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EPIDEMIOLOGY AND CLINICO-THERAPEUTIC STUDIES ON DERMATOMYCOSIS IN DOGS

DEVI. T.

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Department of Veterinary Epidemiology and Preventive Medicine
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680 651
KERALA, INDIA

DECLARATION

I hereby declare that this thesis entitled **" EPIDEMIOLOGY AND CLINICO-THERAPEUTIC STUDIES ON DERMATOMYCOSIS IN DOGS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy **DEVI. T.**

13.09.04

CERTIFICATE

Certified that the thesis, entitled **"EPIDEMIOLOGY AND CLINICO-THERAPEUTIC STUDIES ON DERMATOMYCOSIS IN DOGS"** is a record of research work done independently by **Dr. Devi. T.,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Mannuthy
14.09.04

Dr. K. Vijayakumar
(Chairman, Advisory Committee)
Assistant Professor (SS)
Department of Veterinary
Epidemiology and Preventive Medicine
College of Veterinary and Animal Sciences
Mannuthy

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Dr. Devi. T.,** a candidate for the degree of Master of Veterinary Science in Preventive Medicine, agree that the thesis entitled **"EPIDEMIOLOGY AND CLINICO-THERAPEUTIC STUDIES ON DERMATOMYCOSIS IN DOGS"** may be submitted by Dr. Devi. T., in partial fulfilment of the requirement for the degree.

Dr. K. Vijayakumar
(Chairman, Advisory Committee)
Assistant Professor (SS)
Department of Veterinary Epidemiology and Preventive Medicine
College of Veterinary and Animal Sciences
Mannuthy

Dr. M.R. Saseendranath
Associate Professor and Head
Department of Veterinary
Epidemiology and Preventive Medicine
(Member)

Dr. G. Krishnan Nair
Associate Professor
Department of Microbiology
(Member)

Dr. P. Nandakumar
Associate Professor and Head
University Goat and Sheep farm
(Member)

External Examiner

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Introduction

1. INTRODUCTION

Canine dermatomycosis imposes serious challenges to the practicing veterinarians due to its cosmopolitan distribution, post-treatment relapse and potential public health hazard especially to the children. Dermatomycosis caused by the keratinophilic, filamentous fungi belonging to the genera *Microsporum, Trichophyton* and *Epidermophyton.* The disease assumes increased magnitude recently due to its potential zoonotic implication and rapid mode of transmission by both direct and indirect contact with the contaminated environment.

Dermatomycosis is considered to be a baffling problem in the humid tropics because the infected animals may be presented with a variety of clinical manifestations, which can mimic almost any skin disease. Dermatophytes can only proliferate under the ideal conditions of warmth, moisture and humidity. The presence of increased hydration enhances the ability of dermatophytes to penetrate the skin and favours the germination of spores.

Diagnosis of dermatophytes is seriously hindered by lot of unjust criticism, mostly arising from misunderstanding and also from the difficulties in identifying the organisms. Diagnosis by direct microscopic and cultural examination of the skin scrapings is vital for the confirmation of the organisms and to select the appropriate chemotherapeutic approach. Dermatomycosis, often spontaneously resolve in healthy dogs, however treatment is always recommended due to the highly contagious nature of infection and the infected animals act as a primary and potential source of infection for other in-contact animals. Traditional antifungal agents such as tincture iodine, white field ointment, creosote ointment, resorcinol ointment, etc are of little value in the treatment of dermatomycosis and leaves the opportunity for relapses to occur. Topical therapy including shampoos and cleansing agents may be ideal in animals with mild localised

infections, but not for generalised cases. The chemotherapy of dermatomycosis in animals using oral antifungal agents revolutionised the clinical management of this ubiquitous infection. This approach has reduced the course of treatment considerably and is found to be consistently effective.

Griseofulvin, the fungistatic drug in vogue is used at the dose rate of 20 mg per kg body weight per day orally for a minimum period of 30 days. The drug acts by causing thickening, curling and distortion of the aerial hyphae. The orally active imidazole antifungal drug, approved for use in 1979, ketoconazole is used at the dose of 5 to 10 mg per kg body weight daily orally, for two to three weeks. This prevents the risk of hepatitis and makes the treatment economical. Ketoconazole with increased solubility in an acid environment, good tissue distribution is found to be effective. This drug inhibits P 450 lanosterol c14- demethylase enzyme and resulting in depletion of ergosterol in cytoplasmic membrane of fungus. Triazole group of antifungal agents such as itraconazole and fluconazole are effective against dermatomycosis but not widely used due to technoeconomic constraints.

Allylamine antifungal agent, terbinafine has been developed as an effective and specific drug against dermatophytes in animals and human beings. Terbinafine inhibits squalene epoxidase to suppress the biosynthesis of ergosterol and fungal cell death results from the disruption of cell membrane. Its lipophilic nature results in high concentration in the stratum corneum, hair follicles and nails. Its efficacy in veterinary practice has to be established due to sparse clinical use in animals.

Vaccination with inactivated *Microsporum canis* vaccine ("Biocan M") is best documented in cats. "Biocan M" inactivated vaccine contains an immunogenic, inactivated strain of *Microsporum canis* adsorbed with aluminium hydroxide gel and was introduced as an adjunct to the traditional

therapy in dogs. Fungal vaccines are not protective against challenge exposure and are meant for treatment only (Beale, 2000).

Taking into consideration these facts, the cosmopolitan distribution of the disease and increased number of relapse cases coming to the hospitals, the present study was envisaged with the following objectives.

1. To determine the occurrence of dermatomycosis in dogs.
2. To isolate and identify the dermatophytes by direct microscopic and cultural examination of skin scrapings.
3. To study the haematological and biochemical parameters of dermatophyte infected animals.
4. To assess the comparative efficacy of different antifungal agents such as oral ketoconazole, oral terbinafine and combination of “Biocan-M” vaccine and topical application of ketoconazole.

Review of Literature

2. REVIEW OF LITERATURE

2.1 EPIDEMIOLOGY

2.1.1 Occurrence

Hajsig *et al.* (1975) examined a total of 80 dogs in Zagreb area, Yugoslavia having skin lesions and detected *T. mentagrophytes* in a bitch and three puppies and *M. gypseum* in two dogs.

A total of 4790 skin scrapings from domestic animals were collected and examined mycologically. Most frequently *T. verrucosum* was identified in samples from cattle, followed by *M. canis* isolated from cats, dogs and zoo animals (Weiss *et al.*, 1979).

Pinard *et al.* (1987) isolated dermatophytes from 44 (20. 4 per cent) out of the 198 dogs examined at the Alfort veterinary school in 1983. Among them, *M. canis* was found on 42 dogs and other species isolated were *M. persicolor* and *M. gypseum.*

Wright (1989) reported that *M. canis* accounted for 65 per cent of the total 385 dogs, which were positive for ringworm at Bristol, United kingdom.

In Norway, a survey revealed that culture results were positive from only five per cent of 780 canine specimens suspected of having dermatophytosis (Foil, 1990).

Sidhu *et al.* (1993) conducted a survey on the incidence of mycotic dermatitis in dogs and concluded that maximum incidence was from the month of July to December. This could be due to the hot and humid climate during July to September, which favoured the growth of fungi. Similarly October to December was the period of indoor confinement of animals, which increased the contact among them.

Overall incidence of *Trichophyton* infection in dogs was 0.47 per cent out of the 211 samples examined by cultural examination in Maharashtra (Borikar and Singh, 1994).

Balajee *et al.* (1997) collected 269 soil samples from different habitats in Madras and screened them by hair baiting technique. They isolated three strains of *T. mentagrophytes* and 16 strains of *M. gypseum* from them.

Hatzopoulou and Hatzopoulou (1997) examined 500 healthy dogs by the hairbrush technique and obtained 48 dermatophyte isolates (14.5 per cent) out of 330 dogs from urban areas and 35 (20.5 per cent) out of 170 dogs from rural areas in Thessaloniki, Greece. Majority of the isolates from urban areas were *M. canis* (380), whereas 14 isolates from rural areas were *M. gypseum.*

In a study carried out on 3075 dogs of different breeds, age and sex from November 1992 to April 1993 at Punjab Agricultural University, Ludhiana, the overall frequency of canine dermatitis was about 9.24 per cent (281), of which 14.23 per cent (40) was mycotic dermatitis (Aujla *et al*., 1999).

Pinter *et al*. (1999) examined a total of 3,353 dogs in Croatia and found that 515 dogs were positive for dermatophyte infections. Out of 515 dogs, a significantly higher proportion of infections belong to *Microsporum canis* and 47 were of other species.

Chavez *et al*., (2000) found keratinophilic fungi in higher numbers in the cat's hair coat (67 per cent) than in the dog's hair coat (45 per cent) and the similar observation was noticed in dermatophyte infections with 12 isolates out of 100 samples in cats and seven isolates out of 200 samples from dogs in Mexico city.

Hasegawa (2000) isolated *M. canis, M. equinum, M. gypseum, T. equinum, T. mentagrophytes, T. rubrum* and *T. verrucosum* from cases of animal dermatophytoses in Japan.

Kirubaharan *et al*. (2000) attempted to identify dermatophytes in dogs in Chennai, by taking impressions on agar sausages from the lesions and isolated *Trichophyton, Epidermophyton, Aspergillus* and *Absidia*.

Kumar and Thakur (2001) reported the occurrence of different species of dermatophytes in 193 dogs of different breeds in Ranchi and higher prevalence of *M. gypseum* (52.75 per cent) than other three species such as *T. mentagrophytes* (18.01 per cent), *T. rubrum* (14.64 per cent) and *M. canis* (14.64 per cent) were noticed.

Sturzu *et al.* (2002) confirmed *M. canis* (86 per cent), *M. persicolor* (2.86 per cent), *M. gypseum* (2.72 per cent), *T. mentagrophytes* (3.68 per cent) and *T. simii* (0.27 per cent) from 47 cases of canine dermatitis in Romania by direct microscopic and cultural examination of skin scrapings.

Kozak *et al.* (2003) studied the occurrence of dermatophytes and other micromycetes in skin scrapings and swabs from 100 dogs showing skin problems in Kosice, Slovakia and isolated 12 species of *M. pachydermatis* from 31 cases, *T. mentagrophytes* from two cases and *M. canis* from one case.

2.1.2 Predisposing Factors

2.1.2.1 Age

Jungerman and Schwartzman (1972) reported an age related predisposition for dermatomycosis in dogs and opined that young animals appeared to be infected more often than adults.

Reyes (1972) observed that younger animals were involved with ringworm infections more often than adults, based on clinical signs and cultural examinations.

According to Goldston and Wilkies (1982) occurrence of dermatophytosis was greater among young animals below three years of age than older dogs.

Cutsem *et al*. (1985) observed that isolation of dermatophytes was more successful in younger dogs (less than one-year-old) than older dogs, even though all ages were involved.

Thomsett (1986) reported that susceptibility to dermatophytosis in domestic animals was not age related.

As with many infectious diseases, young animals were predisposed to acquiring symptomatic dermatomycosis, which was partly attributed to the delay in development of adequate host immunity (Foil, 1990).

Young dogs were more prone to dermatophytosis due to difference in non-specific defence or lack of acquired immunity (Lewis *et al*., 1991).

Moriello and DeBoer (1991) and Mignon and Losson (1997) opined that age was not of significance for the occurrence of dermatophytosis in canines and felines.

Sparkes *et al.* (1993) reported that animals less than one year of age appeared to be predisposed to dermatophyte infections.

Dogs less than two years of age were found to be mostly affected with dermatophytosis (Wawrzkienicz *et al*., 1994).

Marchisio *et al. (*1995) surveyed dogs and cats suspected of having dermatophytic lesions in Turin, Italy and observed that animals less than one- year of age were most often affected.

Hatzopoulou and Hatzopoulou (1997) reported that, 148 out of the 220 dermatophyte-infected dogs belong to the 2 to 24 months age group in Greece.

Vishwakarma *et al.* (1997) observed that clinical canine mycotic dermatitis mainly seemed to be a disease of younger age group.

Aujla *et al*. (1999) indicated that the specific proportional frequency of mycotic dermatitis in less than one-year-old dogs was 0.02 out of total 3075 dogs examined, whereas in adults it was 0.01. The frequency of disease declined with the rise in age.

Higher occurrence of ringworm infection was noted in animals less than one year of age (Pinter *et al*., 1999).

Mancianti *et al*. (2002) opined that the dogs less than one year of age were more frequently infected with mycotic dermatitis.

2.1.2.2 Breed

Although there was no breed predisposition, certain individuals or members of a particular family or breeding line might be genetically predisposed to dermatophytosis (Jungerman and Schwartzman, 1972).

Thomsett (1986) did not attribute any breed susceptibility to mycotic infections in domestic animals.

Scott and Paradis (1990) conducted a survey on 558 pet animals in Quebec and reported that, only three breeds appeared to be at increased risk *viz* Boxer, Dachshund and Bichon fries for skin disease. Of these three breeds, only the Dachshunds were at increased risk in the Northern California study.

Sparkes *et al.* (1993) reported that long-haired breeds of dogs and cats had significantly higher prevalence of dermatophytosis, as Chinchilla and Birman breeds of cat and Jack Russell and Yorkshire terrier dogs yielded significantly high proportion of positive cultures.

Aujla *et al*. (1999) observed that purebreds were more susceptible to mycotic dermatitis than non-descript breeds and among the purebreds, the frequency was highest in Doberman and German shepherds, followed by Spitz, Dalmatian, Boxer, Golden retrievers and Grey hounds.

Ringworm was more frequently diagnosed in crossbred dogs when compared to purebreds and was found to be statistically significant (Pinter *et al*., 1999).

Ziony and Arzi (2000) studied dermatophytosis in 129 dogs in Israel and observed that 68 were crossbreds, eight were Golden retrievers, seven were German shepherds, five each were Boxers, Standard poodles and Pointers and the remaining 31 dogs were of other breeds.

Kumar and Thakur (2001) reported the highest occurrence of dermatomycosis in Spitz (45 per cent) in and around Ranchi.

Mancianti *et al.* (2002) showed a significantly higher prevalence of infection by *M. gypseum* in hunting breeds of dogs and by *M. canis* in long-haired breeds, with a ratio of 2 : 1 compared to short-haired breeds.

Moriello (2003) suggested that long-haired breeds might be predisposed to dermatophytosis because their hair might protect spores from mechanical removal through grooming and the longer the contact with skin, greater the possibility that sporulation and infection might develop.

2.1.2.3 Season

No definite seasonal distribution was noticed among *M. canis* infected cats and dogs examined at the Massey University clinic, New Zealand (Baxter, 1973).

Chittawar and Rao (1982) and Aujla *et al*. (1999) opined that the maximum number of cases were presented in January and the minimum in March and suggested that this could be due to prolonged indoor keeping of dogs which might lead to higher humidity.

Jand and Gupta (1989) pointed out that the occurrence of dermatophytosis was greater in warm and humid climates.

Sparkes *et al.* (1993) evaluated a seasonal trend in dermatophyte infections, the submissions were divided into spring (March to May), summer (June to August), autumn (September to November) and winter (December to February) and concluded that there was no seasonal variation in the incidence of dermatophyte infections.

Varghese *et al.* (1994) reported that among 83 dogs with skin lesions brought to B.S.D.P. hospital Bombay, between July and November 1991, 3.61 per cent revealed *T. mentagrophytes* infection.

No significant seasonal difference was noticed among the isolates of dermatophyte in 944 dogs examined for one-year period in Barcelona, Spain (Cabanes *et al.*, 1996).

M. gypseum was found to be more abundant in hot and tropical regions and the lesions usually involved face and feet (BSAVA's News, 1998).

Patwardhan and Dave (1999) reported that maximum number of dermatophyte infections occur in the summer season.

Kumar and Thakur (2001) studied the seasonal occurrence of dermatomycosis in Ranchi and concluded that maximum positive cases were found during summer and lowest in winter.

Mancianti *et al*. (2002) reported a significantly higher occurrence of *M. gypseum* in summer than winter and spring, while the recovery rate of *M. canis* was very high in fall and winter, than in summer and spring. But *T. mentagrophytes* did not show a similar seasonal pattern.

Brilhante *et al.* (2003) reported that dermatophytes were more frequently isolated in March, April and May during a survey on 189 dogs and 38 cats from a city in Northeast Brazil.

2.1.2.4 Sex

Jungerman and Schwartzman (1972) opined that there seemed to be no sex predilection to ringworm infections in animals.

Baxter (1973) concluded that there was no indication of any sex susceptibility in dogs to ringworm infection.

Scott and Paradis (1990) reported that no apparent sex predilections were observed for dermatological disease as a whole in pet animals, by conducting a survey involving 558 cases in Quebec, Canada.

Sex was not of importance in the occurrence of dermatophytes in both dogs and cats. (Moriello and DeBoer, 1991; Mignon and Losson, 1997 and Kozak *et al*., 2003).

Cabanes *et al.* (1996) opined that no significant differences in fungal biota including dermatophytes were observed with respect to the gender of dogs.

Shakir *et al.* (1996) reported that male animals were more prone to skin infection than females. In their study, 83 males were found to be affected with skin disorders out of the total 124 dogs.

Aujla *et al*. (1999) noted that the higher proportional frequency of mycotic dermatitis in males might be due to their stray nature.

Pinter *et al*. (1999) reported that, the highest occurrence of ringworm was observed in male dogs.

Mancianti *et al*. (2002) observed no sexual predisposition for mycotic infections of dogs in Pisa, Italy.

2.1.2.5 Other Factors

Kushida and Watanabe (1975) reported a case of *T. rubrum* infection in a dog in Japan, which probably had acquired from its owner who had athlete's feet.

McAleer (1980) opined that *M. canis* was the chief etiologic agent of ringworm infections in kittens and children whereas *T. verrucosum* was considered as a less frequent cause of ringworm infections.

Quaife (1982) pointed out that *M. canis* from shed hair of naturally infected cats might be viable for more than an year.

Jenkinson (1989) stated that the skin surface air movement was found to be an important factor in the transmission of infected particles including desquamating cells, across the body surface and to other regions such as the mouth and nose.

Glucocorticoid therapy increased the susceptibility in dogs to dermatomycosis by inhibiting the local inflammation (Foil, 1990).

Rycroft and McLay (1991) stated that the household environment of an infected animal was an important potential source for reinfection and use of disinfectant, benzalkonium chloride was found to have good activity against *M. canis.*

Sparkes *et al*. (1994) reported that the infection with *M.canis* might result in the development of a variety of skin lesions, but asymptomatic carriers were considered to be important in the epidemiology of disease.

Mancianti and Papini (1996) collected 400 samples from the floor of 50 private veterinary clinics in Italy, using 55 mm diameter contact plates containing mycobiotic agar and isolated *M. canis, M. gypseum, T. mentagrophytes, T. ajelloi* and *T. terrestre.* They concluded that the keratinic material shed by infected pets might contribute to the development and propagation of dermatophytes in veterinary clinics.

Ranganathan *et al.* (1997-1998) screened 211 dogs in and around Madras city and isolated 89 strains of dermatophytes. Further, the owners of the 11 dogs that yielded *T. mentagrophytes var mentagrophytes* had either *Tinea corporis* or *Tinea pedis*. All the patients responded to antifungal therapy but recurrence was noted. It was evidenced that the reverse transmission of dermatophytes from human to animals might be the reason for the selective predominance of these agents in strictly housed dogs.

It was reported that the pet's environment and grooming tools should also be considered as the important sources of further spread of dermatophyte infection (BSAVA's News, 1998).

M. gypseum was a common geophilic dermatophyte and it was postulated that dogs were exposed to *M. gypseum* by rooting and digging the contaminated soil (Saridomichelakis *et al*., 1999).

Buergelt (2000) opined that guinea pigs might harbour the organisms with out showing clinical signs and the disease could be transmitted by direct or indirect contact.

Bond (2002) concluded that transmission of dermatophytes from wildlife to dogs often resulted in skin disease of the face or limbs, since wild rodents, especially hedgehogs often carried *T. mentagrophytes, M. persicolor* and *T. erinace* infection.

Pal (2002) viewed that the dermatophytosis established the zoonotic significance of zoophilic infection, and the transmission usually occur directly from the diseased animals.

Mancianti *et al*. (2003) opined that dogs seemed to be of lesser importance in the spread of *M. canis*, as they never contaminated the air. On the other hand infected cats appeared to cause substantial environmental contamination and provoke a substantial presence of viable, airborne fungal elements, leading to environmental spread of *M. canis.*

Kozak *et al*. (2003) indicated that the occurrence of mycoses in dogs and other pets was of importance in assessing the risk of acquiring mycotic infections and determining the exposure of human beings to infection.

Moriello (2003) pointed out that increased hydration and maceration of the skin were considered as the important predisposing factors to infection in people. Moisture enhanced the ability of dermatophytes to penetrate the skin and favoured their germination.

Ollhoff (2003) isolated *T. verrucosum* from 32 animals, of which 22 developed lesions from two to eight weeks after the first clinical examination and it showed the importance of latent infection in the epidemiology of ringworm.

2.2 PATHOGENESIS

Jungerman and Schwartzman (1972) reported that the dermatophytes did not invade living tissue, but they might elaborate or excrete toxins or allergens which found their way through the living epidermis into the dermal tissue, where a vascular component was present which was capable of responding to the challenge of toxin through an inflammatory reaction.

Kushida and Watanabe (1975) inoculated *M. canis* and *M. gypseum* into a number of healthy dogs and observed a typical circular lesion at the site of

injection where the epidermis was thickened, inflammed and lesions began to shed their crusts on the day 30, regressing to leave an alopecic scar with dry desquamation by the day 90. New hair growth on the normal skin was found 75 to 120 days after inoculation.

Smith (1988) pointed out that primary lesions were the direct result of a disease process, and secondary lesions resulted from the degeneration of the primary lesions in dermatological disorders.

Wright (1989) opined that dermatophytes invaded only dead keratinised tissues, they elaborated metabolic products which diffused to the living cells of the epidermis and produced inflammatory and hypersensitive reactions which were responsible for the development of lesions.

Gudding and Lund (1995) reported that the glycoprotein of cell wall and proteinases especially keratinase, produced by dermatophytes play a significant role in pathogenesis.

Infections by anthropophilic dermatophytes usually caused the shedding of skin scales, hyphal elements of the fungus, desquamated skin scales which might remain infectious in the environment for months or years (Suhonen *et al.*, 1999).

Dermatophytes produced inflammation, pruritus and desquamation by invading the keratin of the stratum corneum as well as the nails and the hair shaft and their hyphae seldom moved further into the epidermis to damage other tissues (Chavez *et al.*, 2000).

Radostits *et al*. (2000) stated that ringworm fungi chiefly attacked stratum corneum and hair, resulting in autolysis of the hair structure, alopecia, exudation and epithelial debris. The hyphae produced dry crusts, which were characteristic to the disease.

Ahuja *et al.* (2002) explained that formation of thread like hyphae in skin surface, invaded hair shaft, follicles and the stratum corneum of the epidermis in dermatophyte infections. These hyphae did not invade living cells, but their toxic products induced severe local inflammatory reactions.

Kozak *et al.* (2003) stated that mycotic disease in animals manifested as typical changes characteristic to the disease or might persist asymptomatically without evident clinical signs.

2.3 CLINICAL FEATURES

Jungerman and Schwartzman (1972) described that the clinical manifestations of ringworm in domestic animals were extremely variable. At one end of the spectrum was the asymptomatic carrier and at the other extreme was a violently eruptive nodular or tumorous lesion referred to as a kerion. The infinite variety of lesions was related to the interplay of the dermatophytes on one hand and the reactive capacity of the host on the other hand.

Muhammed and Mbogwa (1974) isolated *M. nanum* from a cross bred male dog, aged six years, with skin lesions on the head and back which showed loss of hair and signs of pruritus.

Carman *et al.* (1979) described that the typical lesions in the dog were alopecia, thickening of the skin with slight scaling and pustule formation over the entire muzzle. In some cases, the lesions extended around the eyes, secondary lesions appeared on the forelegs, probably due to the animal's habit of rubbing the muzzles.

Ringworm lesions were reported mostly on head, neck, shoulders, legs, abdomen and the interdigital skin. The lesions were mostly circular, slightly raised and crustaceous. Itching was not a constant feature (Chittawar and Rao, 1982).

Cutsem *et al*. (1985) isolated dermatophytes alone from eight cases out of total 142 (5.6 per cent) asymptomatic dogs and from 33 cases out of total 180 (18.3 per cent) dogs with diffuse hair loss.

Parasitic stages of dermatophytes caused the ring-like lesions and these were the imperfect (asexual) stages of fungi, which were capable of forming spores by asexual process (Wright, 1989).

Cobenas *et al*. (1992) reported that pruritus was the most common finding in all animals affected with dermatomycosis, and in some cases wide areas of alopecia were observed in *T. mentagrophytes* and *T. verrucosum* infections.

Medleau and Ristic (1992) observed that dermatophytosis was usually non-pruritic, but occasionally could be moderately to intensely pruritic. Clinical signs noticed were alopecia, erythema, scales and crust formation. The lesions varied from scaly patches of alopecia to raised erythematous nodules called kerions.

