INVESTIGATION OF BOVINE CUTANEOUS DERMATOMYCOSIS AT DISTRICT VETERINARY HOSPITAL (DVH)

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A THESIS

BY

SHAHRIN RUKHSANA

Semester: September, 2011-March, 2012
Registration No.: 1005111
Session: 2010

MASTER OF SCIENCE (M. S.)

IN

PATHOLOGY

DEPARTMENT OF PATHOLOGY AND PARASITOLOG
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

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Submitted to the
Department of Pathology and Parasitology
Hajee Mohammad Danesh Science and Technology University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE (M. S.)

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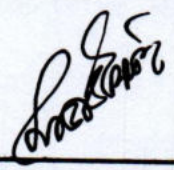

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MARCH 2012


DEDICATED
TO
MY BELOVED
HUSBAND AND CHILD

Acknowledgement

All praises are due to almighty Allah, the supreme authority of the universe, who enabled the author to carry out the whole research work and to build up this thesis.

The author expresses the deepest sense of appreciation, heartiest gratitude and indebtedness to her reverend and beloved teacher and honorable supervisor Dr. Md. Nazrul Islam, Assistant Professor, Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, for his sincere interest, scholastic guidance, continuous encouragement, constructive suggestions and all round help throughout the course of research works and preparation of the manuscript in time.

The author humbly desire to express her deepest sense of gratitude, heartiest respect and sincere appreciation to her respectable co-supervisor Dr. S. M. Harun-ur-Rashid, Professor and Chairman, Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, for his constant help in conduction of experiments and invaluable guidance, scholastic cooperation, constant inspiration and in writing of thesis.

The author also desire to express her deepest sense of gratitude, heartiest respect and sincere appreciation to her respectable teacher Dr. Md. Mostafizur Rahman, Professor and Chairman, and Dr. Mir Rowshan Akhter, Assistant Professor Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, for his help in conduction of experiments in microbiology laboratory.

The author also thankful to veterinary surgeon and other stuffs of district veterinary hospital, Dinajpur for their help in collection of data and samples.

The author also expresses her respect and sincere appreciation to Dr. Md. Mominul Islam and Dr. Md. Hayder Ali, Lecturer, Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, for their constructive criticism.

The author also expresses her cordial thanks to her friends for helping me in laboratory work and to complete my research.

The author also thankful to laboratory technicians and stuffs of the Department of Pathology and Parasitology and Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, for their technical assistant during the experiment.

Thanks and appreciations also extended to my parents, husband, son and friends and well wishers of the author.

The Author
March, 2012

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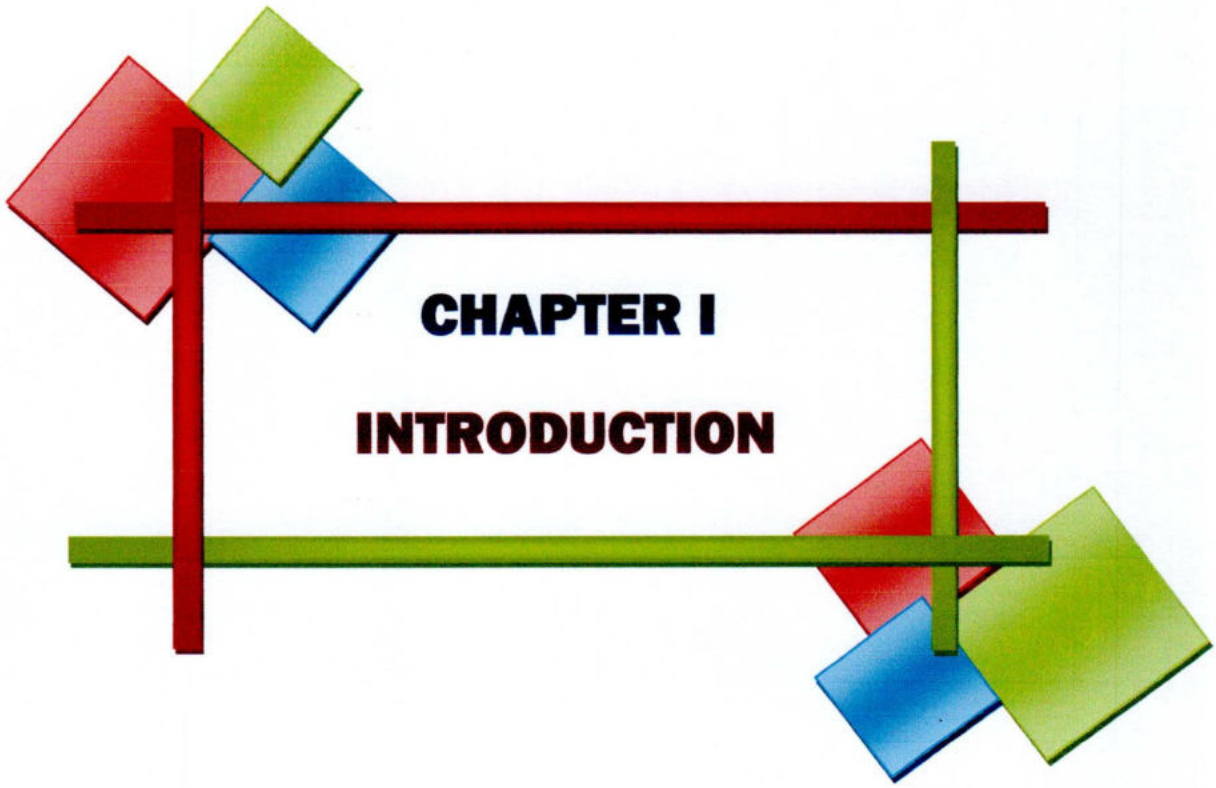
ABBREVIATIONS USED IN THIS TEXT

%	:	Percentage
µm	:	Micrometer
^{0}C	:	Degree centigrade
g	:	Gram
H & E	:	Haematoxylin and eosin
hr	:	Hour
hrs	:	Hours
KOH	:	Potassium hydroxide
ml	:	Milliliter
DVH	:	District Veterinary Hospital
/	:	or
et al	:	and his associates
±	:	Plus minus
<	:	Less than
>	:	Greater than
No.	:	Number
NS	:	Not significant
&	:	and
lb	:	Pound
bwt	:	Body weight
@	:	At the rate of

ABSTRACT

MS Thesis: S. Rukhsana (2013). Investigation of bovine cutaneous dermatomycosis at District Veterinary Hospital DVH) of Dinajpur

This study was done to investigate the Epidemiological, clinicopathological, microbiological and therapeutical response of bovine cutaneous dermatomycosis or ringworm in cattle at DVH, Dinajpur from March-2011 to February-2012. A total of 1681 clinical cases in bovine animal were registered among which 21 were encountered as dermatomycosis. The annual incidences of bovine dermatomycosis were determined based on different epidemiological factors such as season, sex, age, breed, managemental systems. The clinical and pathological features including topographic positions of the lesions and therapeutic strategies were also recorded. The skins of 3 typically affected with dermatomycosis were collected, preserved, processed, embedded with paraffin, sectioned and stained with haematoxylin and eosin for the histopathological study. The annual incidence of dermatomycosis was 1.25% at DVH of Dinajpur. The highest incidence of dermatomycosis were recorded in summer season (1.64%) followed by winter (0.88%) and rainy (0.78%) season. The annual incidences of dermatomycosis were high in female animals (1.35%) than male animals (1.11), in young (2.55%) animal than the calves (0.62) and adult (0.92), in indigenous breed (1.29%) than the crossbred animal (0.92) and in rural housed farms (1.32%) than the intensive farming (0.61%) management. Major lesions were found in head, neck and pelvis regions. Grossly the disease was characterized as circular lesion of scab and crust formation, roughened hair coat with pruritus. Histopathologically the diseases were characterized as hyperkeratosis, parakeratosis, epidermal hyperplasia, densely growth of collagenous tissues with moderate destruction of glandular structures. Identification of fungi under microscope by direct impression smear from the affected skin lesion and growth of grey to white colored colony in Sabouraud's dextrose agar media is main diagnostic features of dermatomycosis. Griseofulvin with topical application of whietfield ointment showed good response in the treatment of dermatomycosis in cattle.



CHAPTER I

INTRODUCTION

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INTRODUCTION

The dermatophytes are fungi which cause diseases of the skin of human, animals or both. They parasitize keratinized tissues, including nails, hair and stratum corneum of the skin, and cause dermatomycoses (**Weitzman and Summerbell, 1995**). These fungi have frequently been treated as special group the 'Dermatophytes' or Ringworm fungi or tinea. It is caused by a variety of zoophilic, anthropophilic and geophilic dermatophytes of the genera *Microsporum, Epidermophyton* and *Trichophyton* (**Weitzman and Summerbell, 1995**). Dermatophytes are cosmopolitan and occur widely in soil and other keratin containing substrate such as bird's nest and thus, the soil serves as a source of infection (**Beneke and Rogers, 1980; Ainsworth, 1971**). The prevalence of dermatomycoses infections have been studied in different parts of the world (**Akpolat *et al.*, 2005; Metin *et al.*, 2001; Devliotou *et al.*, 2000**) The relative occurrence of the etiologic agents of these infections varies from country to country and from one climatic region to another (**Korstanje *et al.*, 1995; Ayadi *et al.*, 1993; Vrocy, 1985**). In

tropical countries, a warm and humid climate, crowded living and poor sanitary conditions all promote the spread of these.

Infections caused by dermatophyte fungi are very serious problem, not only clinical, but also epidemiological and therapeutic. The incidence of skin, hair and nail diseases does not depend on sex, age or social status. There are many species of dermatophytes causing mycoses, so it is very important to assay them properly through mycological examination. Correct identification of the pathogen responsible for disease allows choosing a right treatment for patient. Natural reservoir of dermatophytes is soil and keratin contained in soil is used as main nutrient for these fungi. However, evolutionary progress adapted these microorganisms to a various environments, so they generated ability to metabolize keratin derived not only from soil (**Kaszak, 2004**). For that reason dermatophytes, with regard to their habitat, may be divided into antropophilic, zoophilic and geofilic species. For antrophophilic dermatophytes natural reservoir and carrier is human, zoophilic dermatophytes grow on domestic and stock animals and geophilic dermatophytes are found in soils (**Adamski & Gabryel, 2007**).

In the intensive and extensive mode of keeping cattle trichophytia a health problem not only for animals but, also for the workers and veterinarians on farms. In infected herds, about 95 % of cattle are affected by two years of age, a much rarer older cows. Trichophytia is dermatomycoses in cattle caused by *Trichophyton verucousm*, which lives on the skin surface and its proteolytic and keratolytic enzymes and exotoxins cause parakeratosis and inflammatory changes. The first changes were observed in the form of round no hair fields covered with white scales and appear on the scalp, thickness between 0.5 to 1.0 cm. Dermatomycosis changes occur in the form of individual or collective focus. These clinical cases trichophytia can be complicated with bacterial infection. *Microsporum gypseum* infections have been reported in cattle, horses, sheep, goats, pigs and humans. Sporadic outbreaks appear to be due to the worldwide distribution of *Microsporum gypseum* in soils.

In predisposing factors considered to be an increased percentage of humidity, overpopulation in the facilities, age of animals in the herd as well as the quality of food. The infection is transmitted by direct contact. For favors the emergence of this disease during the winter. Economic losses are

due to decreases the purchasing value of the animals themselves and their skin.

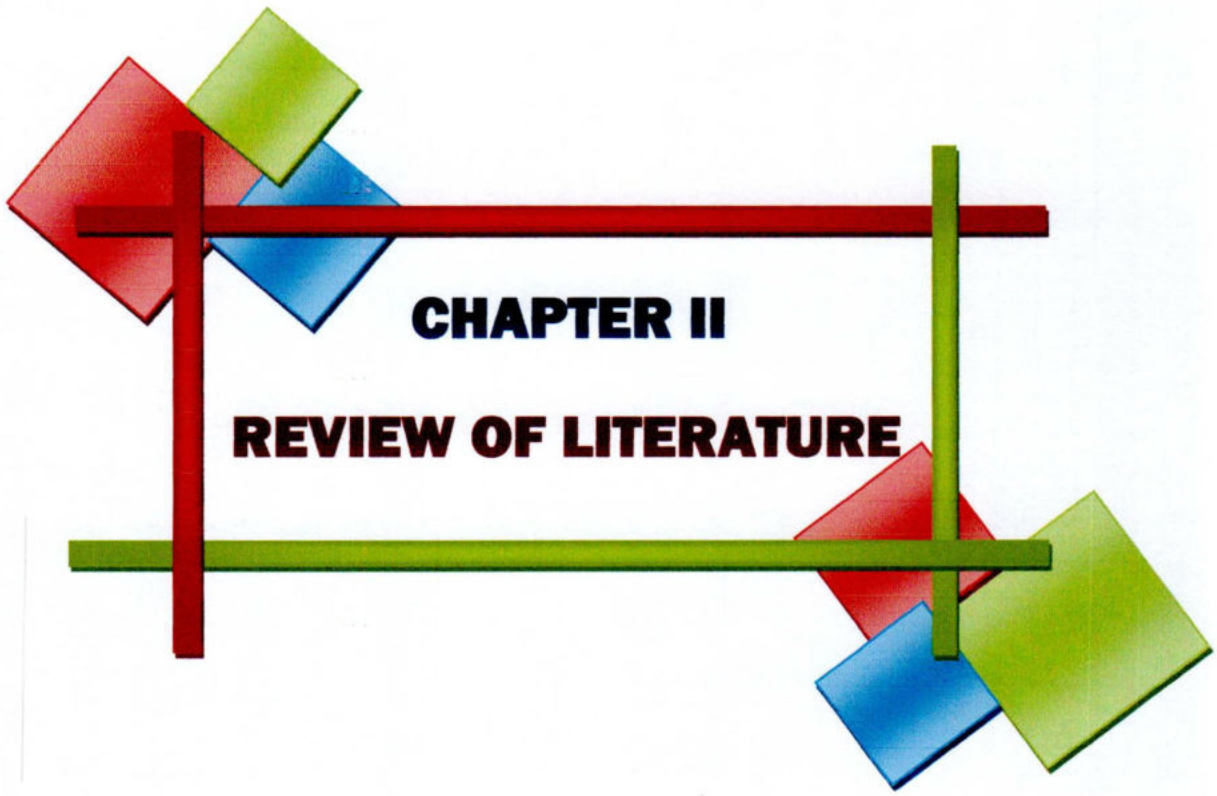
The gross appearance of dermatophytosis is quite variable. The classic ringworm-like lesion is characterized by an annular area of alopecia, stubbled hairs, and variable amounts of scalling, crusting, and dermatitis. Folliculitis and frunculosis are also common manifestations of dermatophytosis. Lesions are most commonly seen on the head, neck, and pelvis and vary from discrete circular areas of alopecia to severe scaling, crusting, suppuration, and ulceration. Pruritus and pain are variable (**Fraser *et al.*, 2008**).

