ISOLATION, ANATOMICAL DISTRIBUTION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF DERMATOPHYTES FROM HORSES IN KADUNA STATE

BY

MAGDALENE BANECHE CHINDO
M.SC/VET-MED/4915/2011-2012

DEPARTMENT OF VETERINARY MICROBIOLOGY,
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

JUNE, 2015

ISOLATION, ANATOMICAL DISTRIBUTION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF DERMATOPHYTES FROM HORSES IN KADUNA STATE

BY

Magdalene Baneche CHINDO, DVM (MAIDUGURI) 2009
(M.SC/VET-MED/4915/2011-2012)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN VETERINARY MICROBIOLOGY

DEPARTMENT OF VETERINARY MICROBIOLOGY,
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

JUNE, 2015

DECLARATION

I declare that the work presented in this thesis entitled ‘‘**ISOLATION, ANATOMICAL DISTRIBUTION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF DERMATOPHYTES FROM HORSES IN KADUNA STATE**’’ has been carried out by me in the Department of Veterinary Microbiology, Faculty of Veterinary medicine, Ahmadu Bello University, Zaria under the supervision of Prof. H. M. Kazeem and DR (Mrs) C.N. Kwanashie.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has previously been presented for another Degree or Diploma at this or any other institution.

DATE

Magdalene Baneche CHINDO

CERTIFICATION

This thesis ‘‘**ISOLATION, ANATOMICAL DISTRIBUTION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF DERMATOPHYTES FROM HORSES IN KADUNA STATE**’’ by Magdalene Baneche Chindo meets the regulations governing the award of the Degree of Master of Science (MSc.) of Ahmadu Bello University, and is approved for its contribution to scientific knowledge and literary presentation.

Prof. H.M. Kazeem ________________ ________________

Chairman, Supervisory Committee SIGNATURE DATE

Dr. (Mrs) C.N. Kwanashie ________________ ________________

Member, Supervisory Committee SIGNATURE DATE

Dr. (Mrs) C.N. Kwanashie

Head of Department, ________________ ________________

Veterinary Microbiology SIGNATURE DATE

Prof. A. Z. Hassan

Dean, Postgraduate School, ________________ ________________

ABU, Zaria, Nigeria SIGNATURE DATE

DEDICATION

This work is dedicated to my husband Dr. Maurice Nanven and My family, The Chindos.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to The Most High for the Grace to carry on with this project, to the members of my Supervisory Committee; Prof. H.M. Kazeem and Dr. (Mrs.) C.N. Kwanashie for their guidance and encouragement throughout the course of this study. I greatly acknowledge Dr. Philip Mshelia from the Department of Veterinary Medicine for allowing me 'tag along,' Dr. Ankali Paul Idoko and Dr Richard Edeh for his help during the period of sampling, Dr. Emmanuel Ngbede O. and Dr. Francis E. Enenche for various advice, Dr L. Allam, Dr K. A. Majiagbe and Dr A. Jibril for their invaluable critic of the manuscript. My gratitude goes to the staff members of the Department of Veterinary Microbiology especially Dr P.H. Mamman, Mrs. Salamatu Garba and Mr. Dodo Bawa, to mention a few for their concern and encouragement during the course of this project and to all the horse owners who allowed me touch their prized animals, and last but not the least Mr. Bamidele of the Department of Human Anatomy for helping with the photography.

Above all, I wish to express my profound gratitude to my husband, who made it all possible.

ABSTRACT

This study was designed to document the site of lesions on the horses associated with fungi, culture and identify the dermatophytes in four Local Government Areas in Kaduna state and to determine the antifungal susceptibility patterns on the isolated dermatophytes. Sites of lesions were recorded, skin scrapings were collected and mycological studies carried out using culture techniques (on SDA, PDA and 5% NaCl SDA media) at room temperature for one to four weeks and isolates were identified using the colour fungal Atlas. Antifungal susceptibility tests on the isolates using five commonly used antifungal agents; Ketoconazole (100mg, 0.03-62μg/ml), Fluconazole (50mg, 0.125-256μg/ml), Griseofulvin (5g, 0.0125-32μg/ml), Terbinafine (50mg, 0.03-64μg/ml) and Amphotericin B. (50mg, 0.03-64μg/ml) were also carried out using the Broth Micro dilution method. Out of 102 horses sampled, 18 (17.6%) were positive for dermatophytes. The skin lesions occurred mostly on the flanks, limbs and saddle areas on the horses. Out of 53 male horses, 12 (22.6%) samples yielded dermatophytes, while 6 (12.24) out of 49 was the case for female horses. There was no significant association between the number of dermatophytes isolated from male and female horses, (p=0.1688). From 40 samples in Zaria LGA 14 (35%) were positive for dermatophytes, 10 from Sabon gari LGA, 1(10%) positive, 1(5.6%) was positive out of 18 from Kaduna North LGA, while 2 (5.9%) of the 34 from Igabi LGA were positive. The association between isolation rate and LGA was statically significant ($p > 0.05$). The species of dermatophytes isolated were; *T. verrucosum* (4, 22.22%), *T. vanbreuseghemii* (2, 11.11%), *T. equinum* (1, 5.56%), *T. soudanense* (1), *T. mentagrophytes* (1, 5.56%), *M. equinum* (3, 16.67%), *M. canis* (2, 11.11%), *M. gypseum* (2, 11.11%), *M. gallinae* (1, 5.56%), *M. fulvum* (1). A total of 16 (88.89%) dermatophytes were susceptible to the antifungal agents while 2 (22.22%) ketoconazole against *M. equinum* and fluconazole against *M. gypseum* were

resistant. Minimum inhibitory concentrations ranged from 0.03µg/ml to 64µg/ml. Terbinafine, Griseofulvin, Fluconazole had the highest MIC of 64µg/ml, 24µg/ml, 19.2µg/ml, respectively against *M. gypseum* (4z), *M. gypseum* (4z) and *M. equinum*. On the other hand, Amphotericin B. against *M. equinum*, *M.gypseum* (2), *T. verrucosum* (2), and *M. canis*, Terbinafine against *T. verrucosum* and Griseofulvin against *M. canis* had the least MIC value of 0.03µg/ml each. This study revealed that a wide range of dermatophytes, covering all ecologic groups infect horses in the study area, all of them with the potential of being zoonotic. Preventive measures should be put in place by Veterinarians and grooms to mitigate the spread of the infectious agent.