Borikar and Singh (1994) noticed that the lesions of ringworm were in the form of grayish-white, roughly circular crusts, raised above the skin in dogs.

Sture (1995) noticed scale and crust formation on the dorsum of the head and greasy seborrhoeic plugs adherent to the pinnal margin of a *Trichophyton* infected dog.

Parker and Yager (1997) mentioned that *Microsporum* spp was the most common cause of dermatophytosis, which produced focal patches of alopecia with erythematous borders and occasional follicular papules. Whereas *Trichophyton* spp produced more severe alopecic papulopustular lesions with crusting and pronounced hyperpigmentation and seborrhoeic scales.

Pinter *et al.* (1999) reported that, among the total 493 dogs, which were positive for dermatophytes by culture examination, a noticeably higher proportion

of dogs (331) showed characteristic lesions whereas 162 dogs did not show evident lesions.

Beale (2000) stated that canine dermatophytosis was characterised by alopecia, scaling, broken hairs, pustules, papules, exudation, crusting and hyperpigmentation. Pruritus was variable and the classical lesion was circular area of alopecia with central healing.

Bond (2002) stated that *M. canis* lesions included patches of alopecia with erythema and grey scales or crusts which often were much less inflammed and thickened than those caused by *T. mentagrophytes* and *T. erinacei*.

2.4 DIAGNOSIS

2.4.1 Direct Microscopic Examination

Muller and Kirk (1969) described that the small spores arranged in masses or in a mosaic pattern were typical of *M. canis*, *M. audouini* and *M. distortum* and these infected hairs might fluoresce, whereas intermediate spores arranged in dense chains that did not cause fluorescence were seen with *T. mentagrophytes*, *T. verrucosum* and *T. rubrum.* Large spores in sparse chains were usually not fluorescent, which were seen in *M. gypseum* infection.

Lloyd (1985) mentioned that detection of arthrospores in scrapings might be facilitated by clearing in alkali such as potassium hydroxide.

Smith (1988a) opined that the direct microscopic examination technique was time consuming and a positive result obtained by this technique was very rewarding.

Foil (1990) reported that dermatophytes never formed macroconidia in tissue but rather formed hyphae and arthroconidia on hair and scales, which could be identified by direct microscopic examination.

Shearer (1991) stated that hair samples required for microscopy or fungal culture should be plucked with forceps, especially from the edge of active lesions and these hairs should be mounted in potassium hydroxide or lactophenol cotton blue for microscopic examination.

Beale (2000) reported that the ectothrix spores appeared as round to oval, greenish translucent beads under direct microscopic examination.

Moriello (2003a) revealed that direct microscopic examination of spores using potassium hydroxide confirmed the diagnosis and shortened the diagnostic time. They also observed that the infected hairs were wider, swollen, frayed, fuzzy and almost filamentous with clear, colourless spores.

2.4.2 Wood's Light Examination

Lloyd (1985) stated that Wood's lamp was a source of ultra-violet radiation at a wavelength, which exhibited a characteristic apple green fluorescence in about 50 per cent of naturally occurring *M. canis* infections.

Smith (1988a) opined that the best use of Wood's light was to identify hairs to be examined microscopically and that could be cultured for fungal growth and hence it was helpful, but not a definite diagnostic tool.

Foil (1990) opined that the *M. canis* infections might be positive on a Wood's light examination, however a negative result should never be used to rule out dermatophytosis since not all infections exhibited fluorescence. True fluorescence was quite bright, apple green and should only be within the shafts of infected hairs.

Quinn *et al.* (1994) stated that *M. canis* and *M. distortum* produced certain metabolites when they grow on hairs and skin that will fluoresce vivid apple green under ultra violet light of 366 nm of Wood's lamp. The animal itself could be examined with the lamp in a dark room to detect inapparent infections.

Beale (2000) reported that ultra-violet light emitted from Wood's lamp might cause fluorescence of hairs infected with *M. canis*. Scales, ointments, creams and bacterial folliculitis might also fluoresce under the light, however they did not give the typical apple green fluorescence and hairs might fluoresce even after the *M. canis* infection had resolved. So positive fluorescence alone should not be used to confirm infection or monitor therapy.

Curtis (2001) stated that the Wood's lamp was a source of ultra-violet light that could be used to induce a yellow-green fluorescence in 80 per cent of *M. canis* strains, when illuminated for three to five minutes and more unusual fluorescent species were *M. audouini*, *M. distortum* and *T. schoenleinii*.

Moriello (2003a) suggested that the Wood's light examination was only a screening tool and could help to identify hairs for further direct and cultural examination.

2.4.3 Fungal Culture

Carter *et al*. (1970) isolated an atypical strain of *M.canis* from a four-year-old standard bred gelding. The most distinguishing feature of this strain was the small number of septations (one to four), in contrast to the smaller sized *M.canis* that normally had six to ten.

Jungerman and Schwartzman (1972) stated that the genus *Microsporum* formed cottony, wooly, matted or powdery colonies of various colours in cultured media and they produced single celled, clavate microconidia and multicelled thick walled macroconidia which were representatives of that genus. On the other hand *Trichophyton* spp formed granular, waxy colonies with variable colours in culture plate. Numerous small microconidia as well as smooth, thin walled, pencil or clavate shaped macroconodia might be visualised microscopically.

Carroll (1974) found that dermatophyte test medium (DTM) was 82 per cent accurate as a diagnostic test for dermatophytes by colour change alone, since

saprophytes usually had dark pigmented colonies and turned DTM red when the growth got established, regardless of colony colour.

Thoday (1981) reported that confirmatory diagnosis of fungal infection relied on the definite identification of the pathogenic organism by fungal culture. Sabouraud's dextrose agar was the most satisfactory culture medium since its slight acidity and added antibiotics inhibited bacterial and saprophytic fungi growth.

DeVroey (1985) reported that the infection to inoculation ratio was found to be low for dermatophytes, but contamination of the environment from clinical cases might be extensive.

Modified method of fungal slide culture was a preferred technique for the observation of the colony and conidia arrangement without disturbing the growth (Harris, 1986).

Smith (1988a) stated that dermatophyte test medium was very helpful in distinguishing pathogenic dermatophytes which metabolised proteins initially, thereby creating alkaline by-products that turned media deep red in the first three to ten days. Non-pathogenic saprophytic fungi, which initially used dextrose, produced no colour change within 14 days.

Harvey (1990) stated that Sabouraud's agar medium was the standard fungal culture medium since it was transparent and colourless and it allowed assessment of the colour of the underside of the colony (reverse pigment).

Mansfield and Stringfellow (1990) isolated *M.vanbreuseghemii* from skin lesions of a female Labrador retriever, using dermatophyte test medium and described that the microconidia were long and tapered with five to twelve septa with thick rough spiny walls and they were of pyriform shape.

Quinn *et al*. (1994) explained that the gross morphology of the fungal colony, type of pigmentation, along with microscopic appearance of fruiting heads and spores from the mount colonies helped in the species identification of dermatophyte cultured.

T. mentagrophytes was isolated from the scaly and crusty lesions on the dorsum of the head and greasy seborrhoeic plugs adherent to the pinnal margins of an immunosuppressed Miniature Yorkshire terrier (Sture, 1995).

Chavez *et al.* (2000) collected samples from 200 dogs and 100 cats by using the Mackenzie's tooth brush technique, processed using routine mycological methods and isolated seven and 12 dermatophytes respectively.

Six cases of *Trichophyton,* one case of *Epidermophyton* and three mixed infections of both *Trichophyton* and *Epidermophyton* were confirmed out of 25 cases examined, by taking impressions on agar sausages from the lesions (Kirubaharan *et al*., 2000).

Guillot *et al*. (2001) assessed the performance of dermatophyte test medium using hair samples collected from experimentally infected guinea pigs. After inoculation, dermatophyte test medium plates were incubated at 18, 21, 24, 27 or 37^{o}c and examined daily for 15 days. It was concluded that rapidity of colour change clearly related to the incubation temperature and species of infected hairs, since at 27^{o}c colour change could be observed only by three days with *M. canis*, four days with *T. equinum* and five days with *T. mentagrophytes* infected hairs.

Moriello (2001) described that spindle shaped microconidia were rare and numerous macroconidia were often present in *M. canis* infections, whereas microconidia were common and cigar shaped macroconidia with spiral hyphae were found in *Trichophyton* infections.

Gromadzki *et al.* (2003) evaluated the new medium, dermatophyte identification medium and reported that it had limited usefulness for presumptive

identification of dermatophytes since only 25 out of 77 dermatophyte isolates made this medium to turn purple after incubation at 37^{o}C and the accuracy of the results could be improved by incubation at 30^{o}C.

Growth of the saprophytic microflora was more rapid than the dermatomycotic agents and hence without actidion it would be impossible to isolate dermatophytes from animal material (Kozak *et al*., 2003).

Moriello (2003a) stated that colour change on dermatophyte test medium should be considered at the same time of colony growth and if the plates were held for long all dermatophytes test medium plates with fungal growth might turn red.

Moriello (2003b) defined the mycological cure as two or three consecutive negative tooth brush fungal culture results at weekly or biweekly intervals and treatment should be continued until all these three negative culture results were obtained.

2.5 CLINICAL PATHOLOGY

Wilkinson (1979) examined the blood sample from two-year old male Australian silky terrier having mutiple dermatophyte infections, which revealed moderate hypochromic microcytic anaemia, mild leucopaenia with lymphopaenia, normal serum proteins and normal liver and kidney function.

Gowda *et al*. (1982) reported leucocytosis, lymphopaenia, eosinophilia, monocytosis and anaemia in non-specific dermatitis.

The serum biochemistry profile in a dog having dermatophytoses with hyperadrenocortism revealed a slight elevation in blood glucose, serum cholesterol and alanine aminotransaminase (Hall *et al*., 1984).

Ibrahim *et al*. (1984) studied haematological and biochemical changes of ringworm infected animals and reported that infected animals had low levels of

erythrocyte count, haemoglobin, packed cell volume, markedly decreased blood glucose level, serum total protein, total lipid, calcium and inorganic phosphorus.

Khosla *et al*. (1989) reported no significant change in haemoglobin, total erythrocyte count, packed cell volume, total leucocyte count and differential leucocyte count in eight dogs experimentally infected with *M. canis*.

Khosla *et al.* (1991) noticed an increased level of blood urea nitrogen, blood histamine and decreased level of ascorbic acid in plasma, before and after the treatment of dogs suffering from experimental *M. canis* infections with various drugs.

Logas *et al*. (1993) stated that various dermatological diseases were associated with low serum zinc level.

Shakir *et al.* (1996) stated that there was an overall decrease in the albumin and alpha globulin fractions and an increase in the beta and gamma globulin fraction in dogs with skin disorders.

Tiley and smith (1997) revealed that results of complete blood count and serum biochemistry could not be used for diagnosing dermatophytosis, but helped to identify an underlying problem which could be a contributing factor in the development of the disease.

Haematological analysis of 40 dogs suffering from mycotic dermatitis showed no significant changes in haemoglobin and total erythrocyte count. But appropriate increase in total leucocyte count, lymphocytosis and eosinophilia were noticed, which might be due to primary and secondary inflammatory response and biochemical analysis also showed no significant changes in total protein and albumin (Aujla *et al* ., 1999).

Benjamin (2001) observed neutrophilia in mycotic infections and eosinophilia in allergic conditions of skin in animals.

2.6 IMMUNOLOGY

Grappel *et al*. (1968) studied the serological reactivities of polysaccharides isolated from five species of dermatophytes *viz M. quinckeanum, T. granulosum, T. interdigitale, T. rubrum* and *T. schoenleinii,* with rabbit antisera, by complement fixation and precipitation. The galactomannans showed differences in their serological reactivities with antisera to *T. interdigitale* and *T. schoenleinii* but no differences were noticed with antisera to other three species.

Jungerman and Schwartzman (1972) opined that the tissues contain a serum factor, which was fungistatic or fungicidal in its effect and apparently restricted the growth of dermatophyte on keratinised tissues.

Wright (1989) pointed out that the development of natural immunity and the spontaneous recovery from infection was a common feature of ringworm. Some infections were remarkably persistent and the symptomless carrier was a common phenomenon, which complicated the control of disease.

Foil (1990) observed that atopic individuals were at increased risk for dermatophytosis due to local inhibition of T-lymphocyte function and inflammation.

Gudding and Lund (1995) described that keratinocytes, which were able to phagocytose and degrade antigens, might process antigens that could be transferred to langerhan’s cells and were directly presented to T cells.

Moriello (2003) stated that the recovery from infection depended on the development of strong cell mediated immunity, which might help to eliminate the infection in tissues and to increase permeability of the epidermal barrier to overcome infection.

2.7 TREATMENT

Wright (1989) found that ringworm caused by *M. canis* usually responded readily to treatment, but when the infection got established in a colony of cats, eradication could be difficult and expensive.

Moriello (2003b) pointed out that the clipping the coat removed infected hairs, minimised the continued shedding of hair fragments and spores and made topical application of drugs easier.

2.7.1 Griseofulvin

Baker *et al.* (1971) stated that griseofulvin therapy resulted in prompt and complete remission of all extensive alopecic lesions and suppurative dermatitis in a rhesus monkey infected with *M. canis*.

Jungerman and Schwartzman (1972) described that griseofulvin impaired the development of the terminal hyphae and caused thickening, curling and distortion of aerial hyphae.

Hitaka and Hamaguchi (1973) reported the case of a 42-year old patient with mutiple scaly lesions, which started after handling mycotic infected dog and was successfully treated with oral griseofulvin.

Oral administration of griseofulvin at the dose rate of 50 mg per kg body weight daily with topical application of etisazole solution on alternate days for five weeks showed considerable improvement in the physical condition with normal blood counts (Wilkinson, 1979).

Scott *et al.* (1980) reported that in *Trichophyton* infection the dosage of griseofulvin was increased to 110 mg per kg body weight, there was marked improvement in clinical condition within 10 days and the animal remained normal, 16 months after discontinuation of treatment.

Shawkat *et al*. (1981) confirmed the efficacy of griseofulvin by treating *M.canis* infected cats and dogs orally and observing the negative culture results and normal histological studies of skin section after treatment.

Wright (1989) reported that daily dosage of 15 to 20 mg per kg body weight of griseofulvin should be given initially for at least four weeks for the treatment of *M. canis* infection in dogs and cats.

It was opined that if two weeks of griseofulvin therapy had given no improvement, the dosage should be increased since griseofulvin got variably absorbed from the gastrointestinal tract and hence a dosage that works in one animal might fail to work in another (Medleau and Kuhl, 1992)

Beale (2000) pointed out that griseofulvin, a fungistatic antimicrobial, was the drug of choice for treating dermatophytosis, unless contraindicated in conditions like pregnancy, infection with feline immunodeficiency virus etc.

Bennett (2001) stated that griseofulvin was fungistatic for various species of the dermatophytes like *Microsporum*, *Trichophyton* and *Epidermophyton* and this drug had no effect on bacteria or other fungi.

Papich *et al*. (2001) stated that within few hours of oral administration, griseofulvin could be detected in the stratum corneum and it got distributed to the keratin of skin, hair and nails.

Ahuja *et al*. (2002) pointed out that griseofulvin was the drug of choice in canine ringworm, at the dose rate of 15 to 20 mg per kg body weight once daily orally, for four to six weeks along with some high fat food, to aid absorption from the gut.

2.7.2 Imidazole

Minagawa *et al*. (1982) reported that ketoconazole had minimum inhibitory concentration of 71.6 µg per ml of the medium against *Candida albicans*; 0.62 to 0.80 µg per ml against other *Candida* spp; 1.76 to 7.94 µg per ml against other yeasts; 0.63 to 20 µg per ml against dermatophytes and 1.25 to 20 µg per ml against *Aspergillus* spp.

Hall *et al*. (1984) stated that ketoconazole was an orally active synthetic imidazole and was very effective in eliminating generalised *T. mentagrophytes* infection in a dog with hyperadrenocortism. However it failed to affect the degree of hyperadrenocortism.

Mahajan (1986) compared the susceptibility of 18 fungi to econazole, ketoconazole and amphotericin-B. Ketoconazole arrested the growth of seven fungi at a concentration of 100 µg per ml. But econazole was the most effective, followed by ketoconazole and amphotericin-B.

Ketoconazole had been shown to be a moderately effective fungistatic drug against *M. canis* and *T. mentagrophytes*, at the dose rate of 10 mg per kg body weight daily for three to four weeks (Foil, 1990).

Medleau and Weithers (1992) noted that ketoconazole at a dosage of 10 mg per kg body weight orally every 12 to 24 hours, was effective in the treatment of generalised dermatophytoses in dogs and cats and treatment should be continued until skin lesions had resolved and fungal cultures yielded no growth.

Weithers and Medleau (1995) evaluated the efficacy of ketoconazole shampoo topically in experimental canine dermatophyte infection, by soaking the infected hairs in ketoconazole twice a week for four weeks and proved that ketoconazole inhibited the fungal growth.

Carlotti and Bensignor (1999) reported that ketoconazole might be given at the dose of 10 mg per kg body weight once daily for the treatment of dermatophyte infection in dogs.

Suhonen *et al*. (1999) pointed out that ketoconazole a diaxolane imidazole compound can be used orally or topically and it interfered with the biosynthesis of ergosterol.

Ketoconazole was the first orally effective broad-spectrum antifungal drug, used in the treatment of dermatophytosis and deep mycosis because it was more soluble at acidic pH (Tripathi, 1999).

The pharmacological action of ketoconazole was the inhibition of sterol 14- alpha demethylase, a microsomal cytochrome P-450 dependent enzyme system and impaired with the biosynthesis of ergosterol and lead to the accumulation of 14 alpha methylase, which inhibited the growth of fungi (Bennett, 2001).

Ahuja *et al*. (2002) stated that ketoconazole shampoo was highly effective when applied topically in the treatment of canine ringworm infection.

Kumar *et al.* (2002) indicated that ketoconazole was effective at the dose rate of 5 to 10 mg per kg body weight twice daily for the treatment of *Malassezia* infection in canine.

Patterson and Frank (2002) revealed that ketoconazole was the most common drug used to treat *Malassezia* dermatitis at the dose rate of 5 to 10 mg per kg body weight orally once daily for 30 days.

2.7.3 Triazole

Medleau and Kuhl (1992) pointed that itraconazole was an approved antifungal compound and the concentrations of itraconozole needed to inhibit fungal growth were much lower than that of ketoconazole.

Moriello and DeBoer (1995) compared itraconazole (10 mg per kg body weight q 24 hours) with griseofulvin (50 mg per kg body weight once daily) for the treatment of dermatophytosis in cats and found that both drugs were equally effective, but itraconazole achieved early cure within 56 days.

Mancianti *et al*. (1998) treated cats suffering from dermatophyte infections with itraconazole at the dose rate of 3 mg per kg body weight once daily.

Bond (2002) pointed out that itraconozole was expensive but might be generally safer than ketoconazole when given at the dose of 5 mg per kg body weight once daily for the treatment of canine dermatophytoses.

Jackson *et al*. (2003) reported that itraconazole (10 mg per kg body weight orally), given every 24 hours, with food or in an empty stomach, was found to be effective in the treatment of dermatophytosis in cats.

2.7.4 Terbinafine

Terbinafine inhibited squalene epoxidase to decrease the synthesis of ergosterol and hence fungal death resulted from disruption of cell membrane (Balfour and Faulds, 1992).

The pediatric dose of terbinafine had been in the range of 4 to 8 mg per kg body weight once a day orally, most often administered at the lower dose of 4 mg per kg body weight (Jones, 1995).

Mancianti *et al.* (1999) reported that 15 cats naturally infected with *M.canis* were treated with terbinafine at the dose of 30 mg per kg body weight orally daily for two weeks and 11 cats (92 per cent) showed complete cure. It was concluded that terbinafine could be an effective alternative to other antifungal drug when fungal resistance or idiosyncratic tolerance were shown.

Terbinafine was fungicidal against dermatophytes and its usual adult dose was 250 mg per day orally with the duration of treatment being dependent on the site and extent of the infection (Suhonen *et al*., 1999).

Kotnik *et al.* (2001) evaluated the effectiveness of terbinafine in the treatment of Microsporosis in cats and suggested that higher dose of drug i.e., 30 to 40 mg per kg body weight orally SID was required for therapy.

The daily dose of terbinafine in human beings was 125 mg twice orally in the treatment of *Tinea* infections (Papich *et al*. 2001).

Atzori *et al.* (2003) observed a complete clinical and mycological recovery from atypical ringworm infection within 30 days of systemic therapy with terbinafine (250 mg per day) in *T. mentagrophytes* infected patients and the lesions completely disappeared in seven days.

It was reported that *T. mentagrophytes* infected patient treated with terbinafine at the dose rate of 250 mg per day orally for 14 days showed good response. After 14 days of therapy, all the lesions healed and cultures were negative. During an one-year follow-up, the patient had been free of cutaneous lesions (Gilaberte *et al.*, 2003).

Moriello (2003b) described terbinafine as the newest and very effective systemic allylamine antifungal agent that suppressed the biosynthesis of ergosterol by inhibiting the enzyme squalene epoxidase.

Pangiotidou and Eremondi (2004) compared the efficacy, safety and tolerability of an eight-week course of oral terbinafine at different doses in *M. canis* infected children. 40 patients were given 6 mg per kg body weight per day, 23 patients with 6 to 7 mg per kg body weight per day and 37 patients were given 7 to 12.5 mg per kg body weight per day and concluded that the administration at a dose of either 6 to 7 or 7 to 12.5 mg per kg body weight per day for eight weeks was safe and effective in the treatment of *M. canis*.

2.7.5 Fungal Vaccines

Mosher *et al*. (1977) treated *M. canis* infected cat with 1 ml intramuscular injections of *M.canis* vaccine, produced by heat inactivation at 60^{o}C and suspended in 0.5 per cent phenol and 0.85 per cent sodium chloride, for five weeks and reported that the lesions regressed and cultures were negative at the end of the treatment.

DeBoer and Moriello (1995) stated that the repeated inoculation of young cats with unadjuvanted cell wall material from *M. canis* induced a measurable humoral and cell mediated immune response to the organism. Despite the induction of immune response, the cats were not protected from experimental exposure since the induction of high antibody titers against *M. canis* were not sufficient to provide protection against infection.

Gudding and Lund (1995) demonstrated that vaccines against ringworm must be capable of eliciting both humoral and cellular immune responses, of which the cellular response was the most crucial for protection.

Beale (2000) opined that the antifungal vaccines were intended as an adjunct to traditional therapy, not a replacement and stated that vaccination had not been demonstrated to eliminate *M. canis* organisms from infected animals and only decreased the severity of the clinical signs.

Radostits *et al.* (2000) stated that vaccination against dermatomycosis had achieved a great deal of success in preventing infection in cattle and horses in most countries of Europe and Scandinavia. Vaccines included those containing highly immunogenic strains, or attenuated strain of fungi or those killed vaccines containing a specific fraction of mycelia.

Seedy *et al*. (2003) assessed the humoral and cellular immune responses of calves and guinea pigs infected with *T. verrucosum*, using ELISA and macrophage migration inhibition test, respectively in both the species, after administration of

four types of vaccines. It was observed that marked positive delayed type skin reactions were clearly observed in all vaccinated calves, with different vaccines after 48 hours, and highest cellular immune response in guinea pigs was observed after the second injection.

Fungal vaccines were not protective against challenge exposure, but they were associated with a temporary reduction in the severity and extend of clinical signs in dermatomycosis (Moriello, 2003b).

Vermout *et al.* (2004) tested a purified recombinant keratinolytic metalloprotease (gamma-MEP3) as a subunit vaccine in experimentally infected guineapigs and evaluated both humoral and cellular immune responses by ELISA and *in vitro* lymphocyte transformation test respectively. Vaccination induced a strong antibody response and transient lymphoproliferative response, but failed to prevent fungal invasion or development of dermatophytic lesions.

Materials and Methods

3. MATERIALS AND METHODS

Dogs presented to University veterinary hospitals at Mannuthy and Kokkalai from April 2003 to March 2004 with clinical history and lesions, which were suggestive of dermatomycosis, formed the experimental animals for the study.

Selection of Cases

Important criteria for the selection were the following

1. Dogs with characteristic ring-like lesions and pruritus.
2. Dogs with clinical signs, which included any combination of pruritus, scaling, hairloss, fragile hairs, frayed hairs, hyperpigmentation and erythema.
3. Dogs with protracted course of skin problems or recurrent infections treated with different treatment regimens.
4. Debilitated dogs with skin problems especially long-haired breeds, which were not given grooming.
5. Animals that were given bath frequently, without grooming.
6. Dogs with skin problems brought to hospital during hot and humid weather conditions.

3.1OUTLINE OF STUDY

3.1.1 Data Collection

Epidemiological data regarding animals were collected as per proforma (Appendix-I)

3.1.2 Collection of Skin Scrapings

3.1.2.1For Direct Microscopic Examination

Hairs and scales were plucked from the periphery of the lesions aseptically, after cleaning the area with 70 per cent alcohol and drying as described by Muller and Kirk (1969) and Quinn *et al.* (1994).