Among fungal zoonoses caused by various fungi species, the most wide spread ones are dermatomycosis (dermatophytosis) of animals and human beings. Dermatophytosis cause a great economic damage to domestic livestock breeding and represent a medical-and-social problem in many countries of the World since diseased animals (cats, dogs, cattle, small cattle, horses, camels and furred animals etc) are often serve as sources of infecting human beings (**Igor *et al.*, 2009**).

Dermatomycosis or ringworm is an important zoonotic disease for animal and man but study on this disease was not done at Dinajpur previously. Therefore the current study was undertaken with the following objectives.

OBJECTIVES OF THE STUDY

- To determine the incidence of the disease based on different epidemiological factors such as age, sex, breed and season at District Veterinary Hospital (DVH), Dinajpur.
- To study the clinical as well as pathological features of skin affected with bovine dermatomycosis.
- Biopsy of the skin scraps and culture for the determination of the dermatophytes in the affected skin
- To study the therapeutical response of dermatomycosis affected bovine animal.



CHAPTER II

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

2.1. TAXONOMIC POSITION OF THE OETIOLOGICAL AGENT

Kingdom: Fungi

Phylum: Ascomycota

Class: Onygenales

Order: Actinomycetales

Family: Arthrodermataceae

Genus: Arthroderma (Includes *Microsporum, Epidermophyton, Trichophyton*)

2.1.1. OETIOLOGY

There are many species of dermatophytes causing mycoses, so it is very important to assay them properly through mycological examination. Correct identification of the pathogen responsible for disease allows choosing a right treatment for patient. Natural reservoir of dermatophytes is soil and keratin contained in soil is used as main nutrient for these fungi. However, evolutionary progress adapted these microorganisms to a various

environments, so they generated ability to metabolize keratin derived not only from soil (**Kaszak, 2004**).

For that reason dermatophytes, with regard to their habitat, may be divided into antropophilic, zoophilic and geofilic species. For antrophophilic dermatophytes natural reservoir and carrier are human, zoophilic dermatophytes grow on domestic and stock animals and geophilic dermatophytes are found in soils (**Adamski & Gabryel, 2007**).

In laboratory practice dermatophyte fungi belonging to three genera (*Trichophyton*, *Microsporum*, *Epidermophyton*), are known. *Trichophyton* and *Microsporum* genera are the most numerous and diverse, there are over 40 species belonging to these two taxonomic groups. *Epidermophyton* genus has only one representative – *Epidermophyton floccosum* species.

Trichophyton mentagrophytes – zoophilic dermatophyte, cosmopolitan fungus, distributed worldwide. It causes infections in animals (cats, dogs, rabbits, guinea pigs, rodents, hedgehogs) as well as in human. Most commonly it causes infections of glabrous skin (*tinea corporis*, *tinea faciei*)

and scalp infections (*tinea capitis*). Lesions often proceed with large inflammatory reactions (**Konsur *et al.*, 2011**).

Trichophyton verrucosum – zoophilic species, the mostly isolated from cattle, it infects people, who work with these animals (farmers). Cosmopolitan fungus, it causes diseases of exposed parts of the body (*tinea corporis*) and face (*tinea faciei*), beard (*tinea barbae*) or head (*tinea capitis*) (**Konsur *et al.*, 2011**).

Microsporum canis – zoophilic species, cosmopolitan, distributed worldwide. It is very contagious aspecially for young cats and dogs. In human it causes infections of the scalp (*tinea capitis*) and beard (*tinea barbae*) or infections of glabrous skin or face (*tinea corporis*, *tinea faciei*) (**Kalinowska *et al.*, 2009b**).

Microsporum gypseum – geophilic species, ubiquitous, isolated from soils worldwide. Most exposed to infections are people, who cultivate the soil (farmers, gardeners), percentage of males prevails over females. Sometimes infection could be transmitted from animals. In human it causes infections of

the scalp (*tinea capitis*) and glabrous skin (*tinea corporis*) (**Kalinowska *et al.*, 2009b**).

Table 1: Fungi reported to cause Dermatophytosis in large animals and humans (Ainsworth and Austwick, 1973; Abdallah *et al.*, 1973; Crestain and Luffair, 1977; Aho, 1980; Abu-Samra and Hago, 1980)

Fungus	Horse	Cow	Sheep	Goat	Pig	Human
Microsporum canis	+	+	+	-	+	+
M. gypseum	+	+	+	-	+	+
M. equinum	+					+
M. nanum		+			+	+
Tricophyton megnini	+	+	+	+	+	+
T. mentagrophytes	+	+	+	+	+	+
T. verrucosums	+	+	+	+	+	+

Microsporum nanuum – zoophilic species, isolated also from soil. It causes infections among breeding cattle, mostly in pigs. In human it causes infections of the glabrous skin (*tinea corporis*) and the scalp (*tinea capitis*) proceeding with or without severe inflammation (**Kalinowska *et al.*, 2009b**).

2.1.2. CYTOMORPHOLOGICAL FEATURES AND CULTURAL CHARACTERISTICS

Spores are the diagnostic feature and appear as round or polyhedral, highly refractive bodies in chains (*Trichophyton* spp), or mosaics (*Microsporum* spp) in hair follicles, epithelial scales and in or on the surface of hair fibers **(Radostits *et al.*, 2004)**

Dermatophytes grow well on Sabouraud's dextrose agar media. *Microsporum* colonies are glabrous, downy, wooly or powdery. The growth on Sabouraud dextrose agar at 25°C may be slow or rapid and the diameter of the colony varies between 1 to 9 cm after 7 days of incubation. The color of the colony varies depending on the species. It may be white to beige or yellow to cinnamon. From the reverse, it may be yellow to red-brown. Variances in colony morphology and color help in inter-species differentiation. Hair perforation test, ability to grow on rice grains and also at 37°C provide useful hints to differentiate *Microsporum* spp.

Microsporuni spp. produce septate hyphae, microaleurioconidia, and macroaleurioconidia. Conidiophores are hyphae-like. Microaleuriconidia are

unicellular, solitary, oval to clavate in shape, smooth, hyaline and thin-walled. Macroaleuriconidia are hyaline, echinulate to roughened, thin- to thick-walled, typically fiusiform (spindle in shape) and multicellular (2-15 cells). They often have an annular frill, Inoculation on specific media, such as potato dextrose agar or Sabouraud dextrose agar supplemented with 3 to 5% sodium chloride may be required to stimulate macroconidia production of some strains. Varieties in shape of macroconidia and abundance of microconidia help in inter-species differentiation.

Trichophyton Characterized by colourless spores (conidia) that is nearly sessile and usually produced at right angles to the fertile filament, or as terminal swellings. Most conidia are 1- or 2-celled (micro-conidia) but at least a few are more than 2-celled (macroconidia). Usually occurring as a skin parasite (dermatophyte) on man and animals but occasionally isolated from soil, leather, feathers, etc.

2.2. EPIDEMIOLOGICAL STUDY OF DERMATOMYCOSIS

Many epidemiological factors have been implicated in the cause, spread and resolution of dermatomycosis.

2.2.1. Geographical distribution

Dermatomycosis is worldwide distributed, but more prevalent in the humid, tropical and subtropical regions, where it constitutes substantial losses in the livestock

The disease has been described cosmopolitanly in different countries of Europe (**Die, 1980**), America, (**Foster *et al.*, 2004**) Africa (**Schmeller, 1997**), India (**Gupta, *et al.*, 1970**), Egypt (**Gabal, *et al.*, 1976**), Middle East, Arabian and African states, Asia (**Devliotou *et al.*, 1995; Claus *et al.*, 2008**), including Bangladesh (**Nooruddin and Dey, 1985, 1986; Nooruddin *et al.*, 1992abc; Neela and Alam, 2000**).

It has been also reported in France (**Foulet, *et al.*, 2007**), Germany (**Seebacher, *et al.*, 2008**), Russia (**Ja¨rv, *et al.*, 2004**), Switzerland (**Manod,**

***et al.*, 2002**), Belgium and Netherlands (**Korstanje and Staats, 1994**), Italy (**Panasiti, *et al.*, 2007**), Finland (**Lehenkari and Kassinen, 1995**), Poland (**Dolenc-Voljc, 2005**), Slovenia (**Dolenc-Voljc, 2005**), Bosnia and Herzegovina (**Prohic, 2008**), Australia (**Merlin, 1999**), Turkey (**Ozkutuk, *et al.*, 2007**), Iran (**Lari, *et al.*, 2005**), Korea (**Jang, *et al.*, 2004**), Singapore (**Tan, 2005**), China (**Tao-Xiang, *et al.*, 2005**), Japan (**Ogasawara, 2003**), Nigeria (**Enweani et al., 1996**), Malawi (**Po¨nnighaus, et al., 1996**), Libya (**Ellabib, *et al.*, 2002**), Jordan (**Abu-Elteen and Malek, 1999**), USA (**Kemna and Elewski, 1996; Foster, *et al.*, 2004**), Canada (**Gupta and Summerbell, 1998**), Brazil (**Fernandes, *et al.*, 2001**), Mexico (**Welsh et al., 2006**).

2.2.2. Susceptible animals/ Host ranges

Dermatomycosis was reported to affect a wide variety of animal species: cattle (**Gupta and Singh, 1969; Edwardson and Andrews, 1979**), sheep and goats (**Pepin and Austwic, 1968; Pier *et al.*, 1994**), horses (**Richard *et al.*, 1994; Pepin and Austwic, 1968; Connole, 1967; Brown and Donald, 1964**), cats and dogs (**Pepin and Austwic, 1968**), rabbits (**Hegen and Gorham, 1972**), pig (**Cameron, 1984; Dunne and Leman, 1975**) and as

well as in humans (**Blank, 1953; Blank and Craig, 1953; Brock, 196; Baxter, 1969).**

Trichophyton verrucosum has been cited as the major agent encountered in cases of bovine, ovine and caprine ringworm. Other species such as *M. canis, M. gypseum, T. mentagrophytes* and *T. equinum* have been isolated from some of these ruminants (**Pepin and Austwick, 1968; Stenwig, 1985; Pier *et al.*, 1994).**

Most authors (**Pepin and Austwick, 1968; Stenwig, 1985**) mentioned that dermatophytosis in horses is mainly produced by *T. equinum*, although other species such as *M. canis, M. equinum, M. gypseum, T. mentagrophytes and T. verrucosum* can usually be found in equine ringworm.

M. Canis and *M. gypseum* are the main spacies affecting dog and cat in the world (**Rebel and Taplin, 1979 and Solans, 1988**). Occasionally, a varicty of other dermatophytic species (e.g. *E.bfloccosum, M. cookei, M. fulvum, M. vanbreuseghemii, T. ajelloi, T. equinum, T. rubrum, T. verrucosum,* etc*)* have been cited as etiological agents of dermatophytosis and/or have been

isolated from the fur of healthy cats and/or dogs (**Rebel and Taplin, 1979 and Solans, 1988**).

Dermatophytosis in pigs is rare and it has little effect on productivity. *Microsporum nanum* is the main cause of ringworm in these animals (**Rebbel and Taplin, 1979**)

2.2.3. Breed, sex, age and season

- *Trichophyton verrucosum* and *Trichophyton mentagrophytes* are the major causes of dermatomycosis (commonly known as ringworm) in cattle in many parts of the world (**McPherson, 1957; Padhye, 1980; Scott, 1994**)
- The infection is mainly spread by contact between infected and susceptible animals or via a contaminated environment such as bedding and walls (**Scott, 1994**).
- High prevalence of dermatomycosis during winter has been attributed to the accumulation of infective material during this period (**Ainsworth, and Austwick, 1975**). In tropical regions like in Africa, where cattle are raised on open pasture for most of the year, the prevalence of the disease is low (**Scott, 1994**).