TABLE OF CONTENTS

CHAPTER THREE: MATERIAL AND METHODS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF ABBREVIATIONS

a2	Alpha2
AIDS	Acquired Immune Deficiency Syndrome
AMB	Amphotericin B
CLSI	Clinical Laboratory Standards Institute
CMI	Cell Mediated Immunity
DMSO	Dimethylsulfoxide
DTH	Delayed- type hypersensitivity
ESCCAP	European Scientific Counsel for Companion Animal Parasites Guidelines
FL	Fluconazole
GRI	Griseofulvin
HI	Humoral Immunity
HIV	Human Immune Deficiency Virus
IFN-γ	Interferon γ
IgA	Immunoglobulin Alpha
IgE	Immunoglobulin Epsilon
IgG	Immunoglobulin Gamma
IgM	Immunoglobulin M
KDSG	Kaduna State Government
KE	Ketoconazole
KOH	Potassium Hydroxide

LCB	Lactophenol Cotton Blue
LGA	Local Government Area
M	*Microsporum*
Mg	Microgramme
MIC	Minimum Inhibitory Concentration
Ml	Millilitre
PDA	Potato Dextrose Agar
pH	Potential hydrogen
Pp	Page
SDA	Sabouraud's Dextrose Agar
S-P	Saprobic- Parasitic
T	*Trichophyton*
TER	Terbinafine
T_H2	Thymic Helper cells2
TRM	*T. rubrum* cell wall mannans

CHAPTER ONE
INTRODUCTION

1.1 Background of the study

Dermatophytes are cited among the most frequent causes of dermatological problems in domestic animals (Nweze, 2011). The superficial mycosis caused by dermatophytes is called dermatophytosis which is commonly referred to as 'ringworm' or 'tinea. In general, dermatophytes do not invade subcutaneous tissue, but pseudotumoral lesions (mycetoma or pseudomycetoma) affecting the subcutaneous tissues have been described in Persian cats (Medleau and Rakich, 1994; Richard *et al.,* 1994.)

Dermatophytes are significant due to their zoonotic potentials and the concern of owners of pets with severe inflammatory diseases (ESCCAP, 2011). They are also important where hides and skins are exported as contributors to the nation's economy especially cattle hides and skin. Dermatophytosis stops the flow of livestock, and devalues the skins (Lehenkari and Silvennoinen-Kassinen, 1995; Chermette *et al.,* 2008).

Dermatophytes are filamentous fungi which are able to use keratin as a source of carbon. Some of these organisms are true parasites. Dermatophytes belong to the Class Ascomycetes which are normally located in the epidermal stratum corneum, hair shaft or hoof where they invade (Samson *et al.,* 2012). Dermatophytosis is recognized as the most infectious dermatosis in pets (ESCCAP, 2011).

Dermatophytosis is caused by fungi in the Genera *Microsporum*, *Trichophyton*, and *Epidermophyton* (Nweze, 2010). There are three ecological groups of dermatophytes: anthropophilic (most associated with humans), zoophilic (associated with animals) and geophylic (found in soil). Some geophilic non-pathogenic dermatophyte species such as *M. cookie, T. ajelloi,* and *T. terrestre* have been isolated from animal fur (Cabanes, *et al.,* 1996; Simpanyan and Baxter, 1996)

Microsporum equinum and *Trichophyton equinum* are frequent causative agents of dermatophytosis in horses (Kwon-chung and Bennett, 1992) and are readily diagnosed by conventional techniques (Samson *et al.,* 2012). Also implicated is *Microsporum canis*, a pathogenic filamentous fungus responsible for dermatophytosis in both humans and animals. This zoophilic dermatophytes are most commonly isolated from infected or carrier cats, its natural host (Sparkes *et al.,* 1994) which can transmit the infection to horses. *Trichophyto*n *verruccossum* can also be transmitted from cows. **The different dermatophytes that can be found in other domestic animals in Nigeria are; in horses; *T. mentagrophytes, T. equinum, T. verrucosum, M. gypseum, M. canis,* and *M. equinum* (Nweze, 2011; Anyawu *et al.*, 2013),** *M. gypseum, T. mentagrophytes, T. equinum, T. verrucosum* (Nweze, 2011) from sheep; *M. audouinii, T. mentagrophytes, M. canis , M. persicolor,* (Nweze, 2011) from dogs and *T. schöenleinii, T. mentagrophytes, M. canis, T. verrucosum, T. equinum* from goats (Nweze, 2011; Chah *et al*., 2012).

Other fungi such as the Genus *Geotrichum* has been implicated in both human and animal dermatomycosis, (Gambale *et al.,* 1987; Rhyan *et al.,* 1990; Sidhu *et al*., 1993; Reppas and Snoeck, 1999; Chahota *et al*., 2001; Conti Diaz *et al.,* 2003; Henrich *et al*., 2009). Keratinopathogenic fungi can cause infections in horses resulting in conditions such as abnormalities of the hoof. Notable examples of such fungi are *Scopulariopsis breviacaulis, Alternata alternate, Geotrichum candidum* and *Accremonium blochii (*Keller *et al.,* 2000; Apprich *et al.,* 2006). In 2004, Riggs *et al*, as reported by *(*Samson *et al.,* 2012*)* discovered the Brittle Tail Syndrome, a novel condition that affected the hair tail of horses. Extensive epidermiological, toxicological and biological investigations revealed that the syndrome was associated with the invasion of affected hairs by a filamentous keratinophylic fungus known as E*quicaphyllimyces hongkongensis.* Other dermatophytes like *T. mentagrophytes, M. equinum, M. canis, and M. gypseum* can also be involved. Although approximately thirty fungal species cause skin infections in various mammals and birds, relatively few species are isolated from clinically overt cases of dermatophytosis.

Clinical signs of dermatophytosis include; scaling, crusting and alopecia, annular plaques and kerion formation on the skin (Nweze, 2011). Diagnosis of dermatophytosis is based on the demonstration of fungal spores in infected samples from direct Pottasium Hydroxide examination, histological examination, culture and identification of dermatophytes (CDC, 1960).

The species of dermatophytes involved in dermatophytosis may be different in similar geographic locations both in humans and animals. In some areas there is a decline in the incidence of animal ringworm where the degree of closeness of animals to human has been lost (Pier, *et al.,* 1994).

In Nigeria, there is much contact between man and domestic animals which may serve as asymptomatic carriers. Also, because of the considerable increase in immunocompromising conditions like cancer, HIV/AIDS diabetes e.t.c. where fungal infections (including dermatophytoses) are reported to produce the most common lesions especially in HIV infections, (Scott, 1988; Macura, 1993).

1.2 Statement of Research Problem

Although dermatophytosis is worldwide in distribution, it is more prevalent in hot humid climates than in cold dry regions (Scott, 1988; Macura, 1993). Ringworm of horses has however received little attention in Nigeria and particularly the north of the country perhaps because the disease is usually self limiting or produces usually benign skin lesions (Mackenzie, 1963; Adekeye, *et al.,* 1989; Macura, 1993). As a result, an actual prevalence figure for tinea in horse is unknown in Kaduna State.

Fungal infections of horses have been described in connection with poor quality hooves and several diseases such as white line disease or laminitis, (Kuwano, *et al.*, 1996; Jahns and

Dietz, 2000; keller, *et al.*, 2000). Several reports indicate that domestic animals constitute important reservoirs of human ringworm epidemics (La Touch, 1955; Blank and Craig, 1977; Gugnani, 1982).