3.1.2.2For Fungal Culture

The skin lesions were cleaned with 70 per cent alcohol to remove bacterial contaminants as completely as possible and the area was allowed to dry. The hairs from several sites were collected into a paper envelope by grasping the hair shafts close to the skin, as suggested by Jungerman and Schwartzman (1972) and Foil (1990).

3.1.3 Blood Collection

3.1.3.1 Whole Blood

Three ml of blood was collected directly from cephalic or saphenous vein of the animal in a clean dry sterile vial with 3 mg of EDTA for the estimation of haemoglobin, total erythrocyte count, total leucocyte count and packed cell volume estimations (Benjamin, 2001).

A drop of blood was placed on a clean, grease free glass slide and a thin smear was drawn for differential leucocyte count.

3.1.3.2 Plasma

For the blood glucose estimation, three ml of blood was collected in a sterile vial containing sodium fluoride (10 mg per ml of blood) and plasma was separated from RBCs as soon as possible.

3.1.3.3 Serum

Three ml of blood was collected in a clean dry sterile syringe for separating serum for biochemical analysis. Sera collected were stored at – 20^0C until further use.

3.1.4 Tab Fungicide : Ketoconazole 200mg (Torrent Pharmaceuticals, Ahmedabad.)

3.1.5 Tab Exifine : Terbinafine 250mg (Dr. Reddy's Laboratories, Ltd, Hyderabad.)

3.1.6 Biocan M inj (BIOVETA PLC, Czech republic)

Composition:

Immunogenic inactivated strain of *M.canis* - at least 500000 vegetative forms

Two per cent Aluminium hydroxide gel - 0.1 ml

Buffered saline solution - 1 ml

Dosage: 1 ml

Route: Intramuscular injection only

3.1.7 Nizral Shampoo : Ketoconazole (2 per cent) shampoo (Johnson and Johnson, Mumbai)

3.2 METHODS

3.2.1 Direct Microscopic Examination

A small amount of hairs and scales were mounted in three drops of 10 per-cent potassium hydroxide for 10 min before being cover-slipped. The sample was heated gently if needed, for further clearance. When the specimen was cleared, the slide was examined using low power of microscope for the presence of arthrospores and hyphae as described by Jungerman and Schwartzman (1972) and Shearer (1991).

3.2.2 Fungal Culture

Sabouraud's dextrose agar, dermatophyte test medium and mycological agar were used for primary isolation of dermatophytes, from clinical materials.

3.2.2.1 Sabouraud's Dextrose Agar (Hi-media)

Ingredients	Gms per liter
Mycological peptone	10
Dextrose	40
Agar	15

Final pH (at 25°c) 5.6± 0.2

3.2.2.2 Dermatophyte Test Medium

Ingredients	Gms per liter
Papaic digest of Soyabean meal	10
Glucose	10

Phenol Red	0.2
Agar	20

Final pH (at 25°c) 5.5± 0.2

3.2.2.3 Mycological Agar, Modified (Hi-media)

Ingredients	Gms per liter
Papaic digest of soyabean meal	10
Dextrose	16
Agar	16

3.2.2.4 Supplements

Chloramphenicol 0.05 g per liter – antibacterial

Cycloheximide (Acti-dione) 0.5 per liter - to inhibit some faster growing saprophytic fungi.

Antimicrobial agents were added after the agar mixture was autoclaved (20 min at 121°c) and cooled to approximately 50°c, and the plates were poured immediately (Leeming and Notman, 1987).

3.2.2.5 Inoculation of Clinical Materials

A light inoculum of sample was scattered over the surface of the agar and gently pressed down on the medium with a swab or sterile forceps. The plates were sealed with parafilm to prevent dessication and incubated aerobically at room temperature. They were examined daily for the presence of growth and not discarded as negative for three weeks (Quinn *et al.,* 1994).

3.2.2.6 Identification of Fungal Culture

Although gross colonial morphology, rate of growth, pigmentation and texture were used as helpful identification aids, confirmation was made by the microscopic examination of the fungal colony.

3.2.2.7 Lactophenol Cotton Blue (LPCB) (Hi-media)

LPCB stain was used for the examination of microscopical aspects of fungal colonies.

3.2.2.8 **Microscopic Examination of Fungal Colonies**

Wet Mount Method

For microscopic examination, a portion of the aerial growth was removed half-way between the center and the periphery of the colony with flamed, curved bacteriologic inoculating needle or sterilized forceps and sample was transferred to a drop of LPCB stain on a microscopic slide. A coverslip was applied and pressure used directly over the colony fragment to spread out the hyphae and other structures.

Sticky Tape Method

A 6 cm long and 2 cm wide cellotape was taken between the thumb and middle finger and the adhesive side of the cellotape was pressed firmly down with the index finger, on the center of the colony to be examined. The inoculated tape was placed over a drop of LPCB on a microscopic slide and free sticky ends folded over each end of the slide (Jungerman and Schwartzman, 1972 and Quinn *et al*., 1994).

3.2.3 Haematology

3.2.3.1 Haemoglobin

Haemoglobin was estimated using acid hematin method as described by Platt (1979).

3.2.3.2 Packed Cell Volume

Packed cell volume of the sample was read by Wintrobe method as detailed by Benjamin (2001).

3.1.3.3Total Erythrocyte Count

Erythrocytes were counted using Hemocytometer and Hayem's fluid as detailed by Wintrobe *et al.* (1981).

3.2.3.4 Total Leucocyte Count

Total leucocyte counting was done by using Hemocytometer and Thomas fluid (Benjamin, 2001).

3.2.3.5 Differential Count

It was carried out by the method described by Meinkoth and Clinkenbeard (2000).

3.2.4. Serum Biochemistry

All biochemical estimations excluding minerals estimation were done by Spectrophotometry in Merck 200 Spectrophometer. Reagents and standards of Agappe's Diagnostics, Maharashtra, were used.

3.2.4.1 Blood Glucose

Blood glucose was estimated by using glucose oxidase method in Agappe's kit as described by Sacks (2001).

3.2.4.2 Cholesterol

Cholesterol oxidase peroxidase method was utilised for cholesterol estimation in enzymatic colorimetric method (Bruss, 1997).

3.2.4.3 Creatinine

Creatinine was estimated by using Jaffe method of analysis by (Newman and Price, 2001).

3.2.4.4 Total Protein

The total protein concentration of the sera was determined by direct Biuret method (Gormall *et al.*, 1949).

3.2.4.5 Albumin

Albumin concentration of the sera was determined by Bromocresol green method (Doumas *et al.*, 1971).

3.2.4.6 Globulin

Globulin concentration was derived from known total protein and albumin values (Benjamin, 2001).

3.2.4.7 A: G ratio

*The A/G r*atio was calculated.

3.2.4.8 Serum Minerals

Serum copper, zinc and iron were estimated using Atomic Absorption Spectrophotometry (AAS) as described by Perkin-Elmer (1982). Sera samples were diluted in deionised water at the ratio of 1:5 and values of copper, zinc and iron were read directly using AAS Perkin-Elmer model-3380, with respective standard solutions diluted in 10 per cent glycerol.

3.2.5 Therapeutic Trials

Animals, which were positive for the presence of arthrospores by direct microscopic examination, were randomly divided into three groups, each consisting of nine animals. Skin scrapings and blood (whole blood, plasma and serum) were collected from the 27 experimental animals on the day of presentation and day 14 post- treatment.

Samples collected from nine apparently normal healthy animals served as control group.

3.2 5.1 Group 1 (Oral Ketoconazole)

In the first group, animals were treated with ketoconazole (Fungicide-Tab 200mg, Torrent Pharmaceuticals, Ahmedabad.) at the dose rate of 5 mg per kg body weight orally daily for 14 days (Kumar *et al*., 2002).

3.2.5.2 Group 2 (Oral Terbinafine)

Terbinafine, Tab Exifine-250mg was administered in group 2 animals at the dose rate of 4 mg per kg body weight orally daily for 14 days (Jones, 1995 and Gilaberte *et al.*, 2003).

3.2.5.3 Group 3 (Combination of "Biocan M' vaccine and Topical Application of Ketoconazole Shampoo)

In the group 3, animals were given inactivated *Microsporum canis* vaccine (Inj. "Biocan M") intramuscularly single dose, along with Nizral (ketoconazole) shampoo as bath at 4 days intervals for two weeks (Beale, 2000 and Ahuja *et al*., 2002).

3.2.6 Statistical Analysis

Epidemiological data were collected and analyzed. In all the clinical trials, efficacy was assessed based on observation of the clinical improvement, culture results and evaluation of haematological and biochemical parameters.

Results

4. RESULTS

4.1 OCCURRENCE

A total of 11016 dogs were brought to the University veterinary hospitals at Kokkalai and Mannuthy from April 2003 to March 2004 different clinical illness. Arthrospores were observed on skin scrapings taken from 94 dogs out of 11016 dogs on direct microscopic examination. These 94 dogs were included in the infected group and subjected to the detailed study. The overall occurrence of dermatomycosis was found to be 0.85 per cent (Fig.1). Among the infected group, 27 animals were randomly selected and divided in to three groups each consisting of nine animals (experimental groups) and the efficacy of three treatment regimens were evaluated.

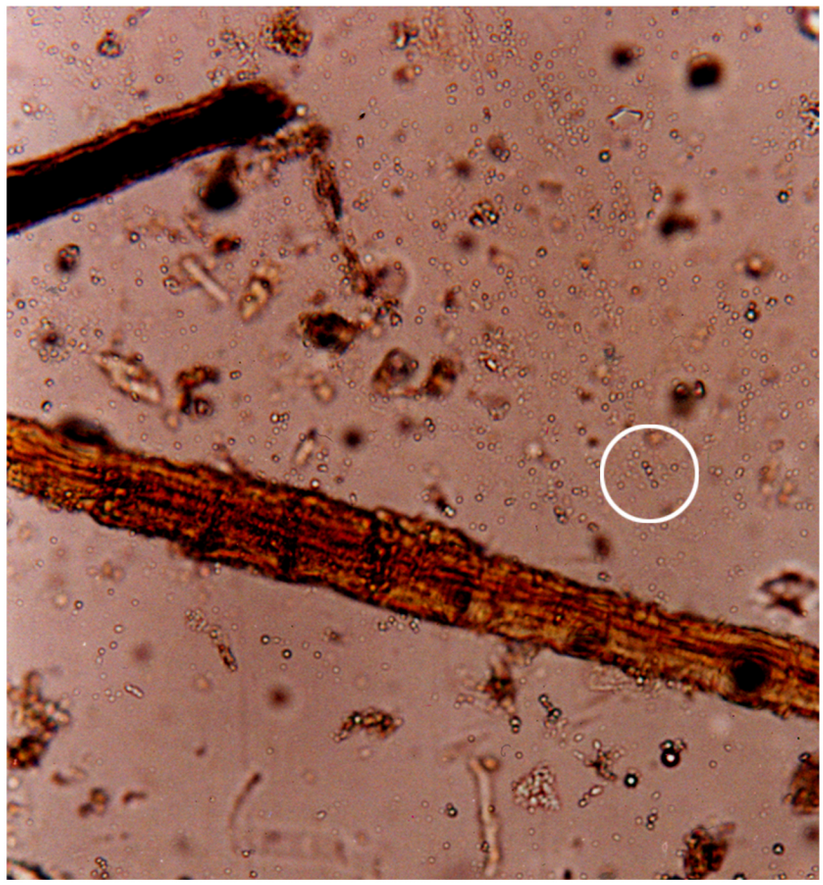
4.1.1 Agewise Occurrence

The agewise occurrence of dermatomycosis is given in Table1 and Fig. 2. The highest occurrence of dermatophyte infection was noticed in dogs of 1 to 6 months of age group (28.72 per cent), followed by 6 months to 1 year (25.53 per cent), 1 to 3 years (23.41 per cent), 3 to 6 years (13.83 per cent) and above 6 years (8.51 per cent) among the total of 94 animals in infected group.

But statistical analysis using chi-square test revealed that there was no significant difference (χ^2 =0.1906 which is less than table value 0.711) between different age groups.

4.1.2 Breedwise Occurrence

Dermatomycosis was observed in various breeds of canines *Viz* Dachshund, Labrador retriever, German shepherd, Spitz, Doberman, Cocker spaniel, Rottweiler, Boxer, Great dane, Bulldog, as well as in non-descript and crossbred dogs. Highest occurrence was observed in Dachshunds (23.40 per cent), followed by Labrador retrievers (18.09 per cent), German shepherds (14.89 per cent), Rottweilers (12.77 per cent), Crossbreds (8.51 per cent), Doberman (7.45 per cent), Spitz (6.39 per



A

B

Plate -1 Direct Microscopic Examination of Skin scrapings

(A)Positive for arthrospores

(B)Negative for arthrospores

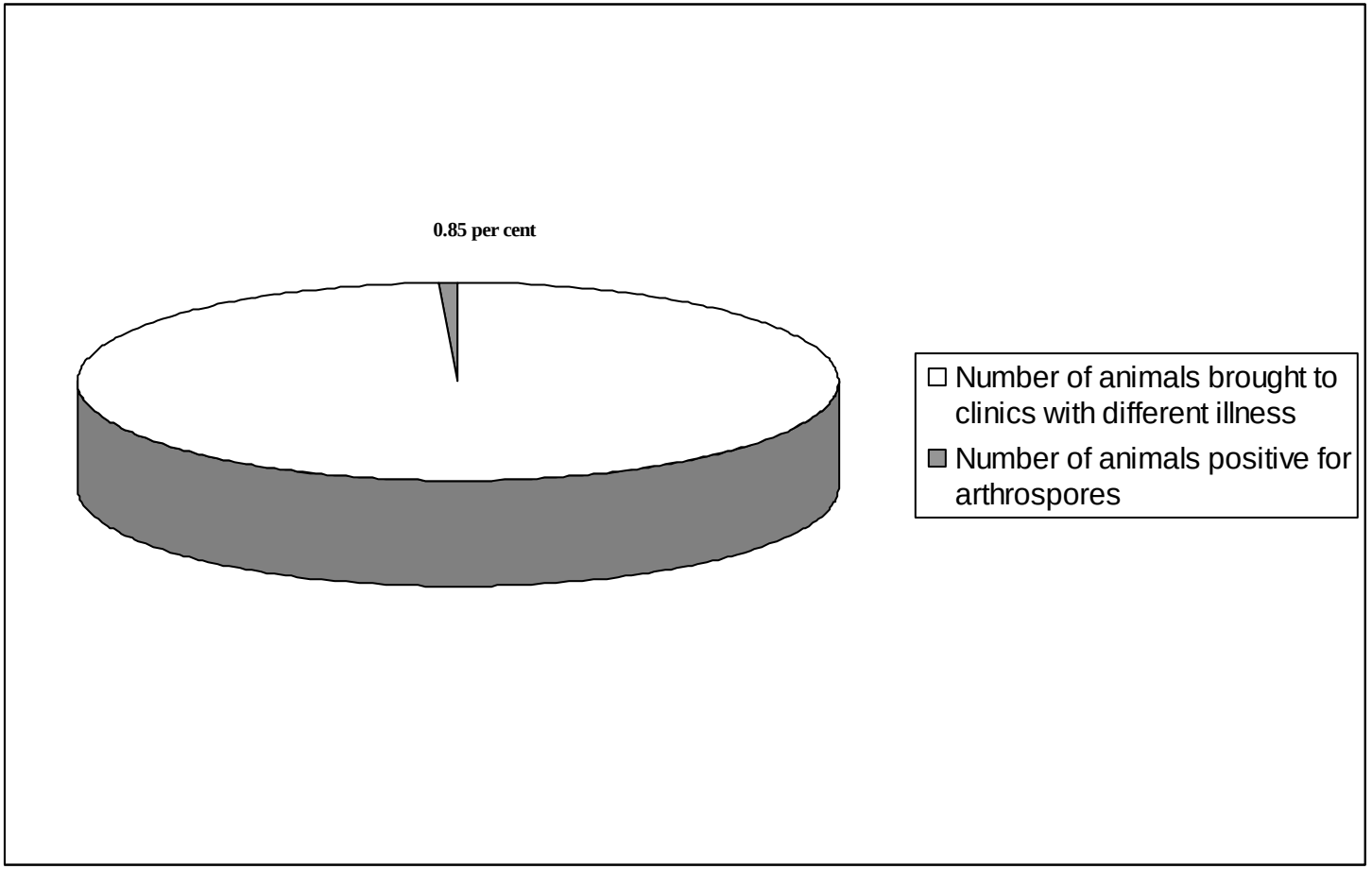


Fig.1. Overall occurrence of dermatomycosis in dogs

cent), Non- descript (3.19 per cent), Cocker spaniel (2.13 per cent) and Boxer, Bulldog and Great dane (1.06 per cent each). Occurrence of dermatomycosis in 94 dogs in the order of decreasing frequency is shown in Table 2 and Fig.3.

Statistical analysis carried out using chi-square test and there was no significant difference (χ^2 =0.00038 which is very less when compared to the table value 4.57) between different breeds of dogs.

4.1.3 Monthwise Occurrence

Dermatomycosis infections had been reported through out the year. However, more number of dermatophyte infected cases brought to the hospitals during the month of November (22.34 per cent), followed by December (18.09 per cent) and lower number of cases reported in March and April (3.19 per cent each). In other months such as May, June, July, August, September, October, January and February rate of occurrence were 9.57 per cent, 7.45 per cent, 5.32 per cent, 6.38 per cent, 6.38 per cent, 8.51 per cent, 5.32 per cent and 4.26 per cent respectively. But infection persisted during almost all months of the year. Monthwise occurrence of dermatomycosis is given in Table 3 and Fig. 4.

Statistical analysis using chi-square test proved that no significant difference was recorded between (χ^2 =0.45 which is less than table value 4.57) different months of the year.

4.1.4 Sexwise Occurrence

Out of a total of 11016 animals, 5861 were females and remaining 5155 animals were males. Among the infected group of 94 animals, 50 animals were female (53.19 per cent) and remaining 44 animals were males (46.81 per cent). Results are presented in Table 4 and Fig. 5.

Table 1. Agewise occurrence of dermatomycosis in dogs

Age groups	Number of animals positive for arthrospores	Per cent	Number of animals brought to clinics with different illness
1 to 6 months	27	28.72	3284
6 months to 1 year	24	25.53	2813
1 to 3 years	22	23.41	2578
3 years to 6 years	13	13.83	1524
Above 6 years	8	8.51	817
Total	**94**	**100.00**	**11016**

Table 2. Breedwise occurrence of dermatomycosis in dogs

Breeds	Number of animals positive for arthrospores	Per cent	Number of animals brought to clinics with different illness
Dachshund	22	23.40	2582
Labrador retriever	17	18.09	1992
German shepherd	14	14.89	1641
Rottweiler	12	12.77	1406
Crossbred	8	8.51	938
Doberman	7	7.45	820
Spitz	6	6.39	703
Non descript	3	3.19	349
Cocker spaniel	2	2.13	234
Boxer	1	1.06	117
Bull dog	1	1.06	117
Great dane	1	1.06	117
Total	**94**	**100.00**	**11016**

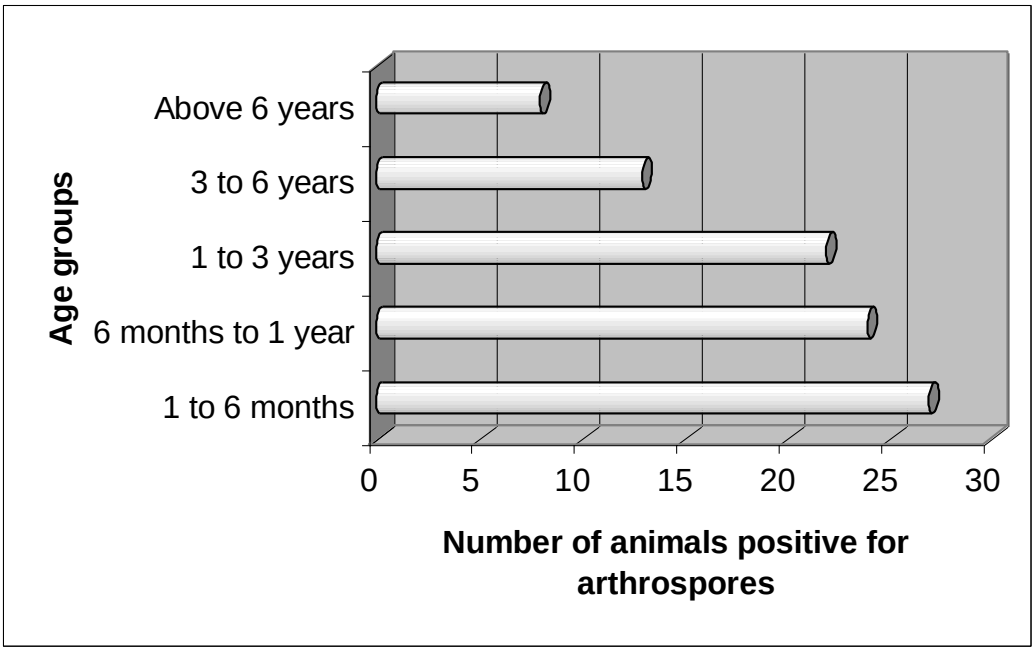


Fig. 2. Agewise occurrence of dermatomycosis in dogs

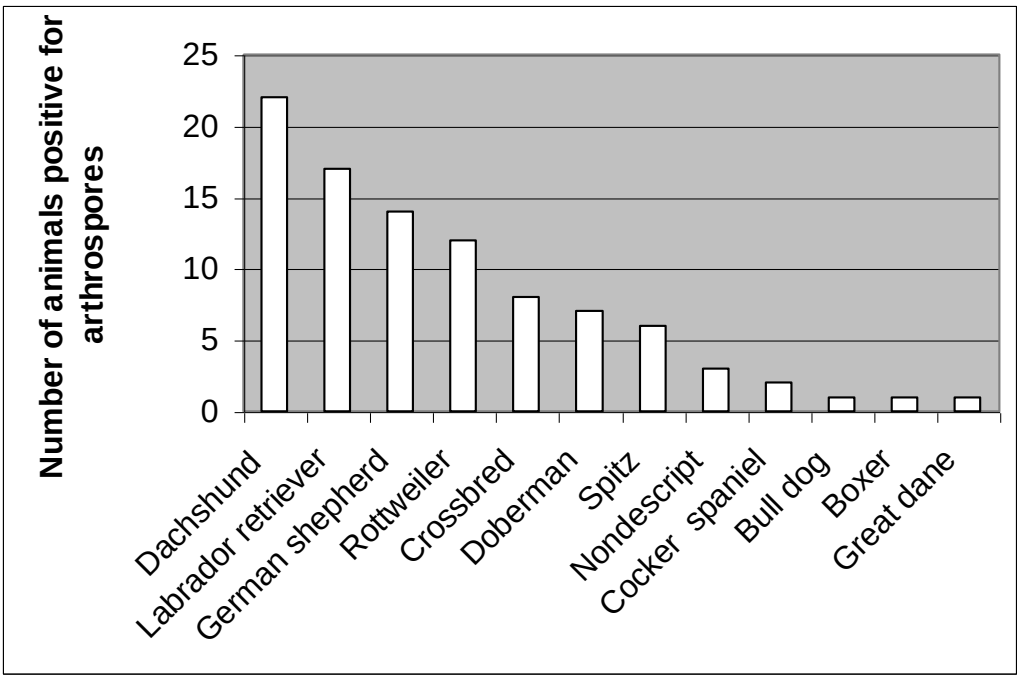


Fig. 3. Breedwise occurrence of dermatomycosis in dogs

Table 3. Monthwise occurrence of dermatomycosis in dogs

Months	Number of animals positive for arthrospores	Per cent	Number of animals brought to clinics with different illness
April 03	3	3.19	349
May	9	9.57	1055
June	7	7.45	820
July	5	5.32	586
August	6	6.38	703
September	6	6.38	586
October	8	8.51	1992
November	21	22.34	2461
December	17	18.09	931
January 04	5	5.32	588
February	4	4.26	479
March	3	3.19	466
Total	**94**	**100.00**	**11016**

Table 4. Sexwise occurrence of dermatomycosis in dogs

Sex	Number of animals positive for arthrospores	Per cent	Number of animals brought to clinics with different illness
Female	50	53.19	5861
Male	44	46.81	5155
Total	**94**	**100.00**	**11016**