- Calves are more susceptible to ringworm infection than older animals (**McPherson. 1957; Gupta and Singh, 1969**). In cattle the lesions are most commonly found on the neck, head and perineum and consist of heavy, grey-white crusts raised perceptibly above the skin, or simply alopecia (**Radostits *et al.*, 1994; Scott, 1994**).
- Transmission of ringworm-causing organisms from infected animals to people has been reported in the past (**Touche, 1955; Mortimer, 1955; Gentles and Sullivan, 1957; Padhye, 1980; Radostits *et al.*, 1994**) and infected animals may act as reservoirs of human infections (**McPherson, 1957**).

2.2.4. Sources of infection and Transmission of organisms

Dermatophytosis is transmitted by direct and indirect contact. Contaminated objects (eg. Housing, fencing, grooming equipments, riding and working gear and dung) are extremely important in the natural dissemination of the disease. Fungal spores may remain viable under natural conditions for years. The incubation period between exposure and clinical disease varies from 1 to 6 weeks.

2.2.5. RISK FACTORS

2.2.5.1. Pathogen factors

M. gypseum, *K. allejoi* and *M. nannum* are soil saprophytes and the reasons for their assumption of pathogenecity are not understood (**Radostits *et al.*, 2004**).

2.2.5.2. Enviroment factors

A high incidence of clinical cases in the winter and of spontaneous recovery in the spring is common but outbreaks also occur during the summer months so that, close confinement and possibly nutrition seem to be more important in the spread of the disease than other environmental factors such as temperature and sunlight. Humidity is known to be important, a high humidity being conducie to multiplication of the fungus (**Radostits *et al.*, 2004**).

2.2.5.3. Host factors

Animal susceptibility is determined largely by immunolologaical status so that young animal is most susceptible (**Radostits *et al.*, 2004**).

2.2.6. PREDISPOSING FACTORS

The predisposing factors in dermatomycosis include (**Hall, 1966; Gugnani, 1972; Dunne and Leman, 1975; Amstutz, 1980**)

- Age : young animals are more susceptible than the adult animals
- Immunity : Prior exposure or immunosuppression
- Environment: contamination, crowding, high humidity, poor ventilation, or darkness
- Poor condition: Poor nutrition or debilitating diseases.

2.2.7. INCUBATION PERIOD

Fungal spores may remain viable under natural conditions for years. The incubation period between exposure and clinical disease varies from 1 to 6 weeks (**Radostits *et al.*, 2004**).

2.2.8. ECONOMIC IMPORTANCE OF DERMATOMYCOSIS

Dermatomycosis usually no effect on animal growth rate or other measures of productivity, unless the eyes or muzzle are severely affected, or secondary

bacterial infections occur (**Ainsworth and Austwick 1973; Amstutz, 1980**). Economic losses may arise because of hide damage or the anima's inability to work or show. Dermatomycosis in all large animal species is a significant public health hazard (**Ates *et al.*, 2008; Igor *et al.*, 2009**).

2.3. METHODS OF PATHOGEN TRANSMISSION

Dermatophytes are keratinophilic fungi, which parasitize on corneous structures, such as stratum corneum, hair or nails (**Kobierzycka *et al.*, 2005**). Dermatophyte species are equipped with numerous enzymes, enabling them to survive on the skin and its appendages, because they have a proteolytic, keratinolytic and lipolytic activity. Furthermore the skin environment is conducive to dermatophytes because the corneal layer lacks blood vessels making it difficult to contact with immunologically competent cells and activate the defense mechanisms. On the surface of the epidermis are proteins, carbohydrates and micronutrients (including iron ions), which may provide substrates for the metabolism of fungi and help them to survive. Of great importance may also be some specific anatomic regions of the skin, greatly facilitating the colonization by fungi. Scalp hair can therefore arrest arthrospores spreaded by air. Similarly, spores are arrested in the

hyponychium under or in the interdigital spaces, or in the folds of the skin where additionally oclusion helps them to develop (**Kaszak, 2004**). The spores are particularly resistant to environmental conditions, such as variable temperature and drying (**Gwozdz *et al.*, 2005; Kobierzycka *et al.*, 2005**). It is known that they can survive outside the host organism and colonize the skin and its appendages under favorable conditions, for example, in warm and humid environment, with increased amounts of CO2, which prevails in the poorly sheered shoes, it can lead to growth of the fungi and invasion of the skin structures.

2.4. SOURCE OF INFECTIONS AND PATHOGENESIS

The sources of dermatophyte fungi infection are: human, animals and soil. Infection with antropophilic dermatophytes may happen through direct contact with infected person, moreover spores of dermatophyte fungi can survive on skin and its appendixes without causing the disease (asymptomatic carrier) (**Adamski & Gabryel, 2007**).

Infection may occur also through some objects on which infectious material can be found (stratum corneum or hair with spores of fungi). The source of trunk, groin or extremities infections may be clothes, underwear or towels

and sponges. Scalp diseases may happen through using the same brushes or combs. Shoes, socks, accessories for feet care or cosmetic pedicure are often the source of infections of feet and toenails.

Nails diseases are often connected with cosmetic manicure, infection may be a result of unsuitable disinfection of nails care accessories in cosmetic salons. Also some public places could be potential source of antropophilic dermatomycoses – for example swimming-pools, toilets, showers, hotels, schools and similar (**Bolinski *et al.*, 2003; Szepietowski & Baran, 2005**).

Zoophilic dermatophytes can be also transmited from human to human. The source of infection in children and adults are mostly domestic animals – cats, dogs, hamsters, guinea pigs, rabbits or even some birds. Farmers also often suffer from dermatomycoses transmitted from breeding cattle (pigs, cows, sheep, horses, goats) (**Adamski & Gabryel H., 2007**).

Infection with geophilic dermatophytes usually happens as a result of contact with soil and it is common among people, who cultivate the soil (gardeners, farmers). The disease more often affect males than females. Working without protective gloves and unsuitable hygiene is conductive for

transmission of pathogen. In literature were also described cases of transmission of geophilic dermatophytes through some animals (for example monkeys, mouses, leopards, rats, tigers) and insects (flies). Infection through direct contact with ill peoples occurs rather rarely (**Kalinowska *et al.*, 2009b**)

2.5. ZOONOTIC IMPORTANCE

Animals serve as reservoirs of the zoophilic dermatophytes, and their infections have considerable zoonotic importance. Zoophilic dermatophytes such as *M. canis, T.mentagrophytes* var. *mentagrophytes* and *T. verrucosum* are significant causal agents of human ringworm in many areas of the world (**Pier *et al.*, 1994; Ates *et al.*, 2008; Igor *et al.*, 2009**).

Spread between species ocuurres readily and in rural areas 80% of human ringworm may derive from animals. *Tricophyton spp.* Infections are commonly contracted from horse and cattle and *M. canis* infections from dogs. Ringworm of animal origin affects adult humans as well as children and diagnosis and treatment are often very difficult (**Radostits *et al.*, 2004**).

2.6. CLINICAL MANIFESTATIONS

The typical lesion is a heavy, gray white crust, raised perceptibly above the skin. The lesions are roughly circular and about 3 cm in diameter. In the early stages the surface below the crust s moist, in older lesion the scab becomes detached and pityriasis and alopecia may be the only obvious abnormalities. Lesions are most commonly found on the neck, head and perineum but a general distribution over the entire body may occur and particularly in calves and in several cases the lesions may coalesce. Itching does not occur secondary acne is unusual (**Radostits *et al.*, 2004**).

Lesions are most commonly seen on the head, neck and pelvis and vary form discrete circular areas of alopecia to severe scaling, crusting, suppuration and ulceration, Pruritus and pain are variable.

2.7. DERMATOPATHOLOGICAL FEATURES

2.7.1. Gross morbid lesions

The gross appearance of dermatomycosis (ringworm, tinea, girth itch) is quite variable. The classic ringworm-like lesion is characterized by annular area of alopecia, stubbled hairs and variable amounts of scaling, crusting and

dermatitis. However, this type of gross lesion is far from diagnostic and may be produced by other disorders, including staphylococcal dermatitis, dermatophilosis, drmodiosis and pemphigus foliaceus. In addition, the

alopecia may be patchy or either scaling o r crusting may predominant in the absence of much alopecia. Folliculitis and frunculosis are also common manifestations of dermatomycosis. In such cases, the predominant lesions may include varying combinations of papules, nodules, ulcers and sinuses. A kerion is a localized, severe inflammatory lesion that is nodular and boggy and oozes pus (**Hoerlein, 1970; Jungerman and Schwartzman, 1972; Ainsworth and Austwick, 1973; Abu- Samra, *et al.*, 1976; Amstutz, 1980).**

2.7.2. Location of the lesions

Lesions are most commonly seen on the head, neck and pelvis and vary from discrete circular areas of alopecia to severe scaling, crusting, suppuration and ulceration. Pruritus and pain are variable. In two large studies of Trichophytosis in cattle (**Pandey and Carbaret, 1980; Nooruddin and Dey, 1985**) the site of lesions was found to be sex and age dependant.

Characteristic location of lesions was the periocular region for calves, the thorax and limbs for cows and heifers and the dewlap and intermaxillary space for bulls. It was postulated that this site differences were basically associated with behavioral differences between the different sex and age groups. In herd outbreaks of dermatomycosis due to *T. metagrophytes and T. equnum* in adult cattle, lesions consisted of generalized areas of circular alopecia with minimal scalling or crusting (**Kral, 1961; Monga *et al.*, 1974**).

2.7.3. Histopathology

The histopathologic features of dermatophytosis are as variable as the clinical lesions. There is no diagnostic histopathologic appearance characteristic of dermatophytosis. The most common istopathologic patterns observed in dermatophytosis (**Aamodt *et al.*, 1982**) , perifolliculitis, and furunculosis, (**Abdallah *et al.*, 1973**), superficial perivascular dermatitis (spongiotic or hyperplastic) with orthokeratotic or parakeratotic hyperkeratosis of the epidermis and hair follicles and (**Gabal *et al.*, 1976**) intraepidermal vesicular (spongiotic) or pustular dermatitis are rare hisstopathologic pattern is that of nodular or diffuse dermatitis (suppurative, pyogranulomatous), with the fungus present as grains and hyphae with in the

derms or subcutis or both. Such unusual tissue reactions to dermatophytes are often referred to as Majocchi's granuloma or pseudomycetoma (**Rinaldi, 1983**). Septate fungal hyphae and spherical to oval conidia may be present with in surface keratin and crust, with in the hair follicles, or in and around the hairs. The numbers of fungal elements present is usually inversely propotional to the severity of inflammatory response. Dermatophytes are often visible in sections staine with H & E but are more readily detected wth periodic acid-Schinn (Pas), acid orcein-Giemsa (AOG) stains

2.8. DIAGNOSIS

- Tentative diagnosis can be made on history and presence of characteristic ringworm lesions.
- Dermatophytes such as *Microsporum* spp. Will fluoresce under an ultraviolet or wood's lamp and this method is commonly used to diagnose ringworm in animals.
- Laboratory diagnosis depends upon the examination of skin scrapings for fungal spores and mycelia by direct microscopic means and culture.
- Skin scrapings are warmed gently in a 20% solution of either potassium or sodium hydroxide.

- Spores are the diagnostic features and appear as round or polyhedral highly refractile bodies in chains or mosaics

2.8.1. Wood lamp examination

For fluorescence causes only certain strains of *M. canis, M. audouinii, M. distortum,* and *Trichophyton schoenleinii* to produce a positive yellow-green colour on infected hairs. The Wood's lamp is an ultraviolet light with a light wave of 253.7 nm that is filtered through a cobalt or nickel filter (**Scott *et al.*, 2001**). The Wood's lamp should be turned on and allowed to warm up for 5 to 10 minutes because the stability of the light's wavelength and intensity is temperature dependent (**Muller *et al.*, 1989; Morriello, 1990**). The animal should be placed in a dark room and examined under the light of the Wood's lamp. When exposed to the ultraviolet light, hairs invaded by M. canis may fluoresce in about 50% of the isolates (**Morriello, 1990; Glabac, 1994; Scott *et al.*, 1995; Zdovc *et al.*, 1997**). Hairs should be exposed for 3 to 5 minutes because some strains are slow to show the obvious yellow-green colour. The fluorescence is due to tryptophan metabolites produced by the fungus (**Gnamusch *et al.*, 1992**). Positive fluorescence should be distinguished from false positive fluorescence due to presence of certain bacteria (Pseudomonas aeruginosa, Corynebacterium minutissimum), kera-

tin, soap, petroleum, and other medication. These fluorescing hairs should be plucked with forceps and used for inoculation of fungal medium or for microscopic examination (**Scott *et al.*, 2001**).

2.8.2. Microscopic examination

One perform microscopic examination by adding 20 % KOH to the hair, scales, and claw material on microscope slide, adding the cover slip and heating (but not boiling) the sample for 15-20 seconds. Instead of heating the preparation may be allowed to stand for 20 minutes at room temperature (**Scott *et., al*, 1995**). Alternatively to KOH, lactophenol can be used without heating (**Zdovc *et al.*, 1997**).