Shah *et al.*, (1988), reported clinical cases of dermatophytosis in which the agents were resistant to the antifungal drugs griseofulvin and the azoles. This may make the eradication of fungal infections difficult especially in Nigeria, where Veterinary services are not solicited until all other avenues of treatment have failed as orthodox treatments of fungal infection usually takes a long course and the cost implication may be prohibitive.

This study is therefore relevant because of the zoonotic effect of dermatophytes and the role played by horses in the socio-economic life in the northern part of the country especially Zaria. This investigation is carried out to determine the infection and the susceptibility of fungal isolates to antifungal agents.

1.3 Justification of the Study

Animals serve as reservoirs for zoophilic dermatophytes, and their infections have considerable zoonotic importance, especially as the international game of polo has found wide acceptance in northern Nigeria. Stabled horses that are groomed together using the same brushes and combs can experience epizootics of dermatophytosis (www.cfsph.iastste.edu). It has been noted that the delineation of the natural foci of zoophilic dermatophytes in each state, country or geographical region may be very important for the understanding of the epidermiology of human dermatophyte infections and help in designing preventive strategies (Ates, *et al*., 2008). Zoophilic dermatophytes such as *M. canis*, *T. mentagrophytes and T. verrucosum* are significant causal agents in human ringworms in many areas of the world but the incidence of dermatophytoses varies according to climate and natural reservoirs. However, in Nigeria, there is a dearth of information pertaining to ringworm of horses as most of the studies carried out use school children as the subject of study (Nweze, 2001;

Ndako *et al*., 2012), and there is no recent work on the subject as the only other work on dermatophytes of animals was by (Adekeye *et al.*, 1989) . This research is therefore geared towards determining the type of dermatophytes encountered in horses in Kaduna State, Northern Nigeria.

1.4 Research Questions

The following research questions guided the study:

1. Are there dermatophytes causing ringworm infections of horses in Kaduna State?
2. If yes, are the dermatophytes susceptible to antifungal agents?
3. Was there any anatomical distribution?

1.5 Aim of the Study

To determine the anatomical distribution, isolation, identification and antifungal susceptibility patterns of dermatophytes from horses in Kaduna State.

1.6 Objectives of the Study

1. To document the site of lesions on the horses associated with fungi.

2. To culture and identify the dermatophytes encountered in horses in Kaduna state

3. To determine the antifungal susceptibility pattern on the isolated dermatophytes.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of Dermatophytosis

Historically, medical mycology, specifically relating to human disease, began with the discovery of the fungal aetiology of favus and centred around three European physicians in the mid-19th Century: Robert Remak, Johann L. Schönlein, and David Gruby (Weitzman and Summerbell, 1995). According to Seeliger (1985), Remak in 1835 first observed peculiar microscopic structures appearing as rods and buds in crusts from favic lesions. He never published his observations, but he permitted those observations to be cited in a doctoral dissertation by Xavier Hube in 1837. Remak credited their recognition to Schonlein, who described their mycotic nature in 1839 (Schönlein, 1939). However, Remak established that the etiologic agent of favus was infectious, cultured it on apple slices, and validly described it as *Achorion schöenleinii*, in honour of his mentor and his initial discovery (Remak, 1845).

According to Weitzman and Summerbell (1995), the real founder of dermatomycology was David Gruby on the basis of his discoveries during 1841 to 1844, his communications to the French Academy of Science, and his publications during this period (Gruby, 1841; 1843; 1844). Independently, and unaware of the work of Remak and Schönlein, he described the causative agent of favus, both clinically and in microscopic details of the crusts, and established the contagious nature of the disease. Gruby also described ectothrix invasion of the beard and scalp, naming the etiologic agent of the latter *Microsporum* (referring to the small spores around the hair shaft) *audouinii*, and described endothrix hair invasion by Herpes (*Trichophyton*) *tonsurans*. In addition to his observations on dermatophytes, he also described the clinical and microscopic appearance of thrush in children.

Raimond Sabouraud, one of the best known and most influential of the early medical mycologists, began his scientific studies of the dermatophytes around 1890, culminating in the publication of his classic volume, Les Teignes, in 1910. Sabouraud's contributions included his studies on the taxonomy, morphology, and methods of culturing the dermatophytes and the therapy of the dermatophytoses. He classified the dermatophytes into four Genera, Achorion, *Epidermophyton*, *Microsporum*, and *Trichophyton*, primarily on the basis of the clinical aspects of the disease, combined with cultural and microscopic observations (Weitzman and Summerbell, 1995). The medium that he developed is in use today for culturing fungi (although the ingredients are modified) and is named in his honour, Sabouraud glucose (dextrose) agar (Odds, 1991). Weiztman and Summerbell (1995) also reported that Sabouraud's treatment of tinea capitis by a one-dose, single-point roentgenologic epilation achieved cures in 3 months as opposed to the therapy of manual epilation and topical application of medications from (Kwon-Chung and Bennett, 1992).

In 1934, Chester Emmons modernized the taxonomic scheme of Sabouraud and others and established the current classification of the dermatophytes on the bases of spore morphology and accessory organs (Emmons, 1934). He eliminated the Genus Achorion and recognized only the three Genera *Microsporum*, *Trichophyton*, and *Epidermophyton* on the basis of mycological principles.

Nutritional and physiological studies of the dermatophytes pioneered at Columbia University by Rhoda Benham and Margarita Silva (Silver and Benham, 1952; Benham, 1953) and at the Centre for Disease Control, in Georgia, by Libero Ajello, Lucille K. Georg, and co-workers (Georg, 1952; Swartz and Georg 1955; Ajello and Georg, 1957; Georg and Camp, 1957) simplified the identification of dermatophytes and led to reduction of the number of species and varieties (Weitzman and Summerbell, 1995). The discovery of the teleomorphs (perfect or sexual state) of *Trichophyton* (Keratinomyces) *ajelloi* in 1959 by Dawson and Gentles

(1961), using the hair bait technique of Vanbreuseghem (Vanbreuseghem, 1952), led to the rapid discoveries of the teleomorphs of many dermatophytes and related keratinophilic fungi.

Griffin in 1960 and Stockdale (1961; 1963) independently obtained the teleomorphs of the *Microsporum gypseum* complex, thereby vindicating Nannizzi's original observation. The discovery of sexual reproduction in the dermatophytes opened the door to classical genetic studies with these fungi, e.g., determining the cause of pleomorphism (Weitzman, 1965) and clarifying the taxonomy and understanding of the incompatibility systems operating in these fungi (Weitzman, 1964). The successful oral therapy with griseofulvin of experimental dermatophytosis in guinea pigs reported by Gentles in 1958 revolutionized the therapy of dermatophytosis and initiated the first major change in the therapy of *tinea capitis* since the work of Sabouraud.