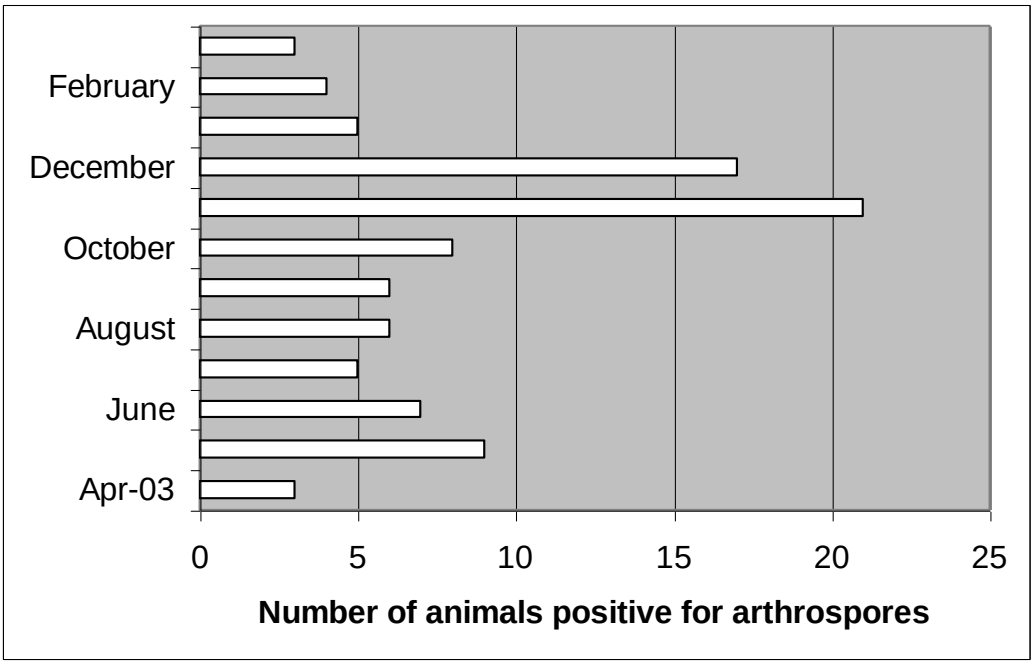


Fig. 4. Monthwise occurrence of dermatomycosis in dogs

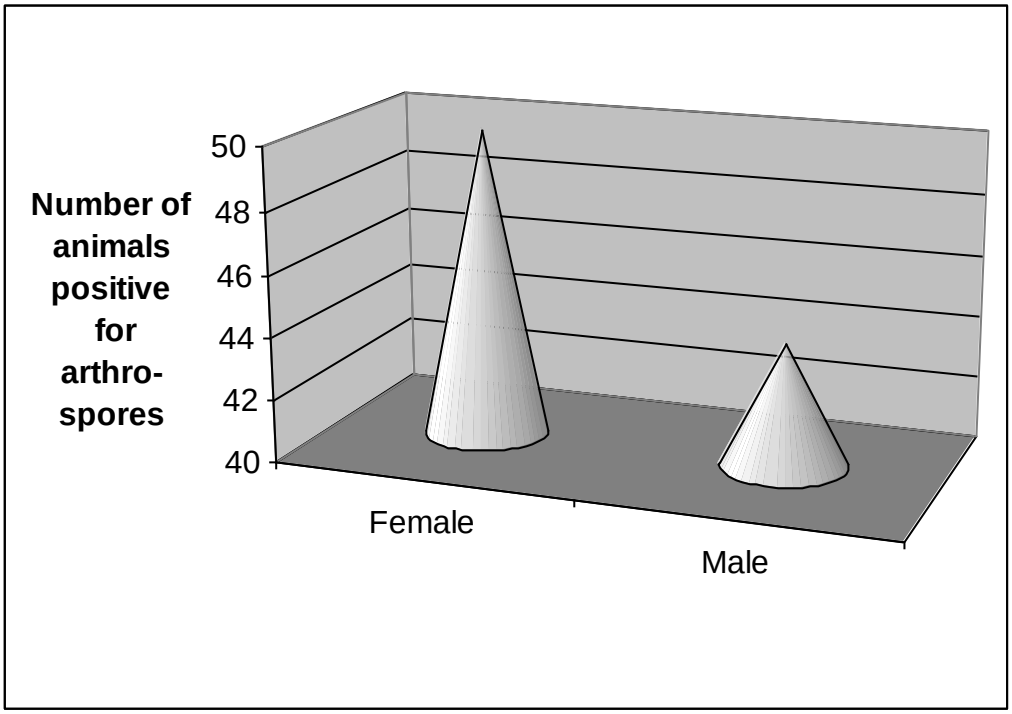


Fig. 5. Sexwise occurrence of dermatomycosis in dogs

Statistical analysis using test of proportion showed no significant difference (Z=0.005 which is less than the table value 1.96) between the two sexes.

4.2 CLINICAL SIGNS

4.2.1 Pruritus

Majority of the animals (67 out of 94) were presented with a history of pruritus. It varied in intensity from mild occasional itching to severe constant itching. Both generalised and localized itching were observed. Owners reported that the animals rubbing their body on wall and other hard objects to relieve irritation. Erythema and dried exudates were noticed on many areas of the animal's body as a consequence to that.

4.2.2 Type of Lesions

Primary lesions were not observed in any of the 94 animals in the infected group. But secondary lesions were commonly noticed. Results are presented in Table 5. Infected animals were presented with a variety of lesions. Alopecia with scales were noticed in 39 animals (41.48 per cent), alopecia combined with exfoliation (shedding of scales) were found in 30 animals (31.92 per cent), erythema in six animals (6.38 per cent), scales formation alone in seven animals (7.45 per cent), exfoliation on five animals (5.32 per cent), crusts (dried exudate) and hyperkeratosis (increased thickness) in two animals each (2.13 per cent each) and three animals (3.19 per cent) with hyperpigmentation (excessive colouration) alone.

4.2.3 Distribution of Lesions

Generalised lesions were observed in 61 animals (64.89 per cent) and localised lesions in 33 animals (35.11 per cent).

Table 5. Occurrence of secondary lesions

Secondary lesions	Number of infected animals positive for arthrospores	Per cent
Alopecia	39	41.48
Alopecia and exfoliation	30	31.92
Scales alone	7	7.45
Scars	0	0
Excoriation	0	0
Hyperpigmentation	3	3.19
Hypopigmentation	0	0
Erosion	0	0
Erythema	6	6.38
Exfoliation alone	5	5.32
Crusts	2	2.13
Ulcers	0	0
Lichenification	0	0
Hyperkeratosis	2	2.13
Abscess	0	0
Callus	0	0
Alopecia alone	0	0
Total	**94**	**100.00**

Table 6. Practice of bathing

Frequency of bath	Daily	Once in 2 days	Once in a week	Once in fortnight	Once in a month	Once in 2 months	Irregular
No. of infected animals	-	-	42	19	-	-	33
Per cent	-	-	44.68	20.21	-	-	35.11

4.3 MANAGEMENT

4.3.1 Practice of Bathing

In infected group of 94 animals, 42 animals (44.68 per cent) were given bath once in a week, 19 animals (20.21 per cent) once in fortnight and 33 animals (35.11 per cent) were given bath irregularly (Table 6).

4.3.2 Practice of Grooming

Grooming was not practised in majority of the animals (55.32 per cent), 34 animals (36.17 per cent) were given occasional grooming and only eight animals (8.51 per cent) were given daily grooming. Results are given in Table 7.

4.4 DIRECT MICROSCOPIC EXAMINATION

On direct microscopic examination of skin scrapings, 80 per cent of samples in infected group revealed the presence of ectothrix small spores, arranged in masses around the infected hair shaft (Plate1-A) and 20 per cent animals of the infected group showed the presence of endothrix spores within the infected hair shafts. All spores appeared as a clear round translucent pearl like beads.

4.5 CULTURE EXAMINATION

Samples taken from dogs, which were positive for the presence of arthrospores were inoculated in to Sabouraud's dextrose agar with chloramphenicol and cycloheximide (CC) supplement and dermatophyte test medium with CC supplement and kept atleast for three weeks at room temperature for incubation. Majority of the inoculated plates showed the presence of contaminants such as *Aspergillus* spp, *Penicillium* spp and *Mucor* spp within a week of time. These were confirmed based on its rapid rate of growth, colony characters and presence of conidiophores. About 40 per cent of the cultured plates showed no growth for more than three weeks of time. Altogether three isolates of *Trichophyton* spp and four isolates of *Microsporum* spp were identified from samples of 94 animals.

Table 7. Practice of grooming

Practice	No. of infected animals	Per cent
Daily practised	8	8.51
Occasionally practised	34	36.17
Not practised	52	55.32
Total	**94**	**100.00**

Table 8. Haematological parameters of dermatophyte infected group with that of control group.

Haematological parameters	Mean values± SD		t values
	Infected group n= 94	Control group n=9	
Haemoglobin (g/dl)	11.986 ± 0.725	13.356 ± 0.639	5.4646^{**}
Packed cell volume (per cent)	35.979 ± 2.342	36.889 ± 1.269	5.1465^{NS}
Total erythrocyte count (10^6/ mm^3)	5.977 ± 0.609	6.244 ± 0.461	0.2026^{NS}
Total leucocyte count ($10^3/mm^3$)	12.841 ± 0.633	14.367 ± 0.464	7.0414^{**}
Neutrophils (per cent)	62.979 ± 6.411	62.333 ± 1.871	0.7107^{NS}
Lymphocytes (per cent)	26.181 ± 5.935	28.333 ± 3.742	1.0651^{NS}
Eosinophils (per cent)	6.660 ± 1.992	5.778 ± 1.563	1.2883^{NS}
Monocytes (per cent)	4.128 ± 1.497	3.333 ± 2.121	0.1465^{NS}

n=Number of animals in each group
** - Significant variation (P<0.01)
NS – Non significant variation

4.5.1 *Trichophyton* spp

Growth obtained nearly after one month of inoculation in to the Sabouraud's dextrose CC agar.

4.5.1.1 Colony Morphology of Trichophyton spp

Obverse side - Whitish fluffy (Plate 2-A).

Reverse side - Yellow to orange (Plate 2-B).

4.5.1.2 Microscopic Identification of Trichophyton spp

Septate hyphae with chlamydoconidia (often in chains) were observed by cellotape method using lactophenol cotton blue staining (Plate 2-C and 3-D).

4.5.2 *Microsporum* spp

Growth was observed after 21 days of inoculation in to a dermatophyte test medium.

4.5.2.1 Colony Morphology of Microsporum spp

Obverse side- Surface was coarse, powdery and yellowish (Plate 2-E).

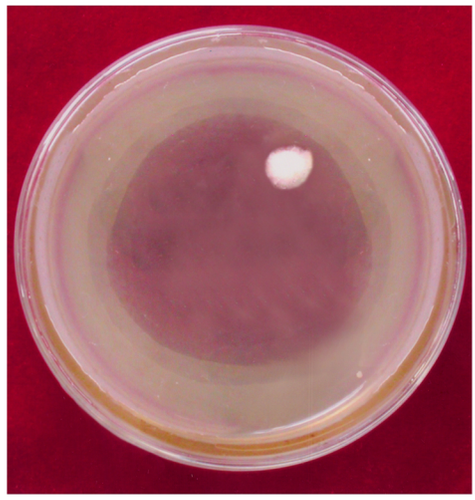
Reverse side- Deep red (Plate 2-F).

4.5.2.2 Microscopic Identification of Microsporum spp

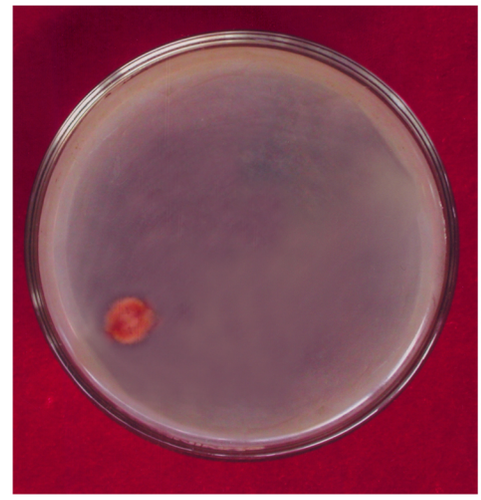
Septate hyphae with club shaped microconidia were identified using cellotape method. But no macroconidia was seen.

4.6 HAEMATOLOGICAL PARAMETERS

Haematological parameters of infected and control group are presented in Table 8.



A



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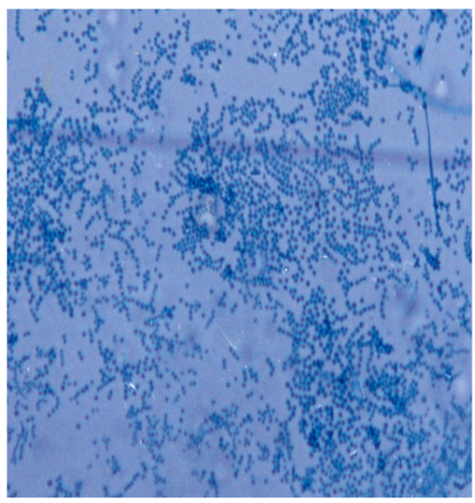

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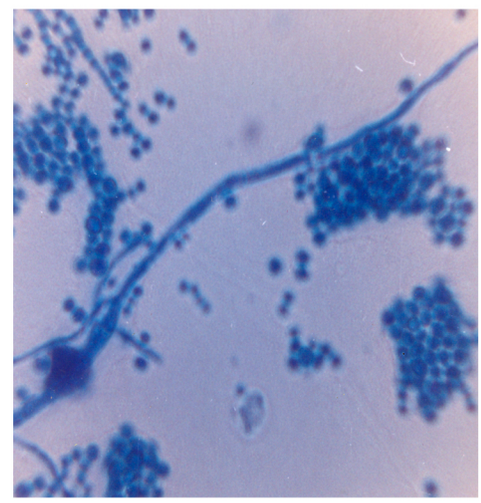

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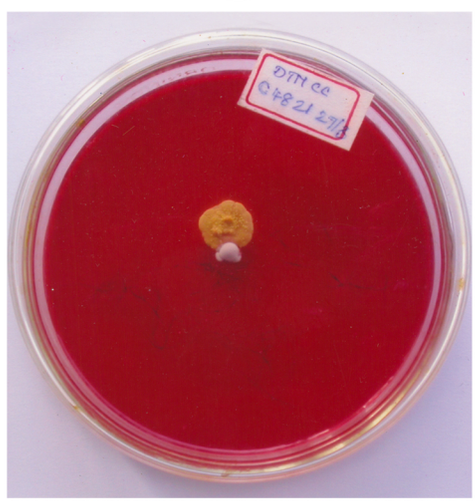

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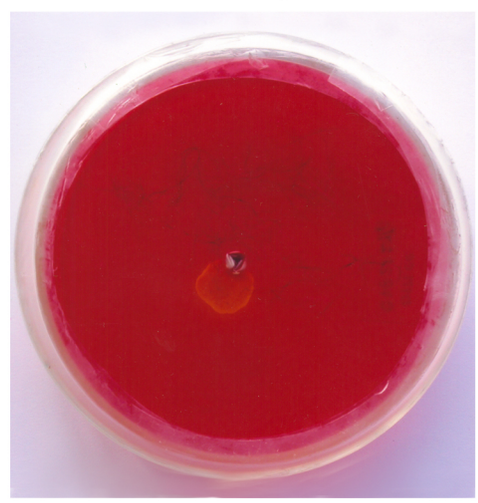


F

Plate - 2 Culture Results

(A) Colony characters of *Trichophyton* spp (Obverse side)

(B) Colony characters of *Trichophyton* spp (Reverse side)

(C) *Trichophyton* spp hyphae with chlamydospores forming chains (10x)

(D) *Trichophyton* spp hyphae with chlamydospores forming chains (40x)

(E) Colony characters of *Microsporum* spp (Oberse side)

(F) Colony characters of *Microsporum* spp (Reverse side)

4.6.1 Haemoglobin

Mean haemoglobin content of infected group (11.986 $\pm$ 0.725 g/dl) was significantly lower than that of control group (13.356 $\pm$ 0.639 g/dl). Statistical analysis using 't' test revealed that a significant difference ($P<0.01$) existed between haemoglobin contents of infected and control group.

4.6.2 Packed Cell Volume

Infected group had a slight lower mean value of packed cell volume (35.979 $\pm$ 2.342 per cent) when compared to the mean value of control animals (36.889 $\pm$ 1.269 per cent), but both the values were within the normal range. The 't' test revealed no significant variation ($P>0.05$) between the packed cell volumes of infected and control group.

4.6.3 Total Erythrocyte Count

Mean total erythrocyte count of infected group (5.977 $\pm$ 0.609 10^6/ mm^3) varied slightly from the total erythrocyte count of the control group (6.244 $\pm$ 0.461 10^6/ mm^3). Statistical analysis by using 't' test showed no significant variation ($P>0.05$) between the total erythrocyte counts of the two groups.

4.6.4 Total Leucocyte Count

Mean total leucocyte count of infected group (12.841 $\pm$ 0.633 10^3 /mm^3) was significantly lower than that of control group (14.367 $\pm$ 0.464 10^3 /mm^3). Statistical analysis using 't' test recorded highly significant difference ($P<0.01$) between total leucocyte counts of the infected and control group.

4.6.5 Differential Count

Only slight differences were noticed between infected and control group on mean values of neutrophils (62.979 $\pm$ 6.411 per cent and 62.333 $\pm$ 1.871 per cent), lymphocytes (26.181 $\pm$ 5.935 per cent and 28.333 $\pm$ 3.742 per cent),

eosinophils (6.660 ± 1.992 per cent and 5.778 ± 1.563 per cent) and monocytes (4.128 ± 1.497 per cent and 3.333 ± 2.121 per cent) respectively and their values were within the normal range. Statistically no significant variation (P>0.05) were observed on neutrophil, lymphocyte, eosinophil and monocyte count of infected and control group by 't' test.

4.7 BIOCHEMICAL PARAMETERS

Biochemical parameters of infected and control groups are presented in Table 9.

4.7.1 Blood Glucose

Infected group had low blood mean blood glucose level (101.106 ± 19.378 mg/dl) compared to the mean vale of control group (105.222 ± 11.032 mg/dl) and both the values were within the normal range. Statistical analysis showed that no significant variation (P>0.05) in blood glucose values of two groups.

4.7.2 Cholesterol

Mean value of cholesterol of infected group (82.138 ± 10.552 mg / dl) was slightly higher than that of control group (78 ± 8.216 mg / dl). However, no significant difference (P>0.05) existed between the values of cholesterol of the two groups.

4.7.3 Creatinine

No significant variation (P>0.05) between creatinine content of two groups were obtained by statistical analysis using 't' test. But slightly low mean value of creatinine was estimated in infected group (0.981 ± 0.227 mg / dl) compared to that of control group (0.944 ± 0.167 mg / dl).

4.7.4 Total Protein

Higher albumin and globulin contents of infected group themselves indicated a higher content of total protein in infected animal (10.842 ± 1.161 g/dl) which was significant (P<0.01) from the total protein content of control group (6.633 ± 0.568 g/ dl) with the higher value in the infected group.

4.7.5 Albumin

Mean albumin value of infected group (4.777 ± 0.776 g / dl) was significantly higher than that of the control group (3.167 ± 0.245 g / dl). There was highly significant variation (P<0.01) between two groups on the albumin content in the sera of two groups.

4.7.6 Globulin

Mean globulin content of infected group (6.066 ± 0.995 g / dl) was also significantly higher than that of the control group (3.467 ± 0.442 g / dl). Statistical analysis indicated highly significant variation (P<0.01) between two groups on the globulin content of sera.

4.7.7 A/G Ratio

The mean A/G ratios of infected and control groups were 0.796 ± 0.111 and 0.925 ± 0.126 respectively. Increased A/G ratio was noted on infected group compared to control group. Statistical analysis showed highly significant variation (P<0.01) on A/G between the two groups.

4.8 SERUM MINERAL STATUS

The mean values of copper, iron and zinc of infected group were 0.143 ± 0.082 ppm, 0.364 ± 0.042 ppm and 0.813 ± 0.204 ppm respectively. On the other hand the mean values of control group were 0.167 ± 0.141 ppm, 0.359 ± 0.033 ppm and 0.876 ± 0.147 ppm respectively. Statistical analysis using ‘t’ test showed no

significant difference (P>0.05) between the serum mineral status of infected and control group. But mean values of copper and iron in both the group were lower than the normal range (0.5 to 1 ppm for copper and 0.6 to 1.9 ppm for iron in dogs). However the mean zinc values of both the groups were in the normal range (Normal range of serum zinc is between 0.8 and 1.2 ppm). Results are given in Table 10.

4.9 TREATMENT TRIALS

4.9.1 Treatment with Oral Ketoconazole (Group 1)

4.9.1.1 Clinical Response to Ketoconazole

The clinical signs, including erythema and alopecia subsided in all the animals by day 7. But complete remission of lesions (Plate 3-A and 3-B) and regrowth of hairs in all the infected areas were noticed only by day 14. However, two animals had mild itching at the end of the treatment (day 14) without any lesions, so the therapy was continued for one more week for the complete cure of the two dogs and owners reported that the animals were free from itching after the one-week treatment. Skin scrapings taken from healed lesions, which were noticed in the treated animals were negative for the arthrospores on day 14 of post-treatment. Hence ketoconazole was considered to be consistently effective against dermatomycosis.

4.9.1.2 Haematological Parameters

Increased mean values of packed cell volume and total erythrocyte count in group 1 animals (37.111 $\pm$ 3.480 per cent and 5.956 $\pm$ 0.665 10^6/ mm^3 respectively) were recorded after the treatment of ketoconazole compared to the pre treatment values 35.556 $\pm$ 3.358 pert cent and 5.789 $\pm$ 0.734 10^6/ mm^3 respectively. The data analysed by 't' test showed a significant variation (P<0.05) between pre and post-treatment values using ketoconazole. Mean values of haemoglobin, total leucocyte count, neutrophils, lymphocytes, eosinophils and monocytes of group 1 animals before treatment were 11.778 $\pm$ 1.160 g/ dl, 12.988 $\pm$ 0.604 10^3/ mm^3, 66.889 $\pm$

Table 9. Biochemical parameters of dermatophyte infected group with that of control group

Biochemical parameters	Mean values± SD		t values
	Infected group	Control group	
Blood glucose (mg/dl)	101.106 ±19.378	105.222 ± 11.032	0.6257 ^{NS}
Cholesterol (mg/dl)	82.138 ± 10.552	78.00 ± 8.216	1.1419^{NS}
Creatinine (mg/dl)	0.981 ± 0.227	0.944 ± 0.167	0.4754^{NS}
Total protein (g/dl)	10.842 ± 1.161	6.633 ± 0.568	16.6901**
Albumin (g/dl)	4.777 ± 0.776	3.167 ± 0.245	14.0790**
Globulin (g/dl)	6.066 ± 0.995	3.467 ± 0.442	14.4816**
A/G ratio	0.796 ± 0.111	0.925 ± 0.126	3.2996**

n=Number of animals in each group
**- Significant variation (P<0.01)
^{NS} – Non significant variation

Table 10. Serum mineral status of dermatophyte infected group with that of control group

Minerals ppm	Mean values ±SD		t values
	Infected group n = 30	Control group n = 9	
Copper	0.143 ±0.082	0.167 ±0.141	0.4719^{NS}
Iron	0.364 ±0.042	0.359 ±0.033	0.0349^{NS}
Zinc	0.813 ±0.204	0.876 ±0.147	0.8561^{NS}

^{NS} – Non significant variation

5.904 per cent, 24.111 ± 6.882 per cent, 5.444 ± 0.89 per cent and 3.556 ± 1.236 per cent respectively. Post-treatment mean values were 11.978 ± 1.290 g/dl, 13.102 ± 0.916 $10^3/mm^3$, 67.667 ± 3.841 percent, 22.667 ± 3.873 per cent, 5.667 ± 0.886 per cent and 4.00 ± 1.323 per cent respectively. There was no significant variation (P>0.05) on haemoglobin, total leucocyte count, neutrophil, lymphocyte, eosinophil and monocyte count between pre and post-treatment with ketoconazole. Results of haematological parameters of group 1 animals are presented in Table 11.

4.9.1.3 Biochemical Parameters

Pre and post-treatment mean values of blood glucose (95.667 ± 18.090 and 98.222 ± 19.142 mg/dl), cholesterol (81.111 ± 7.769 and 83.222 ± 7.855 mg/dl) creatine (1.001 ± 0.210 and 1.073 ± 0.275 mg/dl), total protein (10.022 ± 1.470 and 10.122 ± 1.413 g/dl), albumin (4.378 ± 0.777 and 4.411 ± 0.749 g/dl), globulin (5.644 ± 0.948 and 5.711 ± 0.885 g/dl) and A/G ratio (0.787 ± 0.140 and 0.781 ± 0.128) respectively. There was no significant variation (P>0.05) noted in all biochemical parameters before and after treatment with ketoconazole. Results of biochemical parameters of group 1 animals are presented in Table 12.