Direct examination may reveal hyphae and arthrospores in 40-50% of the cases (**Scott *et al.*, 19956; Zdovc *et al.*, 1997**) but cannot distinguish between different dermatophyte species (**Gnamusch, *et al.*, 1992**). When the result is positive it is a definitive evidence of dermatophytosis (**Scott *et al.*, 1956**).

2.8.3. Microscopic examination with fluorescent microscope

Material (hairs) is placed on the microscopic slide, 2 drops of 10 % KOH solution are added and then mixed. Then 2 drops of calcofluor sre added, mixed and slide covered. Calcofluor is colorless fluorescent stain that fixes to B1-3 and B1-4 polisaccharides that build in the cellulose and chitine. Stained preparation is then exposed to ultraviolet light and green fluorescent fungal elements can be seen. (**Zdovc *et al.*, 1997**).

Microscopic examination with fluorescent microscope is rarely used because of the need of special equipment but it can be useful. According to some data it can be efficient in more than 50% of cases (**Zdovc *et al.*, 1997**).

2.8.4. Fungal culture

Fungal culture is needed for species of dermatophyte to be identificated (**Zdovc *et al.*, 1997**). Collecting the hairs may be done by plucking the damaged hairs from the margin of the alopecic lesion or by brushing the haircoat all over the body, whenever asymptomatic infection. One should avoid taking hairs from all over the body if skin modifications are detected.

Collecting the hairs in this manner encourages contamination of the specimen with saprophitic fungi.

Sabouraud's dextrose agar and dermatophyte test medium (DTM) are traditionally used in clinical veterinary mycology for isolation of fungi (**Scott *et al.*, 2001**). SDA is a classical Sabouraud's dextrose agar containing penicillin and streptomycin that most of fungi grow on it. The antibiotics are added to prevent growing of bacterial contaminants. Sabouraud's dextrose agar containing chloramphenicol and actidion (SCA) is a selective culture plate because chloramphenicol prevents growing of most of the bacteria and actidion prevents growing of most of the saprophytic fungi (**Zdovc *et al.*, 1997**). Dermatophyte test medium (DTM) is essentially a Sabouraud's dextrose agar containing cycloheximide, gentamicin, and chlortetracycline as antifungal and antibacterial agents. The pH indicator phenol was added. Dermatophytes first use protein in the medium with alkaline metabolites turning the medium from yellow to red. When the protein is exhausted the dermatophytes use carbohydrates giving off acid metabolites. The medium changes from red to yellow. The majority of other fungi use carbohydrates first and proteins only later; they too may produce a change to red in DTM – but only after a prolonged incubation (10 to 14 days or longer).

Consequently, DTM cultures should be examined daily for the first 10 days. Fungi such as Blastomyces dermatitidis, *Sporothrix schenkii, H. capsulatum, Coccidioides immitis*, *Pseudoallescheria boydii*, and some Aspergillus species may cause a change to red in DTM, therefore microscopic examination is essential to avoid an erroneous presumptive diagnosis (**Scott *et al.*, 2001**).

Skin scrapings, claws, and hair should be inoculated onto Sabouraud's dextrose agar and DTM. Desiccation and exposure to ultraviolet light hinder growth. Therefore, cultures should be incubated in the dark at 30° C with 30% humidity. A pan of water in the incubator usually provides enough humidity. Cultures should be incubated for 10-14 days and should be checked daily for fungal growth. Proper interpretation of the DTM culture necessitates recognition of the red colour change simultaneously with visible mycelial growth.

2.8.5. DIFFERENTIAL DIAGNOSIS

The major differential diagnosis include staphylococcal dermatitis, dermatophilosis, demodicodis, zinc responsive dermatosis (ruminants) and pemphigus foliaceus (horses and goats). Definitive diagnosis is based on

microscopic examination of hairs and surface debrices, fungal culture and skin biopsy.

2.9. TREATMENT

2.9.1. A. Topical treatment

Individual topical treatment—irrespective of the etiological agent of ringworm, treatment consists of the application of a topical fungicides and the most suitable topical application includes the followings:

- The crusts should be removed by scraping or brushing with a soft wire brush and the medicament brushed or rubbed in vigorously. A weak solution of iodine (7%) and salicylic acid (2%) should be applied on the affected skin on alternate day for 10 days
- Whitefield's (salicylic acid 3%, benzoic acid 5% and Vaseline upto 100g) should be applied once daily for one week. Sevin 50wp 1.5 to 2% in Vaseline ointment daily for 10 days is also effective under field condition.
- Commercially available preparation are miconazole, clotrimazole, econazole or tolnaflate for 4 to 6 weeks.

❖ **Mass topical treatment**

Outbreaks and widespread cases— washes or spray the entire body surface of all animals.

2.9.2. B. Systemic treatment

If the response to treatment is unsatisfactory or in severe cases of dermatophytosis, systemic treatment with fungicides is recommended as an adjunct therapy.

Sodium iodide 1 g/14kg body weight 10% solution IV, more than one injection may be required plus local application of fungicidal agents.

2.9.3. DRUG RESISTANCE

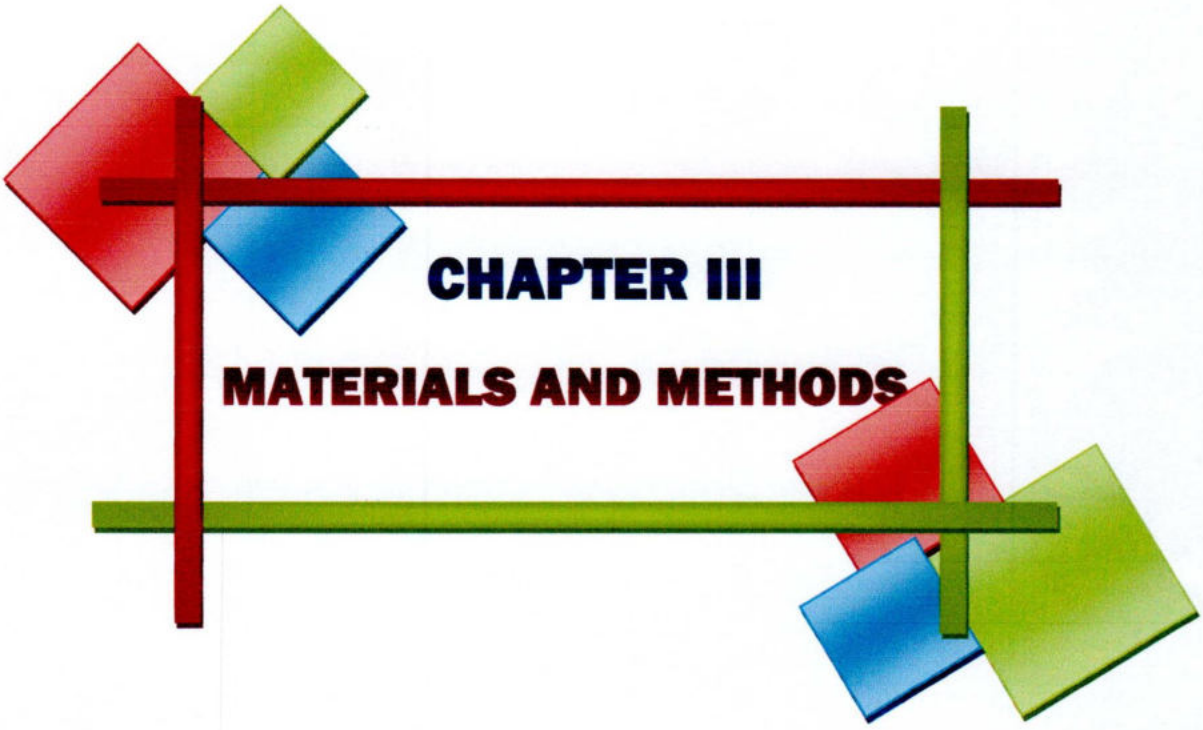
Resistance against ketoconazole has been shown by *Microsporum gyseum*, as well as against miconazole and clotrimazole in certain strains (**Lenhart *et al.*, 1989**). Susceptibility of *Trichophyton mentagrophytes* has been decreasing against fluconazole, and possibly against ketoconazole and griseofulvin (**Barros and Hamdan, 2005**).

2.10. SUSCEPTIBILITY TO DISINFECTANTS

Dermatophytes are susceptible to phenolic compounds, formaldehyde, glutaraldehyde, iodophors and sodium hypochloride (1%) (**Collins and Kennedy, 1999**).

2.11. PHYSICAL INACTIVATION

The infectious substance can be inactivated by UV C (**Menetrez *et al.*, 2010**), gamma (**Silva *et al.*, 2006**) and microwave (aerosol) (**Wu and Yao, 2010**) radiation; moist heat (121°C for at least 20 min (**Csucos & Csucos, 1999**); and dry heat (165-170°C for 2 hours).



CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1. EXPERIMENTAL ANIMALS

Bovine dermatomycosis was studied based on epidemiology, clinical, microbiological, pathological and therapeutical findings at District Veterinary Hospital (DVH) of Dinajpur, Bangladesh.

3.2. EXPERIMENTAL AREA

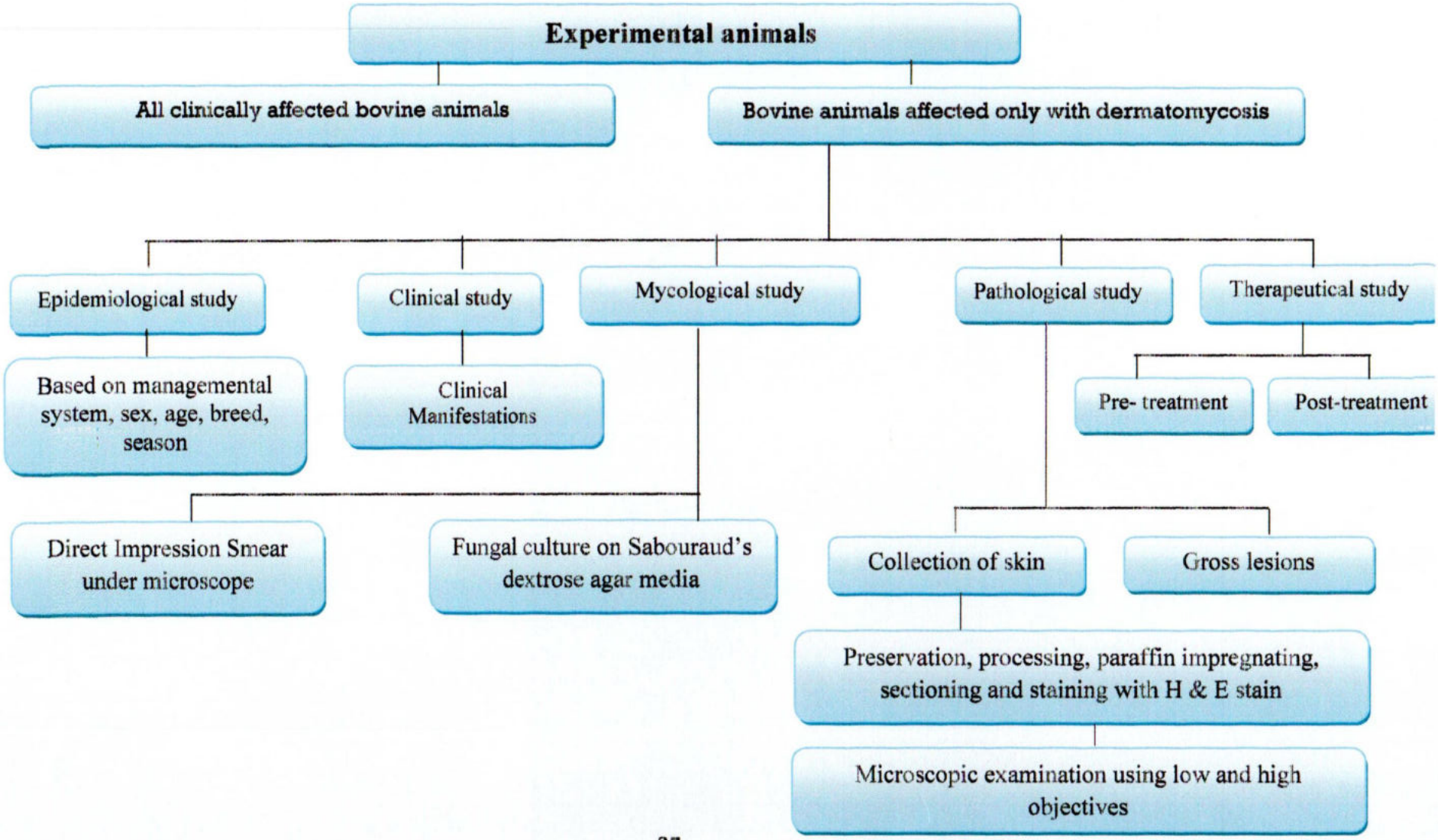
The clinically affected animals visited physically in different locations surrounding the hospital and the animals submitted to hospital for the diagnosis and therapeutic purposes were considered as the experimental animals.

