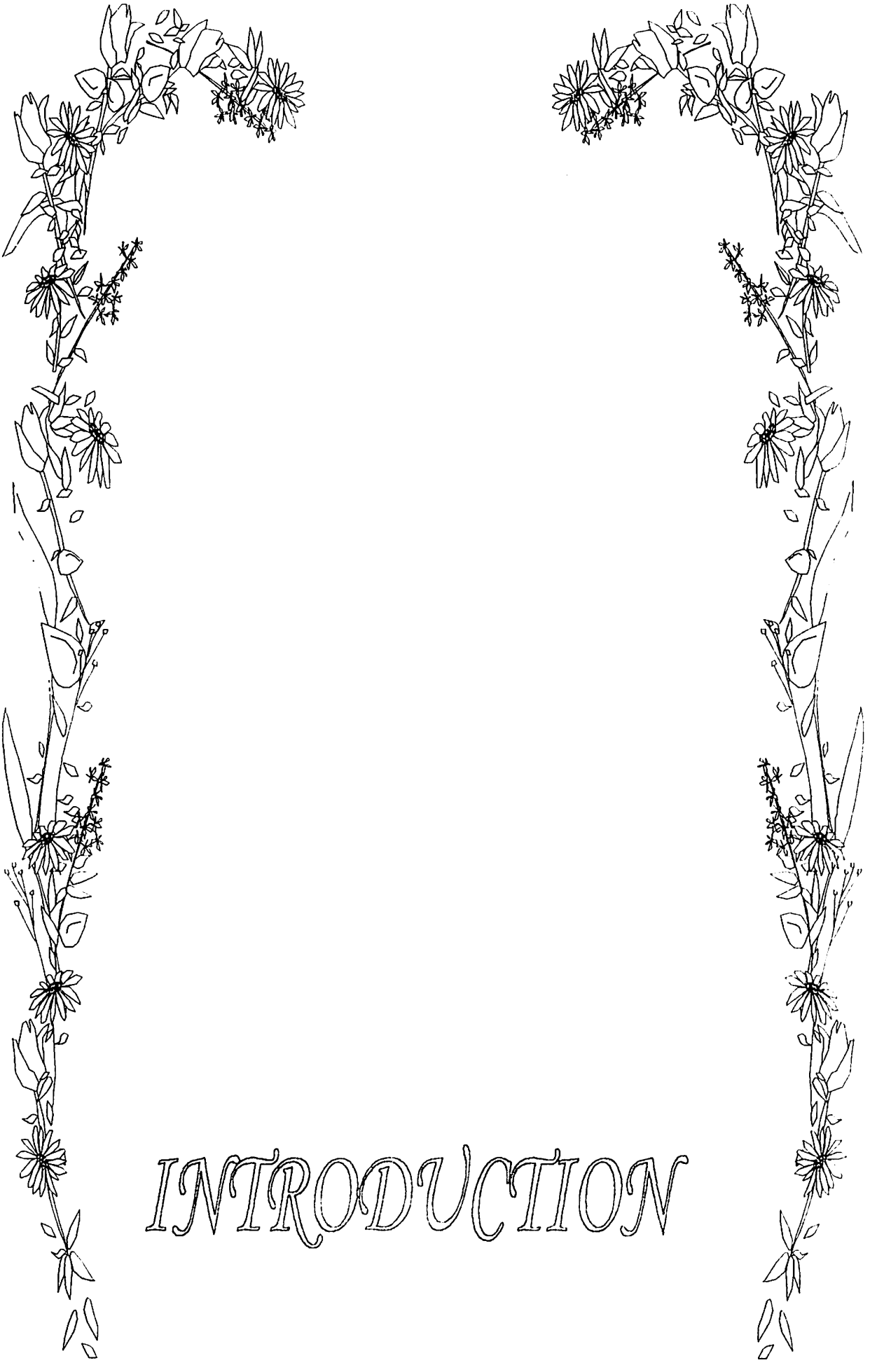

INTRODUCTION

Introduction

Since time immemorial, dog has been portrayed in sculpture and painting invariably shown as a loyal companion of man. In recent times, it has become a necessity and not a luxury to have an animal companion as the trend now is a unit family with one child norm. And then to inculcate a sense of belonging and responsibility in the single child, a companion animal is brought up along with child. The companion animal need not necessarily be a pedigreed dog but also one of many abandoned or stray dog which are neutered and vaccinated against rabies and given for adoption to loving homes in our fast growing cities and towns. Researchers have shown the role of dogs or other pets on human lives and it is ascertained that the pets are an aide to child development or therapy for the old or mentally retarded persons and so on. Scientists of Western countries have found that dogs are of much importance not only to growing children, but also they rendered psychological benefits to adults, specially to the elderly and the house bound.

Dogs act as friends to children or even sibling substitute for the only child and also they are a source of play and learning which help the child development.

Presence of dogs or other pets offer an opportunity for developing a new life-style. A dog is reliable, affectionate, having a sense of consistent routine life. A dog is a stimuli for laughter, play, exercise and keep their owners active. Dog can improve self reliance and may have the ability to reduce stress.

Dogs in their ordinary mode of life, and as a result of domestication are often exposed to many disease. Skin diseases are directly observable and this puts the pet owners in an embarrassing situation. The skin, the first line of defence, does its job well but it sometimes needs a boost, so the wise dog owners regularly monitor their pet's skin condition (Woolf, 1995). In addition to producing specific problems, also serves as the carrier for transmission of infection. Many of the skin diseases are zoonotic in nature i.e. that are shared between man and animals and thus has public health importance. Among all the canine ailments, dermatophytosis is one of the vital problem from both zoonotic point of view as well as for the welfare of dogs as various dermatophytes have been isolated from cases of dermatitis in dogs (Gupta *et al.*, 1968; Chittawar and Rao, 1982; Grant, 1987; Yathiraj *et al.* 1990; Sidhu *et al.*, 1993; Verghese *et al.*, 1994; Vishwakarma *et al.*, 1997). In tropical countries like India the climatic conditions are very conducive to growth and spread of ringworm infections.

In India, dermatomycoses are the most common of all skin infection (Ghosh, 1948). It attracts our attention because it continued to be a difficult problem confronting veterinarians on account of various etiological and clinico-pathological factors.

Dermatophytosis is a superficial fungal infection of the cornified epidermis (hair, nail, feathers) (Chatterjee, 1989). Dermatophytosis is a clinical attribute caused by a group of taxonomically related fungi known as *"dermatophytes"*. The dermatophytes create a change of the invaded structure and this alteration along with immunological reactions are clinically known as *"ring worm"* (Kaplan *et al.*, 1953).

Ringworm, also known as *dermatophytoses* and *tinea*, is the most common superficial and highly contagious mycotic disease of man and a wide variety of animals. The word "*dermatophyte*" literally means "*skin plant*". Ringworm caused by three related genera of filamentous *Fungi imperfecti* namely, *Trichophyton, Microsporum* and *Epidermophyton* (Cambell and Stewart, 1980; Lewis *et al.*, 1991; Sparkes *et al.*, 1993). Dermatophytosis is common infection of domestic animals. Dogs and cats showed the highest prevalence. *Epidermophyton* is almost entirely restricted to man attacks skin and nails. In dogs almost all ringworms are due to species of *Microsporum* or *Trichophyton*.

Dermatophytes are broadly devided into three groups on the basis of their host preference and natural habitat (Ajello, 1962).

a) **Geophilic Dermatophytes** – They are the soil borne fungi and may remain in soil as saprophytes e.g. *M. gypseum, T. terrestre, M. nanum* etc.

b) **Zoophilic Dermatophytes** – They basically live on lower animals e.g. *T. verrucosum, T. equinum. T. mentagrophytes* and *M. canis*.

c) **Anthropophilic Dermatophytes** – They exclusively grow on human beings e.g. *E. floccosum, M. audouinii* etc.

The commonest causal agent *M. canis* is believed to be endemic in many dog population throughout the world, others are *M. gypseum* and *T. mentagrophytes,* and are transmitted from animal to animal. Close confinements and overcrowding of pets make the transmission of dermatophytes easier. However indirect contact with any inmates objects is probably more important. Spread between species occurs rapidly and in rural areas 80% of human ringworm may be derived from animal. The sources of human infections are especially infected cats and dogs (Terragni *et al.* 1993).

Man may get the disease by direct contact with infected one. The dog, being man's universal pet, is considered to be a common source of human infection. Because of the close association between owner and his pet, the possibility of fungal transmission to man can not be ignored. Many human cases of ringworm have been traced to past contact with infected animals. Furthermore, *Microsporum canis* is considered to be the major dermatophyte causing *tinea capitis* in humans (Ahy *et al.* 2000).

A ringworm in dogs may resemble with other skin disorders, such as neoplasia, bacterial dermatitis, seborrhea and parasitic infestation. Dermatophytes often cause progressive diseases which are not easily manageable. Clinical manifestations of ringworm are sometimes inconspicuous, hence, these dogs may pass unnoticed and served as reservoir hosts for human infections. In dogs the lesions are usually located on the face, extremities and lower abdomen. These lesions appear as small pink macules spreading in peripheral direction i.e. dermatophytes have the tendency to grow centrifugally. Sometimes a large areas of the body may be affected. The advanced lesions are circumscribed, discrete or confluent and covered with grayish scales which are attached to the skin. Secondary bacteria or mites may invade the lesions (Chaterjee *et al.*, 1980). Affected animals suffer from constant itching leading to the damage of the skin affecting its sheen (Blank, 1955).

Although considerable information has been disseminated in recent years about dermatophytosis but their successful treatment is still far from satisfactory in many cases. With the introduction of a number of broad spectrum antibiotics and is misuses, this fungal infection pose a problem. The development of specific treatment has emphasized the need for accurate

diagnosis. Today as a result of work in many country dermatophytosis is a rapidly growing subject and knowledge of ins and outs of causative organism in dogs has to be rediscovered. Dermatophytes infections are very common in animals (Jacobs, 1988) and human beings (Terragni *et al.*, 1993). Therefore an exhaustive study on their life cycle appears to be worth interest.

In view of importance of dermatophytes in dogs, their incidence under field condition, lack of proper identification and specific measures, the present research studies on the isolation and identification of dermatophytes in apparently healthy skin and skin with clinical lesions in different breeds of dogs have been carried out in the Department of Veterinary Microbiology, Bihar Veterinary College, Patna – 14 with the following objectives :

Objective :

1. Isolation of dermatophytes in apparently healthy skin and skin with clinical lesions in different breeds of dogs.
2. Identification of dermatophytes in apparently healthy skin and skin with clinical lesions in different breeds of dogs.

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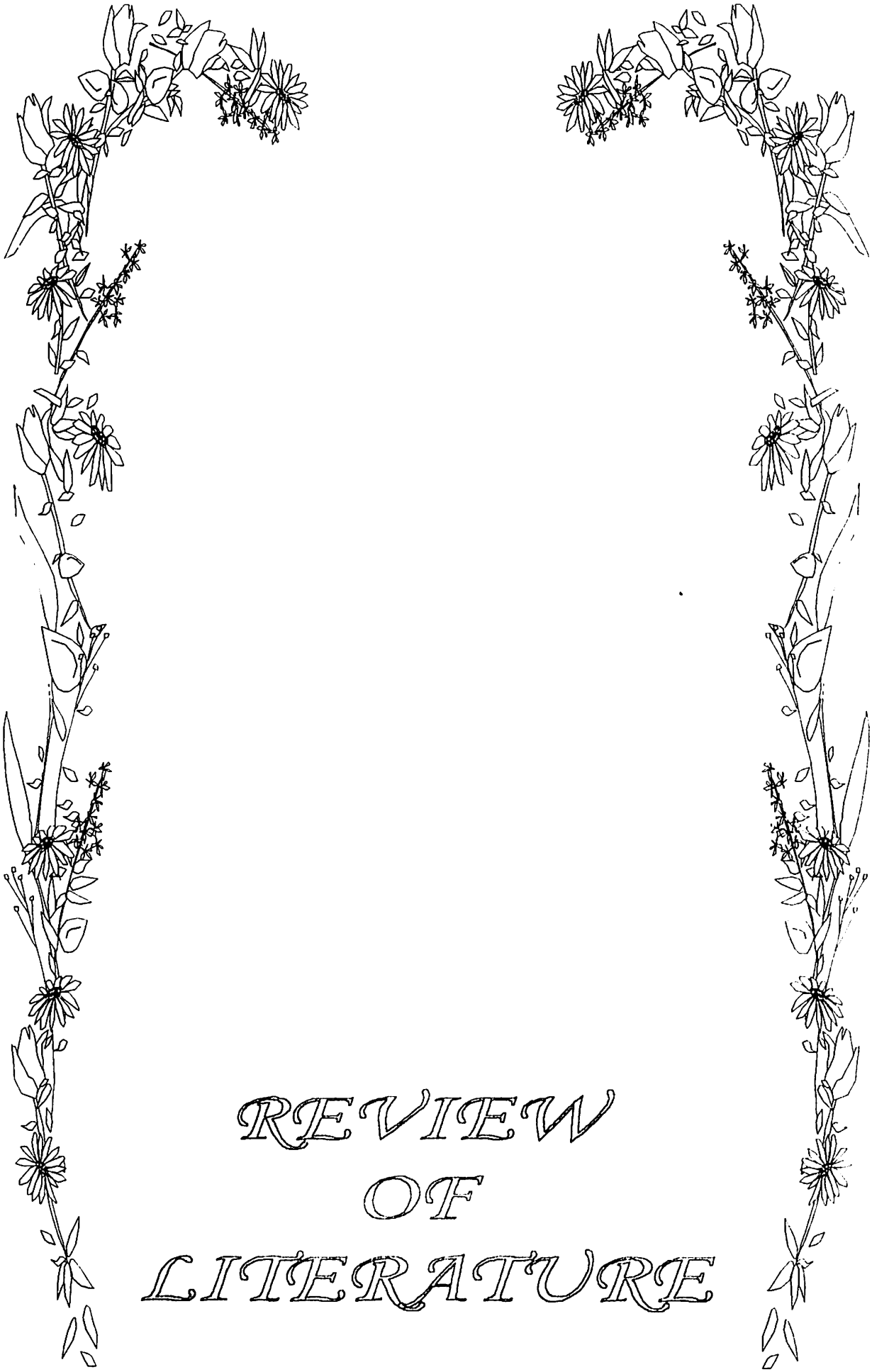

REVIEW OF LITERATURE

Review of Literature

Hoelein (1945) described the involvement of hairs as the principle feature in all the dermatophytes of animal.

Van Der Hoeden (1947) studied the incidence of ringworm in dogs which was largely based on frequencies of positive samples taken from animals clinically suspected to have disease. He demonstrated that animals carry fungal particles on their hair coats without showing any signs of the disease. They may serve as a source of infections.

Ajello (1953) described the saprophytic and parasitic nature of *Microsporum gypseum*.

Fuentes *et al.* (1954) reported the occurrence of *Trichophyton mentagrophytes* and *Microsporum gypseum* on hairs of healthy cats.

Georg (1954) diagnosed *Microsporum canis* and *Microsporum audouinii* and suggested that these were the only dermatophytes which produced fluorescence.

Reiss *et al.* (1954) observed the course of primary infection in dogs in which *Microsporum lanosum* infection was experimentally produced.

Sellers *et al.* (1956) reported that however, but breaks of ringworm were found often to occur during the month of summer and in close confinement but spread of the ringworm was possibly due to lack of nutrition and debilitating condition of the animals than other environmental factors like temperatures and sunlight.

Georg *et al.* (1957) stated that 254 positive specimens of dermatophytes of dog hairs were surveyed by the Communicable disease center, U.S., Public Health service. Of those, 66.9 per cent of dermatophytes infection were due to *Microsporum canis*, 26.0 per cent to *Microsporum gypseum* and 7.1 per cent to *Trichophyton mentagrophytes*.