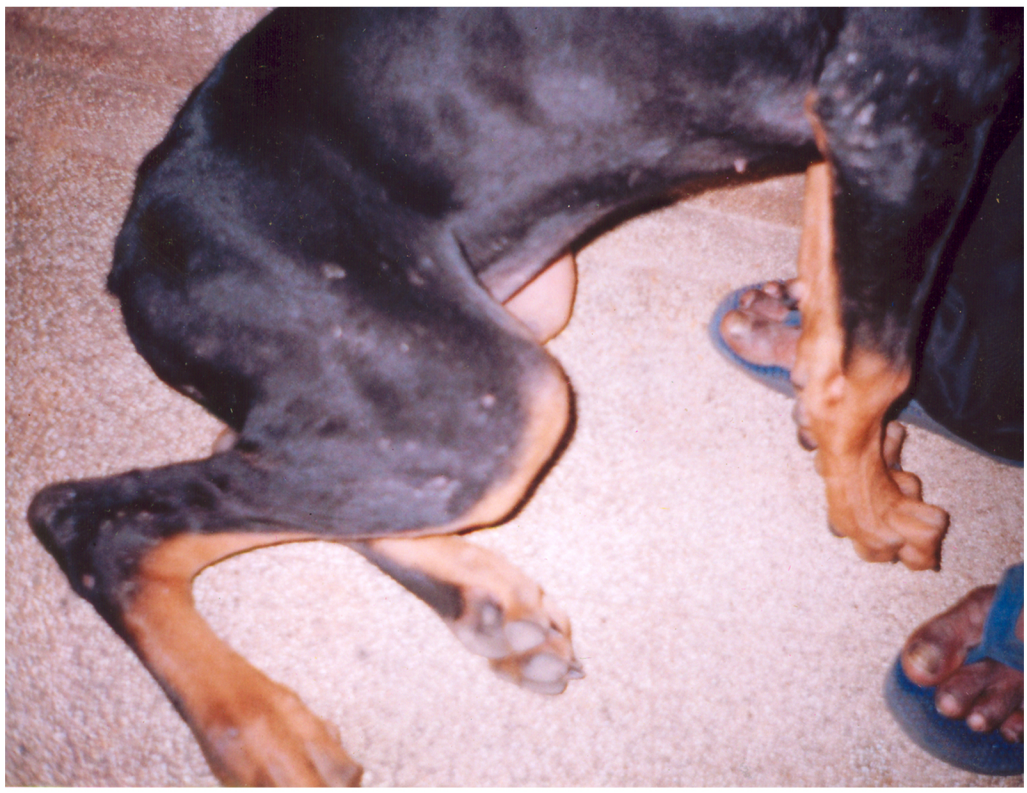
4.9.1.4 Serum Mineral Status

Mean values of copper, iron and zinc of group1 animals before treatment (0.117 ± 0.041 ppm, 0.253 ± 0.039 ppm and 0.827 ± 0.233 ppm respectively) differed slightly from that of the values (0.15 ± 0.055 ppm, 0.273 ± 0.037 ppm and 0.820 ± 0.147 ppm respectively) obtained after the treatment. However, 't' test revealed no significant variation (P>0.05) between the serum mineral contents of pre and post-treatment of ketoconazole (Table 13).

4.9.2 Treatment with Oral Terbinafine (Group 2)

4.9.2.1 Clinical Response to Terbinafine

Terbinafine was found to be effective for the treatment of dermatomycosis in dogs. All the animals in - group 2 showed good recovery after



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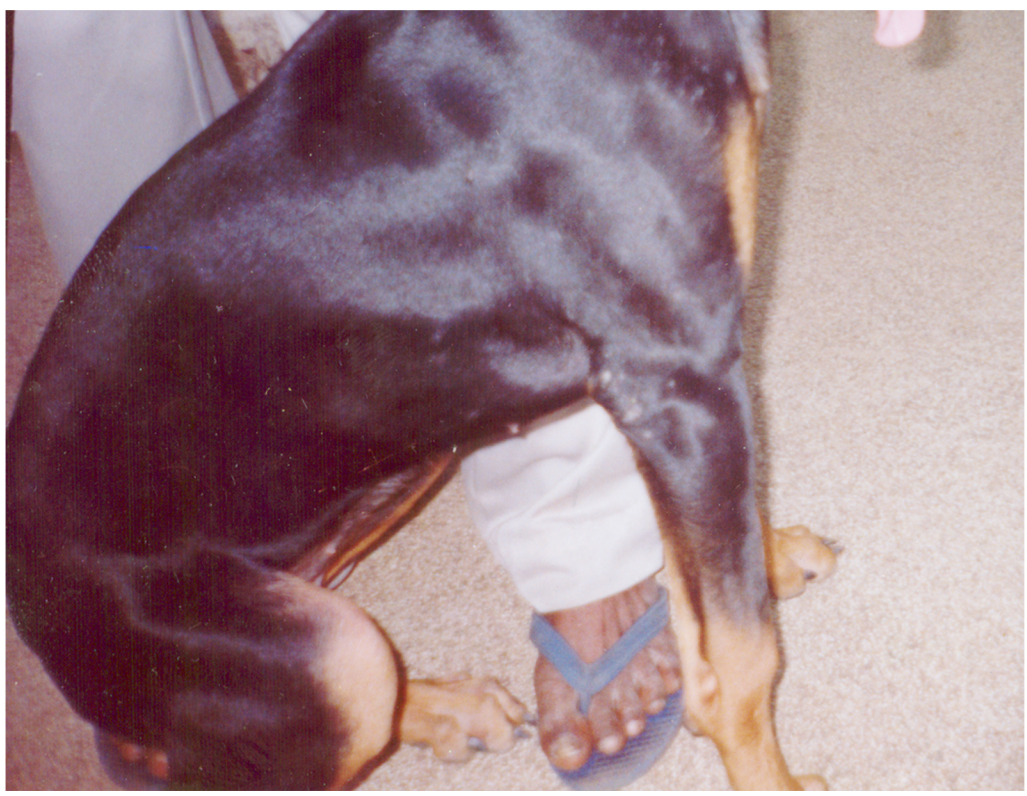


B

Plate - 3 Group 1 Oral Ketoconazole

(A) Before treatment

(B) After treatment (two weeks)

Table 11. Pre and Post-treatment haematological parameters of group 1 animals treated with oral ketoconazole

Haematological parameters	Mean values (Mean ±SD)		t values
	Pre treatment	Post-treatment	
Haemoglobin (g/dl)	11.778 ±1.160	11.978 ±1.290	0.1599^{NS}
Packed cell volume (per cent)	35.556 ±3.358	37.111 ±3.480	2.5776*
Total erythrocyte count (10^6/ mm^3)	5.789 ±0.734	5.956 ±0.665	2.4254*
Total leucocyte count (10^3/mm^3)	12.988 ±0.604	13.102 ±0.916	0.5094^{NS}
Neutrophils (per cent)	66.889 ±5.904	67.667 ±3.841	0.8854^{NS}
Lymphocytes (per cent)	24.111 ±6.882	22.667 ±3.873	1.3200^{NS}
Eosinophils (per cent)	5.444 ±0.89	5.667 ±0.866	0.9961^{NS}
Monocytes (per cent)	3.556 ±1.236	4 ±1.323	0.9363^{NS}

*- Significant variation (P<0.05)
NS – Non significant

Table 12. Pre and Post-treatment biochemical parameters of group 1 animals treated with oral ketoconazole

Biochemical parameters	Mean values (Mean ±SD)		t values
	Pre treatment	Post-treatment	
Blood glucose (mg/dl)	95.667 ±18.090	98.222 ±19.142	2.0655^{NS}
Cholesterol (mg/dl)	81.111 ±7.769	83.222 ±7.855	1.7167^{NS}
Creatinine (mg/dl)	1.001 ±0.210	1.073 ±0.275	1.6052^{NS}
Total protein (g/dl)	10.022 ±1.470	10.122 ±1.413	1.0607^{NS}
Albumin (g/dl)	4.378 ±0.777	4.411 ± 0.749	0.4851^{NS}
Globulin (g/dl)	5.644 ±0.948	5.711 ± 0.885	1.4142^{NS}
A/G ratio	0.787 ±0.140	0.781 ±0.128	0.5198^{NS}

NS – Non significant

the treatment (Plate 4-A and 4-B). There was reduction in skin lesions, pruritus and alopecia by one-week after the onset of treatment and lesions completely resolved by the end of the treatment. Vomiting and regurgitation were the only side effects noticed in two animals during the course of treatment. Few spores were seen on examination of skin scrapings taken from the animals in-group 2 animals at the end of the treatment but they were not of significance to be considered as positive.

4.9.2.2 Haematological Parameters

Mean values of haemoglolin, packed cell volume, total erythrocyte count, total leucocyte count and neutrophils, lymphocyte, eosinophil and monocyte count in-group 2 animals before treatment such as 11.933 $\pm$ 0.808 g/dl, 36.333 $\pm$ 1.732 per cent, 5.811 $\pm$ 0.483 10^6/ mm^3, 12.894 $\pm$ 0.865 10^6/ mm^3, 62.444 $\pm$ 7.038 per cent, 27.111 $\pm$ 6.173 per cent, 6.778 $\pm$ 2.108 per cent and 3.556 $\pm$ 1.333 per cent respectively and post-treatment values are follows 12.022 $\pm$ 0.524 g/dl, 36.667 $\pm$ 2.236 per cent, 5.9 $\pm$ 0.552 10^6/ mm^3, 12.942 $\pm$ 0.996 10^6 /mm^3, 62.889 $\pm$ 7.737 per cent, 26.444 $\pm$ 5.961 per cent 6.444 $\pm$ 1.590 per cent and 4.444 $\pm$ 1.667 per cent respectively. There was significant difference ($P<0.05$) on packed cell volume and total leucocyte count, but no significant difference ($P>0.05$) noted on haemoglobin, total erythrocyte count, and differential count in group 2 animals before and after treatment with terbinafine. Results of haematological values of group 2 animals are shown in Table 14.

4.9.2.3 Biochemical Parameters

Pre and post treatment mean values of cholesterol (89.111 $\pm$ 14.920 mg/dl and 91.667 $\pm$ 15.620 mg/dl), total protein (10.744 $\pm$ 1.408 g/dl and 11.057 $\pm$ 1.194 g/dl), albumin (4.644 $\pm$ 0.749 g/dl and 4.768 $\pm$ 0.765 g/dl) respectively. Statistical analysis showed highly significant difference ($P<0.01$) between pre and post-treatment with terbinafine on cholesterol, albumin and total protein. Where as other values including blood glucose (100.55 $\pm$ 15.150 mg/dl for pre treatment and 99.777 $\pm$ 13.236 mg/dl for post-treatment), Creatinine (1.036 $\pm$ 0.275 mg/dl for pre treatment and 1.113 $\pm$ 0.194 mg/dl for post-treatment), globulin (6.100 $\pm$ 0.707 g/dl for pre

treatment and 6.289 ± 0.571 g/dl for post-treatment) and A/G ratio (0.759 ± 0.070 for pre treatment and 0.758 ± 0.1 for post-treatment), were not of any significant difference before and after treatment with terbinafine (P>0.05). Results of biochemical values of group 2 animals are shown in Table 15.

4.9.2.4 Serum Mineral Status

Mean values of serum minerals such as copper, iron and zinc of group 2 animals before treatment (0.117 ± 0.041 ppm, 0.548 ± 0.055 ppm and 0.748 ± 0.025 ppm respectively) slightly differed from values evaluated after treatment of terbinafine (0.133 ± 0.052 ppm, 0.530 ± 0.050 ppm and 0.765 ± 0.037 ppm respectively). The 't' test showed that there was no significant variation (P>0.05) between the serum mineral status in animals before and after treatment of terbinafine (Table 16).

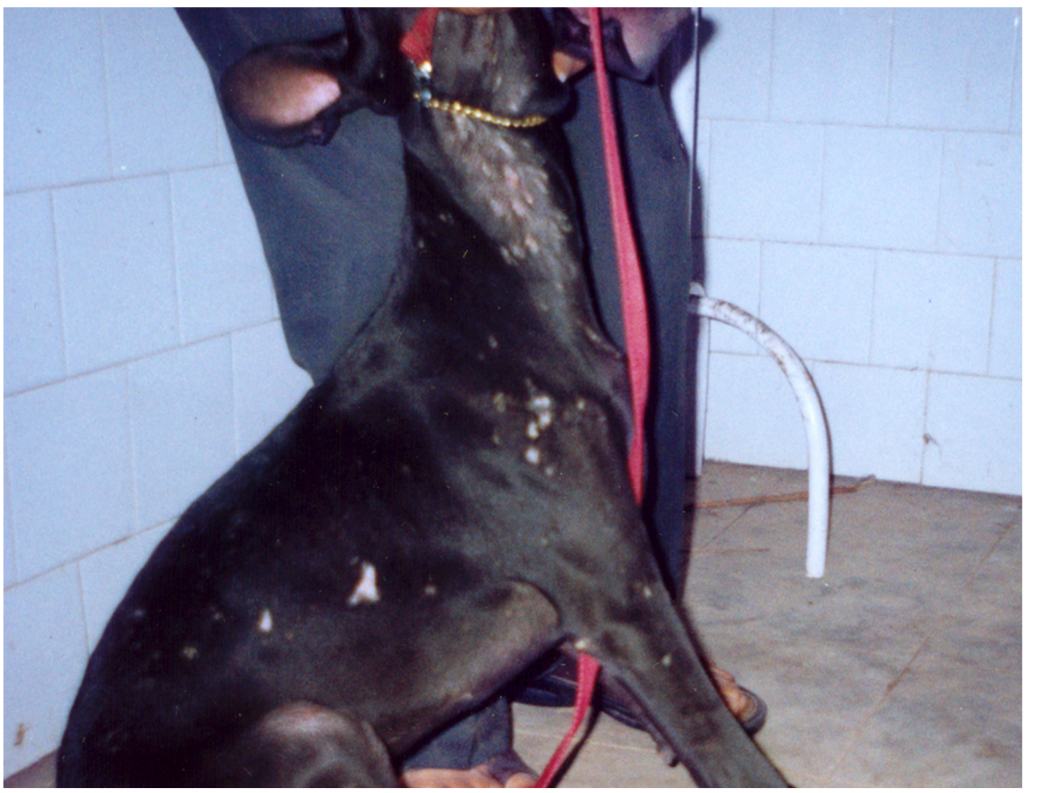
4.9.3 Treatment with "Biocan M" vaccine along with topical application of Ketoconazole Shampoo (Group 3)

4.9.3.1 Clinical Response to Vaccine with Ketoconazole Shampoo

The combination of "Biocan M" vaccine along with topical application of ketoconazole shampoo was not found to be effective. Initially the lesions on the animals seemed to be resolving and later on spreading of infection to other areas was noticed in spite of the continued topical application of ketoconazole shampoo. Although the complete cure had been reported in only one animal out of nine animals, skin scrapings taken from all animals did not show complete mycological cure. However, no side effects were reported in all animals.

4.9.3.2 Haematological Parameters

Total leucocyte count recorded (12.575 ± 0.474 10^3 /mm^3) before treatment in group 3 animals slightly differed from the value (12.85 ± 0.695 10^3 /mm^3) recorded after treatment. Statistical analysis using 't' test revealed a significant difference (P<0.05) on total leucocyte counts between pre and post treatment in-group 3



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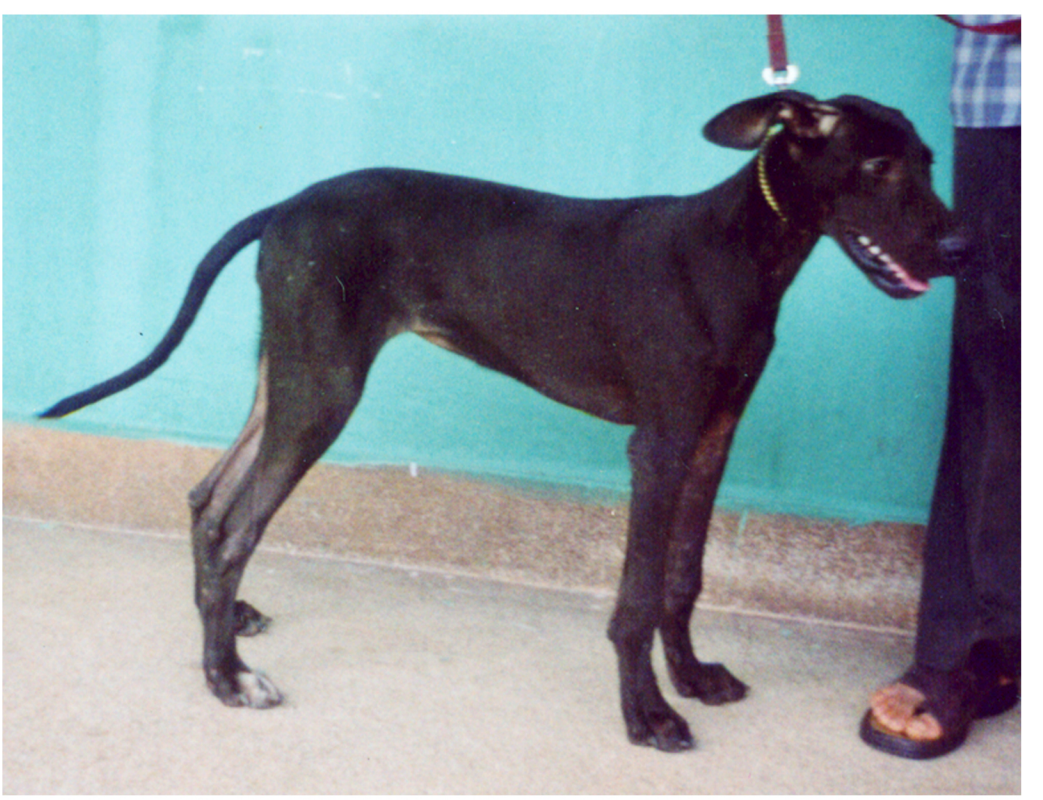


B

Plate - 4 Group 1 Oral Terbinafine

(A) Before treatment

(B) After treatment (two weeks)

Table 13. Pre and Post-treatment serum mineral status of group 1 animals treated with oral ketoconazole

Minerals ppm	Mean values ±SD		t values
	Pre treatment	Post-treatment	
Copper	0.117 ± 0.041	0.150 ±0.055	1.581 ^{NS}
Iron	0.253 ±0.039	0.273 ±0.037	2.3355 ^{NS}
Zinc	0.827 ±0.233	0.820 ± 0.147	0.1851 ^{NS}

^{NS} - Non significant

Table 14. Pre and Post-treatment haematological parameters of group 2 animals treated with oral terbinafine

Haematological parameters	Mean values (Mean ±SD)		t values
	Pre treatment	Post-treatment	
Haemoglobin (g/dl)	11.933 ±0.808	12.022 ±0.524	0.6019^{NS}
Packed cell volume (per cent)	36.333 ±1.732	36.667 ±2.236	0.5447 *
Total erythrocyte count (10^6/ mm^3)	5.811 ±0.483	5.9 ±0.552	0.3592 *
Total leucocyte count (10^3/mm^3)	12.894 ±0.865	12.942 ±0.996	0.4143 ^{NS}
Neutrophils (per cent)	62.444 ±7.038	62.889 ±7.737	0.6447 ^{NS}
Lymphocytes (per cent)	27.111 ±6.173	26.444 ±5.961	0.8000^{NS}
Eosinophils (per cent)	6.778 ±2.108	6.444 ±1.590	0.4714 ^{NS}
Monocytes (per cent)	3.556 ±1.333	4.444 ±1.667	1.5119 ^{NS}

^{NS} – Non significant
* - Significant difference ($P<0.05$)

Table 15. Pre and Post-treatment biochemical parameters of group 2 animals treated with oral terbinafine

Biochemical parameters	Mean values (Mean ±SD)		t values
	Pre treatment	Post-treatment	
Blood glucose (mg/dl)	100.55 ±15.150	99.77 ±13.236	0.7593 ^{NS}
Cholesterol (mg/dl)	89.111 ±14.92	91.667 ±15.62	5.0799**
Creatinine (mg/dl)	1.036 ±0.275	1.113 ±0.194	1.2999^{NS}
Total protein (g/dl)	10.744 ±1.408	11.057 ±1.194	2.6945*
Albumin (g/dl)	4.644 ±0.749	4.768 ±0.765	3.8680**
Globulin (g/dl)	6.100 ±0.707	6.289 ±0.571	1.5801 ^{NS}
A/G ratio	0.759 ±0.070	0.758 ±0.1	0.738 ^{NS}

** - Significant variation (P<0.01)
* - Significant variation (P<0.05)
NS – Non significant

Table 16. Pre and Post-treatment serum mineral status of group 2 animals treated with oral terbinafine

Minerals ppm	Mean values ±SD		t values
	Pre treatment	Post-treatment	
Copper	0.117 ±0.041	0.133 ±0.052	0.5423 ^{NS}
Iron	0.548 ±0.055	0.530 ±0.508	0.8176 ^{NS}
Zinc	0.748 ±0.025	0.765 ±0.037	1.1656 ^{NS}

NS – Non significant

Table 17. Pre and Post-treatment haematological parameters of group 3 animals treated with "Biocan M" vaccine along with topical application of ketoconazole shampoo

Haematological parameters	Mean values (Mean ±SD)		t values
	Pre treatment	Post-treatment	
Haemoglobin (g/dl)	11.667 ±0.961	11.7 ±0.936	0.2773 ^{NS}
Packed cell volume (per cent)	35.667 ±3.011	36.667 ±3.077	2.4533^{NS}
Total erythrocyte count (10^6/ mm^3)	5.767 ±0.983	5.783 ±1.063	0.1910^{NS}
Total leucocyte count (10^3/mm^3)	12.575 ±0.474	12.85 ±0.695	2.6112*
Neutrophils (per cent)	63.167 ±7.782	61.833 ±6.080	0.8414 ^{NS}
Lymphocytes (per cent)	26.167 ±5.345	25.5 ±5.089	0.5976 ^{NS}
Eosinophils (per cent)	6.667 ±2.422	7.5 ±1.643	1.3868 ^{NS}
Monocytes (per cent)	4.167 ±1.722	5.167 ±1.329	1.5811^{NS}

*- Significant variation (P<0.05)
^{NS} – Non significant

Table 18. Pre and Post-treatment biochemical parameters of group 3 animals treated with "Biocan M" vaccine along with topical application of ketoconazole shampoo

Biochemical parameters	Mean values (Mean ±SD)		t values
	Pre treatment	Post treatment	
Blood glucose (mg/dl)	104 ±12.116	101.166 ±11.83	2.0232 ^{NS}
Cholesterol (mg/dl)	74.833 ±7.627	75.667 ±7.339	1.2741 ^{NS}
Creatinine (mg/dl)	1.038 ±0.302	1.083 ±0.194	0.6417 ^{NS}
Total protein (g/dl)	11.950 ±1.546	12.150 ±1.506	1.8787 ^{NS}
Albumin (g/dl)	4.833 ±0.163	4.950 ±0.164	2.4445 ^{NS}
Globulin (g/dl)	7.117 ±1.504	7.200 ±1.407	0.9552 ^{NS}
A/G ratio	0.708 ±0.171	0.711 ±0.148	0.2831 ^{NS}

^{NS} – Non significant

animals. No significant differences (P>0.05) on other haematological parameters were recorded. Mean values of haemoglobin, packed cell volume, total erythrocyte count, neutrophil, lymphocyte, eosinophil and monocyte count of group 3 animals before treatment were 11.667 ± 0.961 g/ dl, 35.667 ± 3.011 per cent, 5.767 ± 0.983 10^6/ mm^3, 63.167 ± 7.782 per cent, 26.167 ± 5.345 per cent, 6.667 ± 2.422 per cent and 4.167 ± 1.722 per cent respectively. Post-treatment mean values were 11.7 ± 0.936 g/dl, 36.667 ± 3.077 per cent, 5.783 ± 1.063 10^6/mm^3, 61.883 ± 6.080 percent, 25.50 ± 5.089 per cent, 7.5 ± 1.643 per cent and 5.167 ± 1.329 per cent respectively. Results of haematological parameters in group 3 animals are presented in Table17.

4.9.3.3 Biochemical parameters

In group 3 animals pre and post-treatment mean values of blood glucose (104 ± 12.116 and 101.166 ± 11.839 mg/dl), cholesterol (74.833 ± 7.667 and 75.667 ± 7.339 mg/dl_), creatinine (1.038 ± 0.302 and 1.083 ± 0.194 mg/dl), total protein (11.950 ± 1.546 and 12.150 ± 1.506 g/dl), albumin (4.833 ± 0.163 and 4.950 ± 0.614 g/dl), globulin (7.117 ± 1.504 and 7.2 ± 1.407 g/dl) and A/G ratio (0.708 ± 0.171 and 0.711 ± 0.148)) respectively. No significant variation (P>0.05) was noted on biochemical parameters such as blood glucose, cholesterol, creatinine, total protein, albumin, globulin, and A/G ratio between before and after treatment in group 3 animals. Results of biochemical parameters in group 3 animals are given in Table18.

4.9.3.4 Serum Mineral Status

Mean values of copper in group 3 animals before and after treatment were exactly same (0.133 ± 0.052 ppm) whereas iron and zinc of group 3 animals before treatment (0.205 ± 0.074 ppm and 0.973 ± 0.169 ppm respectively) slightly differed from values obtained after the treatment (0.207 ± 0.068 ppm and 0.885 ± 0.317 ppm respectively). However no significant variations were observed in iron and zinc values of before and after treatment. Results are given in Table 19.