After the collection of sample, it was preserved for laboratory examination. The histopathological examination was done at the Department of Pathology and Parasitology, and mycological examination was done at the Department of Microbiology of Hajee Mohammed Danesh Science and Technology University, Basherhat, Dinajpur.

3.3. EXPERIMENTAL DURATION

The duration of the experiment was one year from March/2011 to February/2012. The total population of the clinical cases was 1681 among which 21 cases of bovine dermatomycosis were registered in this Hospital during the course of the experimental period.

SCHEMATIC REPRESENTATION OF EXPERIMENTAL DESIGN



3.4. EPIDEMIOLOGICAL STUDY

The annual incidences of bovine dermatomycosis emphasizing on different epidemiological parameters such as farming system, sex, age, season and breed were determined. The topographical positions of the lesions, concurrent infections, nutritional status, previous health history, medications, morbidity and mortality rate, etc. of the patients were also noted carefully and correctly from the farm records and farmer's complaints (Tabl 2, 3, 4, 5, and 6). The seasonal occurrences of the disease were also considered.

3.5. CLINICAL EXAMINATION

The presented clinical manifestations of the bovine dermatomycosis were recorded during the physical visits, following submission in the hospital and the farmer's complaints in relation to the affection were also emphasized (Fig. 2 & 3).

3.6. MYCOLOGICAL EXAMINATION

3.6.1. Collection of samples

Samples of skin scrapping and hair were collected from clinically suspected cases of dermatomycosis. The collected samples were transported using sterilized sample collector vials to laboratory for the microbiological and pathological examination (Fig. 4).

3.6.2. Examination of impression smears

Direct impression smear of the cutaneous exudates was made on a clean glass slide and stained with Gram's stain (Cowan, 1979) to demonstrate the presence of dermatophytes (Fig. 4).

3.6.3. Protocol of Gram's staining

- Exudate from the lesions of the animal typically affected with dermatomycosis was taken by soabing
- Placing of the soab in a dry, cleaned petridish and brought to the bacteriological laboratory with necessary precautions
- An impression smear with the collected exudates was performed

- The slide was held over the flame for a few seconds
- Then the slide was flooded with Gram's iodine solution and allowed to remain for 30-60 seconds
- The stain was poured off and washed in running water
- The film was then decolorized with addition of acetone for 2-3 seconds and washed in running water
- The film was counter stained with safranine solution was allowed to act for about 3 minutes and washed in running water
- Air dried and examined under microscope using oil immersion
- The result was recorded and the image of the film was taken using a digital camera

3.6.4. Culture of the skin scraps

After the collection of skin scraps aseptically, scraping was cultured into Sabouraud dextrose agar media (Ajello *et al.* 1996). The plates or slants were incubated at 28°C for up to 4 days and examined at 2 to 3 days intervals for fungal growth and recorded (Fig. 4).

3.6.4.1.Compositions of Sabouraud's dextrose agar

Ingredients	Gm/ liter
Mycological peptone	10.00
Dextrose	40.00
Agar	15.00

3.6.4.2. Procedure of inoculation

- To make a suspension 65 gms of Sabouraud's dextrose powder was mixed in 1000 ml of distilled water.
- Boiling of the suspension until dissolved the powder
- Autoclaving for the complete sterilization at 15 lb pressure and 121°c for 15 minutes
- Pouring of the suspension in a sterilized Petridis
- Solidification of the agar in room temperature
- Inoculation of the sample with a sterilized stick at 28°c for 48 hours

3.7. PATHOLOGICAL EXAMINATION

The gross morbid lesions of the disease were systematically examined, noted and categorized (Fig.5). The suitable sizes of skins of 3 typically

dermatomycosis affected cattle were collected from the live animals subjected for the diagnosis and treatment for further histopathological study.

The representative cutaneous tissues were collected and preserved at 10% formalin solution and subsequently processed, embedded with paraffin, sectioned and stained with haematoxylin and eosin for histopathological examination (Luna, 1968).

3.7.1. Collection of dermatomycosis affected skin

- Sterilization of surgical instruments by boiling
- Restraining of animals by casting
- Site selection, application of local anaesthetics subcutaneously and wait for few minutes for anaesthetic action
- Folding of skin by artery forceps
- Excision of excess folded portion of skin and subsequently suture with nylon threads
- Locally application of cotton admixing with Tincture of iodine as counter irritant
- Completion of antibiotic course and suggesting to avoid secondary complication

3.7.2. Preservation of skins and Tissue processing

- Preservation of collected skin samples in 10% formalin solution for at least 3 days
- Trimming of preserved samples at suitable sizes
- Watering for overnight to remove formalin
- Dehydration in an ascending grades of alcohol
  - 50% alchol: 1hour
  - 70% alchol: 1hour
  - 80% alchol: 1hour
  - 95% alchol: 1hour
  - 100% alchol: 3 changes and 1 hour for each change
- Chloroform treatment: 2 changes and 1.5 hours for each change
- Impregnation by paraffinization at melting point (56^0C): 2 changes and 1.5 hours for each change
- Blocking
- Sectioning at 6-7μm in thickness, placing on water bath, taking on a glass slide and air dry

3.7.3. Routine haematoxylin and eosin (H&E) staining

3.7.3.1. Preparation of Haematoxylin solution

Haematoxylin crystals	:	4.0 g
Alcohol, 95%	:	200.0 ml
Potassium or ammonium alum	:	6.0 g
Distilled water	:	200.0 ml
Glycerine	:	200.0 ml
Glacial acetic acid	:	20.0 ml

3.7.3.2. Preparation of Eosin stock solution

Eosin Y, water soluble	:	1.0 g
Distilled water	:	20.0 ml
Alcohol, 95%	:	80.0 ml

3.7.3.3. Preparation of eosin working solution

Eosin stock solution	:	1 part
Alcohol, 80%	:	3 part

0.5 ml glacial acetic acid was added to 100 ml of working eosin solution just before use

3.7.4. Protocol of H&E staining

- Xylene treatment: 3 changes and 3 minutes for each change
- Rehydration in descending grades of alcohol
  - 100% alchol: 2 minutes
  - 95% alchol: 2 minutes
  - 80% alchol: 2 minutes
  - 70% alchol: 2 minutes
- Distilled water: 10 minutes
- Haematoxylin: 10-15 minutes
- Distilled water: 15 minutes
- Bluing in lithium carbonate: Few dips
- Eosin: 30 minutes
- Dehydration in ascending grades of alcohol
  - 80% alchol: Few dips
  - 95% alchol: Few dips
  - 100% alchol: Few dips
- Xylene treatment: 3 changes and 3 minutes for each changes
- Mounting with Canada Balsam
- Examined under microscope using both low and high power objectives

3.8. THERAPEUTICAL FINDINGS

For the therapeutical findings, some specific drugs were used in the treatment of affected animals and the results of post-therapeutical managements were also recorded. The registered animals were treated with different types of drugs such as systemic antibiotic (oxytetracycline, gentamycine, combined preparation of penicillin and steptomycine etc), ivermectine (@ 0.2 mg/kg bwt); griseofulvine (@ 10mg/kg bwt) etc and Whitefield ointment (benzoic acid 6%, salicylic acid 3%) locally.

The doses of the drugs were calculated by measuring the approximate body weight of the affected animals following a standard procedure.

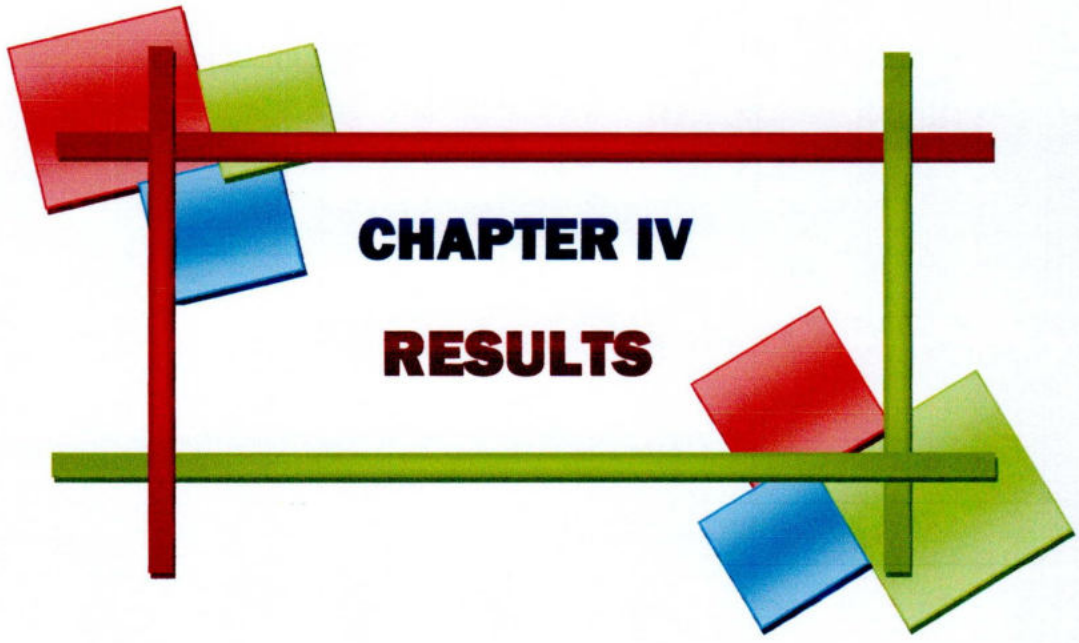
$$\text{Body weight} = \frac{\text{Length} \times (\text{Girth})^2}{300}$$

$$= \text{lb}$$

Considering the general health condition of the patient, fluid therapy, improved diet, proper hygienic measures were also emphasized and suggested for all groups mentioned above. Drugs were applied following recommended dose. Symptomatic treatment was also done.

3.9. PHOTOGRAPHY

The histopathological slides of normal and dermatomycosis affected cutaneous tissues were placed under the microscope (Leica, Germany) and the respective microphotographs were taken directly by a digital camera (Canon IXY, 16.2 MEGA PIXEL, Japan) using both low and high objectives (X4, X10 and X40). The photographs were then placed in computer; image selection and magnification were further modified and placed in this thesis for better illustration of the results.


CHAPTER IV
RESULTS

CHAPTER IV

RESULTS

4.1. ANNUAL INCIDENCE OF BOVINE DERMATOMYCOSIS

A total number of 1681 animals were registered among which 21 were registered as dermatomycosis infection during a year from March 2011 to February 2012.

4.2. EPIDEMIOLOGICAL STUDY OF BOVINE DERMATOMYCOSIS

The epidemiological study of bovine dermatomycosis encountered at District Veterinary Hospital (DVH) was done based on sex, age, season, breed as well managemental systems which were represented in a tabular form (Table 2, 3, 4, 5 & 6) and graphically.

4.2.1. Seasonwise annual incidences of dermatomycosis

There was a great seasonal variation in the occurrences of bovine dermatomycosis encountered at DVH. Highest level of dermatomycosis was

found in the summer season (1.64%) which was followed by winter (0.88%) and rainy (0.78%) respectively (Table 2).

Table 2: Seasonal incidences of bovine dermatomycosis encountered at DVH

Season	Total clinical cases examined	Dermatomycosis affected animals	Percentage (%)
Summer season	849	14	1.64
Rainy season	512	4	0.78
Winter season	339	3	0.88
Total	1681	21	1.25

4.2.2. Sexwise annual incidences of dermatomycosis

The sexwise clinical feature of dermatomycosis was found more in female animals (1.11%) followed by male animals (1.35%) (Table3).

Table 3: Sexwise annual incidences of bovine dermatomycosis at DVH

Categorization of animals	Total clinical cases examined	Dermatomycosis affected animals	Percentage (%)
Male	719	8	1.11
Female	962	13	1.35
Total	1681	21	1.25

4.2.3. Agewise annual incidences of dermatomycosis

The agewise clinical feature of dermatomycosis was found more in young (2.55 %) followed by calves (0.62 %) and adult animals (0.92 %), respectively (Table 4)

Table 4: Agewise annual incidences of bovine dermatomycosis at DVH

Categorization of animals	Total clinical cases	Dermatomycosis affected animals	Percentage (%)
Calf (Below 6 months)	806	05	0.62
Young(> 6 months and below 2years)	549	14	2.55
Adult(Above 2 years)	326	3	0.92
Total	1681	21	1.25

4.2.4. Breedwise annual incidences of dermatomycosis

Breed variation in relation to the occurrences of dermatomycosis in the present study was greatly demarcated between indigenous breed and crossbred which was 1.29 % in indigenous breed whereas 0.98 % was in crossbred animals (Table 5).

Table 5: Breed based annual incidences of bovine dermatomycosis encountered at DVH

Categorization of animals	Total clinical cases examined	Dermatomycosis affected animals	Percentage (%)
Indigenous breed	1475	19	1.29
Crossbred	206	2	0.98
Total	1681	21	1.25

4.2.5. Management based annual incidence of bovine dermatomycosis

Dermatomycosis in cattle was found more in rural housed farms (1.32%) than those reared in intensive or semi intensive farms (0.61%) (Table 6).