Barlow and Chattaway (1958) discussed the parasitism of ringworm groups of fungi. They regarded the ringworm fungi as essentially neutrophytic and the fungi appeared to grow on soft and hard keratin. Dermatophytes had a saprophytic mode of growth unlike that in the host animal when they were removed from the animal body while growing in keratins.

Ainsworth (1959) listed the species of dermatophytes infecting dogs. They were *Microsporum canis* Bodin, *Microsporum audouinii* Gruby, *Microsporum gypseum* Bodin, *Trichophyton Mentagrophytes* (Robin) Blanchard, *Trichophyton gallinae* (Megnin) Selva and Bazihan, *Trichophyton quinkeanum* and *Trichophyton verrucosum* Bodin. Man is susceptible to all these species.

Reyes (1959) shown that culturing pathogenic fungi on plain Sabouraud's dextrose Agar had been unsuccessful due to overgrowth by bacteria and saprophytic molds. But this problem was solved by addition of four tenth (0.4) gram cyclohexamide and five hunderedth (0.05) gram chloramphenicol per litre of media.

Lindqvist (1960) reported that *Microsporum canis* was the main causative agent of ringworm in dogs in Norway.

Ridley (1960) isolated *Microsporum canis* from dogs in Queensland.

Kaplan and Ivens (1961) demonstrated the seasonal variation in the incidence of ringworm in dogs and the patterns of ringworm incidence appeared to vary with the dermatophytes involved.

Georg *et al.* (1962) isolated a new species of *Microsporum vanbreuseghemii* from ringworm of a dog.

Guilhon (1963) reported the presence of *Microsporum canis* in the sebaceous glands of two dogs.

Jaksch (1963) conducted a massive study of skin scrapings and hairs on 200 horses, 100 dogs and 50 cats without skin lesions and 100 horses 450 dogs, 100 cats, 95 rhodents and 26 birds with various skin lesions. He found *Microsporum canis* infection in 6 per cent of horses, dogs and cats without skin lesions and in 6.8 per cent with skin lesions.

Rieth and Dreisorner (1963) examined cases of skin lesions in dogs and stated that the infection was due to *Microsporum canis* but occasionally also due to *Microsporum gypseum* and *Microsporum audouinii.*

Blackemore (1965) observed the relationship between host and dermatophytes was a tenacious one with some factors easily tipping the balance in favour of one or the other. Predisposing causes such as malnutrition, intestinal parasitism and recent physical stress favour the dermatophytes.

James (1965) found that 99 per cent of skin infections in dogs were due to *Microsporum canis* and *Trichophyton mentagrophytes*. He also reported a flat, circular, slowly progressing lesions in the *Microsporum* and

Trichophyton infections whereas *Epidermophyton* caused thick elevated, scaly lesion with exudate underneath.

Keep and pile (1965) reported *Microsporum gypseum* as a causative organism in an outbreak of ringworm in a group of Dachshund dog in the age group of 18 to 48 month old. They also demonstrated the lesions of ringworm in dogs which appeared as raised plaques, 1-3 cm in diameter scattered over the entire body, especially along the back, neck, head, abdomen and inner thighs, and the lesions did not fluoresce Wood's light. After 8 days, the lesion appeared numerous and were devoid of hairs and were covered with a thick grey crusts. A thick yellowish push was observed from the lesion.

Schwarz and Schwarz (1965) stated that the incidence of dermatophytes in dogs was the highest in spring and fall. They further advocated that out of 404 dogs with variety of skin diseases 70 had dermatophytes. They also demonstrated the clinical picture of dermatomycosis. It was found to be polymorphic ranging from alopecia to a form of resembling acanthosis nigricans. There might be small reddish pustules which tended to coalesce into first moist red circular patches (*Trichophyton tonsurans*) and later bare patches developed anywhere on the body coat.

Snyder (1965) isolated *Microsporum canis* from 11 dogs and *M. gypseum* from one dog out of 12 cases of skin infection in dogs.

Olga-Fischman *et al.* (1966) stated that in the past the dogs were rarely infected with *Microsporum gypseum* but in a US survey in 1960, 114 out of 495 dermatophytes isolated were *Microsporum gypseum.*

Kaplan (1967) reported that *Microsporum gypseum* and *Microsporum canis* caused 20 per cent and 10 per cent of ringworm in dogs respectively. He further stated that the incidence was entirely found in summer and fall.

Smith (1967) found that *Microsporum canis* was associated with the ringworm of dogs.

Al-Doory *et al.* (1968) reported that the incidence of dermatophytes were 57 per cent in a ringworm survey from 29 dogs and 6 cats. Among the dermatophytes *M. gypseum* were found in 14 dogs, *Microsporum canis* were found in dogs and 3 cats and *Trichophyton mentagrophytes* was found in one dog.

Dawson (1968) observed the lesions of ringworm in dogs which was usually located on the face, extremities and lower abdomen. The lesions appeared as small pink macules spreading in peripheral direction. Sometimes a large area of the body was affected. The advanced lesions were circumscribed, discrete or confluent and covered with grayish scales which are attached to the skin. He isolated *Microsporum.*

Gupta *et al.* (1968) isolated *Microsporum canis* from single case of dog; *Trichophyton rubrum*, *Trichophyton violaceum*, *Trichophyton tonsurans*, *Trichophyton verrucosum*, *Trichophyton simii* from human beings and *Microsporum nanum* and *Trichophyton mentagrophytes* from pigs. They further stated that *Microsporum nanum* was isolated for the first time in India.

Arambulo (1970) mentioned six human cases of *T. capitis* and *T. corporis* infection reportedly contracted from household pets. *Microsporum*

canis and *Trichophyton mentagrophytes* were isolated from cats and dogs respectively.

Singh and Singh (1970) isolated two strains of *Microsporum canis*, three strains of *Trichophyton violaceum*, two strains of *Trichophyton mentagrophytes* and six strains of *Pseudoarachniotus roseus* from dogs with ring worm.

Jungerman and Schwartzman (1972) stated that transmission of infection of dermatophytes may occur by direct contact with infected animals (with or without skin lesions) or with contaminated premises or inanimate objects. The fungus is inoculated into stratum corneum and either of the following events may occur. The fungus may be brushed off mechanically and may not be able to establish residence primarily because of its inability to compete with normal bacterial flora, or it may establish residence on the skin and produce the clinical disease.

They also claimed that young animals appeared to be infected more often than adults.

Smith *et al.* (1972) stated that lesions of ringworm in dogs were found to be localized on the head and back. Apparently lesions appeared as round, red hairless areas with scales. Sometimes healing of the lesions took place simultaneously.

Baxter (1973) studied the incidence of ringworm in dogs. He found that 60 per cent of the cases of disease were in young dogs. There was no sex or breed disposition but certain individual or members of particular family or breeding might be genetically predisposed.

Reyes (1973) isolated *Trichophyton mentagrophytes* from two puppies whose owners were suffering from ringworm. The puppies shown the typical ringworm lesions and diagnosed and confirmed by fungal culture. Plates of Sabouraud's dextrose agar (with chloramphenicol and actidione) were inoculated with infected hairs to culture the characteristic fungal colonies.

He studied the incidence of a symptomatic carrier animals in the small animal clinic of Massey University, NZ, using hairbrush technique. Of the 120 dogs examined, the incidence of carrier was 8.3 per cent, most of the isolates were *Microsporum canis*.

According to Baxter, hairbrush technique is a useful method in screening animals with minimum symptoms or carriers which are difficult to detect by clinical signs alone. It can also be used for sampling clinically infected animals and assessing the effectiveness of the treatment.

Siegmund (1973) observed that culture on Sabouraud's Dextrose Agar plates took the longest time but was the most effective and specific means of diagnosis. He further stated, it frequently shown infections that had been missed by Wood's lamp and also aided in the identification of the specific aetiological agents.

Baluyut and Constantino (1977) isolated *Microsporum gypseum* from 7 dogs and *Trichophyton rubrum* from one dog while examining the haircoats of 100 dogs, which were randomly selected, with apparently normal haircoats. The incidence of dermatophytes was higher in dogs less than one year old. Breed and sex predisposition were not significant.

Hasegawa *et al.* (1977) isolated dermatophytes including *Microsporum canis* from 48 dogs, *Microsporum gypseum* from 11 dogs and *Trichophyton mentagrophytes* from 3 dogs, during 1962-75, in studies at the Vet. Hosp., Univ. Tokyo.

Kushida (1978) observed that of 123 dogs with ringworm 85 (69.1 per cent) were caused by *Microsporum canis*, 31 (25.2 per cent) by *Microsporum gypseum*, 5 (4.1 per cent) by *Trichophyton mentagrophytes* and 2 (1.6 per cent) by *Trichophyton rubrum*. *Microsporum canis* occurred mostly in animals kept indoors, causing lesions scattered all over the body and showed a high incidence in animals under 6 month old. *Microsporum gypseum* occurred frequently in outdoor breed, exclusively in the autumn, causing lesions restricted to a few parts of the body.

Chatterjee and Sengupta (1979) described *Trichophyton verrucosum*, *Trichophyton mentagrophytes*, *Microsporum gypseum*, *Microsporum distortum* and *Trichophyton rubrum* as aetiological agents involved in the causation of ringworm in dogs.

Fiedler (1979) isolated *Microsporum canis* from 22 out of 327 samples (hairs and skin scrapings) of dogs presented between 1973 and 1978.

Kushida (1979) isolated *Trichophyton rubrum* from an 8-yr-old female poodle. The owner had had dermatomycosis of the feet for many years due to the same fungus, and it was thought the dog contrated the infection from its owner with whome the dog slept.

Aho (1980) isolated *Microsporum canis, Trichophyton mentagrophytes* and *Trichophyton rubrum* from samples of hairs suspected for dermatophytosis from 8 dogs.

Boro *et al.* (1980) observed crust formation and erythema at the root of tail in the lesions of ringworm.

Chatterjee *et al.* (1980) isolated *Microsporum distortum* from ringworm in a four-year-old male Alsatian dog.

Moe (1980) noted loss of hairs, hyperemia and discolouration or pigmentation of hairs in the lesion of ringworm caused by *Microsporum canis. Trichophyton mentagrophytes* and *Microsporum gypseum* in dogs having ringworm.

Morganti *et al.* (1980) found *Trichophyton mertagrophytes, Trichophyton terrestre, Microsporum gypseum* and *Trichophyton ajello* from 16 out of 300 urban dogs in Persaro, Italy and 10 out of 95 rural dogs.

Chittawar and Rao (1982) stated that the body parts which frequently come in contact with the objects or ware easily available for scratching *viz.*, head, neck, legs, shoulders, abdomen and interdigital skin were usually affected with dermatophytes and the lesions were mostly circular, slightly raised and crustaceous though itching was not a constant feature.

They investigated the occurrence of dermatitis in dogs due to fungal agents and observed that out of 211 clinical cases, 39 (18.41 per cent) were positive for fungal infection. Out of these 39 cases, 22 cultures could be isolated. There were 10 *Trichophyton mentagrophytes*, 2 *Trichophyton simii,*

4 *Microspourm canis*, 3 *Microsporum gypseum* and 3 were *Candida albicans*.

Weiss and Weber (1982) isolated the most common strain *Microsporum canis* from the skin scraping cultures of 167 dogs followed by *Trichophyton mentagrophytes* (122 dogs), *Trichophyton rubrum* (7 dogs), *Microsporum gypseum* (7 dogs), *Trichophyton terrestre* (5 dogs), *Microsporum audouinii* (2 dogs), *Trichophyton quinkeanum* (1 dog) and *Microsporum persicolor* (1 dog) in the studies of 2395 dogs having skin lesions.

Petrovich and gorbatov (1984) studied the cultures of 173 skin scrapings which yield 40 isolates of *Microsporum canis* from dogs. The isolates were proved to be pathogenic for guinea pigs, rabbits and hamsters.

Bussieras *et al.* (1984) isolated *Microsporum canis* from 24 dogs and mixed *Microsporum* plus *Trichophyton mentagrophytes* and mixed *Microsporum persicolor* plus *Microsporum gypseum* from two severly affected dogs out of 26 specimens examined.

Aghomo and Adetosoye (1985) described *Microsporum gypseum* acting as an aetiological agent of dermatophytosis in dogs having lesions of acanthosis and hyperkeratosis.

Custem *et al.* (1985) obtained fungi after culturing the specimens from 12 (8.4 per cent) out of 142 of apparently healthy dogs and 40 (20.2 per cent) out of 180 dogs with hair loss. They also isolated 20 strains of *Trichophyton mentagrophytes*, 17 of *Microsporum canis*, 2 of *Microsporum gypseum* mostly from the cases of alopecia.

Lopez *et al.* (1985) isolated dermatophytes by carpet square technique from 27 of 100 apparently healthy dogs at a Canine Asylum. *Microsporum canis* was isolated from 20 and *Microsporum gypseum* from 7 dogs.

Mantelli *et al.* (1988) studied, during 1974-87, 7021 dogs and cats presented for dermatological examination at Milan University Clinic. 1596 yielded dermtophytes (21.4 per cent of dogs and 27.6 per cent of cats). Of the canine cases 94.2 per cent yielded *Microsporum canis,* 3.1 per cent *Trichophyton mentagrophytes*, 2 per cent *Microsporum gypseum*, 0.3 per cent *Trichophyton rubrum* and 0.3 per cent *Trichophyton verrucosum.*

Chatterjee (1989) while studying the skin infection in domestic animals, he found that animals were carrying the dermatophytes on their healthy skin and thus remained as a transmitting agents to susceptible hosts.

Brglez (1991) cultured 284 canine and feline skin scrapings in Sabouraud's medium in the seven-year period 1984-90. 97 (32 dogs and 65 cats) were positive, 87 for *Microsporum canis* and 10 for *Trichophyton mentagrophytes;* 68 of the cases were in August-January and 29 in February-July. Of 71 isolates cultured at both 26^0C and 37^0C, 9 grew only at 26^0C and 11 at 37^0C. Cultures at 37^0C inhibited the growth of saprophytes.

Lewis *et al.* (1991) cultured dermatophytes from 70 of 1824 (3.8 per cent) canine samples submitted over 10 years. *Microsporum gypseum* (31/70) and *Microsporum canis* (30/70) were isolated most frequently from dogs. Both male and female dogs were equally affected by dermatophytosis. There was a higher incidence in dogs below one year of age. Mixed breed dogs (19/70) were most often affected. *Microsporum gypseum* had a greater

incidence of infection in summer and most caused localized dermatophytosis.

Yamada *et al.* (1991) reported a case of ringworm in a 3 years old female Yorkshire Terrier with a history of chronic pyoderma-like skin lesions. Fungal elements were demonstrated in direct microscopy of skin scrapings and *Trichophyton rubrum* was isolated. The isolates grew well at 25°C on Sabouraud's dextrose agar with red pigmentation. It produced many smooth-walled and slender macroconidia and pyriform microconidia.

Sparkes *et al.* (1993) investigated 8349 samples, from dogs and cats, of suspected dermatophytosis, received between 1956 and 1991 and 1368 (16 per cent) yielded positive cultures. Dogs gave 10 per cent positive cultures, and of these *Microsporum canis* accounted for 65 per cent in dogs. Different breeds of dogs had significantly different prevalences of infection and Jack Russel and Yorkshire Terrier dogs having a particularly high proportion of positive cultures. Animals below one year age appeared to be predisposed to infection. But there was no apparent sex predisposition and no conclusive evidence of any seasonal variation in the incidence of the disease. In comparison with the results of dermatophyte culture, direct microscopy had positive and negative predictive values of 93 percent in determining the presence of dermatophytosis. However, cultural examination alone was insufficient for the diagnosis of dermatophytosis owing to the occurrence of false positive and false negative results.