2.2 Aetiologic Agents

2.2.1 Anamorphs

The etiologic agents of the dermatophytoses are classified in three anamorphic (asexual or imperfect) Genera, *Epidermophyton*, *Microsporum*, and *Trichophyton*, of Anamorphic Class Hyphomycetes of the Deuteromycota (Fungi Imperfecti). The descriptions of the Genera essentially follow the classification scheme of Emmons on the bases of conidial morphology and formation of conidia and are updated following the discovery of new species (Ajello, 1968; Ajello, 1977; Matsumoto and Ajello, 1987). The Genera and their descriptions are as follows:

Epidermophyton Species

The Genus *Epidermophyton* includes only two species: *E. flucossum* and *E. stockdaleae* (Weitzman and Summerbell, 1995). The colonies are slow-growing, powdery and unique brownish yellow in colour (Wolfe *et al*., 1979). This Genus is devoid of the infective spores, microconidia. Macroconidia are abundant and produced in clusters (Jagdish, 1995). These macroconidia are thin walled with smooth surface (Wolfe *et al*., 1979). The type species is *Epidermophyton floccosum* and it is the only pathogenic dermatophyte in the Genera (Weitzman and Summerbell, 1995).

Microsporum Species

The Genus *Microsporum* includes 16 species (Weitzman and Summerbell, 1995). The colonial morphology of *Microsporum* species on agar surface is either velvety or powdery with white to brown pigmentation (Jagdish, 1995). Both macroconidia and microconidia are produced but the predominant conidial structures are macroconidia. Microconidia are less abundant. The macroconidia are multi septate with thin to thick wall and rough surface. Rarely some species produce neither microconidia nor macroconidia (St-Getmain and Summerbell, 1996). They do not have any special nutritional requirements .The macroconidia have one to fifteen septa, about 20 to 60 by 4 to 13 mm in size (Weitzman and Summerbell, 1995), from 6 to 16 by 6 to 25mm. (Lakshmipathy *et al*., 2010)

The type species is *Microsporum audouinii* (Weitzman and Summerbell, 1995). Macroconidia are characterized by the presence of rough walls which may be asperulate, echinulate, or verrucose. Originally, the macroconidia were described by Emmons as spindle shaped or fusiform, but the discovery of new species extended the range from obvate (egg shaped) as in *Microsporum nanum* (Fuentes, 1956) to cylindrofusiform as in *Microsporum vanbreuseghemii* (Georg *et al.*, 1962). Microconidia are sessile or stalked and clavate and usually arranged singly along the hyphae or in racemes as in *Microsporum racemosum*, a rare pathogen (Borelli, 1965).

Trichophyton Species

The Genus *Trichophyton* has 24 species (Weitzman and Summerbell, 1995). The colonies on agar media are powdery, velvety or waxy. The predominant spore type is microconidia with sparse macroconidia (Jagdish, 1995). Reverse side pigmentation is characteristic of the species and is used for the identification of the species within the Genus (Wagner and Sohnle, 1995; Larone, 1995). The macroconidia are thin walled with smooth surface and variable shape (Philpot, 1977). Some of the *Trichophyton* species are fastidious in their requirement for amino acid as nitrogen source. *Trichophyton tonsurans* requires ornithine, citrulline and Arginine, whereas *Trichophyton mentagrophytes* requires methionine. This nutritional specificity has been used by many authors in the identification of the *Trichophyton* species (Philpot, 1977). The type species is *Trichophyton tonsurans* (Weitzman and Summerbell, 1995).

Macroconidia, when present, have 1 to 12 septa, are borne singly or in clusters, and may be elongate and pencil shaped, clavate, fusiform, or cylindrical. They range in size from 8 to 86 by 4 to 14 mm. Microconidia, usually more abundant than macroconidia, may be globose, pyriform or clavate, or sessile or stalked, and are borne singly along the sides of the hyphae or in grape-like clusters (Lakshmipathy *et al*., 2010). Since the classification of the dermatophytes by Emmons in 1934, as a result of the discovery of new species and variants, the rigid morphological distinction among the three Genera has become a morphological continuum based on overlapping characteristics; e.g., *Trichophyton kanei* (Summerbell,1987), *Trichophyton longifusum* (Florian and Galgoczy, 1964), and a variant of *T. tonsurans* (Padhye and Weitzman,1994) lack microconidia, and therefore are more suggestive of the Genus *Epidermophyton*, whereas isolates of *Microsporum* spp. producing smooth-walled macronidia are more suggestive of *Trichophyton* spp. (Varsavsky *et al*.,1966).

According to Cabanes, 2000, the following dermatophytes are commonly found in domestic animals (Table 2.1).

Table 2.1 Main Etiological Agents of Dermatophytosis In Different Animal Species

Domestic Animals	Dermatophytes
Cats and dogs	*M. canis,* others*; T. mentagrophytes, M. gypseum, M. persicolor*
Horses	*T. equinum,* others*; M. canis, M. equinum, M. gypseum, T. mentagrophytes, T. verrucosum*
Cattle, sheep and goats	*T. verrucosum,* others*; M. canis, M. gypseum, T. equinum, T. mentagrophyhtes*
Rabbit	*T. mentagrophytes,* others*; M. canis*
Pig	*M. nanum,* others*; M. canis, M. gypseum, T. mentagrophytes*

2.2.2 Teleomorphs

Some dermatophytes, mostly the zoophilic and geophilic species of *Microsporum* and *Trichophyton* are also capable of reproducing sexually and producing ascomata with asci and ascospores. These species are classified in the teleomorphic Genus *Arthroderma* (Weitzman *et al*., 1986), Family Arthrodermataceae of the Onygenales (Currah, 1985), Phylum Ascomycota. Previously, the teleomorphs of the sexually reproducing *Microsporum* and *Trichophyton* species and related keratinophilic fungi had been classified in the Genera Nannizzia and Arthroderma, respectively (Ajello, 1977). However, on the basis of a careful evaluation of the morphological characteristics used to define these two Genera, Weitzman *et al.,* (1986) concluded that the species making up these Genera represented a continuum and that their minor differences did not merit maintaining them in two separate Genera. Nannizzia and Arthroderma are considered to be congeneric, with Arthroderma having taxonomic priority (Weitzman and Summerbell, 1995).

2.3 Ecology of Dermatophytosis

Dermatophytes are among the few fungi causing communicable diseases, that is, diseases acquired from infected animals or birds or from the fomites they have engendered. All but one of the species known to cause disease primarily affects mammals; the exception, *Microsporum gallinae,* is primarily established in gallinaceous fowls (www.cfsph.iastste.edu). Apart from those species usually associated with disease, transitional species exist which appear to be primarily saprobic organisms occasionally or rarely causing infection.

The term dermatophytes should be restricted to designate infectious organisms (Ajello, 1974). Closely biologically related organisms not included in this group include *Chrysosporium* species with teleomorphs in the Genus Arthroderma. Dermatophytes have long been divided into anthropophilic, zoophilic, and geophilic species on the basis of their primary habitat associations (Georg, 1960; Ajello, 1962).