Table 6: Annual incidences of bovine dermatomycosis encountered at different farming systems at DVH

Management system	Total clinical cases examined	Dermatomycosis affected animals	Percentage (%)
Rural household farm	1517	20	1.32
Intensive/semi intensive dairy farm	164	1	0.61
Total	1681	21	1.25

4.3. CLINICAL MANIFESTATIONS

The recorded animals affected with dermatomycosis showed moderate (subacute) to severe (acute) signs. The demarcation between acute and subacute levels of the disease could be made based on the degree of severity, complications or concurrent infections, level of dissemination of lesions, stage of disease as well as general health condition of the animals.

Circular area of crust and scale formation. Reddening of the affected area with pruritus. Roughened hair coat also observed. (Fig. 2, 3)

However, weakness, dullness and depression, reluctant to move, fever, increased respiration rate, pulse rate, disinterested to feed, pruritus, reddening of the skin, general loss of body conditions, reduced production performances and immunity were recorded in both forms of the diseases.

4.5. MYCOLOGICAL FEATURES

Fungal growth was recorded at 4 days after inoculation (Fig. 4) in Sabouraud's dextrose agar media. The colonies were disk shaped white to cream colored. The cultivated fungi were also observed under microscope using 4X objectives (Fig. 4)..

4.6. GROSS PATHOLOGICAL CONDITIONS

4.6.1. Topographical positions of lesions / Distribution of lesions

The topographic positions of the lesions and the types of lesions were enlisted. Lesions were most commonly seen on the head, neck and pelvis and vary from discrete circular areas of alopecia to severe scaling, crusting, suppuration and ulceration. Pruritus and pain were variable (Fig. 2 & 3).

Characteristic locations of lesions were the periocular region for calves, the thorax and limbs for cows and heifers and the dewlap and intermaxillary space for bulls.

4.6.2. Gross lesions

The gross appearance of dermatomycosis (ringworm) was quite variable. The classic ringworm-like lesion was characterized by annular area of alopecia, stubbled hairs and variable amounts of scaling, crusting and dermatitis. Folliculitis and frunculosis were also common manifestations.

4.6.3. Histopathological features

The diagnostic histopathologic features were characterized as perifolliculitis and furunculosis, superficial perivascular dermatitis (spongiotic or hyperplastic) with orthokeratotic or parakeratotic hyperkeratosis of the epidermis and hair follicles and intraepidermal vesicular (spongiotic) or pustular dermatitis (Fig. 5).

4.7. THERAPEUTICAL FINDINGS

Treatment with systemic antibiotic as well as subcutaneous use of ivermectin did not show any positive result but response to antifungal drugs were noticed. Oral administration of griseofulvin along with local use of whietfield ointment in young and calves showed very good response (6 & 7).

Systemic antibiotics, antihistaminics enriched nourishment along with the application of antifungal drug accelerated the healing process. Drugs were selected without sensitivity test.

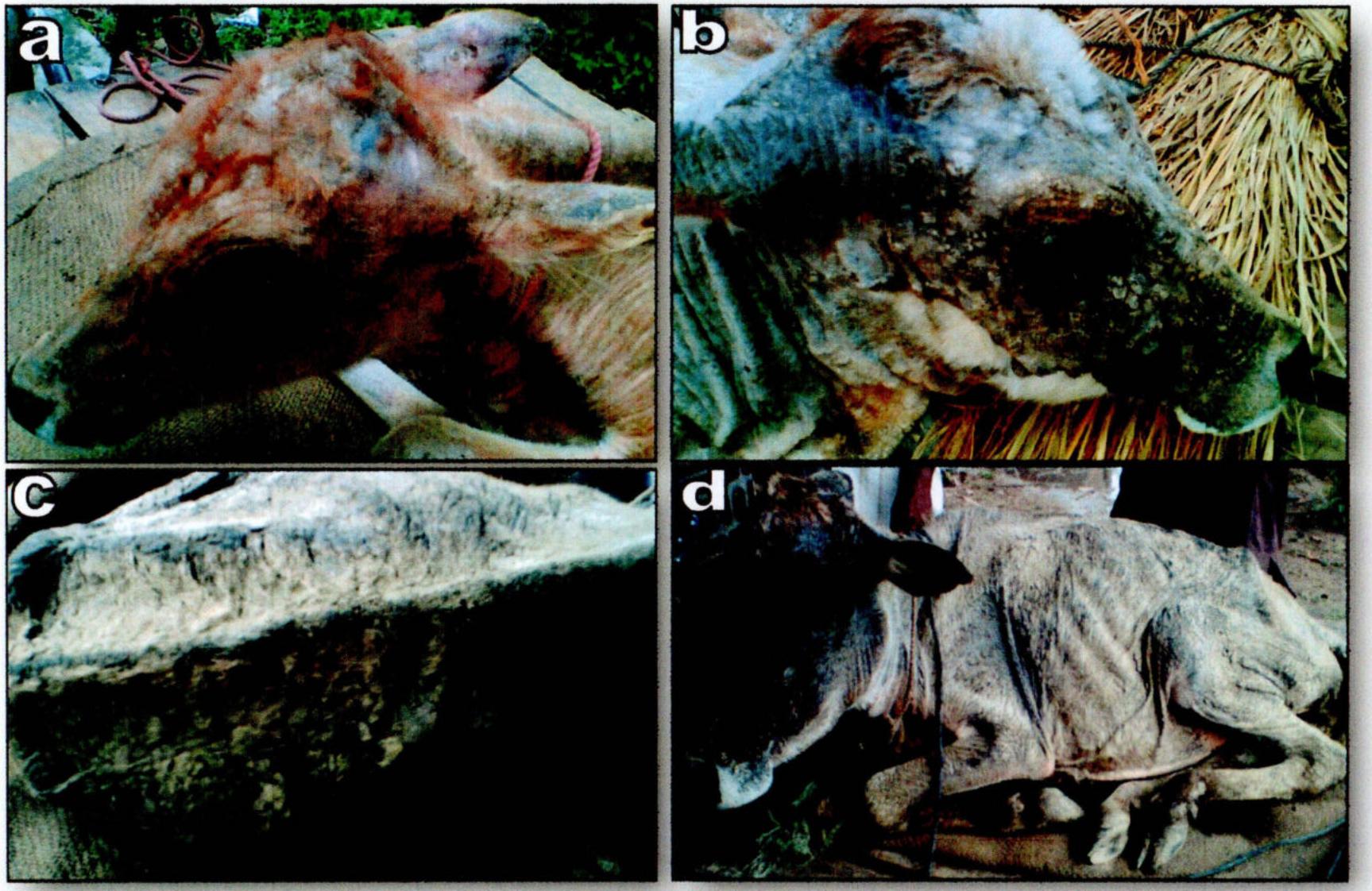


Fig. 2: a) Encrustration with moderate alopecic condition on head and neck regions, b)Thick crusts around eyes, ear and dewlaps c) Piquiliar development and patterns of hairs on the body surface, d) Ill health with severe depression

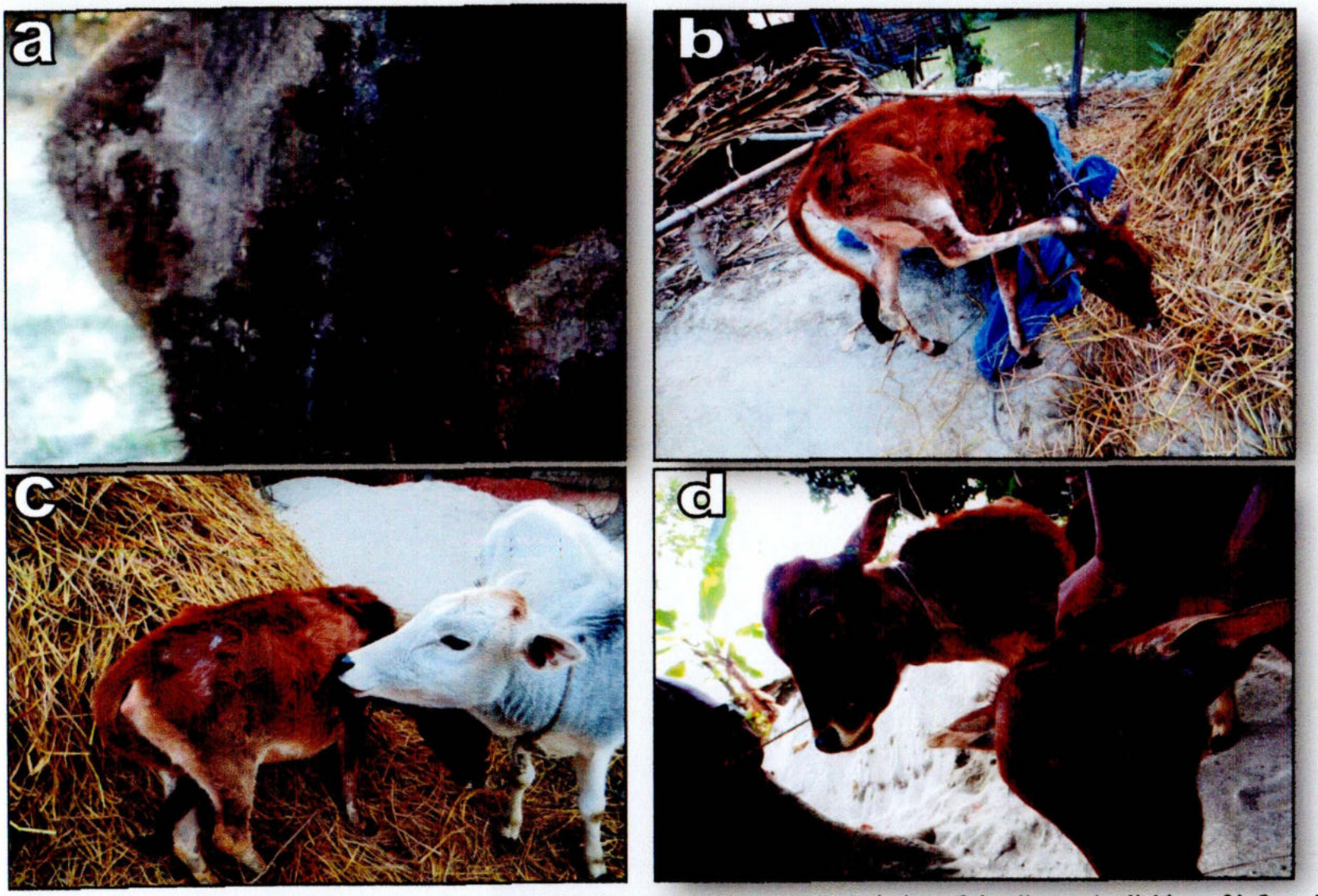


Fig. 3: a) Dry crusts with hairless areas on hump region b Pruritus and itching c) Transmission of the disease by licking of infected animals body, d) Horizontal transmission of the disease of the contact animals.

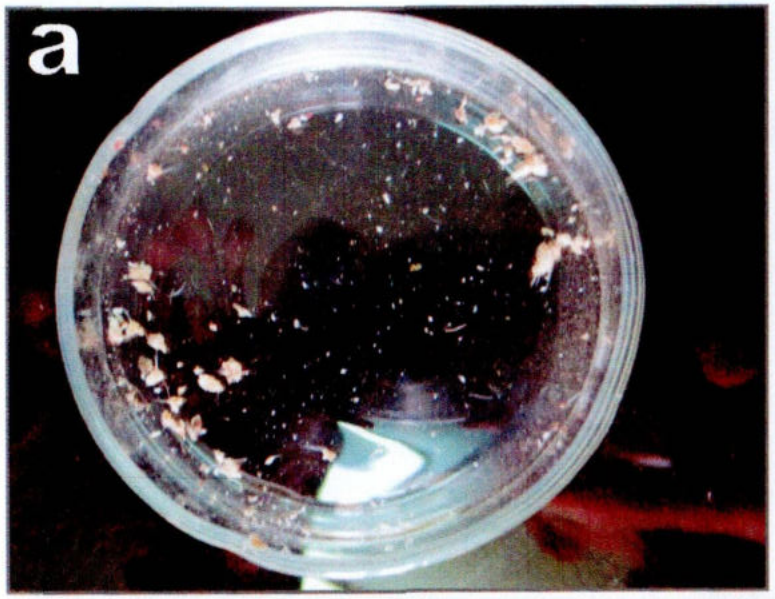


Fig. 4: a) Collected skin scraps for the detection of fungus, b) Gray to white growth of fungus in Sabouraud's dextrose agar media at 5 days postinoculation, c) Threads like growth of fungus observed under microscope