Wawrzkiewicz *et al.* (1994) studied 21 dogs representing different breeds suspected of dermatophytosis, diagnosis was confirmed in 9 dogs (42.9 per cent). Dermatophytosis was found mainly in dogs aged 2 years (71

per cent). Skin lesions caused by *Microsporum canis* were diagnosed in 7 owners.

Bourdzi *et al.* (1996) had reported a case of dermatophytosis in a 3-year-old male German Shephered dog from Greece which had a lesion in the metacarpal region of the left limb. The clinical diagnosis of dermatophytosis was confirmed by direct microscopical examination of hairs and scales and *Trichophyton rubrum* was cultured. The same dermatophyte was isolated from the interdigital area and the nails of owner's feet.

Cabanes *et al.* (1997) did a retrospective study of the main specimens from animals with suspected dermatophytosis examined at the Mycological Diagnostic services of the faculty of Veterinary Science in Barcelona during 1986-95. 136 dermatophytes were identified from dogs and cats, cultures submitted for the identification. The most frequent dermatophytes isolated were *Microsporum canis* (55.9 per cent), *Trichophyton mentagrophytes* (27.2 per cent), *Microsporum gypseum* (7.4 per cent) and *Trichophyton verrucosum* (7.4 per cent). Dermatophytes were cultured from 15 (14.3 per cent) of 105 canine specimens. *Microsporum canis* was the most commonly isolated species (73.3 per cent). There was a high proportion of positive cultures from dogs aged below one year, and in some breeds of dog, but there was no significant differences between sexes. Although dermatophytes were more frequently isolated in autumn and winter months, no significant difference was detected in the seasonal distribution of the canine and feline dermatophytosis.

Vishwakarma *et al.* (1997) studied the dogs brought to the Veterinary College Hospital, Jabalpur. They selected dogs those with clinical signs of

dermatomycosis for further examination. An overall incidence of 15.06 per cent was found with *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Candida albicans* and *Alternaria alternata* being identified in skin serapings.

Batta *et al.* (1999) examined 45 dogs with dermatitis and found fungal infection in 22 dogs. The fungi isolated were *Alternaria* spp. (11), *Microsporum* spp. (4), *Aspergillus* spp. (4) and *Cladosporium* spp. (3).

Saridomichelakis *et al.* (1999) examined 140 privately owned hunting dogs without apparent skin lesions on the nose or elsewhere. They obtained two samples from the nasal bridge of each dog. *Microsporum gypseum* was isolated from one dog which showed no rooting behaviour. They concluded that the rooting behaviour does not seems to play a significant role in contamination of the canine nasal bridge by *Microsporum gypseum*, despite its relatively high yield from the soil in the area.

Carlotti and Bensignor (1999) did a retrospective study of 20 cases of dermatophytosis due to *Microsporum gypseum* and 13 cases due to *Microsporum persicolor* seen at a French dermatology referral practice between 1988 and 1996. The dermatophytosis were diagnosed through fungal culture and in some instances by rapid histopathology with Periodic-Acid-Schiff staining (3/3 cases of *Microsporum gypseum* infection and 5/7 cases of *Microsporum persicolor* infection).

Choi *et al.* (2000) examined specimens of skin lesions obtained from 70 dogs dermatitis in Korea Republic between 1997 and 1998. Dermatophytes were cultured from 15 (21.4 per cent) specimens. Commonest dermatophytes were *Microsporum canis* (12.9 per cent), *Trichophyton mentagrophytes* (4.3 per cent), *Trichophyton rubrum* (2.9 per

cent), *Trichophyton raubitschekii* and *Microsporum gypseum* (each 1.4 per cent). There was a high proportion of positive cultures from dogs below one year age and above three-year old and in some long hair breeds, but there was no significant differences due to sex or living conditions. Although dermatophytes were more frequent in the spring and winter, there was no significant differences in seasonal distribution.

Macianti *et al.* (2000) examined dermatological specimens from 3028 dogs between Jan. 1986 and Dec. 2000. All the animals presented clinical signs of ringworm. They found 566 out of 3028 canines (18.7 per cent) positive for dermatophytes. *Microsporum canis* constituted 83 per cent of the isolated dermatophytes. *Microsporum gypseum* represented 13 per cent and *Trichophyton mentagrophytes* 5.5 per cent.

A sexual predisposition for mycotic infection was not observed. The animals with less than one year of age were more frequently infected. Canine toy breeds showed a significantly higher ($P<0.001$) prevalence of infection by *Microsporum canis*. The annual distribution of the infections in dogs showed a significantly higher incidence for *Microsporum gypseum* in summer verses winter and spring.

Chakrabarti *et al.* (2002) conducted an epidemiological study on 757 dogs with complaints of hairfall and skin lesions during April to September 1996 at Kolkata metropolis and its surrounding. They found that alopecia due to *Microsporum canis* (62.5 per cent) predominated over *Trichophyton mentagrophytes* (25 per cent) or *Microsporum gypseum* (12.5 per cent) among dermatophytes. Of these alopecic dogs, the Spitz (26.79 per cent) and German Shepherd (19.65 per cent) breeds were more affected. Of the

alopecic dogs 38 were males and 18 were females (67.85 and 32.15 per cent respectively). Alopecia was predominantly present within age groups 0-12 months followed by 84-96 months. Alopecia was more common in long hair breeds (53.57 percent) than in short-haired breeds (46.43 per cent).

Cavalcanti *et al.* (2003) examined 471 skin scraping samples obtained from dogs for cutaneous fungal infections by means of unstained KOH preparations and fungal culture in Sabouraud's agar. The frequency of dermatophytes was observed 13.80 per cent of which *Microsporum* corresponded to 63.0 per cent and *Trichophyton* to 37.0 per cent.

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MATERIALS AND METHODS

Materials and Methods

The present investigation was conducted on 200 dogs of different breeds, age and sex. Out of 200 dogs, 100 were naturally infected with skin disease and rest had apparently healthy skin. The present study included animals under investigation from different hospitals of Patna covering different zones, but majority were from Private Dog Health Care Centre where dogs suspected of having dermatophytes were entertained.

A case sheet was prepared for each dog which included, history physical examination, present complaint, differential diagnosis, nutritional status and treatment trials. Records were kept of the age, sex and breeds of dogs. A detailed history was obtained from the owner which included possible source of infections in the master's family and evidence of transmission of skin disease of the pet.

COLLECTION OF MATERIALS :

A total of 200 samples were collected, among which 100 samples were from apparently healthy skins and 100 were from skins with clinical lesions. The clinical samples were collected with all aseptic precautions as described by Muller and Kirk (1969), Booth (1971) and Jungerman and Schartzsman (1972).

Method of collection from Apparently Healthy skin :

Samples were collected using *hairbrush technique* described by Mackenzie (1963). A sterile plastic brush used was oval and slightly less than the diameter of petridish. The animal's head, body and its appendages

were brushed thoroughly. After sampling, the bristles of the brush were cleared and samples were taken on clean sterile paper with the help of forcep. The brush was immersed in a solution of wescidyne (1%) and then kept for later use.

Method of collection from skin lesion :

Sites were selected on area near the margins of a lesion, which were undisturbed, unmedicated, for scraping. The area showing distinct lesions were wiped with 70 per cent alcohol swab to clean surface contaminants, dirts and allowed to dry for five minutes. With help of sterilized blunt scalpel the materials were collected from the periphery of the lesion, slowly and gently for getting sufficient materials on sterilized piece of paper. The scrapped area were then swabbed with damp cotton wool. The scrapings were then put into test tube with cottonplug.

Stubs of lustureless hairs were also taken with the help of forcep. The samples were collected from different places from the same dog to secure the maximum possibilities of infective organisms (Ajello, 1953). Also used small sterile squares of carpet with a short stiff nap, to pickup infected hairs and dermatophyte spores from the scalp or the fur of animals (Mariat and Adan Campos, 1967).

Thus collected materials were brought to laboratory for detailed investigation.

LABORATORY PROCEDURES :

The processing of skin scrapings was done as per the standard method described by Sastry (1985).

DIRECT MICROSCOPICAL EXAMINATION :

Potassium Hydroxide Method : 10 per cent aqueous solution of potassium hydroxide was used as a clearing agent for microscopical examination of the skin scrapings. A small piece of skin scraping was taken on a clean slide and 2-3 drops of 10 per cent potassium hydroxide were added. A clean cover-slip was placed on the preparation. After passing the preparation once or twice over the flame, to eliminate the air bubble, it was kept on the bench at room temperature for 10-15 minutes. The potassium hydroxide preparation was examined under the microscope with reduced light by lowering the condenser's diaphragm for the presence of any fungal mycelia or hyphae and fungal spores as suggested by Beneke (1966). Fungal hyphae were differentiated from a variety of hyphal-like aretefacts such as cottonwool or synthetic fiber and from the so called mosaic fungus. It could be recognized by its outline, abrupt changes in width and the presence of reentrant angles in flat crystalline structures and lack of internal organelles. If the primary KOH mount examination did not reveal the presence of fungal elements, slides were preserved at room temperature overnight and again examined under microscope. Hairs also examined for any ectothrix or endothrix invasion. Both the positive and negative materials were kept for cultural examination to establish a definite diagnosis for dermatophytosis as described by Ajello and Padhye (1985).

CULTURAL EXAMINATION FOR DERMATOPHYTES :

Preparation of Isolation Media :

The media used for the culture examination were Sabouraud's dextrose agar which was fortified with chloramphenicol and cyclohexamide.

The composition of Sabouraud's cyclohexamide-chloramphenicol agar was as follows :

Dextrose	20	gm
Neopeptone	10	gm
Agar	20	gm
Chloramphenicol	40	mg
Cyclohexamide	500	mg
Distilled water	1000	ml.

After the dextrose, peptone and agar were dissolved, it was heated to boiling, added 40 mg chloramphenicol, which has been suspended in 10 ml. of 95 per cent alcohol, and removed quickly from heat. Added 500 mg cyclohexamide and mixed. The above prepared media was sterilized in the autoclave at 120^0C for 10 minutes and adjusted to a final pH of 5.6.

CULTURE :

Tube Culture :

Skin scrapings and hairs were taken and inoculated into the two sets of tubes, containing Sabouraud's dextrose agar with chloramphenicol and actidione, with the help of sterile platinum loop. One set of tubes were incubated at room temperature whereas other sets of tubes were incubated at 37^0C. The tubes were examined at weekly interval for growth of any fungi. No tubes were discarded until four weeks of incubation.

Plate Culture :

During collection of material by hairbrush technique, after sampling the bristles of the brush were pressed firmly into a petridish of mycosel agar containing Sabouraud's dextrose agar with chloramphenicol to inhibit bacterial growth and cyclohexamide to prevent the growth of saprophytic fungi. The plates were then incubated at room temperature. Plates were then examined for typical growth characteristics at seven days interval. They were held for 30 days, before being discarded as negative.

IDENTIFICATION :

Identification on the basis of gross morphology of colony :

When cultures yielded colonies suggestive of a dermatophyte the species were identified by the morphology of the thallus (Rebell and Taplin, 1970). Colony growth on the media was identified with reference to general topography *viz.* flat, heaped, regularly or irregularly folded; texture *viz.* yeast like, powdery, granular, velvety or cottony; surface pigmentation, pigmentation on the reverse side of colony; rate of growth and colony diameter.

In general pathogenic dermatophytes are slow to grow, requiring 10 to 14 days to become visible colony. The three most common dermatophytes infecting dogs have the following distinguishing features as described by Carter (1973). Colonies of *Microsporum canis* are white to buff in colour with yellow to orange-brown reverse.

Microsporum gypseum produces powdery buff to cinnamon brown colonies with fluffy white advancing edge. The underside is pale yellow to tan.

Trichophyton mentagrophytes has a powdery or granular, light buff to rose tan colonies with a buff to deep wine or brown reverse.

Identification on the basis of microscopical morphology :

Preparation of Lactophenol Cotton Blue :

Lactophenol was prepared by dissolving phenol crystals 20 gm (melted in water bath and then weighed), lactic acid 20 gm and glycerine 40 gm in 20 ml of water. Cotton blue was added to yield desired depth of colour.

Lactophenol cotton blue preparation of all cultures were examined, after sporulation, for proper identification (St. Germaine and Summerbell, 1996). When necessary, to induce the production of macroconidia for positive culture, colonies were subcultured on to modified agar media (Van breuseghem and others, 1978).

For this purpose, directly from colonies, a small part was picked up with a stiff wire needle. The material was placed in a drop of lactophenol cotton blue on a slide and teased apart with the help of two sterile steel needles. A cover-slip was kept over it and then examined under low power and then under high power microscope.

Slide Culture (Ridell, 1950; Larone, 1995) **:**

Ridell's Slide Culture technique was adopted to observe the undisturbed relationship between reproductive structures and mycelium for the sake of identification.

10 ml. of melted Sabouraud's agar was poured in a flat bottom, 9 cm petridish so that a layer of agar was formed 3 mm deep. The medium was then cut into 1 cm square after setting using sterile needle. The petridish of agar was stored at 4^0C. When required an agar block was lifted on with a

flattened needle and placed on a sterile slide and subsequently covered with cover slip. The four edges of agar block were inoculated with small pieces of culture under investigation. A sterile cover-slip was then applied to other surface of agar square and this slide preparation was transferred to a closed chamber containing blotting paper soaked in water. This slide culture was left at room temperature and examined every third day.

When adequate sporulation had occurred the coverslip was lifted from the agar and placed aside with the adherent culture uppermost. The agarblock was then carefully removed and discarded, leaving an adherent culture on the slide.

Lactophenol cotton blue preparation of slide cultures :

The fungus growth on both cover slips and slide were treated with a drop of alcohol and before it completely evaporated, drops of lactophenol cotton blue applied. Finally a coverslip was placed on the slide and examined microscopically.

In this procedure the hyphal arrangements, types of hyphae and the presence of macroconidia and microconidia were examined for the identification of dermatophytes.

In general most dermatophytes produce two kinds of conidia ; large, multicellular (multiseptate) macroconidia and unicellular smaller conidia, called microconidia. The presence or absence of these two types of conidia and appearance of the macroconidia (rough or smooth) are of generic significance; species identification is largely based on the morphology and arrangement of the conidia (Padhye *et al.*, 1979).

Genus *Microsporum* has following microscopical features as described by Gruby (1843). They produce both macroconidia and

microconidia. The essential distinguishing feature is the presence of macroconidia that have rough walls ranging from spiny to warty. The macroconidia vary in shape from egg-shaped to cylindrifusiform; they may have thin to thick cell walls and 1-15 septa depending upon the species. The roughness of the cell walls may not be readily apparent in some isolates or species. Microconidia are clavate (Club shaped).

The distinguishing features of genus *Trichophyton* has been described by Malmsten (1845). Smooth-walled macroconidia and microconidia are produced by members of this group. The macroconidia may range in shape from elongated to pencil shaped, clavate, fusiform or cylindrofusiform, multiseptate and may be thin or thick walled. Microconidia are usually produced in abundance than macroconidia and their shape varies from pyriform, clavate, spherical or elongated. Microconidia are borne along the hyphae singly or in clusters and are sessile or borne on short stalks. Their arrangement on fertile hyphae is one of the important factors in their identification.

STATISTICAL ANALYSIS OF DATA :

The data were analysed by using the χ^2 test and comparing samples that yielded a positive culture with those that were negative. A P value of (<0.05) was considered significant as per methods suggested by Snedecor and Cochran (1967). The predictive values of positive tests and negative tests and specificity of the tests were calculated by using standard formulae (Gertsman and Cappucci, 1986).

The percentage of dermatophytes recoveries were examined in relation to age, gender and breeds of dogs.