2.3.1 Geophilic dermatophytes

Geophiles exist as saprophytes in the soil and have the ability to competitively colonise keratinous substrates successfully. Their distribution appears to relate to the distribution of available keratin (Marples, 1965; Mantovani, 1978; De Vroy, 1984). But the distribution is also influenced by the pH of the soil and generally they prefer a near neutral pH (Bohme, 1972).

Geophilic dermatophytes are primarily associated with keratinous materials such as hair, feathers, hooves, and horns after these materials have been dissociated from living animals and are in the process of decomposition. These species may cause human and animal infection when they come in contact with them. Geophilic species, are thought to be ancestral to the pathogenic dermatophytes, pre-adapted to cutaneous pathogenesis by their ability to decompose keratin and their consequent close association with animals living in hair and feather-lined nests in contact with soil (Chmel, 1980). Certain species known to be zoophilic may be isolated more often from soil and from fur of apparently healthy animals (Feuerman, *et al*., 1975; Ozegovic, 1980) than from animals with frank disease.

A few geophiles do have the additional capacity to cause ringworm in some species of animals, including man. These dermatophytes are generally contracted directly from soil containing a high number of spores and are only rarely transmitted from man to man or lower animals to man (Ajello, 1974; De Vroy, 1984). For example, *M. nanum*, which causes ringworm in animals, especially pigs, is mainly associated with surroundings having pigs (Baxter and Pearson, 1969; De Vroy, 1984; Connole, 1990). The proof for its geophilic existence was provided by observation of macroconidia in soil, (Ajello, 1974) since it is well known that these spores are not formed on infected animals.

The principle virulent geophilic dermatophytes are members of the *M. gypseum-fulvum* complex. This complex has been well documented as a pathogen in man and animals. According to Georg (1960), soil isolates of *M. gypseum* compared to animal isolates have a low pathogenicity and only very virulent strains are able to establish infection. Alternatively, strains of low infectivity may increase in virulence after "passage" through a host of low resistance. *Arthroderma fulva* is known to be the least pathogenic.

The distribution of the *M. gypseum-fulvum* complex is world-wide (Ajello, 1953; Durie and Frey, 1962; Marples, 1965; Londero *et al.,* 1985). For pathogenic geophilic dermatophytes, infective propagules originate from saprobic sources and are transmitted either directly or indirectly, and are referred to as saprobic-parasitic (S-P) infections (De Vroy, 1984; 1985). This mode of infection is common for *M. gypseum*, where the source of most infections in man and animals is the soil (Ajello *et al.*, 1966).

In children, facial ringworm by *M. gypseum* can follow recreational exposure to soil-borne propagules of this fungus (De Vroy, 1985). Occupational exposure is illustrated by reported cases in gardeners (De Vroy, 1985) and small epidemics observed in, for example, cucumber growers (Alsop, 1961; Gentles, 1962). The macroconidia and microconidia, ascospores and other propagules are produced during the saprophytic growth of dermatophytes on keratin in soil or other biotopes (e.g. birds' nests in the case of *M. ripariae*) (De Vroy, 1984) and it is these which form the potential inoculum.

The *T. terrestre* complex is considered to be non- pathogenic (Howard, 1983), although human infections by *T. terrestre* have been reported (Marples, 1960) and experimental animal infections have also been successfully induced (Bakerspigel, 1974).

Other geophilic dermatophytes include *M. cookei and T. ajelloi* which are non-pathogenic. *Microsporum cookei* is a geophile with a global distribution, often isolated from soil and also from rodents and other animals not showing any clinical symptoms of ringworm (Ajello, 1959; Simpanya and Baxter, 1996). Human infections by *M. cookei* have rarely been reported (Lundell, 1965; Frey, 1971). *Trichophyton ajelloi* is commonly found in colder climates but is sporadic in hot climates (Marples, 1965), possibly because higher temperatures inhibit its growth. The fungus has been found to be more often associated with acid soils than with alkaline soils (Marples, 1965).

2.3.2 Zoophilic dermatophytes

Zoophilic species are basically animal pathogens, often with a single preferred animal host or very limited host range, outside which they are found only in exceptional circumstances (English, 1972). Zoophilic dermatophytes rarely grow actively as saprophytes but survive in a dormant state on contaminated materials of animal origin. *Microsporum canis, T. verrucosum* and *T. mentagrophytes* are common agents of ringworm in animals but are also frequently associated with human infection. The amount of literature on human infections due to the three dermatophytes is enough evidence of their human affinity. Of the three, *M. canis* is the best documented (Baxter, 1973; Weitzman and Summerbell, 1995; Roberts and Friedlander, 2005; Nweze, 2011; Ndako *et al.*, 2012). This is mainly because it is the most frequent causes of scalp ringworm in children (English, 1972; Ndako *et al*., 2012). *Microsporum canis* commonly infects pet animals and especially cats and dogs which shed infective particles into the domestic environment and contact with this results in familial infections (De Vroy, 1985).

Like other types of ringworm, young children particularly in the age range 5-14 years are more susceptible to infection than adults. Similarly, kittens and puppies are more susceptible to ringworm than adult animals (Philpot, 1977). This is attribtable to the lack of development of various protective long chain fatty acids that protects against the establishment of dermatophyte spores on the host. *Microsporum canis* is also known to cause ringworm in horses, monkeys, apes and chinchillas (Vanbreusegehem *et al*., 1978; Weitzman and Summerbell, 1995).

Another dermatophyte species closely related to *M. canis* is *M. distortum*, known to cause ringworm infections in monkeys, dogs and cats. It has been reported to occur mainly in New Zealand (Di Menna and Marples, 1978), Australia and the United States (Vanbreuseghem *et al.*, 1978) although it had also been reported in Nigeria by Adekeye *et al*., (1989). It is now regarded as a variety of *M. canis*.

Trichophyton verrucosum, on the other hand, is a common cause of tinea in cattle. It has also been reported in donkeys, dogs, goats, sheep and horses (Beneke and Rogers, 1980; Weitzman and Summerbell, 1995; Anyawu *et al*., 2013). Close contact by man with infected animals and their fomites leads to contracting the fungus. It is also generally accepted that in countries with cold winters where housing of the animals is required, the incidence of *T. verrucosum* rises in both animals and humans at that time of the year (English, 1972). Cattle breeders and Veterinarians occasionally suffer from tineas due to *T. verrucosum*, which is mainly an agent of inflammatory skin and scalp lesions (kerion).

Members of the *T. mentagrophytes* complex (with the exception of *T. mentagrophytes var. interdigitale)* are transmitted from wild rodents and the prevalence of human infections due to this fungus is known to be higher in rural areas where there is a reservoir of rodents e.g. Africa, North America and Europe (Georg, 1956; Gentles and Sullivan, 1957; Kaben, 1967; Chermette *et al*., 2008). *T. mentagrophytes* has occasionally been isolated from the soil (Baxter, 1966; Padhye and Carmicheal, 1968) where it can survive for several months.

