Chapter-1

Introduction

Dermatophytosis (also known as ringworm) is a superficial fungal skin infection of keratinized tissues, claws, hair, and stratum corneum in dogs and also in other companion, domestic and wild animals, caused by zoophilic, geophilic or anthropophilic fungal organisms, most commonly *Microsporum canis, M. gypseum and Trichophyton mentagrophytes*, where majority of cases are due to *Microsporum canis* (Mantelli and Sommariva, 1988; Mancianti et.al., 2002; Cabanes et al., 2003) while very rare, other species isolated from dogs is *Trichophyton mentagrophytes*(Vokoun andKucera, 1991). In dogs, nearly 70% of cases are caused by *Microsporum canis*, 20% by *M. gypseum*, and 10% by *Trichophyton mentagrophytes*. The prevalence of dermatophytoses in dogs range from 4% to 10% however, few studies show a higher prevalence (Cabañes, 2000).Predisposing factors to dermatophytoses are age of the dog (first 2 years of life),immuno-suppression (including immunosuppressive treatment), nutritional deficits (especially proteins and vitamin A), high temperature and high humidity, season, skin trauma resulting from increased moisture, playing or aggressive behavior, clipping, poor hygiene, other diseases etc. (Nichita and Marcu, 2010; Pier et al., 1994; Lewiset al., 1991).Dermatophytosis is one of the most common contagious diseases but not life-threatening, treatable and curable, easily contracted by direct contact and of zoonotic importance (Moriello 2014). The epidemiology of the dermatophytes is closely connected to its environment (Mattei et al. 2014). Most cases of ringworm are spread by direct contact with infected animals or indirect with contaminated objects such as furniture or grooming tools, broken hairs with associated spores. Spores attach to the epidermis and germinate to produce hyphae that invade stratum corneum and hair. The incubation period of canine dermatophytosis to the onset of the skin lesions is normally seven to 14 days (Newbury et al., 2010).Clinical signs of Canine dermatophytosis are often characterized by typical round alopecic lesions and brittle hairs. Single or multi- focal scaly crusted lesions were observed, which is covered by a crust and the edges swollen (Moretti et al., 2013).

Chapter-2

Materials and Methods

A female Dachshund dog(*Canis lupus familiaris*) approximately4 years old was presented to Shahedul Alam Quadary Teaching Veterinary Hospital(SAQTVH)of Chattogram Veterinary and Animal Sciences University (CVASU)with a history of one month duration of multifocal areas of hair loss with scaling on the trunk and limbs (fig.1) and it had been treated with various drugs including a combination of topical and systemic antifungals and antibiotics with incorrect dosage. General physical examination was normal of the dog with poor body condition. Samples were collected by plucking hair with forceps and by scraping epidermal scales with a sterile surgical blade from the affected areas for mycological and parasitological examination. No mites were found in a microscopic examination of skin scrapings. Wood's lamp examination was applied for fluorescence on the hair shafts and infected hairs showed an apple-green fluorescent on the infected area (fig.2). After placing a drop of KOH solution on a microscopic slide, the specimen (small pieces of hairs and skin scales and crusts) was added and gently heated the preparation over the flame of a spirit lamp. As soon as the specimen was cleared, then examined it microscopically using the 10x and 40x.Hyphae, microconidias and macroconidias were absent while examined in direct microscopy with 10% potassium hydroxide (KOH).Blood was collected from cephalic vein. Complementary laboratory blood tests showed that the dog had no blood abnormalities that showed there was no other evidence of disease. Samples taken from scraping lesions were inoculated onto Sabouraud dextrose agar(SDA) with 0.05% chloramphenicol and 0.5% cycloheximide and incubated at room temperature for one week (fig.3). After 7 days incubation, fungal cultures on Sabouraud dextrose were taken for microscopic examination. The conidia were identified after lactophenol cotton blue staining on the basis of their size, shape, presence of septa, thickness of conidial wall and arrangement of conidial cells around the hyphae to confirm the genera of fungus for definitive diagnosis (fig.4).In that patient, we used combination of oral and topical antifungal in two phases. The dog was prescribed firstly Fluconazole (Tab. Canazole) dosage 10 mg orally for a period of 7 days and Whitfield ointment (Oint. Fungalin) topically two times daily for a period of 14 days .After confirmation of microsporosis the dog was treated with Itraconazole (Cap. Itra) 10 mg orally, once daily for 21 days and Ketoconazole (Nizoder shampoo2%) topical for two times/ week for a month.