RESULTS AND DISCUSSION

Results and Discussion

DIRECT MICROSCOPICAL EXAMINATION :

The details of the direct microscopical examination of skin scrapings, from dogs with apparently healthy skin and skin with clinical lesions, in 10 per cent KOH are presented in table – 1.

Total 200 samples were examined, 100 each from dogs with apparently healthy skin and skin with clinical lesions, of which 15 per cent and 25 per cent yielded positive respectively (Fig. 1). The potassium hydroxide preparation of the positive samples showed numerous mycelial fragments. The hyphae were branched or unbranched (Fig. VI). The affected hairs showed ectothrix type of invasion with different sizes of spores. Examination of the fungus in and on the surface of a hair gave a reliable clue to generic identity of a dermatophyte. But it did not permit specific identification and in lesions of glabrous skin most of the dermatophytes were indistinguishable. Therefore it was essential to isolate a dermatophyte in culture.

CULTURE EXAMINATION :

The details of culture examination of skin scrapings, from dogs with apparently healthy skin and skin with clinical lesions, are presented in table –2 .

Total 200 samples were cultured. 100 each from dogs, with clinical lesion on skin and with apparently healthy skin, of which 17 per cent and 8 per cent showed positive culture in the present study. Out of 15 samples

positive on direct examination from apparently healthy skin of dogs 8 were yielded growth on culture. Of 25 KOH positive samples from skin with clinical lesions only 15 of them yielded growth on cultural examination. Two samples which were negative on direct examination were found positive on culture. The overall incidence of dermatophytes was found to be 8 per cent in healthy dogs. This finding is almost similar to that obtained by Baxter (1973) (8.3 per cent). The overall incidence of dermatophytes was 17 per cent in affected dogs in this study. The difference in the number of positive cultures of dermatophytes from apparently healthy and affected dogs appeared to be striking (Fig. 2).

The disease was more prevalent in debilitating animals and they were clinically normal otherwise.

The cutaneous lesions were mostly localized and were situated on the face, ears, back tail, thighs and legs. Most of the lesions were apparently circular in appearance, having alopecia in the center and erected or matted hairs with vesicular or granular encrustated active lesions at the periphery. Few lesions were of nonspecific types having brans, scabs or encrustations only on the body coat without producing any demarcated skin lesions. In some foreign breeds the lesions were circular raised plaques scattered all over the body specially along the back, neck, head, abdomen and inner thigh. Thick elevated and scaly lesions with exudate underneath were also observed in the affected dogs having intense pruritis. (Fig. I - V).

Of the 100 samples from dogs with apparently healthy skin examined, 8 carried dermatophytic fungi on the hair coats *M. gypseum* was isolated most frequently from 6 dogs and *M. canis* and *T. mentagrophytes* were isolated from rest two in this study (Table – 3).

Of the 100 samples from dogs with skin lesion, examined 17 carried dermatophytic fungi. In this case *M. canis* was the most frequently recovered dermatophyte showing the prevalence, of 76.47 per cent. followed by *M. gypseum* (17.64 per cent) and *T. mentagrophytes* (5.88 per cent) (Table – 3).

In both cases there was a significantly different prevalence of various species of dermatophytes in dogs (Fig. 3).

Of the 25 positive culture from dogs. Only 3 species were identified. The identity of these dermatophytes was *M. gypseum* (9), *M. canis* (14) and *T. mentagrophytes* (2).

The colony of *M. gypseum* was first seen three to four days after inoculation of Mycosel agar as a downy, feather like projection. It gradually developed into a flat disc like colony within 14 days. The surface colour was cinnamon brown with a powdery texture terminating in a fluffy white advancing edge. The undersurface of the colony was pale yellow. As the culture ages, tufts of white fluffy thalli developed rapidly on the colony surface.

Microscopically, the picture of the colony mount of *M. gypseum* was dominated by numerous ellipsoid roughwalled macroconidia containing from 3-5 cells. A few single celled, club shaped microconidia attached to the sides of the hyphae were also observed (Fig.VII).

Culture of *M. canis* developed developed rapidly as a whitish, coarsely fluffy spreading colony of aerial mycelia. It developed a deep yellow pigment on the underside. This pigment appeared during the first week of growth, but turned dark and dull with ageing.

Microscopically the lactophenol cotton blue preparation of the culture showed numerous macroconidia which were fusiform with thick, rough

walls and knobbed apex. They were multiseptate. A few microconidia were also observed. (Fig. VIII).

In the culture of *T. mentagrophytes* floccose and granular type of growth with a yellowish buff powdery surface were seen. The powder appeared to be sprinkled in concentric rings or rays. The underside was tan or dark brown. The growth was rapid.

On microscopical examination in lactophenol cotton blue mount, round thin walled clusters of microconidia attached to hyphae with stigmata were observed. Few smooth walled, clavate to pencil shaped macroconidia were also observed. (Fig. IX).

In this work *M. gypseum* (75 per cent) was the most common fungus isolated from dogs with apparently healthy coats (Fig. 3). It is a geophilic fungus and widely distributed in soil and thus soil serves as a common source of infection (Emmons *et al.*, 1974). Dogs may get when rooting soil. Whitelock (1968) claimed that *M. gypseum* is not an uncommon infection for man. Man transmits the infection to lower animal and the possibility of man-to-animal transmission might not be unlikely.

In dogs with suspected lesions of dermatophytosis, *M. canis* (76.47 per cent) was the most common species isolated (Fig. 3) as in most other studies of canine dermatophytosis (Kaplan *et al.*, 1957; Kaplan and Ivens 1961; Weiss and other 1979; Kristensen and Krogh, 1981; Stenwig 1985). With few exception Lewis *et al.* (1991) also isolated *M. canis* as most common species in several cases of canine dermatophytosis. The findings of this study are also corroborated with findings of Rieth and Dreisorner (1963), James (1965), Snyder (1965), Weiss and Weber (1982) and Cabanes *et al.* (1997).

M. canis, M. gypseum and *T. mentagrophytes* were isolated in this study. This finding is similar to the findings of Kaplan and others (1957), Kaplan and Ivens (1961), Blackemore (1974), Scott and Others (1980). The incidence of these dermatophytes appeared quite similar during the study period to those of Ainsworth (1959), Chatterjee and Sengupta (1979) and Aho (1980).

The details about the material collected and the total no. of isolates from apparently healthy skin of different breeds of dogs are presented in table – 4.

Out of 30 German Shephered breed of dogs screened, 5 (16.6%) were found positive. One spitz dogs out of 10 was affected. Out of 50 native dogs screened only 2 (4%) was affected with dermatophytoses. 5 Dachshund, 3 Doberman and 2 Labrador were also examined but they were found negative in the study (Fig. 4).

The details about the material collected and the total no. of isolates from different breeds of dogs with skin lesions are presented in table – 5.

25 each of crossbred, native and German Shephered breeds of dogs were subjected to study. Of which 4 (16%) crossbred, 5 (20%) each of native and Germanshephered dogs were found positive for dermatophytosis. Out of 13 Doberman breed 2 (15 %) and out of 12 spitz dogs 1 (8.3 %) was found to harbour dermatophytes (Fig. 5).

In both the cases different breeds of dogs had non significant difference of prevalence of dermatophytosis.

There was a greater occurrence of dermatophytosis in foreign breeds than in native dogs. This result agrees with the findings of Keep and Pile (1965) and Lewis *et al.* (1991).

According to Baxter (1973), no correlation exists between the occurrence of dermatophytosis with the breeds of animals, however certain individuals or members of a particular family or breeding may be genetically predisposed. Based on study no definite statement could be made wheather or not these breeds from which the dermatophytes have been isolated are nearly more susceptible than the other breeds. Further studies covering more number of animals equally distributed among the different breed should be undertaken to established the true picture of the occurrence of dermatophytes in dogs in relation to breed.

Considering the way these animals are taken cared of, native dogs have more chance of contact with other dogs and soil because they are allowed by their owners to room or sleep outside the house or even to go astray in the streets; hence, the probability of contact with the source of dermatophytes is greater. On the other hand, pure breed dogs are confined most of the time in the house or kennels, therefore, the chance of getting contact with the reservoir of the infection is slimmer with lesser chance of getting the dermatophytes. Contrary to these expected results, the present study showed a greater prevalence of fungus in foreign breeds than native dogs.

The fact that more foreign breeds carry dermtophytes than native dogs may be related to the type of haircoat. In long haired foreign breed, the fungus can be lodged on the haircoat for an indefinitely longer period. In native dogs with very short hair coat, the dermatophytes can be easily shrugged off by animals. Chittawar and Rao (1982) reported that long hair breed are more prone to ringworm infection.

The details about the different species of dermatophytes isolated from the different breeds of dogs with skin lesions are presented in table-6.

A total of 13 isolates of *Microsporum canis* were obtained in the study. Out of 13 isolates 3 from crossbred 4 each from native and German Shephered and one each from spitz and Doberman and 3 *Microsporum gypseum* were isolated one each from native, Doberman and German Shephered. Only *Trichophytom mentagrophytes* isolated was from crossbred.

The breeds of dogs showed a non significant difference in the type of dermatophyte infection, most notable was the high prevalence of *M. canis* infection in all breeds of dogs under study (Fig. 6).

Out of 100 dogs with apparently healthy skin, 25 per cent were in the age group of below one year and rest 75 per cent were in the age group of one year and above, (Table – 7).

It was found that 5 affected dogs (20 per cent) were less than one year of age and 3 affected dogs (4 per cent) were in the age group of one year and above. Rest dogs gave negative findings for dermatophytosis (Fig. 7).

Altogether 45 adult, 20 young and 35 unspecified dogs with skin lesions were screened and found that 6 affected dogs (30%) were young, 7 affected dogs (15.55 per cent) were adult and 4 dogs (11.42 per cent) affected were of unknown age. Rest dogs were found negative for dermatophytosis, (Table – 7).

In both the cases there was a significantly different prevalence of dermatophytosis in different age groups ($P<0.05$); The incidence of ringworm was higher in pups below one year old than in adult.

In this study there appeared to be greater occurrence of dermatophytes in the haircoats of the younger than older dogs. The present observation is in

agreement with those of Ainsworth and Austwick (1973); Baxter (1973); Chatterjee and Sengupta (1979). Age was considered predisposing factor also by several authors. (Lewis *et al.*, 1991; Sparkes *et al.*, 1993; Filipello *et al.*, 1995; Cabanes *et al.*, 1997 and Faggi *et al.*, 1999). It is possible that during the time these young animals were brushed, some of them might have been suffering from subclinical infection while the others might just harbour the dermatophytes on their coats simply as contaminants.

In common with previous studies and clinical observations (Kaplan and Others, 1957; Keep, 1963; Kushida, 1978; Muller *et al.*, 1989; Lewis and Other, 1991), the data strongly suggests that the young dogs are predisposed to the development of dermatophytosis with the prevalence of infection in animals less than one year old being more than the adult.

Susceptibility of younger animals to ringworm might be due to lack of adequate immunological response as compared to great immunity acquired by older dog. (Jungerman *et al.*, 1972). It may also be attributed to the pH of the skin which varies according to age. In humans, susceptibility to ringworm infections is greater before puberty than afterwards when the skin pH falls from about 6.5 to about 4. This change is largely due to the excretion of fatty acids in the sebum which are highly fungistatic (Blood and Henderson, 1971). This theory might also hold true in animals, but further study on this matter is necessary.

A total of 100 samples from dogs of different breeds with apparently healthy skin were examined in this study. Among which 60 per cent and 40 per cent were males and females respectively. Dermatophytes were isolated from 8 of 100 (8 per cent) (5 male, 3 female) samples. Rest 92 of 100 (55 male and 37 female) were found negative for dermatophytes, (Table – 8).

A total of 100 samples from 55 male and 45 female dogs with skin lesions were subjected to examination. Out of 100 samples 17 (17per cent) were found positive (9 male, 8 female) for dermatophytosis. Rest dogs were found negative (46 male, 37 female) (Table-8).

In both the cases there was no significant difference in the distribution of culture positive and culture negative between the sexes.

In this study, both male and females were almost equally developed dermatophytosis. The slight fluctuation of the occurrence is negligible (Fig. 8). Both males and females were equally susceptible. This result agrees with the finding of Cabanes *et al.* (1997). However there is no evidence to support any sexual predisposition. (Sparkes *et al.*, 1993).

In two previous studies (Gregor, 1965 and Baxter, 1973) it has been suggested that males are more likely to develop dermatophytosis. The high incidence in male dogs could be due to more exposure to infection, by the behaviour of male dogs, selective preference of keeping male dogs by dog owners. But in this study there was no any significant difference in evidence to support any sex predisposition. This result is also corroborated with that of Lewis *et al.* (1991).

The seasonal incidence of dermatophytes isolated from dogs with skin lesions is reported in table-9.

To evaluate any seasonal trend in dermatophyte infection the samples were devided into spring, summer, autumn and winter. The seasonal distribution of the infection in dogs showed a significantly higher incidence for *Microsporum canis* in autumn (61.5 per cent) verses winter, summer and spring, while the recovery rate of *Microsporum gypseum* was higher in summer. The only *Trichophyton mentagrophytes* was isolated in winter.

Lewis *et al.* (1991) and Sparkes *et al.* (1993) showed no direct evidence of any seasonal trend in dermatophytosis, although several studies has suggested a prevalence peak for the disease in the autumn and winter month. In this respect, Sparkes *et al.* (1993) pointed out in their studies that although proportion of total positive culture was highest in autumn and winter month. However, Lewis *et al.* (1991) mentioned that the probability of having a *M. gypseum* was significantly higher in summer. In the present study also the positive culture was higher in autumn followed by winter (Fig. 9).

Seasonal variations have been recognized in the incidence of *M. gypseum* in dog population (Kaplan and levns, 1961; Cabanes and others, 1996). In particular, this was high from July to Nov. (Scott and other,1995), which may reflect the higher ambient temperature and humidity during the warm season along with the longer periods dogs spend out doors, hence the greater opportunity they have to be in contact with soil.

The incidence of these dermaptophytes appeared quite similar during the study period, while the seasonal distribution showed a significantly higher incidence of *M. canis* in fall. In a previous report an increase of *M. canis* infection in May to June and in July to Aug. with a further peak in Nov. was observed (Filipello *et al.* 1995). Several previous studies of canine dermaptophytes have suggested that the peak incidence for the disease in general or *M. canis* in particular, is in autumn and winter month (Ainsworth and Austwick, 1983). Present study also shows the direct evidence of the seasonal trend on dermatophytes isolated.

****

Table – 1 : Showing the overall result of direct microscopical examination of skin scrapings, from dogs with apparently healthy skin and skin with clinical lesions in 10% KOH.

Animal	No. of samples examined	Direct examination		Positive samples (in per cent)
		+ve	-ve	
Apparently healthy	100	15	85	15
Affected	100	25	75	25
Total	**200**	**40**	**160**	**20**

Table – 2 : Showing the overall result of culture examination of skin scrapings, from dogs with apparently healthy skin and skin with clinical lesions.

Animal	No. of samples cultured	Culture growth		Positive samples (in per cent)
		+ve	-ve	
Apparently healthy	100	8	92	8
Affected	100	17	83	17
Total	**200**	**25**	**175**	**12.5**

Fig. 1 : Histogram showing the overall occurrence of positive samples in Direct microsopical examintion from apparently healthy and affected dogs.