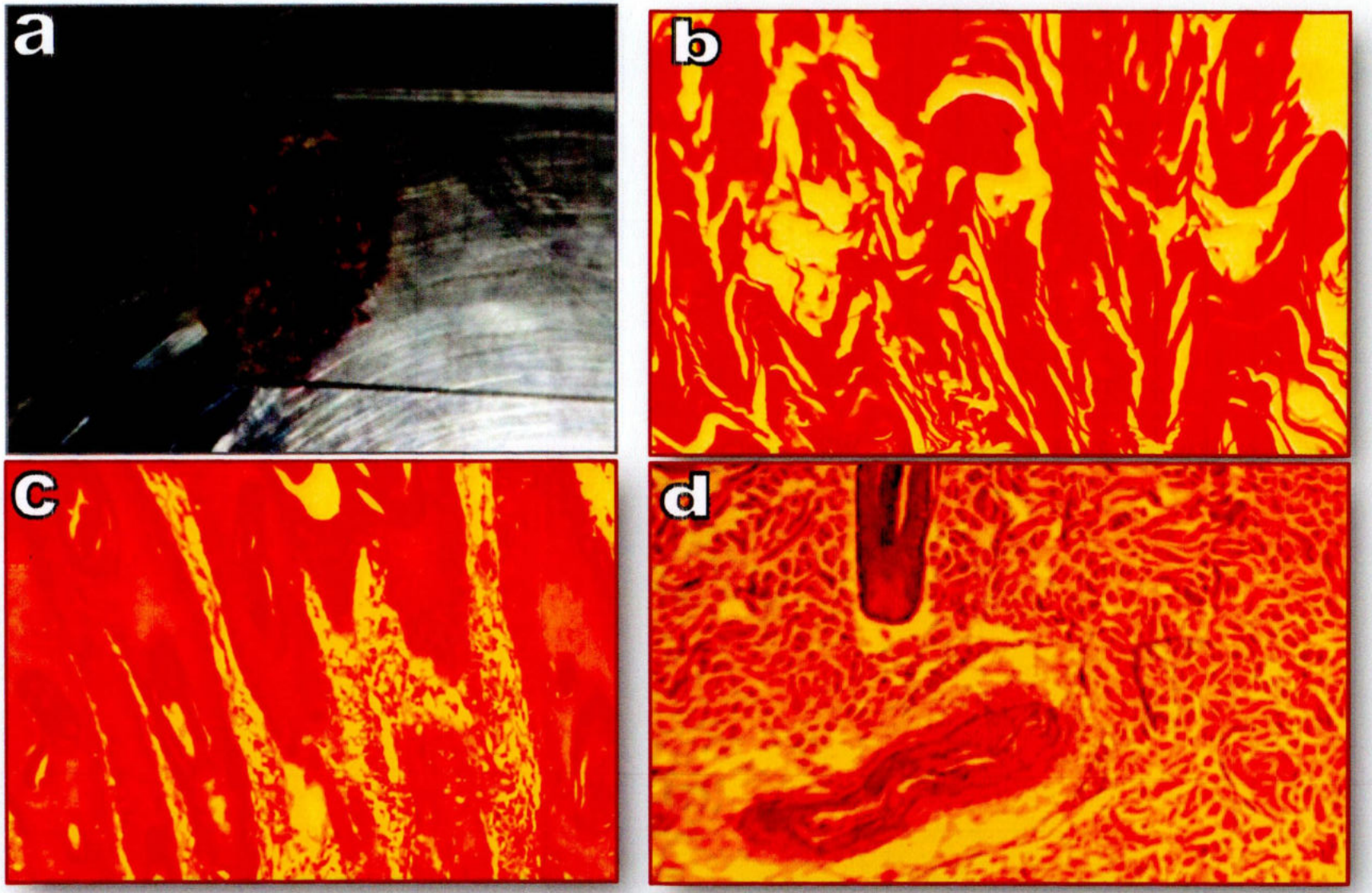


Fig. 5: a) Surgically collected skin from the affected animal, b) Hypekeratosis and parakeratosis, c) Acanthosis with reactive cells infiltration, d) Heavily deposition of collagenous fibers.

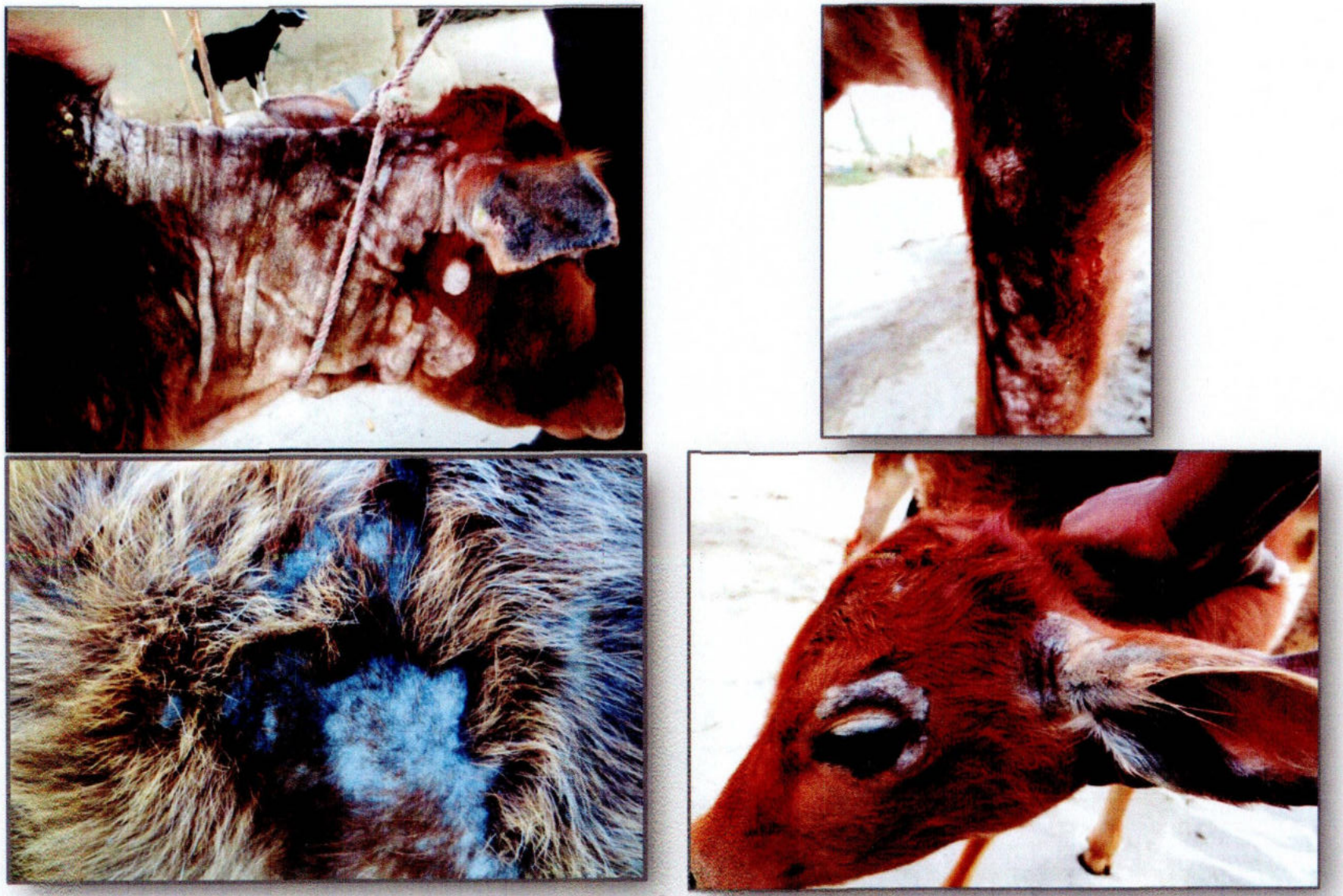


Fig. 6: Clinical signs detected prior to treatment

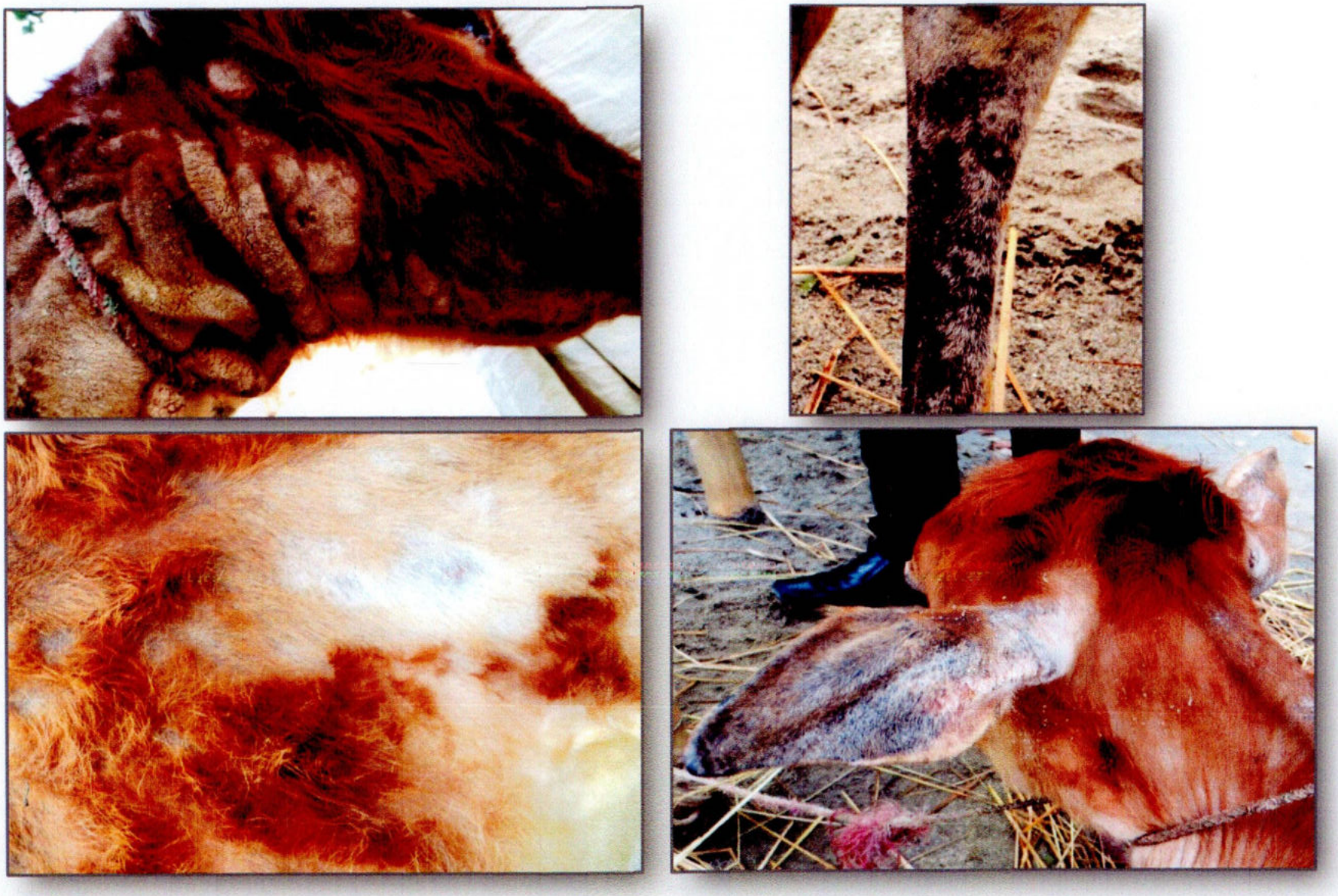
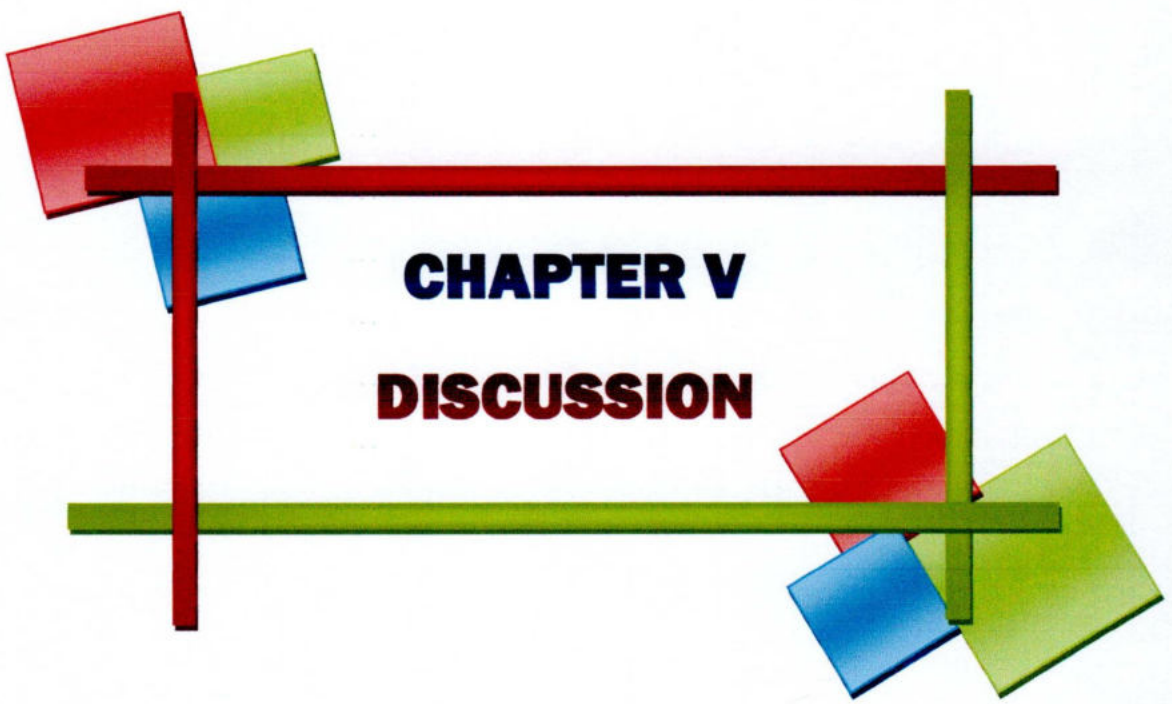


Fig. 7: Recovery of the clinical signs after treatment with griseofulvin and topical application of whietfield ointment


CHAPTER V
DISCUSSION

CHAPTER V

DISCUSSION

5.1. ANNUAL INCIDENCE OF BOVINE CUTANEOUS DERMATOMYCOSIS ENCOUNTERED AT DVH

Bovine cutaneous dermatomycosis or ringworm in cattle was studied at DVH, Dinajpur from March-20011 to February-2012. A total of 1681 clinical cases were registered among which 21 were found infected with dermatomycosis. The annual incidence of dermatomycosis in cattle was 1.25%.