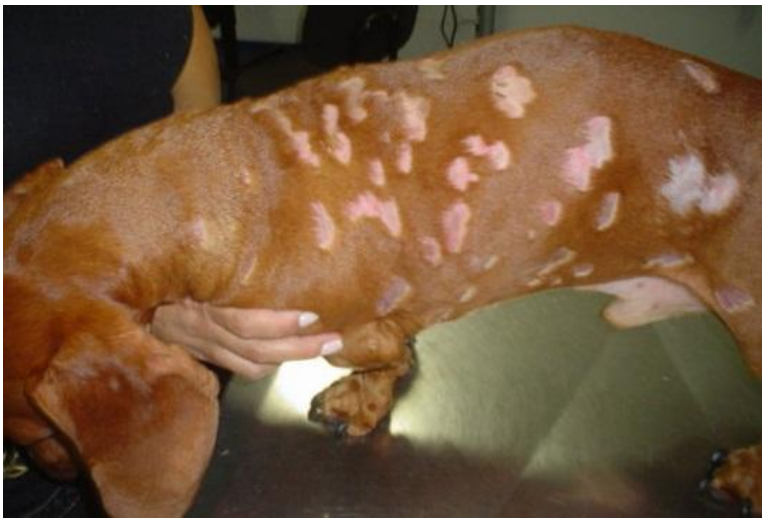


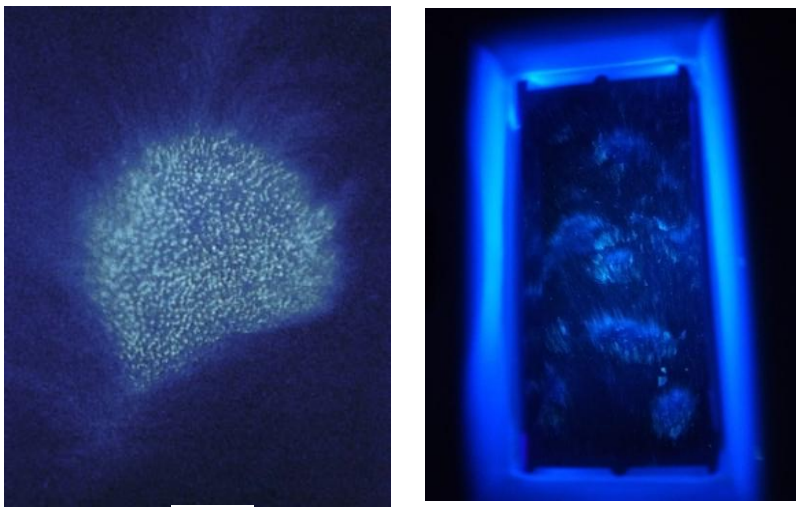
Figure 1: Patches of scaling, scratching and alopecia on the trunk and limbs

Chapter-3

Results and Discussion

Physical examination revealed hair loss, scaling, scratching, crusting and alopecia on the trunk and limbs (Fig. 1). Classical lesions include one or more areas of partial alopecia with scaling and crusting most commonly on the trunk and limbs and lesions may be hyper-pigmented (Moriello 2004). In this case Diagnosis of dermatophytosis was based on history, clinical examination and complementary tests, such as Wood's lamp, light microscope and fungal culture.

In this case, Wood's lamp examination showed apple-green fluorescent on the infected area (Fig.2) which indicated that the fluorescence probably due to dermatophyte species including *M. canis*. According to Outerbridge (2006), hairs invaded by most of *M. canis,* when ex-posed to the light showed yellow green glow. However, the green fluorescence came not only due to *M. canis* but also from other materials. According to the Wood's lamp is used to establish a tentative diagnosis of dermatophytosis in dogs but cannot be used to exclude this type of infection since some skin ointments and other materials will fluoresce and may give a false positive result (Gupta and Singh 2004). Therefore, examination with Wood's ultra-violet light was only used for screening method for dermatophytosis. Mycological Culture remains the most reliable technique for confirming dermatophytosis in dogs.



(a) (b)

Figure 2: Apple-green fluorescent on the affected area in skin (a) and Wood's lamp (b)

The samples were subjected to for direct microscopy detection of fungal elements (hyphae and arthrospores) after preliminary treatment with 10% KOH and gentle heating over the flame of a spirit lamp. The result of that examination was negative for hyphae, microconidias and macroconidias. Nevertheless, these results could be false negative. Identification of fungal elements directly in clinical samples using potassium hydroxide 10% (KOH) by microscopic is a quick method, but its specificity and sensitivity is low. Moreover, false negative results are possible. Levitt *et al*. (2010) suggested that the sensitivities of direct microscopy using 10% KOHwere73.3%.

In this case, fungal culture was definitive diagnosis which was considered the "gold standard" for diagnosis (Moriello, 2001). Sabourauds dextrose agar (SDA) was the most commonly used for fungal culture media. Clinical specimen (Skin scrapings and plucked hair) placed on Sabouraud dextrose agar and incubated at room temperature for one week. Growing fungal Culture showed a white, coarsely fluffy spreading colony with a distinctive "hairy" or "feathery" texture. On the underside of the medium, a characteristic deep yellow pigment developed due to the metabolites secreted by the fungus. The intensity of this yellow pigmentation peaked on the 6th day of colony growth. (Fig. 3). These characters were like *Microsporum canis* (Moriello KA., 2014). Hyaline hyphae and large thick walled spindle shaped macroconidia were detected by lactophenol cotton blue (LPCB) staining of culture (Fig. 4) (Ilhana et al., 2016). Although urease test was not performed, however, from microscopic examination the culture was suggested as *Microsporum canis*.