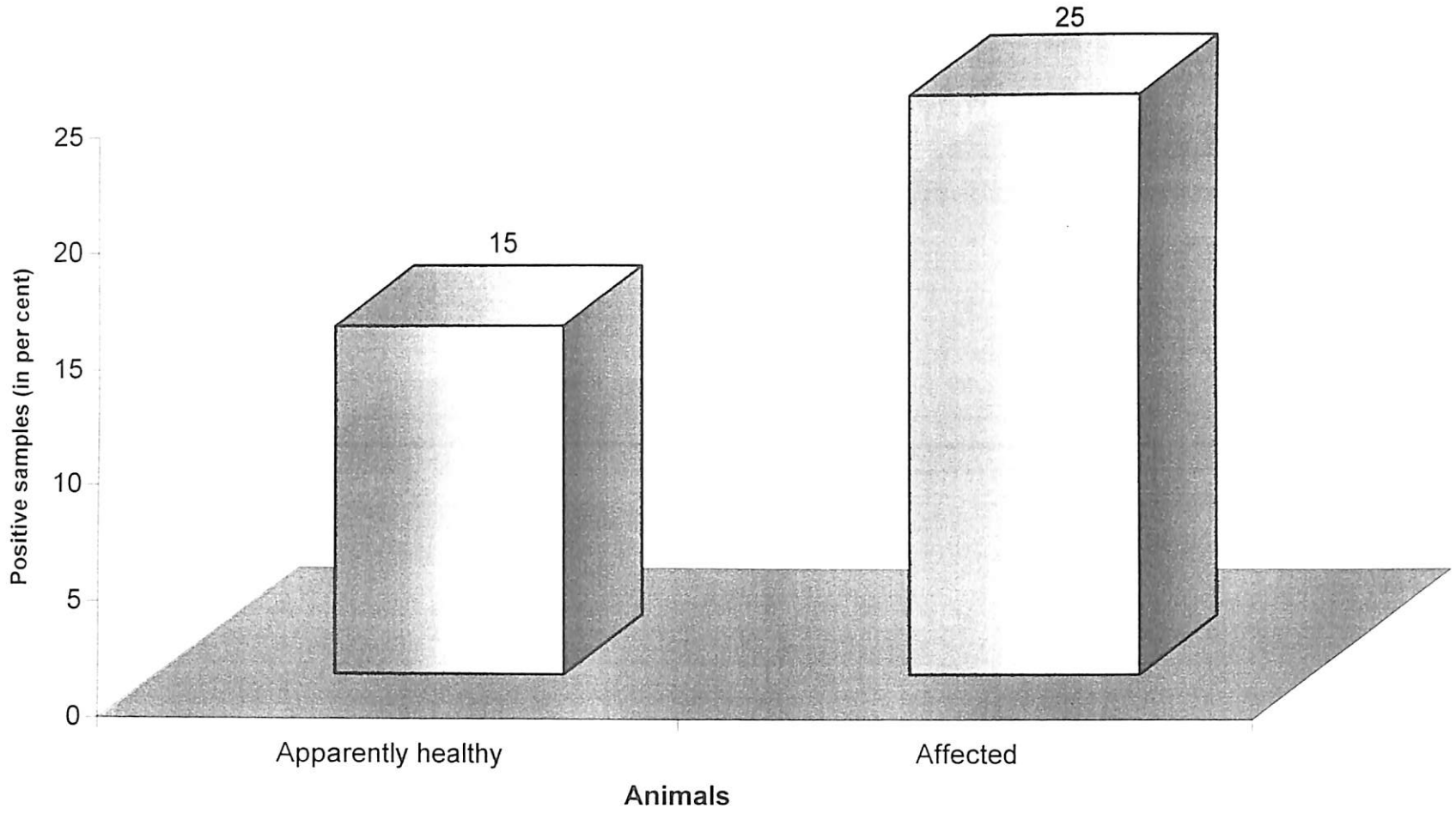


Fig. 2 : Histogram showing overall occurrence of positive samples in culture examination from apparently healthy and affected dogs.

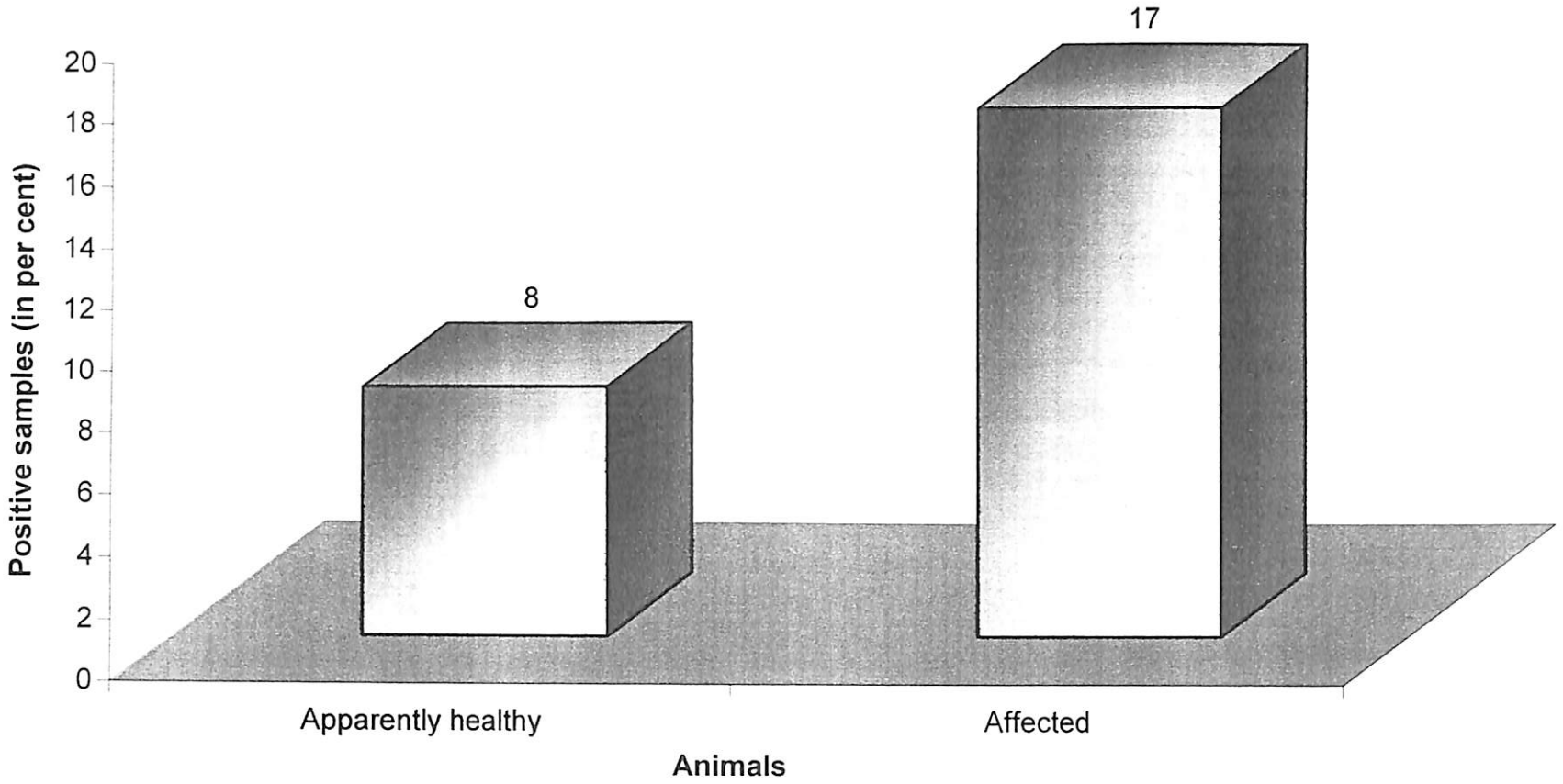


Table – 3 : Showing details about the different species of dermatophytes isolated from the dogs with apparently healthy skin and skin with clinical lesions.

Animal	Total no. of isolates	Name of the dermatophytes isolated	No. of isolate	Incidence (in per cent)	χ^2 at 2_{df}
Healthy	8	*M. canis*	1	12.50	6.263*
		M. gypseum	6	75.00	
		T. mentagrophyte	1	12.50	
Affected	17	*M. canis*	13	76.47	14.59**
		M. gypseum	3	17.64	
		T. mentagrophyte	1	5.88	

N.B. - * Significant at 5% level (P<0.05)

** Significant at 1% level (P<0.01)

Table – 4 : Showing details of the samples and number of dermatophytes isolated from different breeds of dogs with apparently healthy skin.

Sl. No.	Breeds	Total no. of samples collected	Total no. of samples found positive for dermatophytes	Overall incidence (in per cent)	χ^2 at 5_{df}
1.	German Shephered	30	5	16.6	4.66^{NS}
2.	Spitz	10	1	10.0	
3.	Doberman	3	0	0	
4.	Dachshund	5	0	0	
5.	Labrador	2	0	0	
6.	Native	50	2	4.0	
	Total	**100**	**8**	8.0	

N.B. – NS = Non-significant

Fig. 3 : Histogram showing over all incidence of different dermatophytes in apparently healthy and affected dogs.

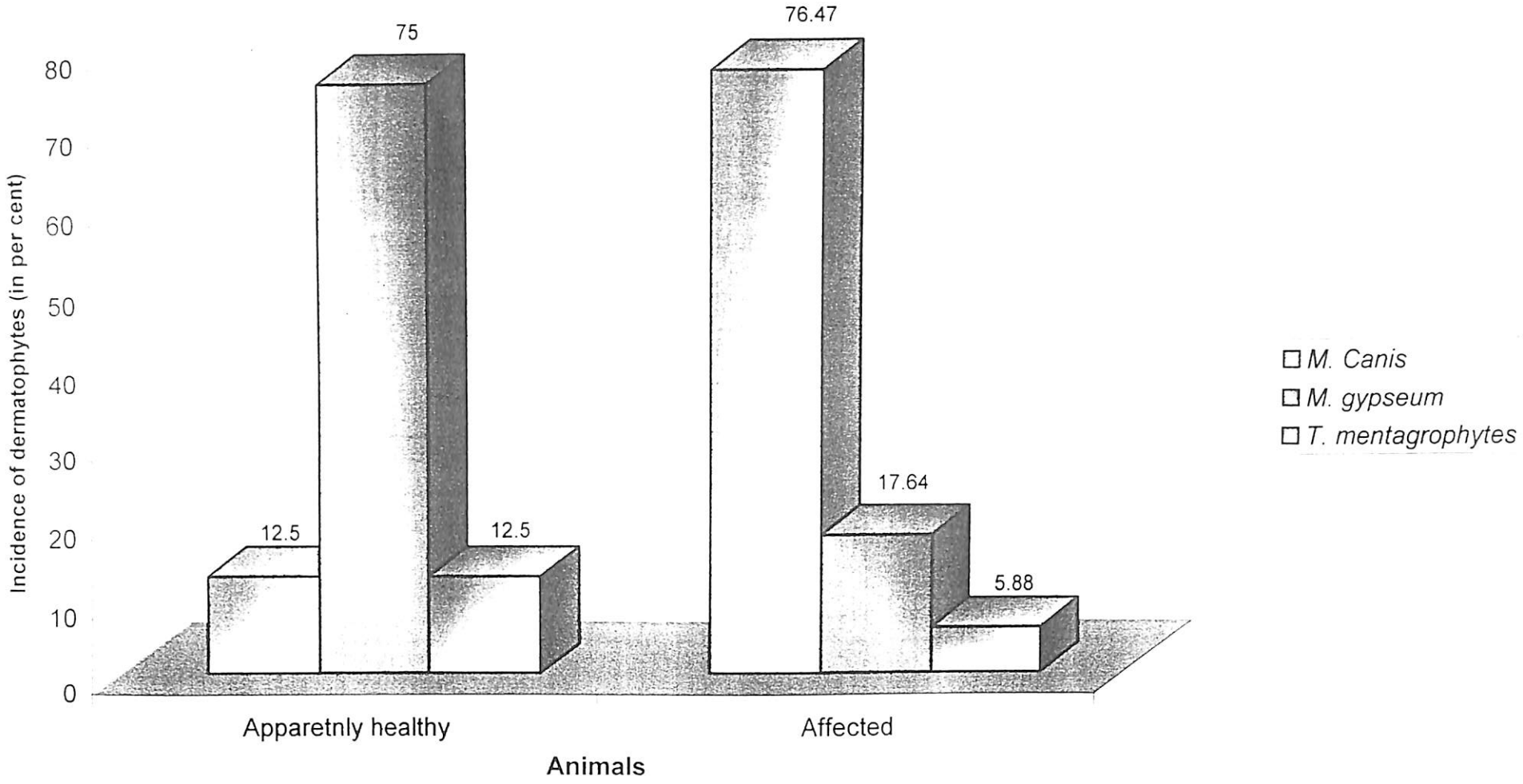


Fig. 4 : Histogram showing breed-wise incidence of dermatophytes in apparently healthy dogs.

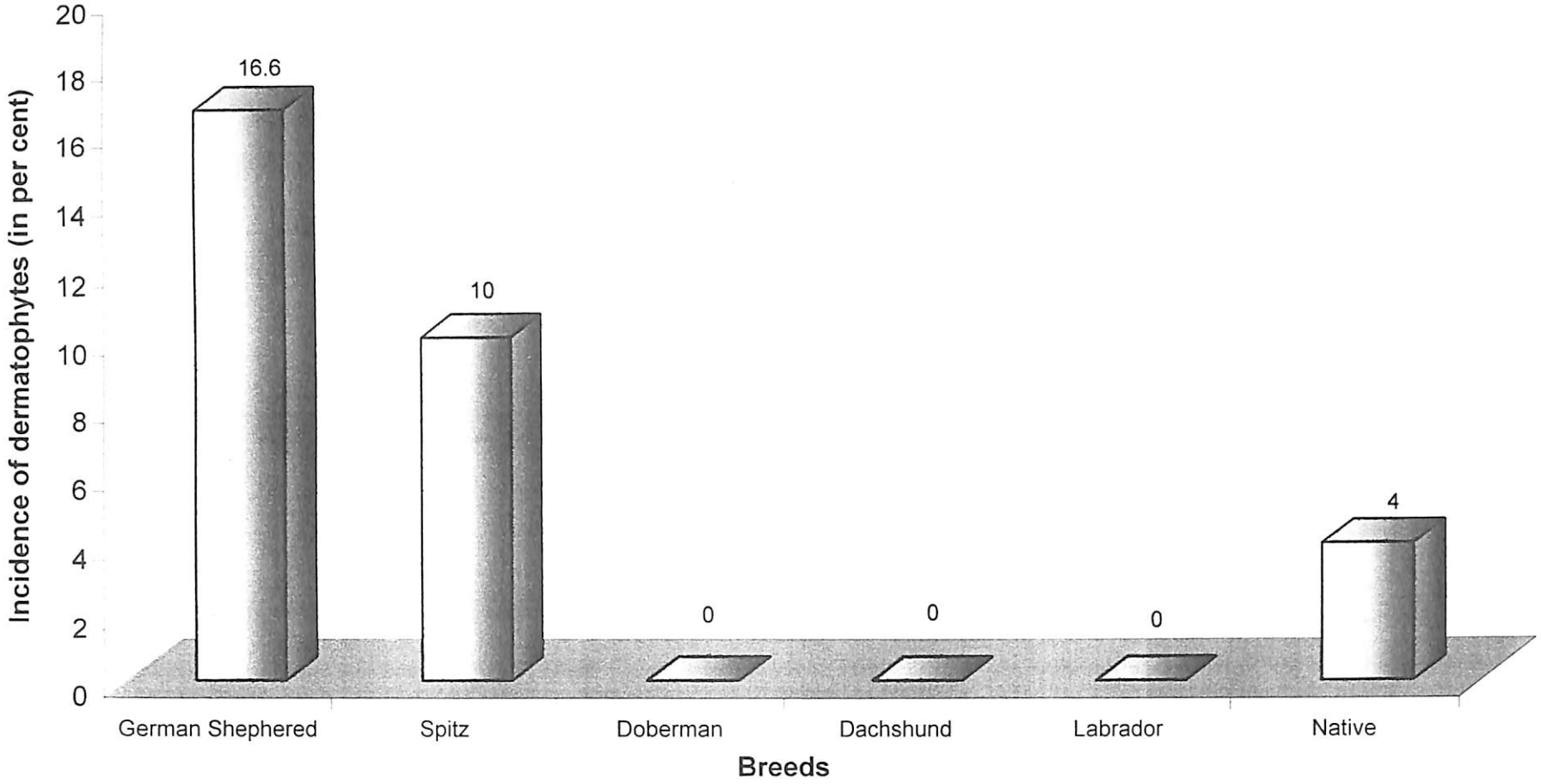


Table – 5 : Showing details of the samples and number of dermatophyte isolated from different breeds of dogs with skin lesions.