Table 19. Pre and Post-treatment serum mineral status group 3 animals treated with "Biocan M" vaccine along with topical application of ketoconazole shampoo

Minerals ppm	Mean values ± SD		t values
	Pre treatment	Post-treatment	
Copper	0.133 ±0.052	0.133 ±0.052	0.000 ^{NS}
Iron	0.205 ±0.074	0.207 ±0.068	0.222 ^{NS}
Zinc	0.973 ±0.169	0.885 ±0.317	0.8133 ^{NS}

^{NS} – Non significant

Discussion

5. DISCUSSION

Canine dermatomycosis is a potentially contagious disease. The disease has a protracted course and it often creates annoyance to pet owners due to recurrence. This problem demanded greater attention of the veterinarians and hence a detailed systematic study was conducted to study the epidemiology of dermatomycosis and the efficacy of three therapeutic regimens in the clinical management of the disease in dogs.

5.1 OCCURRENCE

A total of 11016 dogs were presented to the University Veterinary Hospitals at Kokkalai and Mannuthy with different clinical illness for the period of one-year from April 2003 to March 2004. Among them, the dogs, which were showing clinical symptoms suggestive of dermatomycosis, were selected and their skin scrapings were examined by direct microscopic method. Samples collected from 94 dogs were found to be positive for the presence of arthrospores, which formed the infected group and were subjected to the detailed study. Total number of animals presented were 11016 and number of animals in infected group were 94. The occurrence of dermatomycosis based on direct microscopic examination was 0.85 per cent. Similar findings correlate well with findings of Borikar and Singh (1994) and Kozak *et al.* (2003). Borikar and Singh (1994) found 0.47 per cent of dermatophyte incidence out of 211 dogs in Maharashtra. Kozak *et al.* (2003) stated that three dogs were positive for dermatophytosis out of 100 samples from dogs, which exhibited skin problems.

Baxter (1973) and Lewis *et al*. (1991) found that incidence of dermatomycosis in dogs was two times lesser than that of cats. Thomsett (1986); Wright (1989) and Sparkes *et al*. (1993) confirmed that dermatophyte infections were more frequent in dogs than in cats. This low occurrence of infection indicates that the fungus may either be brushed off mechanically or may not be able to establish a disease process due to its inability to compete with the normal bacterial flora. Sometimes, it may persist asymptomatically without exhibiting clinical evidence of infection. Another

reason for this low occurrence is that the disease is self-limiting and this may relate to the shorter hair cycle in animals than in human beings.

5.1.1 Agewise Occurrence

In this present study, infected group were divided in to 5 groups based on the age such as 1 to 6 months of age group, 6 months to 1 year, 1 year to 3 years, 3 to 6 years and above 6 years. In general, young dogs were susceptible to dermatomycosis because of its poor development of immunity (Thomsett, 1986 and Sparkes *et al*., 1993). There is a possibility that within the one-year of age, dogs between 1 to 6 months had increased susceptibility due to lack of previous exposure to the dermatophytes. Hence dogs below one-year were further divided in to two groups such as 1 to 6 months and 6months to 1 year, were included in this present study. According to the findings of Cutsem *et al* (1985), Thomsett (1986), Moriello and DeBoer (1991), Larsson *et al*. (1994) and Mignon and Losson (1997) all age groups were found to be affected with dermatomycosis and this was the reason to include all age groups in this study. Aujla *et al*. (1999) pointed out that the frequency of disease declined with the increase of age. In the present study also the frequency of disease decreased as age advanced.

Although no significant difference was observed between different age groups, per cent of infection was highest in dogs of 1 to 6 months (28.72 per cent), followed by 6 months to 1 year (25.53 per cent), 1 to 3 years (23.41 per cent), 3 to 6 years (13.83 per cent), and above 6 years (8.51 per cent). This concurs with the earlier reports of Cutsem *et al*. (1985), Thomsett (1986), Foil (1990), Medleau and Ristic (1992) and Sparkes *et al*. (1993), Marchisio *et al*. (1995) and Aujla *et al*. (1999).

Cutsem *et al.* (1985) noticed the high prevalence of dermatophyte infection in younger animals compared to older groups. Thomsett (1986) reported that the younger animals had poor cell mediated immunity as they may suffer from nutritional deficiencies. Foil (1990) stated that delay in the development of host immunity predisposed the young animals to dermatophyte infections. Medleau and Ristic (1992) observed that zoophilic dermatophyte infections were more common

in young age group of animals. Sparkes *et al*. (1993) examined the skin scrapings taken from different age groups by culture method and isolated 100 per cent of isolates from age group between 1 to 12 months. Marchisio *et al*. (1995) mentioned that animals having less than one-year old were frequently infected with dermatophytes.

This difference in occurrence of dermatomycosis may be explained by the greater susceptibility of younger animals to dermatomycosis due to their naïve immunological status, closer and more frequent contact with an infected dam and bad zoohygienic conditions.

5.1.2 Breedwise Occurrence

In this present study, almost all the breeds of dogs were affected with dermatomycosis but increased rate of affection was noticed in four breeds such as Dachshund (23.40 per cent), Labrador (18.09 per cent), German shepherd (14.89 per cent) and Rottweiler (12.77 per cent) and lowest occurrence in Boxer, Bull dog and Great dane (1.06 per cent each). This agrees with the findings of Scott and Paradis (1990) who reported that Dachshund was at the highest risk for getting dermatological diseases in a Northern California study. The reason for the apparently highest occurrence in Dachshund is not clear and it might be due to individual or genetical susceptibility of a particular family to the occurrence of disease and host response to dermatomycosis may vary with the coat type of the animal. But these findings are contradictory to the findings by Thomsett (1986), Sparkes *et al* (1993), Ziony and Arzi (2000), Mancianti *et al* (2002) and Moriello (2003). Thomsett (1986) opined that dermatophytosis infection may be extensive in long-haired dogs and he also mentioned that in some long-haired breeds, chronic infection occurred confined entirely to individual hair shaft, which gave rise to asymptomatic carrier state. Sparkes *et al*. (1993) revealed that prevalence of *M. c**anis* was significantly higher in long-haired breeds than short-haired breeds. Ziony and Arzi (2000) reported that German shepherds and Golden retrievers were affected with dermatophytosis more frequently than other breeds. Mancianti *et al*. (2002)

isolated twice the number of isolates of *M.canis* from long-haired breeds compared to short-haired breeds. Moriello (2003) stated that hairs in long-haired breeds may protect fungal spores from mechanical removal through grooming and longer the contact with skin, greater the infection.

However statistical analysis of the findings in this study revealed that there was no difference observed between different breeds of dogs. These findings agree with the findings of Jungerman and Schwartzman (1972) and Cabanes *et al*. (1996). Jungerman and Schwartzman (1972) opined that any breed can get the dermatophyte infection and Cabanes *et al.* (1996) stated that no significant difference was observed with respect to breeds of dogs.

5.1.3 Monthwise Occurrence

Statistically no significant difference in occurrence was observed among the different months of the year. It is rarely reported. However this finding concurs with finding of Baxter (1973) and Cabanes *et al.* (1996) where they opined that there was no seasonal distribution among dermatomycosis infected animals. Long time survival of arthrospores in an environment might be attributed to this persistence of the disease through out the year.

But highest occurrence of dermatomycosis was noted in the month of November (22.34 per cent), followed by December (18.09 per cent) and lowest in the months of March and April (3.19 per cent each). November and December are the months of winter season in Kerala and closed confinement during winter months favoured the growth of fungi and lead to the increased incidence. The same findings recorded by Sparkes *et al*. (1993), where he reported that *M.canis* infection rate was at peak particularly during autumn and winter months. The main reason for the highest occurrence of dermatomycosis from October to December and lowest in April and March can be attributed to the presence of increased humidity and moisture which are considered as the most important predisposing factors, since most of the fungi that affect the skin only proliferate under the ideal conditions of warmth, moisture and humidity.

In the current study, 7.45 per cent and 5.32 per cent of occurrence were recorded in the months of June and July respectively. These are the main rainy months in Kerala. This agrees with the findings of Sidhu *et al*. (1993) and Varghese *et al*. (1994). Sidhu *et al*. (1993) recorded the incidence of mycotic dermatitis in dogs between July and December. Varghese *et al.* (1994) reported 33 per cent of incidence of dermatitis between July and November in Bombay. Household environment during rainy reason with associative factors such as lower perceived value of animals in societies, poor housing prolonged exposure to other infected animals, poor ventilation, darkness, overcrowding and dampness may predispose animals to higher infection. Hence comparatively higher occurrence of disease was noted during June and July (rainy season) than March and April (summer season in Kerala).

5.1.4 Sexwise Occurrence

More females (53.19 per cent) were found to be affected with dermatomycosis compared to males (46.81 per cent). The reason for the highest occurrence in females might be a consequent to stress, hormonal changes, nutritional deficiencies and debilitating disease during oestrum, parturition and lactation period. In contrast, Baxter (1973) reported that males were likely to develop dermatomycosis than females due to their stray nature. Shakir *et al*. (1996) opined that male dogs were more prone to skin disorders than females. Higher frequency of mycotic dermatitis was noticed in males due to their stray nature (Aujla *et al*., 1999). Pinter *et al.* (1999) suggested that males were more susceptible to mycotic infection than females.

But statistical analysis revealed that no significant difference between the two sexes. Similar findings reported by Jungerman and Schwartzman (1972), Moriello and DoBoer (1991), Larsson *et al*. (1994) and Mignon and Losson (1997) who suggested that sex was not of significance in dermatophyte infection in dogs. Baxter (1973) revealed that ringworm infection in dogs was not sex related. Scott and Paradis (1990) stated that sex was not an important predisposing factor for the

occurrence of dermatomycosis. Mancianti *et al*. (2002) pointed out that sexual predisposition was not considered to be important criteria for mycotic infection.

5.2 CLINICAL SIGNS

5.2.1 Pruritus

Ringworm was named so, since it produces classical ring-like lesions, but the classical lesions cannot be expected in all the cases of dermatomycosis and symptoms are extremely variable and non-specific. Hence it is very difficult to diagnose dermatophytosis solely on the basis of clinical signs. However, the common complaint reported in the current study was pruritus. Muhammed and Mbogwa (1974) isolated *M.nanum* from the lesions of animal, which exhibited signs of itching and scratching. Carman *et al.* (1979) described that skin lesion of the dermatomycosis infected animal was thickened and pruritic. Smith (1988a) stated that fungal growth on keratin layer of the skin damaged the keratin and hair shaft, which resulted in inflammation and pruritus. Cobenas *et al*. (1972) pointed out that pruritus was common clinical finding observed in animals with *Trichophyton* infection. Beale (2000) mentioned the pruritus was variable in canine dermatophytosis infection. In contrast, Medleau and Ristic (1992) pointed out that dermatophytosis was usually non-pruritic, but occasionally it was intensely pruritic. BSAVA News (1998) reported that lesions of ringworm in dogs non-pruritic to moderately pruritic.

Although clinical sign that typical of dermatomycosis is pruritus, presence of pruritus would not be suspected for dermatophytosis since it is not constant feature of dermatophytosis.

5.2.2 Type of Lesions

Primary lesions including macula, plaque, nodule, pustule and papule absent in all 94 cases and secondary lesions such as alopecia, scales, exfoliation (shedding of scales), hyperkeratosis (increased thickness) crusts (dried exudates), erythema and

hyperpigmentation (excessive colouration) were observed among 94 animals in different combinations. This variability in lesions depends on the host-fungus interaction and thus the degree of inflammation (Foil, 1990). Primary lesions are the direct result of disease process, and secondary lesions that result from degeneration of primary lesions in dermatological disorders.

Carman *et al*. (1979) described the typical lesions of canine dermatomycosis as alopecia, thickening of the skin and presence of scales on entire infected areas. Medleau and Ristic (1992) noticed alopecia, erythema, scales and crusts as the clinical signs of dermatophytosis. Parker and Yager (1997) observed alopecia with scales and hyperpigmentation in various parts of the area in dog infected with dermatophytes. BSAVA News (1998) described that lesions of ringworm in dogs were often classical foci of alopecia with scales and crusts. Ahuja *et al*. (2002) stated that marked degree of inflammation and erythema in canine ringworm infection. Bond (2002) pointed out that dermatophytes should be suspected in any dog showing lesions comprising combinations of alopecia, erythema, scaling and crusting. As these lesions were suggestive of dermatomycosis, materials were collected from them for further cultural examinations.

5.2.3 Distribution of Lesions

Generalised lesions were observed in 61 animals (64.89 per cent) where as localised lesions were in 33 animals (35.11 per cent). The reason for the higher percentage of generalised lesion could be the infective particles containing spores, which transmit the infection across the various body regions, and the organism in infected material may be viable for more than a year. The findings of the present study agree with those of Cutsem *et al*. (1985), Parker and Yager (1997) and BSAVA News (1998). Cutsem *et al.* (1985) reported that dermatophytes were isolated from 18.3 per cent of dogs with generalised hair loss compared to 5.6 per cent of dogs with out any lesions. Parker and Yager (1997) opined that focal patches of alopecia were typical of *Microsporum* spp in canines. BSAVA News (1998)

stated that lesions of ringworm in dogs were produce foci of alopecia with scales and crusts.

5.3 MANAGEMENT

5.3.1 Practice of Bathing

Animals which were given bath once in a week (44.68 per cent) were more infected with dermatophytes than animals given bath irregularly (35.11 per cent) and once in fortnight (20.21 per cent).

This may be explained by the fact that frequent bathing increases the water absorption in to the skin, which softens keratin (Muller and Kirk, 1969). Soulsby (1982) stated that frequent use of alkaline soap or shampoo during bath acted as a predisposing factor to the skin diseases. Moreover, use of detergents like aromatic quaternary ammonium compound increased the occurrence of dermatomycosis because of its good wetting properties (Rycroft and McLay 1991). Moriello (2003) reported that presence of moisture enhanced the ability of dermatophytes to penetrate the skin and favoured the germination of fungal spores.

5.3.2 Practice of Grooming

Higher rate of dermatophytes infection was observed in animals, which were not given grooming (55.32 per cent), followed by animals groomed occasionally (36.17 per cent) and given daily (8.51 per cent). Muller and Kirk (1969) stated that animal should be groomed few minutes regularly than many hours sporadically for healthier good hair coat. Grooming stimulated the anagen stage (actively growing stage) of hair growth by removal of dead shedding hairs (Heiden, 1994). Therefore it can be inferred that grooming improves circulation in skin and removes the matted hairs, scales and infected debris, which helps to make the coat less susceptible to fungal infection.

5.4 DIRECT MICROSCOPIC EXAMINATION

Samples were taken from the lesion of an infected area after cleaning with 70 per cent alcohol to reduce the bacterial contamination, submitted for direct microscopic examination, using 10 per cent potassium hydroxide, under low power of microscope. The time required for the clearing process depended on the density of the specimen (Quinn *et al*., 1994). Jungerman and Schwartzman (1972) mentioned that excessive amount of hair or scale on the slide for direct microscopic examination may impede visualizing the fungal elements.

About 80 per cent of skin scrapings taken from the 94 dogs (infected group) revealed the presence of ectothrix spores in low power of direct microscopic examination and rest 20 per cent of the samples showed the presence of endothrix spores. This indicates that majority of the infection in 94 dogs could be due to *Microsporum* spp, since small ectothrix spores (two to three microns) arranged in a mosaic pattern were typical of *Microsporum* species where as intermediate endothrix spores (three to seven microns) arranged in a linear pattern were characteristic to *Trichophyton* infection. Hence direct microscopic examination is one of the best methods, not only for the identification of dermatophytes but also for the differentiation between *Microsporum* and *Trichophyton.*

Thoday (1981) stated that fungal hyphae might be seen as filaments, sometimes fragmenting in to arthrospores on microscopic examination. Thomsett (1986) suggested that arthrospores in small groups or in chains around the infected hair, along with fungal hyphae, could be identified if the spore mass was not dense. Smith (1988) opined that, although the positive result obtained from direct microscopic examination was more rewarding, the technique of identifying spores was possible only by expert dermatologist. The aim of doing direct microscopic examination was to identify the fungal elements (hyphae and spores) on or within hairs and it could lead to misinterpretation as saprophytic fungal spores may be present (BSAVA News, 1998). Moriello (2001) reported that veterinary

dermatophytes produce ectothrix spores, where as human dermatophytes produce endothrix spores.

One of the most difficult aspects of diagnosing canine dermatophytosis is the marked variability in clinical signs. So direct microscopic examination is considered to be a method of diagnosis with highest sensitivity of detecting dermatophytes, compared to the cultural examination, which requires minimum of three weeks time, which delay the treatment.

5.5 CULTURAL EXAMINATION

Skin scrapings collected from the lesion of animals in the infected group were cultured using Sabouraud's dextrose with CC supplement and dermatophytes test medium with CC supplement. Three isolates of *Trichophyton and* four isolates of *Microsporum* were identified based on the pigmentation of the obverse and reverse sides of culture plates. Sabouraud's dextrose agar is the most satisfactory culture medium since its slight acidity and added antibiotics prevents the contamination (Chloramphenicol - antibacterial, Cycloheximide - inhibit the saprophytic fungal growth). The saprophytic microflora grows more rapidly than the dermatophytes hence without actidione it would not possible to isolate the dermatophytes from the samples. Similar results noted by Weiss *et al*. (1979), Wright (1989), Cabanes *et al*. (1996) and Kozak *et al*. (2003). Weiss *et al.* (1979) examined a total of 4790 skin scrapings mycologically and isolated 887 strains of dermatophytes over a period of 13 years. Wright (1989) pointed that very large number of negative samples (92 per cent) received from ringworm-like lesions made the diagnosis of *M.canis* very difficult. Cabanes *et al*. (1996) screened 944 dogs with lesions, which were suspected of having dermatophytes from the municipal kennel of Barcelona and found that none of them yielded dermatophytes by culture examination. Kozak *et al.* (2003) isolated two species of *T. mentagrophytes* and only one *M canis* from 100 samples. One of the main difficulties in isolation of dermatophytes is that the contamination with saprophytic fungi is very extensive and secondly, the infection

to inoculation ratio is low for dermatophytes during cultural examination (DeVroey, 1985).

5.5.1 Trichophyton spp

Growth was noticed after one month of inoculation in to the Sabouraud's dextrose CC agar. Septate hyphae with chlamydoconidia (often in chains) were identified on microscopic examination of colony growth. Jungerman and Schwartman (1972) stated that growth was very slow to rapid and surface of *Tichophyton* was heaped and cottony white to cream and reverse side of colony was orange or deep red on SDA. Microconidia were very numerous elongate borne singly along hyphae or in clusters. Spindle shaped macroconidia are rare and abundant in some strains. Hence the linear arrangement of cluster of spores along with septate hyphae confirmed that the isolate obtained, was *Trichophyton* species.

5.5.2 Microsporum spp

Growth was obtained after 21 days of inoculation in to a dermatophyte test medium. Septate hyphae with club shaped microconidia were observed. No macroconidia was seen on microscopic examination of colony growth. Red colour change occurred on DTM after eight days of inoculation. Carroll (1974) reported that deep yellow-pigmented colonies on DTM were a characteristic of *Microsporum canis.* Muller and Kirk (1969) stated that growth of *Microsporum* species appeared as white fluffy spreading colony, which developed a deep yellow-pigment. Single celled micro conidia and septate hyphae were common in *Microsporum* spp. So it can be inferred that the isolate was *M.canis* and the diagnosis is mainly based on microscopic appearance of microconia with septate hyphae.

5.5.3 Comparison Between Sabouraud's Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM)

Dermatophyte test medium is not much confirmatory for dermatophytes by its colour alone, since many of the saprophytic contaminants such as *Aspergillus,*

Mucor, Penicillium also changes the dermatophytes test medium with in the period of 10 days. But growth of dermatophytes in DTM can further be identified by their septate hyphae with micro and macroconidia under microscopic examination.

Carroll (1974) found that DTM was 82 per cent accurate as a diagnostic test for dermatophytes by colour change alone. Results must be read with in 14 days because prolonged incubation of non-pathogenic glucose fermenting strains will turn it red. Harvey (1990) pointed out that the culture should be examined daily for the signs of early colour change, in association with early colony growth and he also mentioned that SDA was the standard medium for confirmation of dermatophytes because of its transparency showed a clear picture of reverse pigment. Salkin *et al.* (1997) reported that non-dermatophytic fungi may also cause a colour change on DTM and gave false positive results. Hence, using DTM is a preliminary method of examination. Moriello (2003) reported that colonies obtained from DTM should be subcultured on to a plain SDA since the colour indicator may alter the gross and microscopic appearance of the fungal colonies or depress macroconidia growth (Moriello, 2003a).

So it can be inferred that SDA is considered as the ideal medium for the confirmation of dermatophytes since it will inhibit the growth of contaminants without altering the gross and microscopic colony morphology of pathogens.

5.6 HAEMATOLOGICAL PARAMETERS

Infected group had significantly lower mean values of haemoglobin content, packed cell volume and leucocyte count (11.986 $\pm$ 0.725 g/dl, 35.979 $\pm$ 2.342 per cent and 12.841 $\pm$ 0.633 $10^3/mm^3$, respectively) when compared to respective mean values of control animals (13.356 $\pm$ 0.639 g/dl, 36.889 $\pm$ 1.269 per cent and 14.367 $\pm$ 0.464 $10^3/mm^3$ respectively). Where as, not much variation was noted in other haematological parameters such as total erythrocyte count, packed cell volume and differential count from that of control group and values of both the groups were within the normal range. These findings correlate well with findings of Wilkinson (1979) and Ibrahim *et al.* (1984). Wilkinson (1979) revealed that moderate

hypochromic microcytic anaemia and mild leucopenia were evident with multiple dermatophyte infections in dogs. Ibrahim *et al.* (1984) stated that ringworm infected animals had significantly low haemoglobin level. So in present study, the reason attributed for leucopaenia and low haemoglobin could be due to less food intake caused by clinical illness and discomfort in mycotic infections. Furthermore serum mineral estimation revealed a low level of copper and iron in infected group, hence it can be concluded that the animals may be anaemic.

On the other hand, Koshla *et al*. (1989) reported no significant changes in total erythrocyte count, packed cell volume and differential count in experimental *M.canis* infection.

5.7 BIOCHEMIAL PARAMETERS

Mean total protein values (10.842$\pm$ 1.161 g/dl for infected group and 6.633 $\pm$ 0.568 g/dl for control group), albumin values (4.77 $\pm$ 0.776 g /dl for infected group and 3.167 $\pm$ 0.245 g/dl for control group), globulin values (6.066 $\pm$ 0.995 g/dl for infected group and 3.467$\pm$ 0.442 g/dl for control group) and A/G ratios (0.796 $\pm$ 0.111 for infected group and 0.925 $\pm$ 0.126 for control group) showed that mean values of total protein, albumin, globulin and A/G ratio of infected group were significantly higher ($P<0.01$) from that of control group. Similar findings recorded by Shakir *et al.* (1996) and Aujla *et al.* (1999) where globulin content was higher in skin disorders and mycotic infection. This increased serum protein may be due to inflammatory response produced by dermatophyte infection. The antigens were trapped in epidermis by Langerhan's cells, which were the prominent antigen presenting cells of the skin immune system and presented the antigens to T-lymphocytes. Migration of antigen presenting cells was initiated by cytokines. So a dense infiltration of inflammatory cells in infected area and subsequent increase in serum protein content (Gudding and Lund, 1995).

Other than protein change, no significant changes were observed in cholesterol, blood glucose and creatinine in infected group and they were within the normal range. The serum biochemistry estimation was only used for identifying an underlying problem, which might be a contributing factor in the development of disease, and not for the diagnosis of disease.

5.8 SERUM MINERAL STATUS

5.8.1 Copper

No significant difference was noted between copper contents of infected and control group (0.143 $\pm$ 0.082 ppm for infected group and 0.167 $\pm$ 0.141 ppm for control group). But both the values were lower than the normal range of copper in canine (0.5 to 1 ppm). The lower level of copper in infected and control group indicates that animals in both the groups were anaemic, since the lowering of the copper content in blood was a constant finding of anaemia (Maynard *et al*., 1979). Furthermore, copper deficiency decreased the activity of copper dependent enzyme cytochrome oxidase, which facilitated the reduction of $Fe^{3\pm}$ to $Fe^{2\pm}$ before incorporation in to porphyrin molecule, which is involved in the biosynthesis of heme (McDowell, 1992). Copper is required for keratinisation and tissue pigmentation, hence in infected group, decreased copper level may aggravate the skin lesions caused by dermatophytes.