2.3.3 Anthropophiles and other human associated dermatophytes

Anthropophilic species are primarily adapted for parasitism of man, but some species occasionally cause ringworm in animals. For example, *T. rubrum* has been reported to have caused an infection in dogs (Kaplan, 1958; Georg, 1960). Anthropophilic dermatophytes are mainly associated with community life. Since transmission is man to man, contracting the disease therefore requires human contact (Weitzman and Summerbell, 1995). The spread of anthropophiles is more common in communities like schools, barracks, prisons and the family (Philpot, 1977; Rippon, 1982). In concentrated communities, the use of facilities such as shower- rooms, and common headgear leads to rapid spread of infection.

Four of the *Microsporum* species, according to Vanbreuseghem, can be distinguished from each other on clinical, epidemiological and mycological grounds: *M. audouinii, M. langeroni, M. rivalieri and M. ferrugineum* (Weitzman and Summerbell, 1995). *M. langeroni* (Vanbreuseghem, 1950; 1963a; 1963b) has been separated from the classic *M. audouinii* by its geographic region (restricted to Central Africa) and unlike *M. audouinii* can cause tinea corporis (ringworm of the glabrous skin) and can be inoculated to produce experimental lesions in guinea pigs. However, most mycologists consider *M. langeroni* and *M. rivalieri* as variaties of *M. audouinii* (Weitzman and Summerbell. 1995).

Among the anthropophilic *Trichophyton* species, *T. rubrum* is a very common cause of tinea unguium, cruris, and pedis (Rippon, 1985; De Vroy, 1985). *Trichophyton rubrum* very rarely invades hair *in vivo* (Weitzman and Summerbell. 1995). The distribution of *T. rubrum* is global, cutting across all populations and ethnic groups (De Vroy, 1985; Rippon, 1985). It is a dermatophyte becoming more prevalent among urban populations, especially in developed countries, due mainly to the “modern” way of life such as the wearing of occlusive shoes, which maintain heat and humidity (Philpot, 1977). It is also able to adapt to its environment in a way other species cannot (English, 1980). In India, *T. rubrum* causes tinea corporis in women and tinea cruris in men due to the sari (worn by women around the waist) and the dhobie (loin cloth) worn by men, both of which are tight-fitting (Fulton ,1975) . *Trichophyton rubrum* is also known to cause chronic forms of infections and it has been suggested that the amino acid composition of perspiration may predispose individuals to chronic infection. Certain amino acids are considered “inducers” of *T. rubrum* infections (Rippon, 1983).

Trichophyton mentagrophytes var. *interdigitale*, a member of the *T. mentagrophytes* complex, is essentially a cause of tinea pedis and tinea cruris, and does not invade hair *in vivo* (De Vroy, 1988). It causes the infection of the skin of the foot usually. Consequently, of the three dermatophyte strains, pathogenicity studies with laboratory animals have shown anthropophilic dermatophytes are primarily associated with humans and rarely infect other animals (Mayr, 1989).

Zoophilic dermatophytes usually infect animals or are associated with animals but occasionally infect humans. Many infections by zoophilic dermatophytes appear to be acquired indirectly from keratinous fomites, often deriving from apparently healthy animal carriers (De Vroy, 1985).

Potentially infectious geophilic dermatophytes such as members of the *M. gypseum* complex, growing on similar keratinous debris, overlap in ecology with these zoophiles. They differ mainly by their greater persistence in soil and are found regularly in habitats not strongly modified by the constant presence of animal associates (Weitzman and Summerbell, 1995).

Rippon, (1985) has pointed out a correspondence between soil association and conidial production in dermatophytes: the less significant the growth on dissociated keratin in the ecology of a dermatophyte, the less likely is the dermatophyte to produce conidia abundantly. Soil association also tends to correlate with the ability to form heterothallic teleomorphs in nature (Kwon-Chung and Bennett, 1992), ability not found in most anthropophilic dermatophytes and some zoophiles. Many anthropophilic and certain zoophilic species appear to consist predominantly or exclusively of isolates of a single mating type, as determined by the induction of infertile ascomata with *Arthroderma simii* mating type testers (Stockdale, 1968).

Tanaka *et al*. (1992) has pointed out that burrowing and denning animals tend to be associated with dermatophytes possessing a full roster of soil association characters, such as conidial abundance and dimorphism, heterothallic mating, osmotolerance, and the possession of typical arthropod predation deterrent structures such as conidial ornamentation, helical septea (spirals), vitamin and amino acid autotrophy, the elaboration of a urease enzyme, and the formation of perforating organs in dissociated hair. Dermatophytes primarily associated with humans or with non-burrowing, non-denning animals such as ungulates and equines tend to lack some or all of these characters.

Several specialized anthropophilic species (e.g., *Trichophyton concentricum* and *Microsporum ferrugineum*) consist of highly morphologically simplified, asexual isolates with little or no ability to produce conidia. The dermatophyte structure most commonly associated with contagion, especially in the poorly conidial anthropophilic dermatophytes, is the arthroconidium,'' or chlamydospore found within or attached to the exterior of infected hairs and within skin scales (Weitzman and Summerbell, 1995). These structures, particularly in certain species, may persist for years in the environment (Rippon, 1985; Shimura, 1985) and are highly heat resistant (Sinksi *et al*., 1980), particularly when embedded in hair or skin scales (Stockdale, 1953). In some anthropophilic species studied in detail, arthroconidia have a tendency to adhere *in vitro* to corneocytes derived from particular body sites (Aljabre *et al.*, 1993). It is possible that they may dissociate from skin cells in the environment and come in contact with new potential hosts as disseminated arthroconidia. Their persistence as an environmental source of con- tagion may lead to recurrent outbreaks of dermatophytosis in individuals and in institutions (Kane *et al.,* 1988).

According to Rippon (1985), the arthroconidia of *T. rubrum* do not survive as long as do those of other species, e.g., *E. floccosum*. The transition from potentially sexual to asexual life histories in the non-soil- associated dermatophytes appears to have led to adaptive radiation, at least in the anthropophilic dermatophytes (Tanaka *et al*., 1992). By most estimates, approximately two-thirds of the recognized dermatophyte species primarily associated with mammalian pathogenesis is anthropophiles (Tanaka *et al.*, 1992). Within the anthropophiles, polymorphous morphological variation is common, and numerous atypical and variant types are recognized (Rebell and Taplin, 1970; Young, 1972; Kane and Smitka, 1980), probably indicating further genetic drifts. Allopatric speciation appears to have been common in anthropophilic dermatophytes but rare in zoophiles, and several anthropophilic species have well-defined areas of endemicity (Rippon, 1988) while others, such as *T. rubrum* and *T. tonsurans*, are now cosmopolitan but appear to have had a more restricted distribution in the past, having been transported widely as a result of human migration (the anthropophiles travel with their human hosts) (Rippon, 1988).