Sl. No.	Breeds	Total no. of samples collected	Total no. of samples found positive for dermatophytes	Overall incidence (in per cent)	χ^2 at 4_{df}
1.	Cross bred	25	4	16	1.297^{NS}
2.	Native	25	5	20	
3.	German Shephered	25	5	20	
4.	Doberman	13	2	15	
5.	Spitz	12	1	8.3	
	Total	**100**	**17**	17	

N.B. – NS = Non-significant

Fig. 5 : Histogram showing breed-wise incidence of dermatophytes in affected dogs.

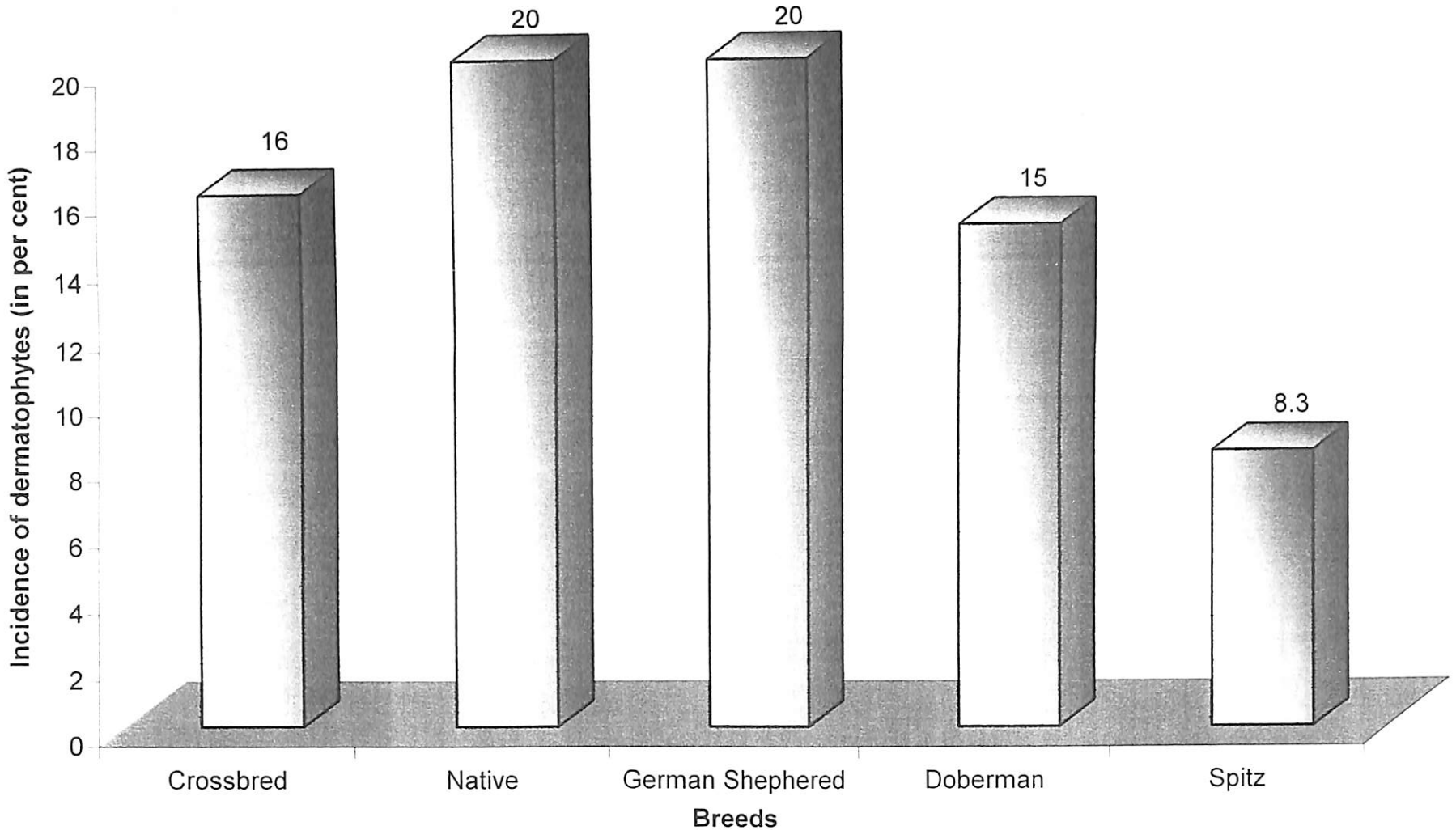


Table – 6 : Showing details about the different species of dermatophytes isolated from the various breeds of dog with skin lesion.

Breed	Overall isolation	Species of dermatophytes isolated			χ^2 at 4_{df}
		M. gypseum	*M. canis*	*T. mentagrophytes*	
Crossbred	4	-	3	1	1.0^{NS}
Native	5	1	4	-	1.8^{NS}
German Shephered	5	1	4	-	1.8^{NS}
Doberman	2	1	1	-	-
Spitz	1	-	1	-	-
Total	**17**	**3**	**13**	**1**	**-**

N.B.- NS = Non-significant

Table – 7 : Showing age-wise incidence of dermatophytes in apparently healthy and affected dogs.

Animal	Total no. of animals studied	Age groups of animals	No. of animal studied	No. of positive animal	Incidence (in per cent)	χ^2 at
Apparently healthy	100	Below 1-yr-old	25	5	20	1_{df} 3.96*
		1-yr and above	75	3	4	
Affected	100	Below 1-yr-old	20	6	30	2_{df} 6.87*
		1 yr and above	45	7	15.55	
		Unspecified	35	4	11.42	

N.B. * Significant at 5% level ($P<0.05$)

Fig. 6 : Histogram showing breed-wise isolation of different dermatophytes in affected dogs.

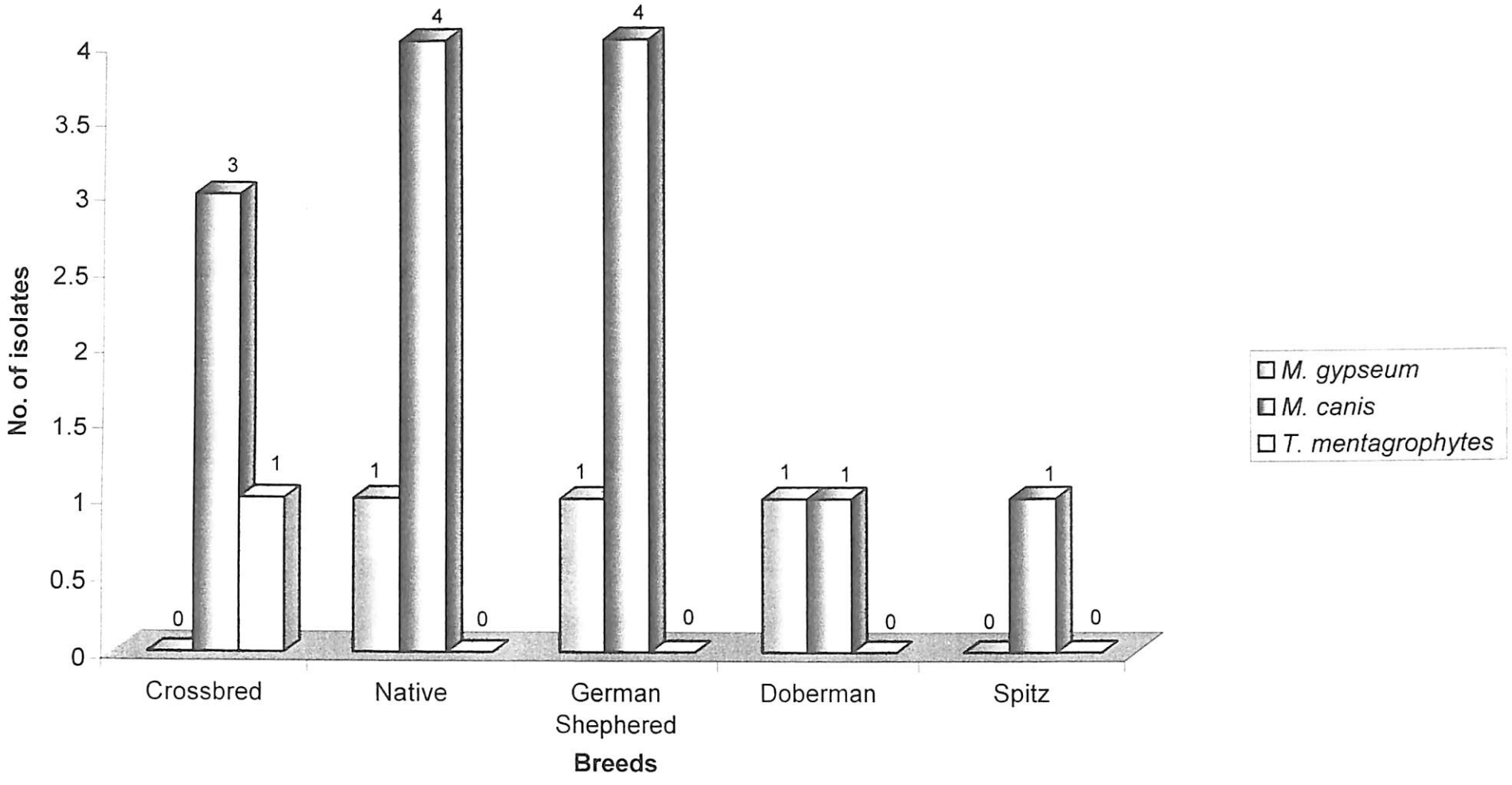


Fig. 7 : Histogram showing age-wise incidence of dermatophytes in apparently healthy and affected dogs.

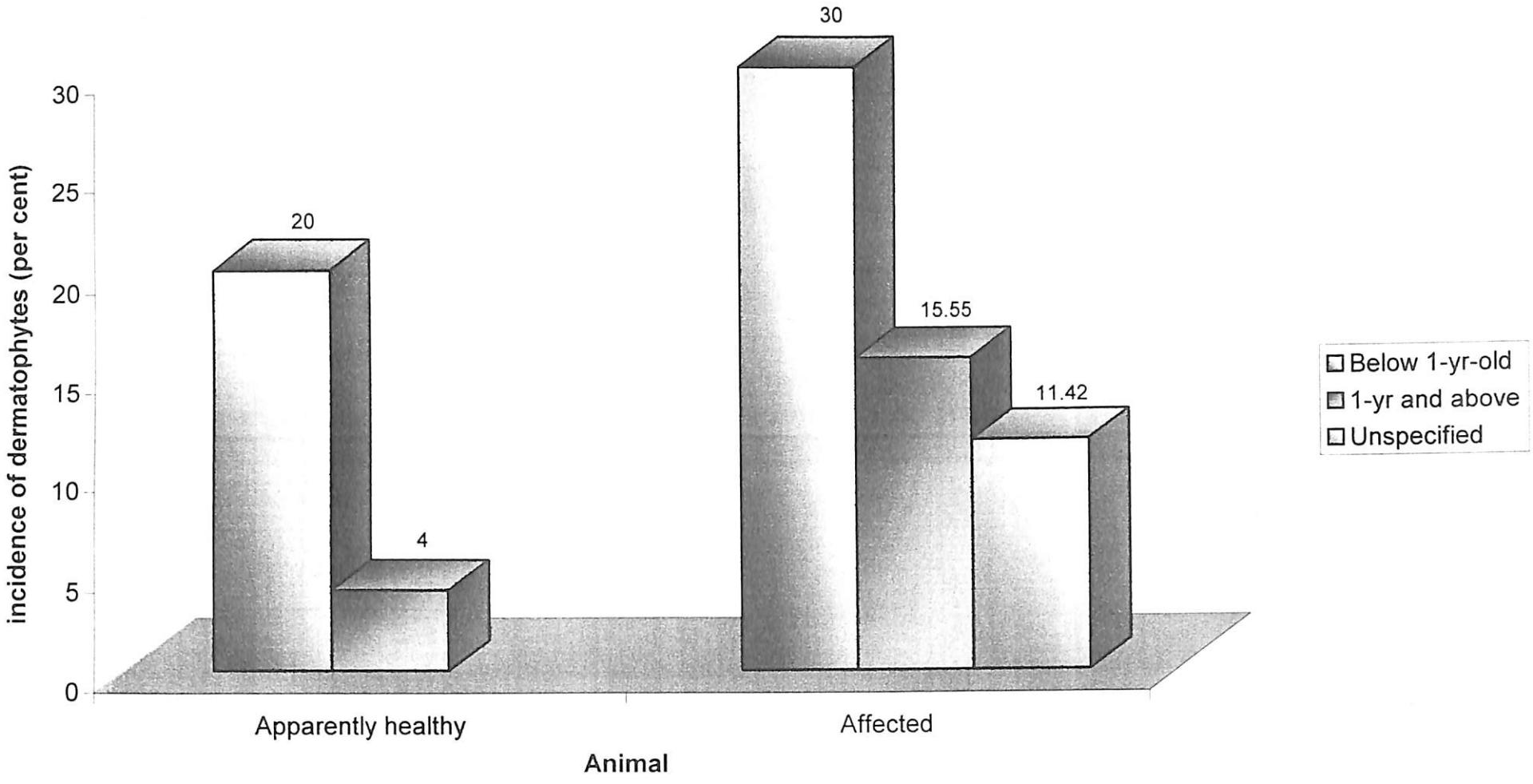


Table – 8 : Showing sex-wise incidence of dermatophytes in apparently healthy and affected dogs.

Animal	Total no. of animals studied	Sex groups of animal		No. of positive	Incidence (in per cent)	χ^2 at 1_{df}
		Sex	No. of animals studied			
Apparently healthy	100	Male	60	5	8.3	0.050^{NS}
		Female	40	3	7.5	
Affected	100	Male	55	9	16.3	0.206^{NS}
		Female	45	8	17.7	

N.B. - NS : Non-significant

Table – 9 : Showing season-wise isolation of dermatophytes from dogs with skin lesions.

Name of the dermatophyte isolate	Total no. isolation	Seasonal distribution of isolated dermatophyte				χ^2 at 3_{df}
		Spring	Summer	Autumn	Winter	
M. canis	13	1	1	8	3	10.05*
M.gypseum	3	-	2	1	-	
T.mentagrophytes	1	-	-	-	1	-
Total	**17**	**1**	**3**	**9**	**4**	

N.B. * Significant at 5% level ($P<0.05$)

Fig. 8 : Histogram showing sex-wise incidence of dermatophytes in apparently healthy and affected dogs.