5.8.2 Iron

Mean values of serum iron in infected and control groups were 0.364 $\pm$ 0.042 ppm and 0.359 $\pm$ 0.033 ppm respectively. No significant difference ($P>0.05$) between iron contents of infected and control group was observed. But these values were lower than the normal level of iron in dogs (0.6 to 1.9 ppm is normal and values between 0 to 0.78 ppm is considered as deficient). Iron requirement for domestic animals was influenced by age, growth rate and availability of dietary iron source (Smith, 1997). Anaemia may occur at any time when the available supply of iron become deficient relative to the need for haemoglobin formation (Maynard *et*

al., 1979). Hence deficiency of iron also revealed the anaemic status of animals in both the groups.

5.8.3 Zinc

Statistical analysis revealed no significant difference between the zinc contents of infected (0.827 ± 0.204 ppm) and control group (0.820 ± 0.147 ppm) and both were with in the normal range and these findings are contradictory to the findings of Underwood (1971), Maynard *et al*. (1979) and McDowell (1992). Underwood (1971) stated that alopecia accompanied by gross epithelial lesions of the skin was observed in zinc deficiency. Maynard *et al*. (1979) pointed out that zinc deficiency was characterised by specific skin lesions. McDowell (1992) mentioned that dramatic disappearance of the excess keratinisation of the epidermis was noticed when the diet was supplemented with zinc.

5.9 TREATMENT TRIALS

Treatment trials were conducted in 27 animals of the infected group and these animals were randomly divided in to three groups. Response to treatment was assessed on the basis of clinical improvement, examination of skin scrapings and estimation of haematological and biochemical parameters and serum mineral level on the day of presentation and on day 14 after the onset of treatment.

5.9.1 Treatment with Oral Ketoconazole (Group 1)

Clinical response after treatment of ketoconazole was encouraging. Nine animals in the group 1 treated with 5 mg per kg body weight of ketoconazole once daily orally for 14 days showed a dramatic clinical improvement. On the day of presentation, all animals brought with skin lesions, mild itching, erythema and alopecia over the body coat. Clinical examination of dogs on day 14, revealed complete remission of lesions and signs of regrowth of hairs in almost all affected areas in all the animals. The appetite and temperature were normal. The direct examination of skins scrapings on day 14 showed negative result for the presence of

arthrospores. It was reported that pruritus had been reduced much in seven animals. Two animals had mild itching without any gross lesion over the body on day 14 and treatment was extended to one more week. Subsequent examination of the animals revealed that they were free from itching. Thus, ketoconazole is a good drug of choice for the treatment of dermatomycosis and ketoconazole was included in this current study since it has increased solubility in an acid environment and good tissue distribution. Furthermore, use of ketoconazole at the dose of 5 mg per kg body weight is considerably economical and reduces the risk of liver disorders. Hence, ketoconazole at the dose of 5 mg per kg body weight once daily for two weeks is consistently effective. However, similar results reported by Kumar *et al*. (2002) and Patterson and Frank (2002) where they used 5-10 mg per kg body weight of ketoconazole once daily for two weeks in the treatment of *Malassezia* infection in canine. On the other hand, Foil (1990), and Carlotti and Bensignor (1999) noted that administration of 5 to 15 mg per kg body weight of ketoconazole orally considered to be an effective in treating canine dermatomycosis.

5.9.1.1 Haematological Parameters

Mean values of packed cell volume and total erythrocyte count of group 1 animals before and after treatment of ketoconazole were 35.556 $\pm$ 3.358 per cent, and 5.789 $\pm$ 0.734 10^6/ mm^3, 37.111 $\pm$ 3.480 per cent and 5.956 $\pm$ 0.665 10^6/ mm^3 respectively. Statistical analysis revealed that higher levels of packed cell volume and total erythrocyte count were observed in animals after the treatment with ketoconazole than before treatment. The reduced packed cell volume and total erythrocyte count indicates that anaemic status of the infected animals, which might be due to the deteriorated bodily condition caused by dermatophytes. Subsequent to the treatment, improvement in food intake was reported which might be due to the remission of clinical signs such as pruritus and alopecia. These findings correlate well with findings of Wilkinson (1979) who reported that a moderate anaemia in dermatophyte infection in dogs. Other values such as haemoglobion, total leucocyte count, differential count of group1 animals before and after treatment with ketoconazole were within the normal range. Gowda (1982) and Khosla *et al*. (1989)

revealed that there were no significant changes in haemoglobin, packed cell volume, total leucocyte count and differential count. This is contradictory to the findings of Aujla *et al.* (1999) who reported that leucocytosis in dermatomycosis and pointed that leucocytes might be due to the inflammatory response.

5.9.1.2 Biochemical Parameters

No significant differences were noticed (P>0.05) in biochemical parameters such as blood glucose, serum total protein, albumin, globulin and A/G ratio in group 1 animals before and after treatment with ketoconazole. Aujla *et al.* (1999) recorded no significant changes in total protein and albumin in ringworm infections in dogs. On the other hand, Ibrahim *et al*. (1984) noted decreased glucose level and total protein in dermatophyte infected animal whereas Shakir *et al*. (1996) recorded low albumin level in skin infection of dogs.

5.9.2 Treatment with Oral Terbinafine (Group 2)

Though many studies of terbinafine use in dermatophytes infected human beings were conducted in countries like United States, United Kingdom and Italy, not much work has been taken up in India so far. Moriello (2003b) reported that terbinafine was a systemic allylamine antifungal agent to be used for the treatment of dermatomycosis in felines. The drug is specifically active only against dermatophytes and hence can be considered as the drug of choice. In the current study, the drug terbinafine used at the dose rate of 4 mg per kg body weight once daily orally for 14 days was found to be effective against dermatophytes in dogs. No side effects except temporary vomiting and regurgitation were reported during the course of treatment in two animals. But these were mild transient. Skin scrapings taken from these group 2 animals after 14 days of treatment showed few spores but which were not of significance to consider as a positive result. Hence, terbinafine is considered to be an effective drug for the treatment of dermatomycosis in dogs and the present study agrees with the findings of Jones (1995) which stated that the pediatric dose of terbinafine is in the range of 4 to 8 mg per kg body weight once a day and most often it was at the dose of 4 mg per kg body weight. It was reported

that complete clinical and mycological recovery of ringworm infection occurred within 30 days of oral therapy with terbinafine at the dose rate of 250 mg once daily in human beings (Suhonen *et al*., 1999; Papich, 2001 and Atzori *et al*., 2003).

5.9.2.1 Haematological Parameters

Mean values of haematological parameters such as haemoglobin, packed cell volume, total erythrocyte count, total leucocyte count and differential count were within the normal range before and after the treatment with terbinafine in animals of group 2. No significant variations (P>0.05) were noticed in any haematological findings before and after treatment with terbinafine. This findings agree with reports of Khosla *et al.* (1989) who reported no significant changes in any of the haematological parameters such as haemoglobin, packed cell volume, total leucocyte count and differential count in *M. canis* infection in dogs. Toxins produced by dermatophytes may alter the blood parameters by passing through the vascular component, but we cannot expect haematlogical changes to occur in dermatophyte infections.

5.9.2.2 Biochemical Parameters

Pre and post treatment biochemical values such as cholesterol (89.111 $\pm$ 14.920 mg/dl and 91.667 $\pm$ 15.620 mg/dl), total protein (10.744 $\pm$ 1.408 g/dl and 11.057 $\pm$ 1.194 g/dl), albumin (4.644 $\pm$ 0.749 g/dl and 4.768 $\pm$ 0.765 g/dl respectively). Mean values of cholesterol, albumin (P<0.01) and total protein (P<0.05) of experimental group after treatment of terbinafine were increased than values taken before the treatment, but both the values were within the normal level. But when there is an inflammation there will be an increased serum protein, however, this finding correlates well with the findings of Shakir *et al.* (1996) who reported the decreased total protein and albumin levels in dermatomycosis. The slight decrease in levels of cholesterol, total protein and albumin before treatment might be due to nutritional deficiency caused by discomfort of itching or other clinical signs and debilitated condition.

5.9.3 Treatment with "Biocan M" vaccine along with topical application of Ketoconazole Shampoo (Group 3)

5.9.3.1Clinical Response to Vaccine with Ketoconazole Shampoo

The combination of "Biocan M" and topical application of ketoconazole was not found to be effective in the treatment of the dermatomycosis infection in dogs. Initially slight reduction in the clinical signs was reported in all the treated animals, but complete cure was not noticed by the end of the treatment. Infection recurred in eight animals and good improvement in the condition was reported in only one animal but skin scrapings of that animal was also positive for the presence of arthrospores. The failure of this treatment regimen in majority of the animals might be due to the failure in development of sufficient antibody titers against *M. canis* in order to ensure complete protection. Vaccine is considered only an adjunct to the traditional therapy and may not afford complete protection and hence its application should always be coupled with an antifungal drug. DeBoer and Moriello (1995) reported that the induction of high antibody titers produced by inoculation of cell wall *M.canis* vaccine were not sufficient for the protection of animals against *M.canis* infection. It was suggested that *M.canis* vaccination was not for the elimination of *M.canis* organism but would help in the reduction of the severity of clinical signs (Beale, 2000). Moriello (2003b) stated that fungal vaccines were not for prophylaxis and were involved with the temporary reduction in clinical signs of dermatomycosis. Skin scrapings were positive for the presence of arthrospores after the completion of the treatment in all the nine animals in this study, which concur the findings of earlier workers.

5.9.3.2 Haematological Parameters

Total leucocyte count recorded ($12.575 \pm 0.474\ 10^3$ /mm^3) before treatment in group 3 animals slightly differed from the value ($12.85 \pm 0.695\ 10^3$ /mm^3) recorded after treatment. Total leucocyte count was increased ($P<0.05$) after the treatment. This might be consequent to the fact that keratinocytes, which were able to phagocytose, degrade the antigens and may process antigens that are transferred to

Langerhan's cell which produces cell mediated immune response and the toxins produced by the organisms may cause abnormalities in the blood when they passes through the vascular component. Gudding and Lund (1995) reported that, in ringworm vaccination cellular response was the crucial for protection.

5.9.3.3 Biochemical Parameters

No significant variation (P>0.05) was noted on biochemical parameters such as blood glucose, creatinine, total protein, albumin, globulin and A/G ratio before and after treatment in-group 3 animals. Generally when there is a cell-mediated response, there will be increased level of serum protein. This normal level itself indicates that cell-mediated response, which was produced by vaccine, may not be sufficient for the complete protection.

5.9.4 Serum Mineral Status in Experimental Groups

In all the treatment trails, the copper, iron and zinc levels before treatment slightly differed from that of after treatment. No significant variations were observed on serum mineral contents of experimental groups between pre and post treatment. But copper and iron level were in the lower level compared to the normal level. Low copper level in dermatophytes infected animals may act as a contributing factor for the lesions and hypopigmentation to develop. Because conversion of thyrosin to melanin catalysed by copper containing enzyme poly phenyl oxidase (Underwood, 1971). The low level of iron may be due to chronic inflammatory condition. When an inflammation became chronic, serum iron decreased, as iron is sequestered in macrophage and is made unavailable to the erythropoietic tissue (Kaneko, 1997). Zinc contents in serum of experimental groups before and after treatment were with in the normal range. Conversely, Logas *et al*. (1993) reported that low zinc level in serum was associated with dermatological disorders. Randhava *et al*. (1994) recorded decreased copper and zinc levels in buffaloes with skin diseases. Zinc non-responsive dermatosis like acrodermatitis of bull terrier is a lethal autosomal recessive disease with decreased serum zinc levels and no response to zinc supplementation. Whereas rapidly growing large breed dogs, a relative zinc

deficiency may lead to dermatitis and a short course of zinc supplementation may resolve the disease condition and so serum zinc levels may be helpful but are unreliable (Stannard *et al*., 2000).

5.9.5 Comparison Between Ketoconazole and Terbinafine Treatment Regimens

Both ketoconazole and Terbinafine were found to be equally effective in the treatment of dermatomycosis based on the clinical response and mycological cure by 14 days of treatment. At the same time, cost of treatment is the most important factor in deciding the practicability of the treatment in any disease condition, hence it is important to assess the economical variability of both the treatment regimens. It was calculated that, only 10 per cent extra amount was required for the treatment with Terbinafine compared to that of ketoconazole as per the current market cost. Therefore it is concluded that there is no much difference in the cost of the Terbinafine treatment regimen compared to that of ketoconazole and furthermore Terbinafine is the specific drug against dermatomycosis. Whereas one advantage in using ketoconazole is that, even before the confirmation of diagnosis in skin disease, ketoconazole can be given since it is effective not only against dermatophytes and also against yeast.

Summary

6. SUMMARY

The present study was carried out to get an understanding on the epidemiological determinants, diagnostic techniques and assessment of the efficacy of three different regimens in dermatophyte infections in dogs. A total of 11016 dogs were presented to the University Veterinary Hospitals at Kokkalai and Mannuthy with different clinical illness for a period of one year from April 2003 to March 2004. Among them, 94 animals were positive for the presence of arthrospores on direct microscopic examination of skin scraping and these 94 dogs were included in the infected group. Epidemiological data of infected animals were collected as per the proforma. The control group comprising nine apparently healthy animals presented to the hospitals during the period. Three different therapeutic trials were conducted in three different experimental groups, which were selected randomly among the infected group.

No significant differences were noticed among age, breed, sex and season in the occurrence of dermatomycosis among the infected animals. However, highest percent of infection noticed in dogs of one to six months of age group and in the Dachshund breed of dogs. Females were affected more when compared to males. Increased number of infected cases were reported in the month of November followed by December compared to the remaining months of the year.

Detailed clinical examination of infected animals revealed the presence of alopecia, scales, exfoliation, lichenification, crusts, erythema and hyperpigmentation with pruritus. Diagnosis was mainly based on direct microscopic examination of skin scrapings using 10 per cent potassium hydroxide and cultural identification of isolates. Direct microscopic examination was one of the most accurate and effective methods of diagnosis and it revealed that 80 per cent of the samples from infected animals were positive for ectothrix spores and 20 per cent showed endothrix spores. Culture examination had been undertaken using Sabouraud' dextrose agar (SDA) with chloramphenicol and cycloheximide supplement and concluded that SDA was the most standard and satisfactory culture medium for the identification of

dermatophytes, whereas culture method using dermatophyte test medium can be employed as a primary method of diagnosis. Microscopic examination was carried out using lactophenol cotton blue by either cellotape method or wet mount method. Presence of single celled microconidia with septate hyphae was observed in *Microsporum* species and presence of linear arrngement of cluster of spores along with septate hyphae was identified in *Trichophyton* species.

In haematological evaluation, low haemoglobin content (11.986 $\pm$ 0.725 g/dl) and low leucocyte count (13.356 $\pm$ 0.639 10^3 /mm^3) were recorded in infected group compared to control group (12.841 $\pm$ 0.633 g/dl and 14.367 $\pm$ 0.464 10^3 /mm^3 respectively). Serum biochemistry estimations revealed that total protein, albumin, globulin and A/G ratio were higher in infected group than that of control group. No significant differences were observed on minerals such as copper, iron and zinc between infected and control groups. Copper level of infected group was 0.143 $\pm$ 0.082 ppm and for control group was 0.167 $\pm$ 0.141 ppm. Mean values of serum iron in infected and control groups were 0.364 $\pm$ 0.042 ppm and 0.359 $\pm$ 0.033 ppm respectively. Serum zinc level of infected group was 0.813 $\pm$ 0.204 ppm and control group had 0.876 $\pm$ 0.147 ppm of serum zinc level. Copper and iron levels were low in both the experimental and control groups compared to the normal range of copper and iron in canine. Whereas zinc levels in both the groups were within the normal range.

Treatment trials were conducted in 27 infected animals by grouping the animals in to three groups each with nine animals. Group 1 animals were treated with 5 mg per kg body weight of ketoconazole once daily orally for 14 days and ketoconazole treatment gave promising results. Complete recovery of infection was observed. Pruritus was reduced and regrowth of hairs was noticed. Skin scrapings taken after the treatment were negative for the presence of arthrospores. Packed cell volume and total erythrocyte count level were increased after the treatment with ketoconazole.

Terbinafine was administered at the dose rate of 4 mg per kg body weight once daily orally for 14 days in group 2 animals. Terbinafine was found to be highly effective in the treatment of dermatomycosis in dogs. Lesions disappeared very rapidly. Mild transient side effects like vomiting and regurgitation was reported in two animals. Mean values of cholesterol, albumin ($P<0.01$) and total protein ($P<0.05$) of experimental group after the treatment of terbinafine were increased than the values taken before treatment, but all values within the normal range.

The combination of “Biocan M” and topical application of ketoconazole was not found to be effective in treating dermatomycosis infections in dogs. Although, slight reduction was noticed in all animals, no complete cure was reported. In these trials, spreading of lesions and recurrence of infection were the major complications. But no other side effects were reported.

In the current study, ketoconazole and terbinafine were found to be very effective for the treatment of canine dermatomycosis and no remission of lesions were noticed within two weeks of treatment.

References

REFERENCES

Ahuja, A., Chahar, A. and Verma, D. 2002. Dermatophytosis (Ringworm) in canine. *J. Canine Dev. Res*. 2: 95-97

* Atzori, L., Pau, M. and Aste, M. 2003. Erythema mutiforme ID reaction in atypical dermatophytosis: a case report. *J. European Acad. Dermatol. Venereol*. 17: 699-701

Aujla, R.S., Singh, N., Jand, S.K., Sodhi, S., Sood, N. and Gupta, P.P 1999. Mycotic dermatitis in dogs in Punjab: Clinico pathological studies. *Indian. J. Comp. Microbiol. Immunol. Infect. Dis*. 20: 134 -136

Baker, H.J., Bradford, L.G. and Montes, L.F. 1971. Dermatophytosis due to *Microsporum canis* in a rhesus monkey. *J. Am. Vet. Med. Assoc*. 158: 1911

*Balajee, S.A., Ranganathan, S. and Menon, T. 1997. Soil dermatophytes in Madras, India, in relation to human ringworm. *Mycoses* 40: 317-320

*Balfour, J.A. and Faulds, D. 1992. Terbinafine: a review of its pharmacodynamic properties and therapeutic potential in superficial mycoses. *Drugs* 43: 258-284

BSAVA News. 1998. Ringworm (dermatophytosis). *J. Small Anim. Pract*. 39: 362-366

*Baxter, M.1973. Ringworm due to *Microsporum canis* in cats and dogs in New Zealand. *N. Z. Vet. J*. 21: 33-37

Beale, K.M. 2000. Dermatophytosis. *Saunders Manual of Small Animal Practice.* (eds. Birchard, S.J. and Sherding, R.G.). Second edition. W.B.Saunders Company, Philadelphia, pp. 317-329

Benjamin, M.M. 2001. *Outline of Veterinary Clinical Pathology.* Third edition. Kalyani Publishers, New Delhi, p.351

Bennett, J.E. 2001. Antifungal agents. *The Pharmacological Basis of Therapeutics*. (eds. Hardman, J.G. Limbird, L.E.). Tenth edition. McGraw-Hill Medical Publishing division, Texas, pp.1295-1312

Bond, R. 2002. Canine dermatophytosis associated with *Trichophyton* species and *Microsporum persicolor*. *In Pract*. 24: 388-395

Borikar, S.T. and Singh, B. 1994. A note on clinical ringworm in domestic animals. *Indian Vet. J*. 71: 98-99

*Brilhante, R.S., Cavalcante, C.S., Junior, S.F.A., Cordiro, R.A., Sidrim, J.J. and Rocha, M.F. 2003. High rate of *Microsporum canis* feline and canine dermatophytoses in Northeast Brazil: epidemiological and diagnostic features. *Mycopathologia* 156: 303-308

Bruss, M.L. 1997. Lipids and Ketones. *Clinical Biochemistry of Domestic Animals.* (eds. Kaneko, J.J., Harvey, JW. and Bruss, M.L.). Fifth edition. Academic Press, London, pp .83-115

Buergelt, C. D. 2000. Guinea pig dermatophytosis. *Vet. Med*. 775

*Cabanes, F.J., Abarca, M.L., Bragulat, M.R. and Castella, G. 1996. Seasonal study of the fungal biota of the fur of dogs. *Mycopathologia* 133: 1-7

*Carlotti, D. W. and Bensignor, E. 1999. Dermatophytosis due to *Microsporum persicolor* (13 cases) or *M. gypseum* (20 cases) in dogs. *Vet. Derm*. 10: 17-27

*Carman, M.G., Munro, R.F.M. and Carter, M.E. 1979. Dermatophytes isolated from domestic and feral animals. *N. Z. Vet. J.* 143-144

Carroll, H.F. 1974. Evaluation of dermatophyte test medium for diagnosis of dermatophytosis. *J. Am. Vet. Med. Assoc.* 165: 192-195

Carter, G.R., Beneke, E.S. and McAllister, H.A. 1970. Ringworm of the horse caused by an atypical form of *Microsporum canis*. *J. Am. Vet. Med. Assoc.* 156: 1048-1050

*Chavez, R.E.G., Zaragoza, C.S., Olivares, R.A.C. and Perez, G.T. 2000. Presence of keratinophilic fungi with special reference to dermatophytes on the hair coat of dogs and cats in Mexico and Nezahualcoyotl Cities. *Revista Latinoamericana de Microbiologia* 42: 41-44

Chittawar, D.R. and Rao, K.N.P. 1982. Incidence of canine dermatitis of mycotic origin in central India. *Indian Vet. J*.59: 675-677

*Cobenas, M.M.E., Nalazco, J., Iribarren, F., Penas, M. and Guida, N. 1992. Wide spread dermatophytosis in cattle caused by *Trichophyton mentagrophytes*. *Veterinaria-Argentina* 9: 246-249

Curtis, C.F. 2001. Diagnostic techniques and sample collection. *Clin. Tech. Small Anim. Pract*. 16: 199-206

Cutsem, J.V., Keyser, H.D., Rechette, F. and Flaes, M.V.D. 1985. Survey of fungal isolates from alopecia and asymptomatic dogs. *Vet. Rec*. 116: 568-569

DeBoer, D.J. and Moriello, K.A. 1995. Investigations of a killed dermatophyte cell-wall vaccine against infection with *Microsporum canis* in cats. *Res. Vet. Sci*. 59:110-113

*DeVroey, C. 1985. Epidemiology of ringworm (Dermatophytosis). Seminars in dermatology 4: 185-200

*Doumas, B.T., Watson, W.A. and Biggs, H.G. 1971. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin. Chem. Acta*. 31: 87-96

Foil, C.S. 1990: Dermatophytosis. *Greene Infectious Diseases of the Dog and Cat*. (eds. Greene, C.E.). Second edition. W.B. Saunders, Philadelphia, pp.362-370

Gilaberte, Y., Coscojuela, C., Prats, G.M.D. and Mairal, M.P. 2003. Erythema multiforme associated with inflammatory ringworm on the hand. *Br. J. Dermatol*. 49: 1078-1079

Goldston, R.T. and Wilkies, R.D. 1982. Veterinary medical mycology. *Vet. Med. Small Anim. Clin*. 10: 1447-1451

Gormall, A.G., Bardawill, C.J. and David, M.M. 1949. Determination of serum protein by the biuret reaction. *J. Biol. Chem*. 177: 751-756

Gowda, B.K.K., Rao, P.M. and Ganesh, T. 1982. Biochemical and haematological studies in non specific dermatitis. *Indian Vet. J.* 2: 29-33

Grappel, S.F., Blank, F. and Bishop, C.T. 1968. Immunological studies on dermatophytes. *J. Bact*. 96: 70-75

Gromadzki, S., Ramani, R. and Chaturvedi, V. 2003. Evaluation of new medium for identification of dermatophytes and primary dimorphic pathogens. *J. Clin. Microbiol.* 41: 467-468

Gudding, R. and Lund, A. 1995. Immunoprophylaxis of bovine dermatophytosis. *Can. Vet. Med*. 36: 302-306

Guillot, J., Latie, L., Deville, M., Halos, L. and Chermette, R. 2001. Evaluation of the dermatophyte test medium Rapid Vet –D. *Vet. Dermatol*. 12: 123-127

*Hajsig, M., Sertic, V., Naglic, T. and Bauer, M. 1975. First findings of *Microsporum gypseum* and *M.canis* infections in the dog and cat in Zagreb, Yugoslavia. *Veterinarski Arhiv* 45: 117-121

Hall, E.J., Miller, W.H.and Medleau, L. 1984. Ketoconazole treatment of generalized dermatophytosis in a dog with hyperadrenocortism. *J. Am. Anim. Hosp. Assoc*. 20:597-602

Harris, J.L. 1986. Modified method for fungal slide culture. *J. Clin. Microbiol.* 460-461

*Hasegawa, A. 2000. Dermatophytes from animals. *Jap. J. Med. Mycol*. 41: 1-4

Harvey, R. 1990. Fungal culture in small animal practice. *In Pract*. January: 12-15

*Hatzopoulou, M.E. and Hatzopoulou, B.E. 1997. Epidemiological study of ringworm in dogs in the Thessaloniki area. *Bulletin of the Hellenic Veterinary Medical Society* 48: 87-92

*Heiden, V.D.C.A. 1994. *Management of Kennels and Catteries Veterinary Nursing.* (eds. Lane, D.R. and Cooper, B.). Pergamon, London, pp.137-185