Also, Kane *et al.* in 1990 discovered that spatial and ecological sympatric isolation appeared to be a predisposer to speciation in the anthropophiles: human-associated dermatophytes, unlike zoophiles, often have marked affinities for particular body sites. Most recognized asexual anthropophilic dermatophyte species are distinctive in morphology, physiology, and body site preference.

Recognition of dermatophyte taxa is clinically relevant. The need for species identification of dermatophytes in clinical settings is often related to epidemiological concerns. Especially relevant in human dermatophyte epidermiology is the identification of dermatophytes that; May have animal carriers, are linked to recurrent institutional or family outbreaks, such as *T. tonsurans* and *T. violaceum* (Mackenzie, 1961; Klokke *et al*., 1966; Kane *et al.*, 1988; Arnow *et al*., 1991; Stiller *et al.,* 1992), may cause rapidly progressing epidemics, such as *M. audouinii* and *T. tonsurans* (Bronson *et al*.,1983),and are geographically endemic, reflecting exposure during travel or residence in the area of endemicity or contact with a person with such a history (Badillet, 1988; Viguie *et al.,* 1992). These are important in the control of the disease.

2.4 Epidemiology of Dermatophytosis

Epidemiology is important in infection control and public health issues related to the different types of dermatophytosis.

In tinea capitis, the predominant agents in North America are *T. tonsurans* and *Microsporum canis*. In Maiduguri, Nigeria, (Nweze, 2001) tinea capitis was the most common form of infection followed by tinea coporis. The causative agent was *T. schöenleinii*, followed by *T. verruccosum* and *M. gallinea*. *Microsporum canis* is usually acquired from infected humans or their fomites and has caused a progressive, continent-wide epidemic now of some 40 years in duration (Georg, 1952; Bronson *et al*., 1983; Rippon, 1988). Urban areas and their communities of minorities have been particularly strongly affected. *M. canis* is usually acquired from infected cats or dogs, although limited human-to-human transfer leading to outbreaks can occur (Shah *et al*., 1988; Snider *et al.*, 1993). It is the predominant agent of tinea capitis in rural areas and in some parts of Europe, the eastern Mediterranean, and South America (Caprilli *et al*., 1980; Vidotto *et al*., 1982; Sinski and Flouras, 1984; Alteras *et al*., 1986; Rippon, 1988).

Tinea capitis in general is a condition most commonly seen in children (Rippon, 1988). In tinea capitis caused by *T. tonsurans*, however, a proportion of sufferers become long-term carriers of a subclinical scalp infection and may intermittently shed viable inoculum for decades (Pipkin *et al*., 1952; Hebert *et al*., 1985; Kane *et al*., 1988). When encountered in symptomatic adults, *T. tonsurans* is more frequently seen as an agent of tinea corporis (Bronson *et al*., 1983), and other infections, such as tinea manuum and onychomycosis, occur uncommonly. Similar patterns of age and body site preferences are found in other more geographically concentrated agents of endothrix tinea capitis such as *T. violaceum* (Scott and Scott, 1972).

Tinea corporis caused by *T. tonsurans* and other agents of endothrix tinea capitis may be more common in persons, particularly women, in close contact with children than among other adults. In institutional outbreaks, staff may transmit the fungus among immobile patients (Kane *et al*., 1988; Shah *et al*., 1988). Contact sports may distribute the disease among adolescents and young adults (Stiller *et al*., 1992). Among children, *T. tonsurans* is transmitted primarily by the sharing of combs, hats, bedding, and other materials contacting the scalp. Its environmental persistence on these fomites is noteworthy (Mackenzie, 1963; Kane *et al.*, 1988). Zoophilic and geophilic dermatophytes in general tend to form lesions that are more inflammatory than those formed by anthropophilic dermatophytes but are also more likely to resolve spontaneously (Rippon, 1988). This pattern is seen in tinea capitis caused by *M. canis* (Klingman, 1955; Klingman, 1956; Rippon, 1988).

The closely related anthropophile *M. audouinii*, once common in North America but now mainly restricted to parts of Africa and Asia (Rippon,1988), and appears particularly specialized as an agent of juvenile tinea capitis (Rippon,1988). Adult infections are rare, and spontaneous resolution usually occurs upon attainment of puberty (Klingman, 1955).

Tinea other than tinea capitis, when caused by anthropophilic fungi, tends to be associated with adults and adolescents, although infection of children may occur. *T. rubrum*, *E. floccosum*, and the anthropophilic *T. mentagrophytes* (i.e., cottony and velvety forms (Kane and Fischer, 1973; Kane and Smitka, 1980) known as *T. mentagrophytes var. interdigitale*) show a common pattern of association with tinea corporis, tinea cruris, and tinea pedis (English, 1980). In addition, *T. rubrum* and *T. mentagrophytes* are associated with tinea manuum and onychomycosis (Sinski and Flouras, 1984; Summerbell *et al*., 1989). It is likely that exposure to these dermatophytes is a common occurrence. Although the ecological and host factors involved in developing symptomatic infection are poorly known, known risk factors include foot dampness and abrasion combined with likely exposure to high fungal inoculum in communal aquatic facilities, such as swimming pools and showers (Detandt and Nolard, 1988; Auger *et al*., 1993). Exchange of clothing, towels, and linen, either directly or via substandard communal laundering, is another recognized risk (Rippon, 1988) which may lead to outbreaks. Asymptomatic infection is common, especially in tinea pedis (Auger *et al*., 1993). Damp foot conditions may lead to aggravated symptoms due to mixed infection by dermatophytes and bacteria (Hay *et al*., 1988; Auger *et al*., 1993).

Zoophilic dermatophytes, apart from causing tinea capitis, most commonly cause tinea corporis (including tinea faciei) in persons of any age group (Sinski and Flouras, 1984). Tinea of the extremities, tinea cruris, and onychomycosis caused by zoophiles are uncommon to rare (Sinski and Flouras, 1984).