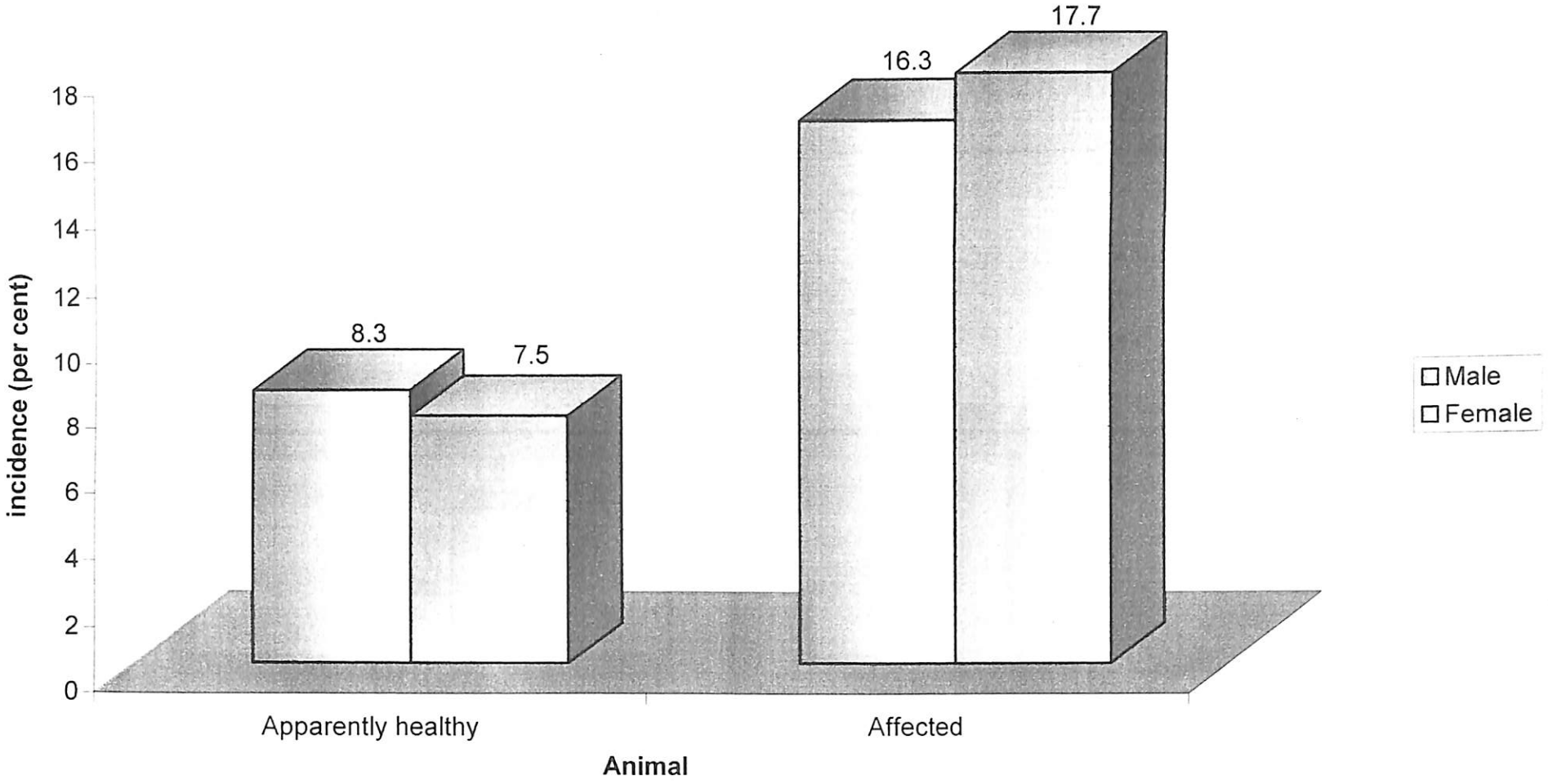


Fig. 9 : Histogram showing season-wise isolation of dermatophytes from affected dogs.

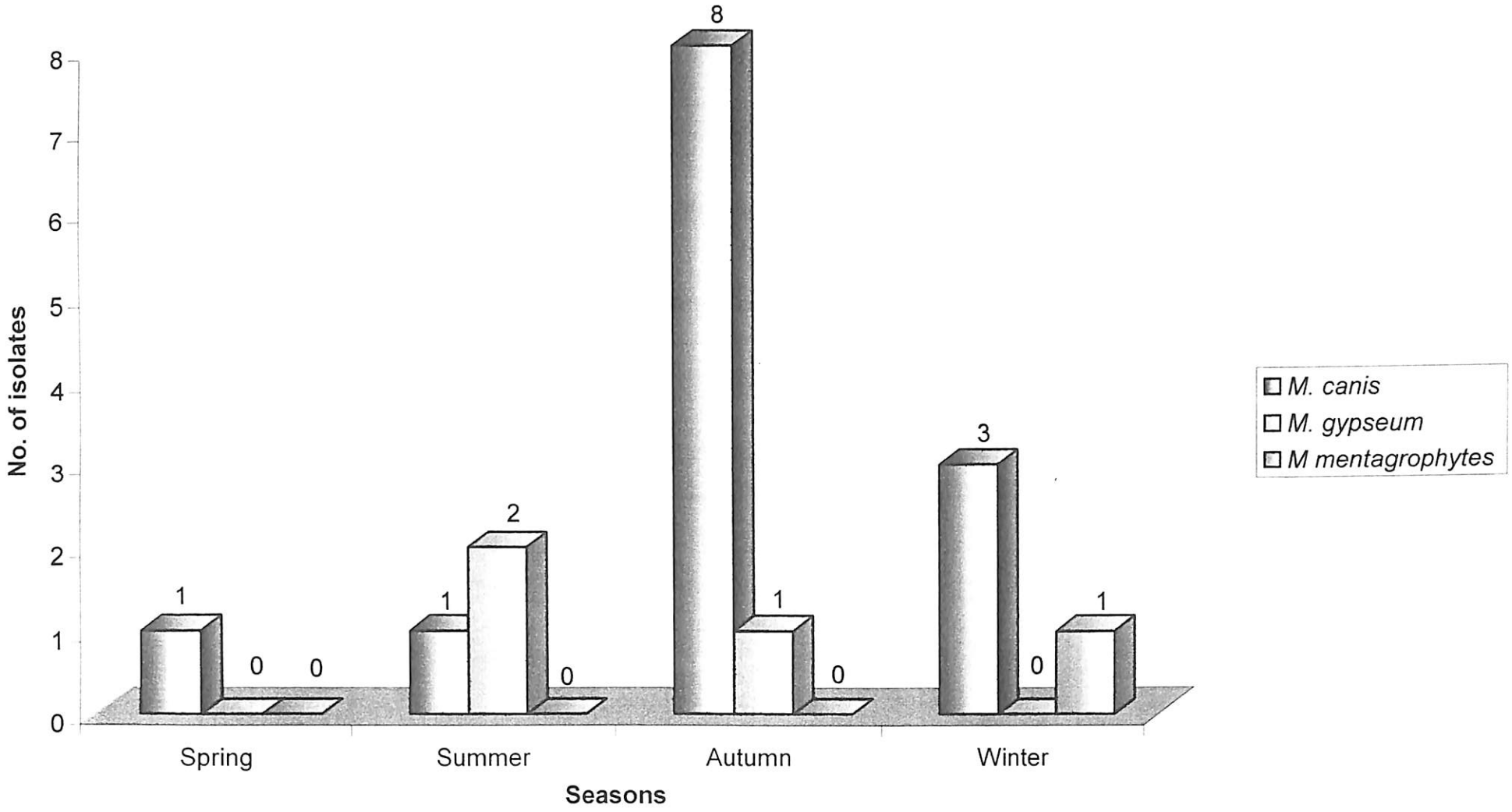
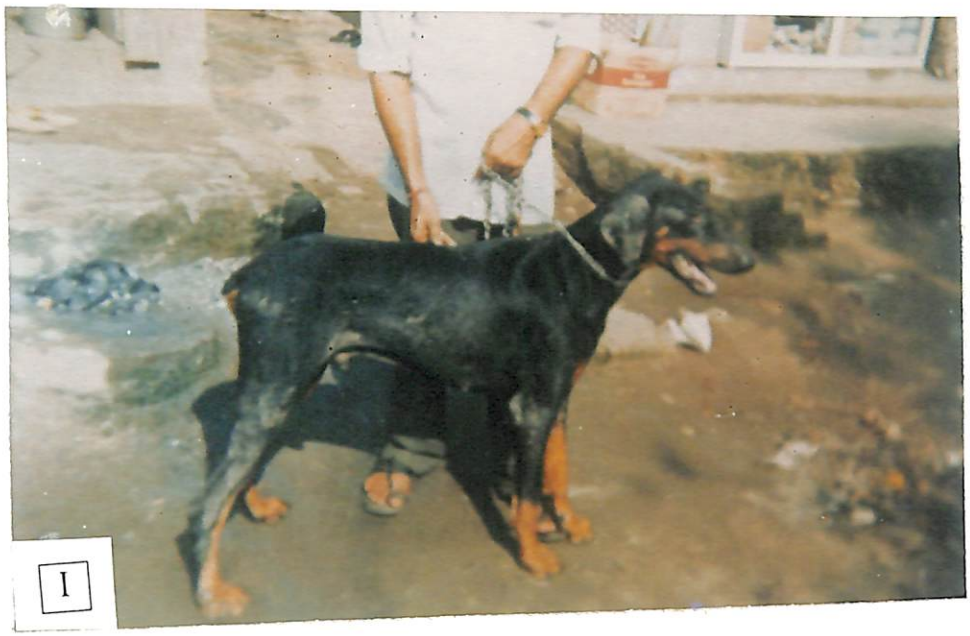


Fig. 1 : Doberman dog showing diffused, hairless, circular and irregular ringworm lesion with crust formation on head, ear, chest and thigh



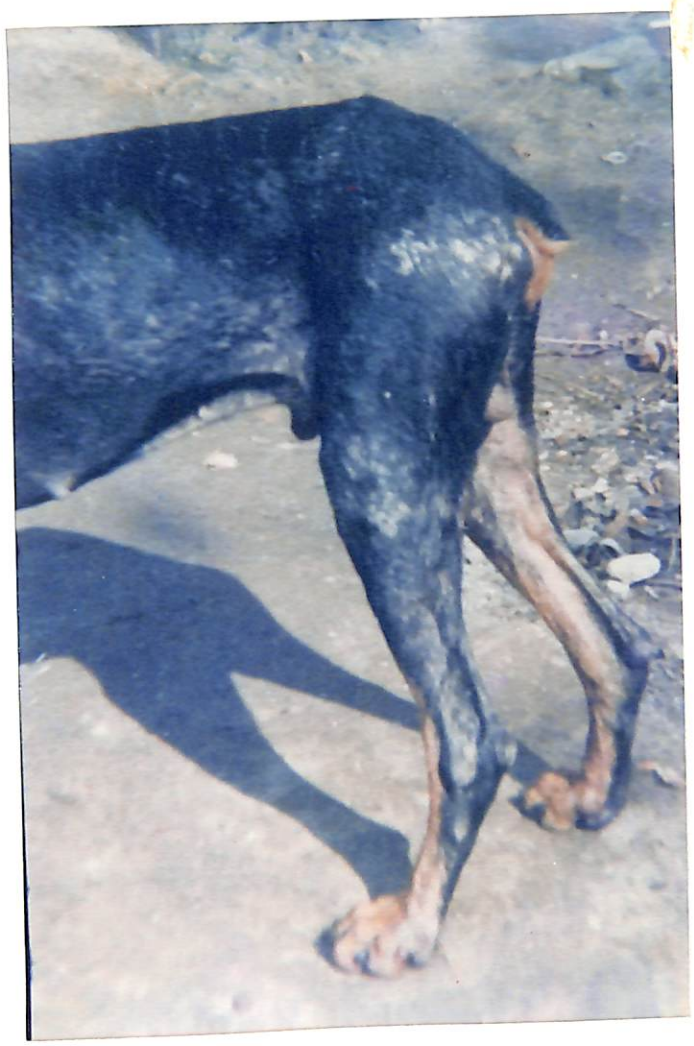


Fig. I : Doberman dog showing diffused, hairless, circular and irregular ringworm lesion with crust formation on head, ear, chest and thigh.

Fig II Alsatian dog showing diffused, hairless and erythematous lesions with encrustation of left thigh.

Fig. III : Doberman dog exhibiting diffused, desquamated, circular and irregular ringworm lesion with alopecia and crust formation on chest, back, thigh and hind legs.

Fig IV Alsatian dog showing diffused hairless, circular ringworm lesion on the back.

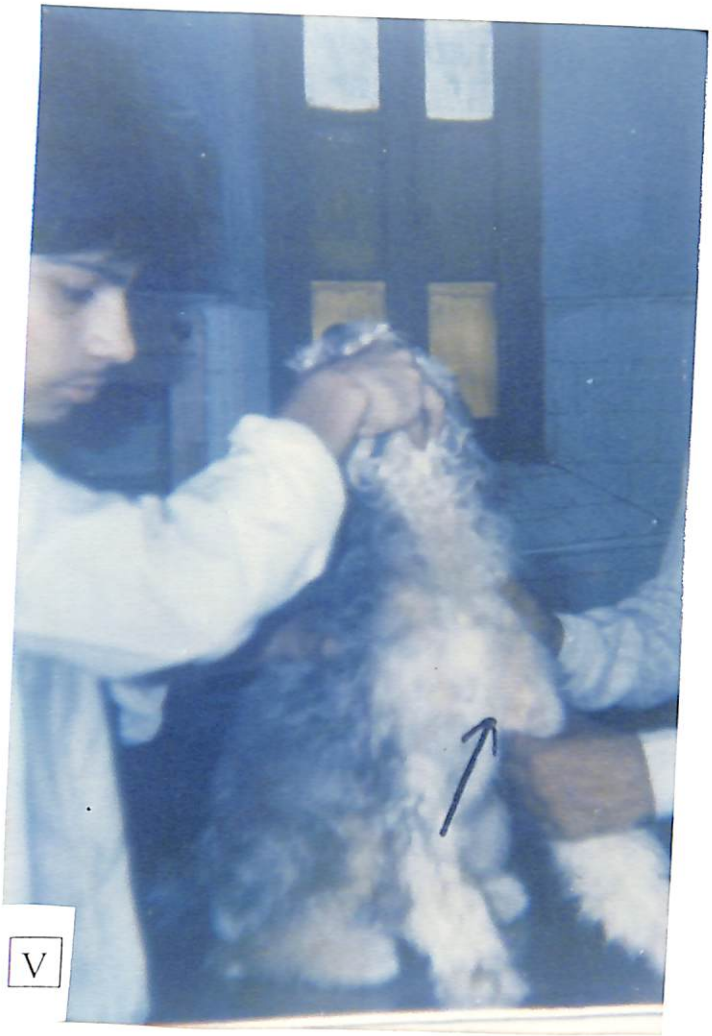
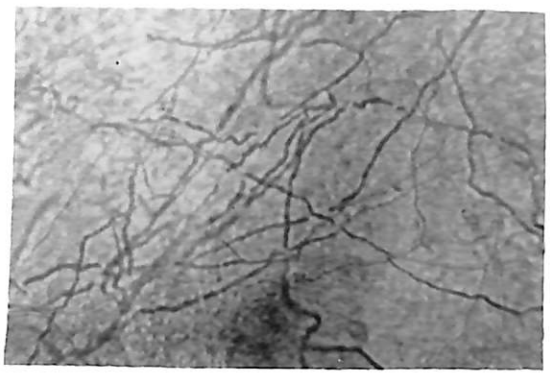
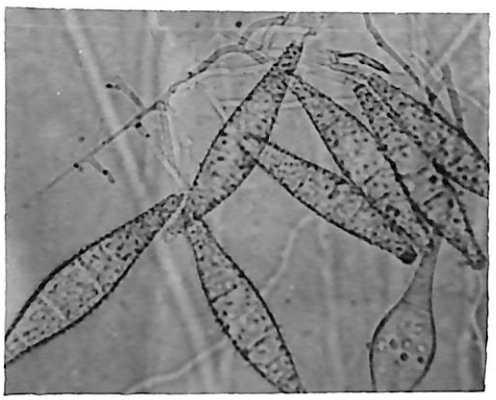


Fig. V : Pomerarian dog showing ringworm infections at the ventral aspect of neck indicating sticky crust.