*Hitaka, Y. and Hamaguchi, T. 1973. Two cases of tinea corporis caused by *Microsporum canis. Mie Medical Journal* 23: 89-94

*Ibrahim, Hafez, S.M., Hassan, M.S. and Hassan, N.K. 1984. Haematological and biochemical changes of ringworm infected buffaloes. *Assiut Vet. Med. J.*12: 161-165

Jackson, D. Medleau, L. and Hensel, P. 2003. Chronic, recurrent dermatophytosis in a Persian cat. *Vet. Med*. 216-220

*Jand, S.K. and Gupta, M.P. 1989. Dermatomycosis in dogs. *Mycoses* 32: 104-105

Jones, T.C. 1995. Overview of the use of terbinafine (Lamisil) in children. *Br. J. Derm.* 132: 683-689

Jenkinson, D.M. 1989: Skin surface responses to external challenge. *In Pract*. 11: 207-210

Jungerman, P.F. and Schwartzman, R.M. 1972. Microsporosis and Trichophytosis. *Veterinary Medical Mycology*. Lea and Febiger, Philadelphia, p.200

Kaneko, J.J. 1997. Porphyrins and the porphyrinas. *Clinical Biochemistry of Domestic Animals.* (eds. Kaneko, J.J., Harvey, J.W. and Bruss, M.L.). Academic Press, California, pp.205-221

Khosla, R. Gupta, M.P., Dhablania, D.C. and Jand, S.K. 1989. Clinico-diagnostic features of dermatophytosis in dogs with particular reference to therapeutic measures. *Indian Vet. J.*66: 1157-1159

Khosla, R., Rai, P. and Saxena, S.C. 1991. Certain blood biochemical studies during experimental dermatomycosis in dogs. *Indian J. Anim. Sci*. 62: 559-560

Kirubaharan, J.J., Nagarajan, B., Chandran, M. Palanisamy, K.S., Chandramathi, K., Geetha, M. and Navis, H. 2000. Isolation and identification of fungi from dogs with dermatomycosis. *Indian Vet. J.* 77: 161-162

*Kozak, M., Bilek, J., Beladicova, V., Beladicova, K., Baranova, Z. and Bugarsky, A. 2003. Study of the dermatophytes in dogs and the risk of human infection. *Bratisl Lek Listy* 104: 211-217

Kotnik, T., Erzen, N.K., Kuzner, J. and Kosorok, M.D. 2001. Terbinafine hydrochloride treatment of *M. canis* experimentally induced ringworm in cats. *Vet. Microbiol*. 83: 161-168

Kumar, A., Singh, K. and Sharma, A. 2002. Treatment of dermatitis in dogs associated with *Malassezia pachydermatis*. *Indian Vet. J*. 79: 730-732

Kumar, V. and Thakur, D.K. 2001. Incidence of mycotic skin disease in dogs in and around Ranchi. *Indian Vet. J*. 78: 159-160

*Kushida, T. and Watanabe, S. 1975. Canine ringworm caused by *Trichophyton rubrum;* probable transmission from man to animal. *Sabouraudia* 13: 30-32

*Larsson, C.E., Nahas, C. R. and Ledon, A.L.B.P. 1994. Ringworm in domestic cats in Sao Paolo, Brazil, between 1981-1990. *Feline Pract*. 22: 11-14

Leeming, J. P. and Notman, F. H. 1987. Improved methods for isolation and enumeration of *Malassezia furfur* from human skin. *J. Clin. Microbiol*. p.2017-2019

Lewis, D.T., Foil, C.S. and Hosgood, G. 1991. Epidemiology and clinical features of dermatophytosis in dogs and cats at Loussiana State University 1982-90. *Vet Dermatol*. 53-58

Lloyd, D.H. 1985. Diagnostic methods in dermatology. *Br. Vet. J*. 141: 463-471

Logas, D., Kunkle, G.A. and McDowell, L. 1993. Comparison of serum zinc levels in healthy, systemically ill and dermatologically diseased dogs. *Vet. Dermtol*. 4: 61-64

*Mahajan, V.M. 1986. Further studies on antimycotic agents. *Mycoses* 29: 407-412

*Mancianti, F., Nardoni, S., Cecchi, S., Corazza, M. and Taccini, F. 2002. Dermatophytes isolated from symptomatic dogs and cats in Tuscany, Italy during a 15-year-period. *Mycopathologia* 156: 13-18

*Mancianti, F., Nardoni, S., Corazza, M., D'Achille, P. and Ponticelli, C. 2003. Environmental detection of *Microsporum canis* arthrospores in the households of infected cats and dogs. *J. Feline Med. Surg*. 5: 323-328

*Mancianti, F. and Papini, R. 1996. Isolation of keratinophilic fungi from the floors of private Veterinary Clinics in Italy. *Vet. Res. Commun.* 20: 161-166

*Mancianti, F., Pedonese, F., Millanta, F. and Guarnieri, L. 1999. Efficacy of oral terbinafine in feline dermatophytosis due to *M. canis. J. Feline Med. Surg*. 1: 37-41

Mancianti, F., Pedonse, F. and Zuillino, C. 1998. Efficacy of oral administration of itraconazole to cats with dermatophytosis caused by *Microsporum canis*. *J. Am. Vet. Med. Assoc*. 213: 993-995

Mansfield, P.D. and Stringfellow, J.S. 1990. Isolation of *Microsporum vanbreuseghemii* from skin lesions of a dog. *J. Am. Vet. Med. Assoc.* 197: 875-876

*Marchisio, V.F., Gallo, M.G., Tullio, V., Nepote, S., Piscozzi, A. and Cassinelli, C. 1995. Dermatophytes from cases of skin disease in cats and dogs in Turin, Italy. *Mycoses* 38: 239-244

Maynard, L.A., Loosli, J.K., Hintz, H.F. and Warner, R.G. 1979. *Animal Nutrition.* Seventh edition. Tata McGraw-Hill publishing Company Limited, New Delhi, p.602

*McAleer, R. 1980. Zoophilic dermatophytes and their natural hosts in Western Australia. *Med. J. Aust*. 2: 506-508

McDowell, L.R. 1992. *Minerals in Animal and Human Nutrition.* Academic Press, London, p.524

Medleau, L. and Kuhl, K. A. 1992. Dealing with chronic recurring dermatophytosis. *Vet. Med.* 1101-1104

Medleau, L. and Ristic, Z. 1992. Diagnosing dermatophytosis in dogs and cats. *Vet. Med.* 1086-1097

Medleau, L. and Weithers, W.N.E. 1992. Treating and preventing the various forms of dermatophytosis. *Vet. Med.* 1096-1100

Meinkoth, J.H. and Clinkenbeard, K.D. 2000. Normal haematology of the dog. *Schalm's Veterinary Hematology*. (eds. Feldman, B.F., Zinkl, J.G. and Jain, N.C.). Fifth edition. Lippincott Williams and Wilkins, A. Wolters Kluwer Company, Philadelphia, pp.1057-1063

*Mignon, B.R. and Losson, B.J. 1997. Prevalence and characterization of *Microsporum canis* carriage in cats. *J. Med. Vet. Mycol*. 35: 249-256

*Minagawa, H., Kitaura, K., Mineura, K. and Marumo, H. 1982. Studies on antifungal activity of ketoconazole (KW-1414). I. *In vitro* antifungal activity. *Jap. Med. Mycol.* 23: 171-188

Moriello, K.A. 2001. Diagnostic techniques for dermatophytosis. *Clin. Tech. Small Anim. Pract*. 18: 219-224

Moriello, K.A. 2003. Important factors in the pathogenesis of feline dermatophytosis. *Vet. Med*. 845-854

Moriello, K.A. 2003a. Practical diagnostic testing for dermatophytosis in cats. *Vet. Med*. 859-874.

Moriello, K.A. 2003b. Feline dermatophytosis: Topical and systemic treatment recommendations. *Vet. Med.* 877-884

*Moriello, K. A. and DeBoer, D.J. 1991. Fungal flora of the coat of pet cats. *Am. J. Vet. Res.* 52: 602-606

*Moriello, K.A. and DeBoer, D.J. 1995. Efficacy of griseofulvin and itracononazole in the treatment of experimentally induced dermatophytosis in cats. *J. Am. Vet. Med. Assoc.* 207: 439-444

Mosher, C.L., Langendoen, K. and Stoddard, P. 1977. Treatment of ringworm *(Microsporum canis*) with inactivated fungal vaccine. *Vet. Med. Small Anim. Clin*. 72 : 1343-1345

Muhammed, S.I. and Mbogwa, S.1974. The isolation of *M.nanum* from a dog with skin lesions. *Vet. Rec*. 21&28: 573

Muller, G. H. and Kirk, R. W. 1969. *Small Animal Dermatology*. W. B. Saunders, Philadelphia, p. 487

Newman, D.J. and Price, C.P. 2001. Nonprotein nitrogen metabolites. *Tietz Fundamentals of Clinical Chemistry.* (eds. Burtis, C.A. and Ashwood, E.R.). Fifth edition. W.B.Saunders Company, Philadelphia, pp.414-426

*Ollhoff, R.D. 2003. Mycological examination and clinical evaluation of cattle with latent infection of *Trichophyton verrucosum. Archives of Veterinary Science* 8 : 47-50

Pal, M. 2002. A view on mycotic infections in India. *The Veterinarian.* 26: 3-6

*Panagiotidou, D.D. and Eremondi, K.T.H. 2004. Efficacy and tolerability of 8 weeks treatment with terbinafine in children with tinea capitis caused by *Microsporum canis*: a comparison of three doses. *J. European Acad. Dermatol. Venereol.*18 : 155-159

Papich, M.G., Heit, M.C.and Riviere, J.E. 2001. Antifungal and antiviral drugs. *Veterinary Pharmacology and Therapeutics.* (eds. Adams, H.R.) Eighth edition. IOWA State University Press, pp.918-946

Parker, W.M. and Yager, J.A. 1997. *Trichophyton* dermatophytosis-A disease easily confused with pemphigus erythematosus. *Can. Vet. J*. 38: 502-505

Patterson, A.P. and Frank, L.A. 2002. How to diagnose and treat *Malassezia* dermatitis in dogs. *Vet. Med*. 612-622.

*Patwardhan, N. and Dave, R. 1999. Dermatomycosis in and around Aurangabad. *Indian J. Pathol. Microbiol*. 42: 455-462

Perkin-Elmer 1982. Analytical methods for atomic absorption spectrophotometry (Instrument manufacture's Manual). Model-3380

*Pinard, M., Chermette, R. and Bussieras, S. 1987. Diagnosis and prophylaxix of ringworm in dogs and cats. The 1983 survey at the Alfort veterinary school. *Recueil de Medicine Veterinaire* 163: 1107-1116

*Pinter, L. Jurak, Z., Ukalovic, M. and Susic, V. 1999. Epidemiological and clinical features of dermatophytoses in dogs and cats in Croatia between 1990 and 1998. *Veterinarski Arhiv* 69: 261-270

Platt, W.R. 1979. *Colour Atlas and Textbook of Hematology*. Second edition. Philadelphia, p. 644

Quaife, R.A. 1982. *Microsporum canis* isolations from show cats. *Vet. Rec.* 3:333-334

Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.R. 1994. *Clinical Veterinary Microbiology*. Wolfe Publishing Ltd, Spain, p.468

Radostits, O.M., Gay, C.C., Blood, C.D. and Hinchcliff, K.W. 2000. *Veterinary Medicine. A textbook of the diseases of Cattle, Sheep, Pigs, Goats and Horses*. Ninth edition. W.B.Saunders Company, Ltd, p.1877

*Randhava, S.S., Arora, C.L., Randhawa, C.B. and Joshy, B.P. 1994. Therapeutic evaluation and hair mineral profile of leucodermic buffaloes. *Societa Italina dibuijatria.* 2: 1545-1548

*Ranganathan, S., Balajee, S.A. and Raja, S.M. 1997-1998. A survey of dermatophytosis in animals in Madras, India. *Mycopathologia* 140: 137-140

*Reyes, A. C. 1972. Notes on ringworm. *Philippine J. Vet. Med*. 11: 16-27

Rycroft, A.N. and McLay, C. 1991. Disinfectants in the control of small animal ringworm due to *Microsporum canis*. *Vet. Rec.* 14: 239-241

Sacks, D.B. 2001. Carbohydrates. *Tietz Fundamentals of Clinical Chemistry.* (eds. Burtis, C.A. and Ashwood, E.R.), Fifth edition. W.B.Saunders Company, Philadelphia, pp.427-461

Salkin, I.F., Padhye, A.A. and Kemna, M.E. 1997. A new medium for the presumptive identification of dermatophytes. *J. Clin. Microbiol*. 35: 2660-2662

Saridomichelakis, M.N., Koutinas, A.F., Hatzopoulou, B.E., Petridou, E., Hatziefremidis, I. and Leontides, L. 1999. Recovery of *Microsporum gypseum* and *Malassezia pachydermatis* from the nasal bridge in various dog groups. *Vet. Rec*. 7:171-172

Scott, D.W., Kirk, R.W. and Smith, J.B. 1980. Dermatophytosis due to *Trichophyton terrestre* infection in a dog and cat. *J. Am. Anim. Hosp. Assoc*. 16:53-59

Scott, D.W. and Paradis, M. 1990. A survey of canine and feline skin disorders seen in a university practice. Small animal clinic, University of Montreal, Saint-Hyacinthe, Quebec. (1987-1988). *Can. Vet. J*. 31: 830-835

*Seedy, E.F.R., Radwan, I.A., Shawki, H.M. and Hassan, W.H. 2003. Experimental evaluation of different vaccines against trichophytosis in guinea pigs and calves. *Veterinary Medical Journal Giza* 51: 83-94

Shakir, S.A., Ahmed, N.M., Thanikachalam, M. and Sundararaj, A. 1996. Serum proteins in canine non-specific skin disorders. *Indian Vet. J.* 73: 396-401

*Shawkat, M. E., Fayed, A.A., Tayed, F.A. and Arab, R.M. H. 1981. A study on ringworm in dogs and cats. *J. Egyptian Vet. Med. Assoc*. 41 : 127-133

Shearer, D. 1991. Laboratory diagnosis of skin disease. *In Pract*. 13: 149-156

Sidhu, R.K., Singh, K.B., Gupta, M.P. and Jand, S.K. 1993. Incidence of mycotic dermatitis in dogs. *Indian Vet. J.* 70: 885-888

Smith, E.K. 1988. Planning the workup for dermatologic patients. *Vet. Med*. 34-49

Smith, E.K. 1988a. Dermatophytosis in pets: Avoiding misdiagnosis. *Vet. Med*. 554-565

Smith, J.E.1997. Iron metabolism and its disorder. *Clinical Biochemistry of Domestic Animals.* (eds. Kaneko, J.J., Harvey, J.W. and Bruss, M.L.). Academic Press, California, pp.205-221

Soulsby, E.J.L. 1982. *Helminths, Arthropods and Protozoan of Domestic Animals*. The English language book society and Bailliere Tindall, London, p.809

Sparkes, A.H., Jones, G.T.J., Shaw, S.E., Wright, A.I. and Stokes, C.R. 1993. Epidemiological and diagnostic features of canine and feline dermatophytosis in the United Kingdom from 1956-1991. *Vet. Rec*. 17: 57-61

Sparkes, A.H., Werrett, G., Stokes, C.R. and Jones, G.T.J. 1994: *Microsporum canis:* Inapparent carriage by cats and the viability of arthrospores. *J. Small Anim. Pract*. 35: 397-407

Stannard, A.A., Cannon, A.G. and Olivry, T. 2000. Scaling and crusting dermatoses. *TextBook of Veterinary Internal Medicine.* Fifth edition. (eds. Ettinger, S.J. and Feldman, E.C.) W.B. Saunders Company, Philadelphia, pp.47-51

Sture, G. 1995. Scaling dermatoses of the dog. *In Pract*. 276-285

*Sturzu, S., Bucur, E., Draghici, A. and Niculescu, A.L. 2002. Aspects concerning diagnosis and treatment of dermatomycoses in the dog. *Revista Romana de Parazitologie* 12: 77-78

Suhonen, R.E., Dawber, R.P.R. and Ellis, D.H.1999. *Fungal Infections of the Skin, Hair, and Nails.* Martin Dunitz Ltd, p.123

Thomsett, L.R. 1986. Fungal diseases of the skin of small animals. *Br. Vet. J*. 142: 317

Thoday, K.L. 1981. Investigative techniques in small animal clinical dermatology. *Br. Vet. J*. 137: 133-154

*Tiley, F.P. and Smith, F.W.K. 1997. The 5 minutes veterinary consult. *Canine and Feline*. Williams and Wilkins, USA, 506-507

Tripathi, K. D. 1999. Antifungal agents. *Essentials of Medical Pharmacology*. Fourth edition. Jaypee brothers. Medical Publishers Ltd, New Delhi, p.935

Underwood, E.J. 1971. *Trace Elements in Human and Animal Nutrition.* Third edition. Academic Press, New York, p.543

Varghese, M.A., Jagadish, S. and Bhalerao, D.P. 1994. Studies on the hospital incidence of dermatitis in dogs in Bombay. *Indian Vet. J*. 71: 948-949

*Vermout, S.M., Brouta, F.D., Descamps, F.F., Losson, B.J. and Mignon, B.R. 2004. Evaluation of immunogenicity and protective efficacy of a *Microsporum canis* metalloprotease subunit vaccine in guinea pigs. *FEMS Immunol. Med. Microbiol.* 40: 75-80

Vishwakarma, S.K., Bandopadhyay, A.C., Nair, N.R., Rao, K.N.P. and Singh, S.M. 1997. Canine dermatomycosis on Jabalpur region and evaluation of its therapy. *Indian Vet. J.* 74: 167-169

*Wawrzkienicz, K., Ziolkowska, G. Czajkowska, A. and Wawrzkienicz, J. 1994. *Microsporum canis* - the major pathogen of canine and feline ringworm. *Medycyna Weterynaryjna* 50: 319-322

[*]Weiss, R., Bohm, K.H., Mumme, J. and Nickdas, W. 1979. 13 years of Veterinary mycological routine diagnostics. Isolation of dermatophytes in the years 1965-1977. *Sabouraudia* 17: 345-353

[*]Weithers, W.N. and Medleau, L. 1995. Evaluation of topical therapies for the treatment of dermatophyte infected hairs from dogs and cats. *J. Am. Hosp. Assoc*. 31: 250-253

Wilkinson, G.T.1979. Multiple dermatophyte infections in a dog. *J. Small Anim. Pract*. 20:111-115

Wintrobe, M.M., Lee, G.R., Boggs, D.R., Bithell, T.C., Foester, J. Athens, J.W. and Lukens, J.N. 1981. *Clinical Hematology*. Eight edition. Lea and Febiger, Philadelphia, p.2021

Wright, A.I. 1989. Ringworm in dogs and cats. *J. Small Anim. Pract*. 30: 242-249

Ziony, Y. and Arzi, B. 2000. Use of Iufenuron for treating fungal infections of dogs and cats: 297 cases (1997-1999). *J. Am. Vet. Med. Assoc*. 217 : 1510-1513

[*] Originals not consulted

Appendices

Appendix-I

PROFORMA

Sl.No: Case No: Date:

Name and address of the owner: Phone No:

Details of the animal:

Breed: Colour: Age: Sex:

Weight: Parity: Identification mark:

Complaint:

History:

General appearance : Good | Fair | Poor | Thin | Stunted

Behaviour : Frenzy | Mania | Active | Restlessness | Dullness | Depressed

Expression : Anxious | Wobegone | Lethargic

Bodily condition : Normal | Obese | Thin | Emaciated | Hide bound

Skin changes

Elasticity : Present | Absent

Epilation : Present | Absent

Haircoat : Good | Poor | Rough | Others (Broken, easily pulled off)

Configuration : Annular | Linear | Central healing | Grouped

Distribution : Generalised | Localised | Asymmetrical | Bilateral | Symmetrical | Patchy | Scattered

Depth of lesion : Elevated | Surface | Deep

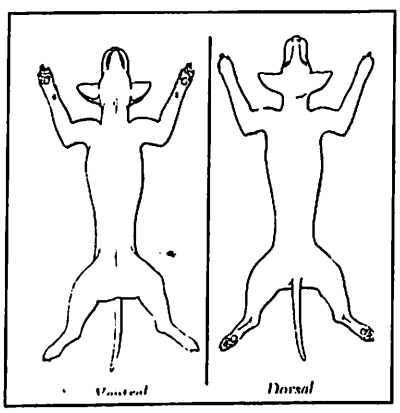
Consistency : Soft | Fluctuant | Atrophied

Quality : Dry | Moist | Greasy | Bleeding | Purulent

Colour of lesion : .....

Alopecia : Present | Absent

If present : Localised | Generalised | Symmetrical | Asymmetrical



Pruritus: Present | Absent | Constantly | Occasionally | Only at night

Food consumption : Normal | Less | Anorectic

Whether it was purchased : Yes | No

If purchased : Kennel | Pet shop | Private

TYPE OF LESIONS

Primary		*Secondary*	
Macule	Tumour	Scales	Crusts
Plaque	Wheal	Scars	Ulcers
Nodule	Papule	Excoriation	Lichenification
Pustule	Patch	Hyperpigmentation	Hyperkeratosis
Vesicle	Bulla	Hypopigmentation	Abscess
		Erosions	Callus
		Erythema	Alopecia
		Exfoliation	

Clinical signs:

When the problem began?
Has it spread? : Yes | No If Yes, where .....
Date of last whelping :
Mating history :
Are the symptoms seasonal : Yes | No If Yes, Year round | Seasonal | Non seasonal

In which season? : Summer | Rainy | Winter
Whether the animal is kept indoor?
Duration in indoor : ..... Duration in outdoor .....
Whether any other contact animals affected? Yes | No

Whether any contact human affected? Yes | No

Management details:
Whether it is given bath : Yes | No

Frequency of bath : Daily | Once in 2 days | Once in week | Once in forth night | Once in a month | Once in 2 months

Soaps/shampoos used : Used | Not If Yes, Name:

Do you groom the dog? : Yes | No Occasionally | Just before bath | After bath
Diet :

Frequency of feeding : Time of feeding:
Type of food : House hold | Pet foods
Dewormed/Not : Date of last deworming:
Drug and dose :
Vaccinated/Not :
Details about vaccination :
Ectoparasites present : Biting flies | Lice | Ticks | Fleas

Result of scraping : Sarcoptes | Psoroptes | Others
1. Direct method using KOH:
2.Lactophenol Cotton Blue staining:
3.Culture results :

EPIDEMIOLOGY AND CLINICO-THERAPEUTIC STUDIES ON DERMATOMYCOSIS IN DOGS

DEVI. T.

Abstract of the thesis submitted in partial fulfilment of the requirement for the degree of

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Kerala Agricultural University, Thrissur

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Department of Veterinary Epidemiology and Preventive Medicine
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680 651
KERALA, INDIA

ABSTRACT

The present study was mainly focused on epidemiological, diagnostic and clinico-therapeutic aspects of dermatomycosis in dogs. A total of 94 animals showed positive results for the presence of arthrospores on direct microscopic examination, out of the total 11016 animals brought to University Veterinary Hospitals Kokkalai and Mannuthy from April 2003 to March 2004, were included in the infected group. No age, sex, breed predilection and seasonal variation for dermatophyte infection were observed. Clinical signs of the infected group are non-specific and quite variable, hence the diagnosis mainly relied on the examination of skin scrapings by direct microscopy and cultural method. Direct microscopic examination is considered to be an ideal method of diagnosis for detecting dermatophytes, since cultural method requires minimum of three weeks time and delays the treatment, even though, it is conclusive.

Isolates of *Microsporum* spp and *Trichophyton* spp were identified by cultural method using Sabouraud's dextrose agar with chloramphenicol and cycloheximide supplement. Haematological abnormalities recorded were low haemoglobin content and leucopaenia and serum biochemistry revealed increased levels of total protein, albumin, globulin and A/G ratio in infected group compared to that of control group. No significant variations were observed on serum minerals such as copper, iron and zinc contents in infected group compared to control group. But mean values of copper and iron in infected and control groups were lower than the respective normal range in canine, where as zinc levels in both the groups were within the normal range. Three different therapeutic trials were conducted in three different experimental groups. Group 1 animals treated with ketoconazole at the dose rate of 5 mg per kg bodyweight daily orally for two weeks, showed a dramatic clinical response. Clinical examination of dogs at the end of treatment, revealed complete remission of lesions and signs of regrowth of hairs in almost all affected areas in all the animals. Terbinafine at the dose

rate of 4 mg per kg body weight once daily orally given in-group 2 animals was proved to be very effective in treatment of dermatophyte infected dogs. Combination of intramuscular injection of "Biocan M" vaccine adsorbed with aluminium hydroxide and topical application of ketoconazole shampoo in-group 3 animals was not found to be effective. Hence the results of present study confirms the importance of the diagnosis of dermatophyte infections by direct microscopy, applicability of the cultural method of isolation of dermatophytes and proved the efficacy of ketoconazole and terbinafine as the good choice of drugs for the effective treatment of dermatomycosis.