5.2. EPIDEMIOLOGICAL STUDY OF BOVINE DERMATOMYCOSIS

Bovine dermatomycosis has worldwide distribution and more prevalent in the tropical and subtropical regions (**Nooruddin and Day, 1984; Fraser *et al.*, 2008**). The disease has been considered as a public health problem all over the world (**Kane *et al.*, 1997; Igor *et al.*, 2009**). Bangladesh is geographically placed in the subtropical regions of the world and

dermatomycosis in cattle has been diagnosed in the context of this environment of Bangladesh (**Nooruddin and Dey, 1984, 1985, 1990, Nooruddin *et al.*, 1986b, 1992abc; Hassan 1988**).

Trichophyton verrucosum is the usual zoophilic dermatophyte involved in cattle ringworm throughout the temperate regions of the world (**Wabacha, 1998; Weber, 2000**). It also affects, but with lower prevalence, sheep, goat and other ruminants (**Stenwig, 1985; Pier, 1994**)

In the present study the highest annual incidence of bovine dermatomycosis was recorded in indigenous breed (1.29%), in the summer season (1.64%), in female animals (1.11%), in young animal (2.55%) and in rural housed farms (1..32%). **Nooruddin and Day, 1984; Thakur *et al.*, 1983; Nooruddin and Day, 1990; Nooruddin *et al.*, 1992c; Neela and Alam, 2000**, determined the prevalence of bovine dermatomycosis and their results more or less similarly correlate with the results of the present study.

Nevertheless, sex, age and breed do not seem to affect host susceptibility, but increased prevalence of dermatomycosis in young animals with

longstanding exposure to moisture and immunosuppression is explained (**Fraser *et al.*, 2008; Shams *et al.*, 2009**). Prevalence and incidence of dermatomycosis is more in summer season than the winter and rainy season. It is due the hot and humid climate and at summer season increase population of tick, mite, and flies also somewhat responsible to increase the prevalence of the disease (**DeBoer and Moriello, 2006**).

Breed and managemental differences played greater role than the differences in sex and age (**Wabacha, 1998; Weber, 2000**).

The incidences of bovine dermatomycosis were found in higher levels in summer season (15.03%) followed by winter (3.33%) and rainy (8.29%) seasons, and this could proportionally be related with the degree of humidity, hot and dry weather and insect's population. Moreover, proportionally greater levels of fly infestation, ticks and lice infestations were also observed throughout the length of the experimental period.

5.3. CLINICAL FEATURES

Characteristics clinical signs were circular area of crust and scab formation. Scales were found in severe form. Reddening, alopecia and itching with pruritus also characteristics clinical signs.

5.4. MYCOLOGICAL EXAMINATION

Detection of fungal hyphae after impression smear and Gram's stain under microscope confirms the dermatomycosis infection. Grey to white coloured colony of fungal growth in Sabouraud's Dextrose Agar media is the diagnostic features of dermatomycosis infection. This characteristics were also observed by **Nooruddin *et al.*, 1992b and Shahitha, *et al.*, 2013.**

5.5. PATHOLOGICAL FINDINGS

Annular area of alopecia, stubbled hairs and variable amounts of scaling, crusting and dermatitis. Folliculitis and frunculosis are also common manifestations of dermatomycosis. The predominant lesions included varying combinations of papules, nodules, ulcers and sinuses.

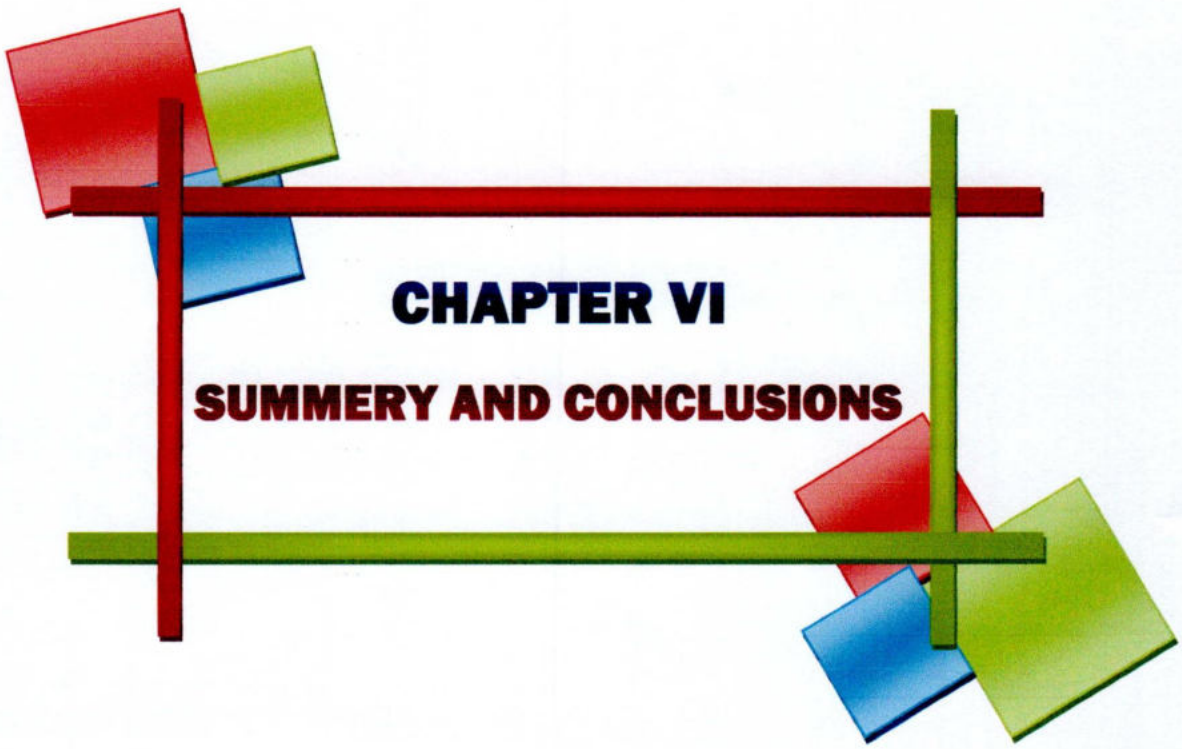
The most common histopathologic patterns observed in dermatophytosis were perifolliculitis, furunculosis, superficial perivascular dermatitis (spongiotic or hyperplastic) with orthokeratotic or parakeratotic hyperkeratosis of the epidermis and hair follicles and intraepidermal vesicular (spongiotic) or pustular dermatitis. These histopathologic features also observed by **Abdallah *et al.*, 1973; Gabal *et al.*, 1976; Aamodt *et al.*, 1982.**

5.6. TOPOGRAPHICAL POSITIONS OF THE LESIONS

The topographic positions of the lesions and the types of lesions were variable among the different body regions. Lesions are most commonly seen on the head, neck and pelvis. Characteristic location of lesions was the periocular region for calves, the thorax and limbs for cows and heifers and the dewlap and intermaxillary space for bulls. These observation were similar with **Pandey and Carbaret, 1980; Nooruddin and Dey, 1985; Radostits *et al.*, 1994; Scott, 1994; Fraser *et al.*, 2008.**

5.7. THERAPEUTICAL EFFICIENCIES

The treated animals showed no response to systemic antibiotic and subcutaneous use of ivermectine but when it was treated with antifungal drugs showed good response. Oral administration of griseofulvin along with local whietfield ointment in cattle showed very good response. Grisofulvin along with the whietfield ointment is the choice of drug for the treatment of ringworm in cattle. It is well established by different authors (**Nooruddin and Day, 1985; Radostits *et al.*, 1994; Hill *et al.*, 1995; Foil, 2005; Fraser *et al.*, 2008**). In case of concurrent infection with bacteria or ectoparasits, systemic antibiotics and ivermectin along with griseofulvine show good response.



CHAPTER VI

SUMMERY AND CONCLUSIONS

CHAPTER VI

SUMMERY AND CONCLUSIONS

Dermatopathology in respect to bovine cutaneous dermatomycosis with clinical observations was studied on multiple parameters. The annual incidences of bovine dermatomycosis were studied based on different epidemiological parameters. The topographic positions of the lesions, histopathological examination as well as therapy efficiencies in relation to the bovine dermatomycosis were also included.

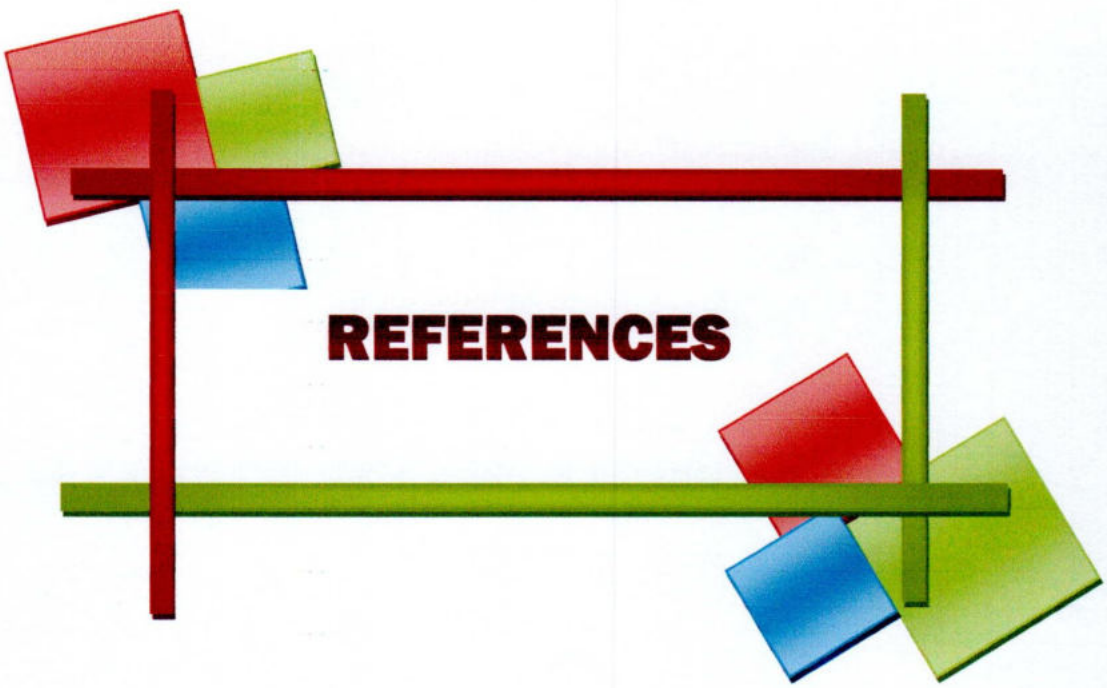
The highest clinical cases of dermatomycosis were recorded in summer season (1.64%) followed by winter (0.88%) and rainy season (0.78%). Annual incidences of dermatomycosis were higher in female (1.35%) than male animals (1.11), in young (2.55%) animal than the calves (0.62) and adult (0.92), in indigenous breed (1.29%) than the crossbred animal (0.92) and in rural housed farms (1.32%) than the intensive farming (0.61%) management. Major lesions in head, neck and pelvis regions were also noted.

The disease was clinically and pathologically characterized as circular lesion of scab and crust formation, roughened coat with pruritus and histopathologically by hyperkeratosis, parakeratosis, epidermal hyperplasia, densely cellular dermis with moderate destruction of glandular structures. Identification of fungus under microscope by impression smear from the affected skin lesion and growth of grey to white colored colony in Sabouraud's dextrose agar media is main diagnostic features of dermatomycosis. Griseofulvin with topical application of whietfield ointment showed good response.

The following conclusion was drawn based on the facts and findings studied throughout the course of the research experiments.

- Dermatomycosis was more prevalent in indigenous cattle than the crossbred.
- Incidences were found higher in summer season, in rural housed farms, in young and female animals.
- Hyperkeratinzation, parakeratinization, severe encrustation and epidermal epithelial hyperplasia were the general histopathological features.

- ❖ Detection of fungus under microscope from direct impression smear from the affected skin lesion and growth of grey to white colored colony in Sabouraud's dextrose agar media was main diagnostic features of dermatomycosis.
- ❖ Griseofulvin with topical application of whietfield ointment was the drugs of choice for the clinical improvement.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