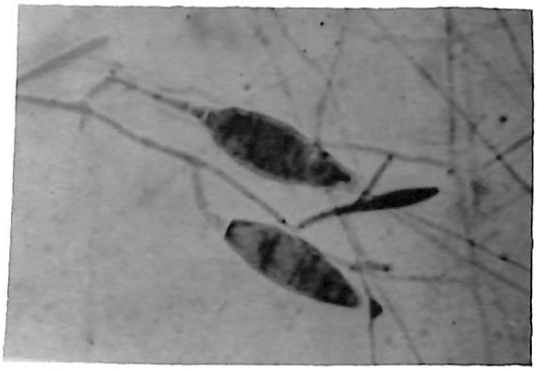
VI

Fig. VI : Photomicrograph showing numerous mycelial fragments in KOH preparation.



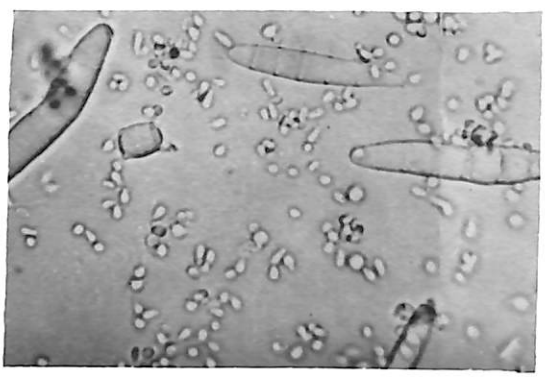
VII

Fig. VII : Photomicrograph showing lactophenol cotton blue preparation of the culture with numerous macroconidia and a few microconidia.



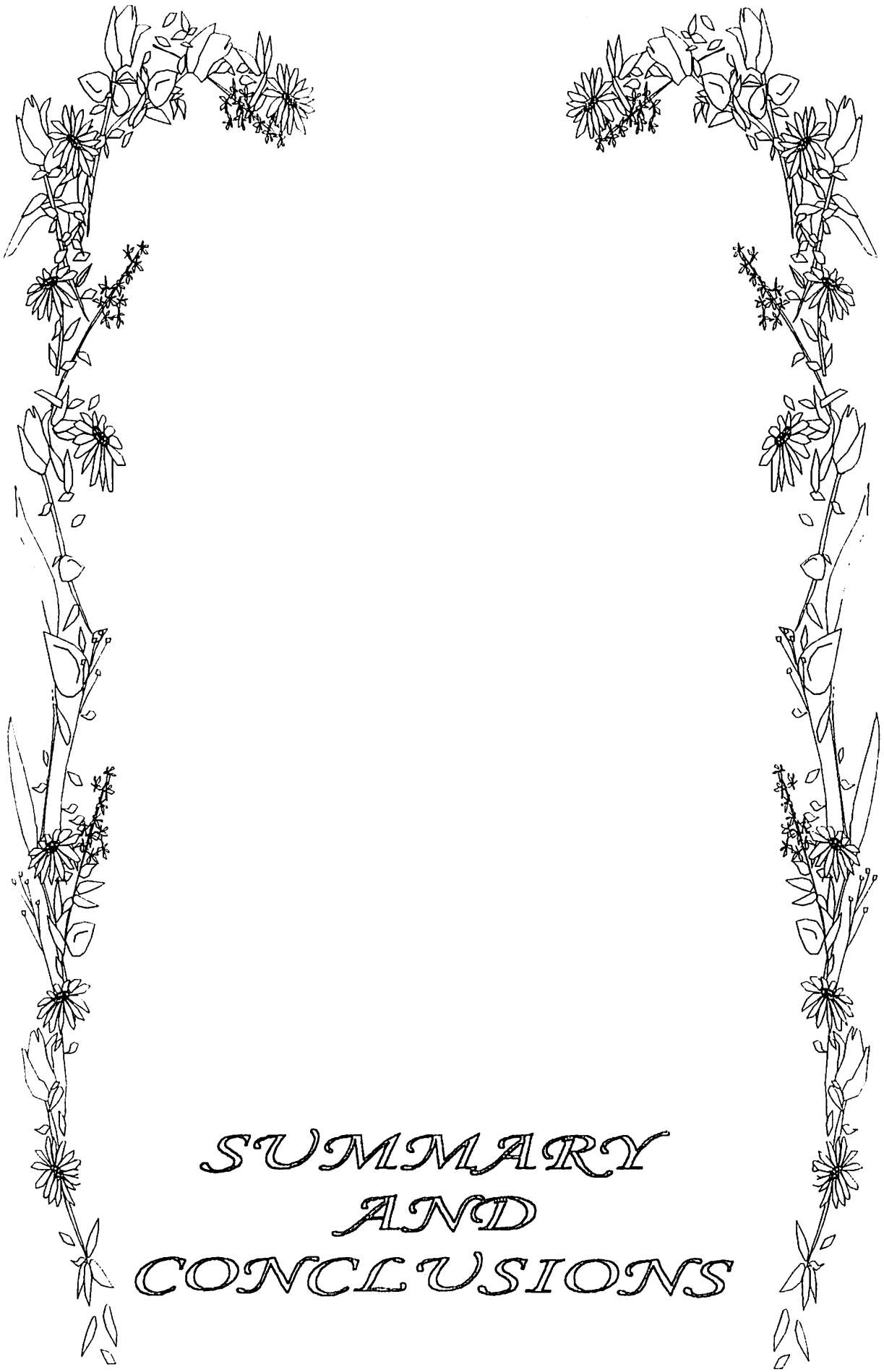
VIII

Fig. VIII : Photomicrograph showing lactophenol cotton blue preparation of the culture with numerous macroconidia and a few microconidia.



IX

Fig. IX : Photomicrograph showing lactophenol cotton blue preparation of the culture with numerous microconidia and a few macroconidia.



SUMMARY AND CONCLUSIONS

Summary and Conclusions

Dogs are closely associated with human beings as such diseases of dogs are of much importance from both zoonotic point of view as wall as for the welfare of dogs. Among all the canine ailments, dermatophytosis is one of the vital problem.

Dermatophytosis is a superficial fungal infection of cornified epidermis caused by dermatophytes which create a change in invaded structure and this attention along with immunological reaction is clinically know as Ringworm.

Ringworm of dog is very common and cause a major problem for dogs as well as for human beings as it can be transmitted both directly and indirectly, but there is lack of proper identification and specific measures to alleviate the problem.

In the present study investigation attempts were made to isolate and identify dermatophytes from skin of both apparently healthy dogs and affected dogs of various breeds. Altogether 200 skin samples were collected, 100 each from apparently healthy skin and skin with lesions, by hairbrush technique and scraping method for cultivation of zoonotic dermatophytes. The samples were collected from different hospitals of Patna but majority were from Private Dog care centre.

A total of 50 foreign breeds (25 Alsation, 13 doberman and 12 spitz) and 25 native and 25 crossbred clinical cases of specific skin deseases in dogs were taken for present study. Fifty apparently healthy foreign breeds (Alsation 30, spitz 10, Doberman 3, and Labrador 2) and 50 native dogs were subjected to study.

Out of 200 samples put to direct microscopical examination in 10% KOH for the presence of any fungal mycelia or hyphae and fungal spores and any ectothrix and endothrix invasion of hairs, 40 (20%) scrored positive for dermatophytes, 15 out of 100 healthy dogs (15%) and 25 out of 100 samples (25%) from affected dogs.

Skin sciapings were cultured for isolation of dermatophytes on Sabouraud's dextrose agar fortified with chloramphenicol and cyclohexamide.

Dermatophytes were cultured from 17 of 100 (17%) specimens from affected dogs and 8 of 100 (8%) specimens from apparently healthy dogs.

The identification of ringworms from skin scrapings of dogs was done on the basis of gross morphology of colony, rate of growth, general topography, texture, and pigmentation. While for microscopical identification Lactophenol cotton blue preparations of culture after sporulation were examined.

Various species of dermatophytes isolated from the dogs belonged to two genera : *Microsporum* and *Trichophyton*. In this study 25 of 200 (12.5%) isolates were identified. Of the 17 isolates from dogs with skin lesion, the most frequent dermatophytes were *Microsporum canis* 13 (76.47%) followed by *Microsporum gypseum* 3 (17.64%) and *Trichophyton mentagrophytes* 1 (5.88%). Out of 8 isolates from dogs with apparently healthy skin, the most frequent dermatophytes were *Microsporum gypseum* 6 (75.00%) followed by *M. canis* 1 (12.50%) and *Trichophyton mentagrophytes* 1 (12.50%).

In both the cases there was a significantly different prevalence of various species of dermatophytes, ($P<0.05$).

Of the various breeds of apparently healthy dogs screened, German Shephered (16.6%) headed the list followed by spitz (10.00%) and native (4%) for dermatophytes while from affected dogs native (20%) and German Shephered (20%) shared the top position followed by crossbred (16%), Doberman (15%) and spitz (8.3%). However, different breeds of dogs had non significant difference of prevalence of dermatophytosis. Of the 13 isolates of *Microsporum canis* from affected dogs 4 each was from native and German Shephered, 1 each was from Doberman and spitz and 3 was from crossbred. Of 3 isolates of *Microsporum gypseum* 1 each was from native, German Shephered and Doberman. The only isolates of *Trichophyton mentagrophytes* was from crossbred.

There was a high proportion of positive cultures from healthy and affected dogs less than 1 year of age. In healthy animals : 5 of 25 dogs (20%) less than 1 year of age were positive compared to 3 of 75 dogs (4%) aged one year and above were containing dermatophytes. In affected group : 6 of 20 dogs (30%) less than one year age were positive compared to 4 of 35 dogs (11.42%) of unknown age and 7 of 45 dogs (15.55%) aged one year and above were harbouring dermatophytes. In both the case there was a significantly different prevalence of dermalophytosis in different age groups ($P<0.05$); The incidence of ringworm was higher in pups below 1 year of age.

There was no significant difference in incidence of dermatophytosis between the sexes. 5 of 60 males, or (8.3%) and 3 of 40 female, or (7.5%) were positive in healthy group. And 9 of 55 males, or (16.3%) and 8 of 45 females, or (17.7%) were positive from affected dogs. A sexual predisposition for dermatophytes infection was not observed.

Annual distribution of infection in dogs showed a significantly higher incidence of *Microsporum canis* in autumn verses summer, spring and winter while the recovery of *Microsporum gypseum* was higher in summer. Only *T. mentagrophytes* obtained in winter.

Some of the conclusions drawn from the present investigation were :

(i) The dermatophytosis is more prevalent in debilitating animals.

(ii) *Microsporum gypseum* is mostly prevalent in apparently healthy dogs.

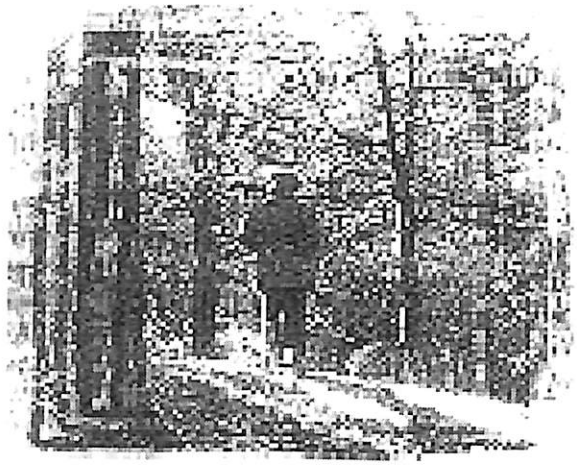
(iii) *Microsporum canis* is mostly prevalent in clinically ill dogs.

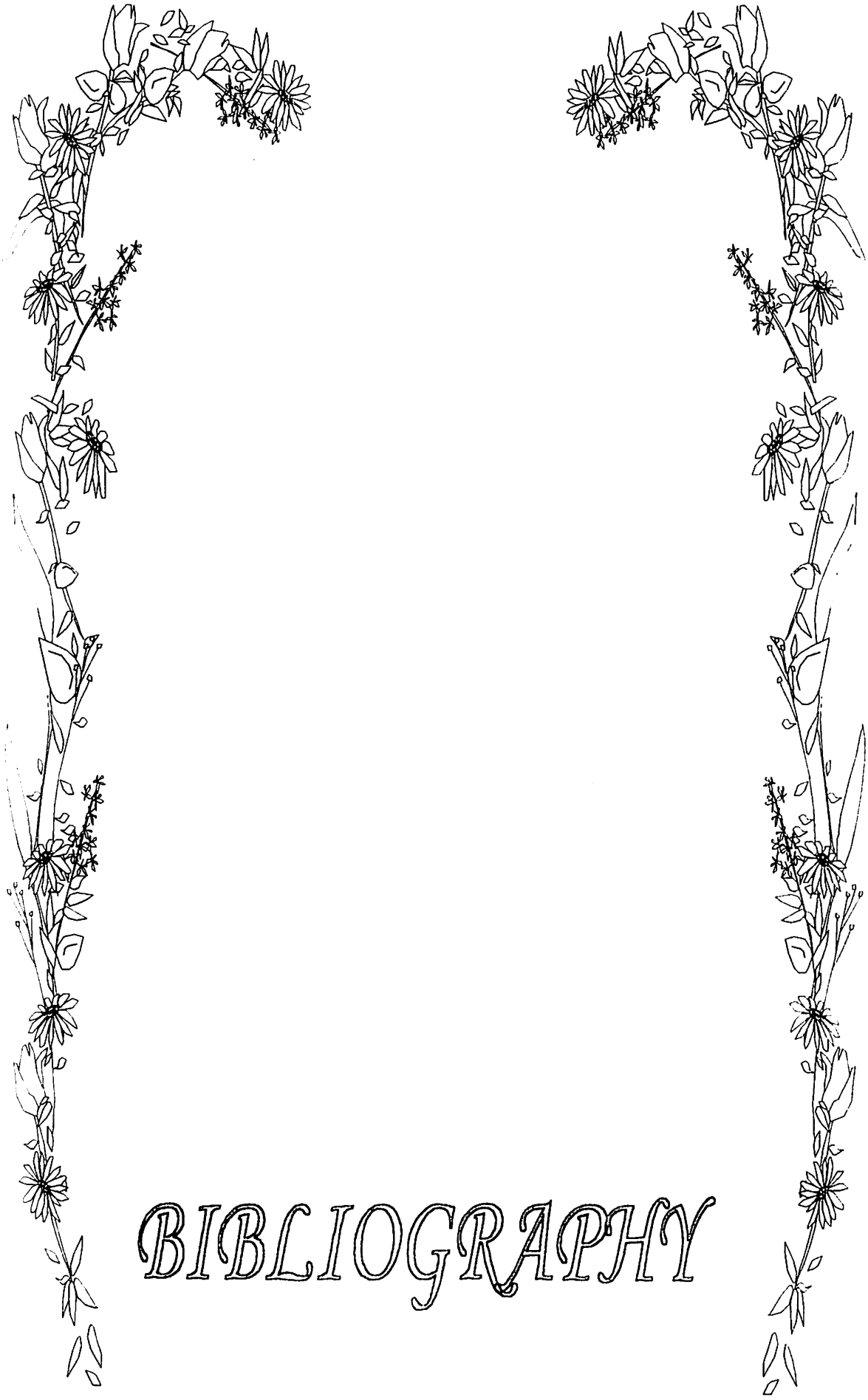
(iv) Foreign breeds of dogs are comparatively more susceptible to dermatophytosis.

(v) Young dogs are predisposed to the development of dermatophytosis with the prevalence of infection in animals less than 1 year of age.

(vi) Both males and females are equally susceptible to dermatophytosis and there is no any sexual predisposition.

(vii) There is a seasonal trend is incidence of dermatophytosis with a prevalence peak for the disease in autumn.





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