Figure 3: Sabouraud dextrose agar showed a white, cottony spreading colony with yellowish pigmentation

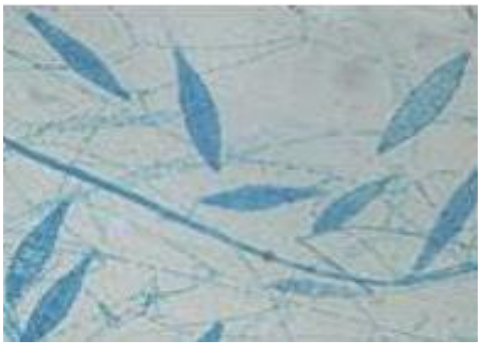


Figure 4: Lacto phenol cotton blue mount showing macroconidias

In this case, the dog was treated in two steps with combination of systemic and topical antifungals. Firstly, the dog was prescribed Fluconazole (Tab. Canazole) dosage 10 mg orally for 7 days and Whitfield Ointment (Oint. Fungalin) topically for 14 days on the basis of clinical signs. After mycological confirmation, a systemic oral therapy with itraconazole (Cap. Itra) at a dose of 10 mg/kg for a period of 21 days and topical treatment with (Nizoder shampoo2%)two times/ week for a month were given. *M. canis* invade the hair follicle and hair shaft. According to Moriello KA. (2004), most commonly effective dose of itraconazole was 5–10 mg kg−1 orally every 24 hours interval which works by altering fungal cell membrane permeability through inhibition of ergosterol synthesis .Topical therapy alone does not adequately penetrate the hair follicle so that optimal treatment of dermatophytosis requires systemic therapy for effective penetration to this site (Borgers *et al*., 1993). After treated with itraconazole for 21 days and ketoconazole for one month, the dog showed reduction of lesions (Fig. 5). Bond (2010) suggested thatthe treatment must be extended over 2 to 4 weeks after clinical cure and after obtaining two or more negative fungal cultures. Complete resolution of lesions was achieved after 75 days of itraconazole and ketoconazole treatment.

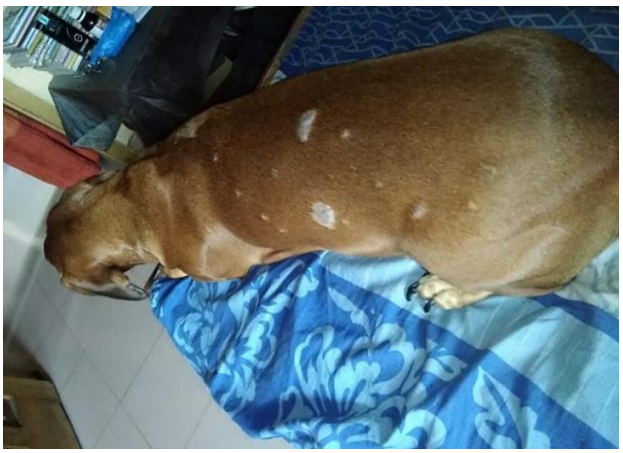


Figure 5: After use of combination treatments with systemic and topical antifungal for one month

Chapter-4

Conclusion

Dermatophytosis is one of the most common superficial fungal infections in dogs. This report provides an example of a cutaneous fungal infection in a female Dachshund dog. The dog suffered from *Microsporum canis* dermatophytosis and showed a successful response to systemic itraconazole and topical ketoconazole antifungal therapy. Attention is focused mainly on *Microsporum canis* due to veterinary and public health importance.

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I first praise to my creator the Supreme Personality of Godhead for his causeless compassion and enable me to pursue this study in this field of science and to complete this clinical report writing for the Degree of Doctor of Veterinary Medicine (DVM).

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Biography

I am Avi Das, son of Mr. Madhusudan Das and Mrs. Bijali Das. I passed my Secondary School Certificate (SSC) from Poroikora Noyontara High School, Anwara, Chattogram in 2012 and Higher Secondary Certificate (HSC) from Anwara Govt. College, Anwara, Chattogram in 2014 from Chattogram board, Bangladesh. I enrolled for Doctor of Veterinary Medicine (DVM) degree in Chattogram Veterinary and Animal Sciences University (CVASU), Khulshi, Chattogram in 2014-2015 sessions. Now I am doing my internship program which is obligatory for awarding my degree Doctor of Veterinary Medicine (DVM), from Chattogram Veterinary and Animal Sciences University (CVASU). This study was the inauguration of me in the era of research and I have a strong intention to involve myself in these types of activities in future. I want to be a researcher as well as a veterinary practitioner in future.