2.4.1 Dermatophytoses in Africa

In various studies carried out in west Africa, *T. soudanense, M. audounii, T. rubrum, T. mentagrophytes, T. tonsurans, T. violaceum*, play the dominant role in human dermatophytoses (Ellabib *et al*., 2002; Menan *et al*., 2002; Adou-Bryn *et al*., 2004; Adeleke *et al*., 2008; Nweze, 2010; Efuntoye *et al*., 2011). Efuntoye and Fashanu, (2002) carried out 2 different studies on the dermatophytes of poultry and animal pens in Oyo state, Nigeria and isolated various dermatophytes with *M. gallinae* and *M. gypseum* being the dominant species isolated. Chineme *et al*., 1980, and Adekeye *et al.*, 1989 carried out surveys on the dermatophytes of domestic animals in Kaduna State and isolated various dermatophytes from the Trichophyton and Microsporum Genera. In another study carried out by Nweze (2011) in seven states in Nigeria, (Enugu, Anambra, Ebonyi, Abia, Imo, Kogi and Delta) in different domestic animals, a large spectrum of dermatophytes consisting of 10 species was isolated. They were mostly zoophilic species and include *M. canis, T. mentagrophytes, T. verrusosum, M. gypseum, M. gallinae, T. equinium, M. nanum, M. equinium, M. persicolor* and *T. gallinae*. Out of these, *M. canis* was the most predominant species consisting of 37.4% of all positive samples. Chah *et al*., (2012), carried out a study on the dermatophytes from skin lesions of four species of domestic animals; dogs, goats, sheep and pigs and isolated various dermatophytes including *T. mentagrophytes, T. schöenleinii, M. gypseum and M. audounii.* A case of mixed infection of *T. mentagrophytes* and *T. verrucosum* in a part Arab horse was reported by Anyawu *et al*., (2013) in Nsukka, Enugu state. Dalis *et al.*, (2014) reported an outbreak of bovine dermatophytoses in calves in Vom, Plateau state with *T. verrucosum* as the the causal organism in all the calves.

2.5 Transmission

People and animals become infected with dermatophytes after contact with spores (conidia) (Lakshmipathy *et al.*, 2010). Dermatophytes growing in a vertebrate host normally form only arthrospores (arthroconidia), asexual spores that develop within the hyphae (Weitzman and Summerbell, 1995). In the environment (e.g., in laboratory culture), they can also produce microconidia and macroconidia, asexual spores that develop outside the hyphae (Weitzman and Summerbell, 1995). Initially, the dermatophyte infects a growing hair or the stratum corneum of the skin. These organisms do not usually invade resting hairs, since the essential nutrients they need for growth is absent or limited (Weitzman and Summerbell, 1995). Hyphae spread in the hairs and keratinized skin, eventually developing infectious arthrospores (www.cfsph.iastste.edu).

Anthropophilic and zoophilic dermatophytes are mainly transmitted between hosts by arthrospores in hairs or skin scales. Other asexual or sexual spores formed by the environmental stages may also be infectious. Fomites such as brushes and clippers are important in transmission. Spores may remain viable in suitable environments for up to 12-20 months, and some spores were also reported to persist for at least a year in salt water (Chermette *et al*., 2008). Certain types of spores (e.g., microconidia) might be dispersed by airborne means (www.cfsph.iastste.edu).

2.6 Pathogenesis and Clinical Manifestations

Dermatophytes can survive solely on outer cornified layers of the skin (Rippon, 1988, Samdani, 2005). The ability of certain fungi to adhere to particular host arises from numerous mechanisms and host factors, including the ability to adapt to the body (Rippon, 1988). Natural infection is acquired by the deposition of viable arthrospores or hyphae on the surface of the susceptible host (Hay and Moore, 2004). After the inoculation in the host skin, suitable conditions favour the infection to progress through the following stages (Verma and Herffernan, 2008).

2.6.1 Adherence

After overcoming obstacles (ultraviolet light, temperature, and moisture variation) and competing with the normal flora and sphingosines produced by keratinocytes and the fatty acids produced by the sebaceous glands, the arthroconidia (infectious element) adhere to the keratinized tissue (Verma and Heffernan, 2008). The germination of arthroconidia and hyphal growth adherence proceeds radially in multiple directions (Sandy, 2008; Aljabre *et al*., 1993). It has been hypothesised that dermatophytic-secreted proteases could facilitate or must even be necessary for efficient adherence (Lakshmipathy *et al*., 2010). The ability of *T. rubrum* to adhere to epithelial cells has been attributed to carbohydrate-specific adhesins, expressed on the surface of microconidia.

From a morphological point of view, fibrillar projections have been observed in *T. mentagrophytes* during the adherence phase. At the skin surface, long and sparse fibrils connect fungal arthroconidia to keratinocytes and to each other, while in the inner skin layers, newly formed arthroconidia show thin and short appendices covering their entire surface; the latter begin to vanish as a large contact area is established between conidia and skin tissue (Sandy, 2008).

2.6.2 Penetration

Dermatophytes are provided with an arsenal of proteases aimed at the digestion of the keratin network into assimilable oligopeptides or amino acids (Sandy, 2008). Once established, the spores must germinate and penetrate the stratum corneum at a rate faster than desquamation. Penetration is accompanied by dermatophytes secreting multiple serine-subtilisins and metallo-endoproteases (fungalysins) formerly called keratinases that are found almost exclusively in the dermatophytes (Sandy, 2008; Dahl, 1994).

A direct relationship between keratinases and pathogenicity has been established. However, little information is available about hydrolases, such as lipases, and a ceramidase, produced by these fungi (Sandy, 2008). The mechanism by which mucolytic enzymes, which help in penetration, also provide nutrition to the fungi is unknown (Verma and Heffenan, 2008). These dermatophytic keratinolytic proteases cannot act before disulfide bridges are reduced within the compact protein network constituting keratinized tissues. This was recently shown to depend from a sulfite efflux pump encoded by the *Ssu1 gene* (Verma and Heffeman, 2008). Sulfite excretion by this transporter allows sulfitolysis of proteins, rendering them accessible for proteases, and functions in the same time as a possible detoxification pathway, a future target for new antifungal treatments (Sandy, 2008).

The protease production in *T. rubrum* is highly host specific showing reduced physiological activity when growing on their preferred host (Rippon, 1988; Rippon and McGinnis, 1995). This would explain the well-established anthropophization of these species. Ranganathan reported a similar finding on the relationship between chronicity and low-protease profile of *T. rubrum* isolates (Venkatesan *et al.,* 2007).

Fungal mannans in the dermatophyte cell wall have immuno-inhibitory effects and *T. rubrum* cell wall mannans (TRM) seem to be involved in an immunosuppression phenomenon, inhibiting lymphoproliferative response of mononuclear leukocytes in response to several antigens (dermatophytic or not) and mitogens. *Trichophyton rubrum* mannans may also decrease the keratinocyte proliferation rate, directly or via lymphocyte function alteration, contributing significantly to the chronicity of *T. rubrum* infection (Dahl, 1994; Chander, 1995; Verma and Heffenan, 2008; Sandy, 2008). However, clinical heterogenicity in substrate preference, with all dermatophyte species invading the stratum corneum of the skin and wide variation in their capacity to invade hair and nail, has been seen (Wagner and Sohnle, 1995).

2.6.3 Development of host response

Although the cornified layers of the skin lack a specific immune system to recognize this infection and rid itself of it, nevertheless, both humoral and cell-mediated reactions and specific and nonspecific host defense mechanisms respond and eventually eliminate the fungus, preventing invasion into the deeper viable tissue. However, the disease process is greatly influenced by the host response to dermatophyte infection. Hosts infected with dermatophytes usually develop cellular hypersensitivity (Cox, 1989).

Several studies have demonstrated that animals experimentally infected with dermatophytes, generally develop delayed hypersensitivity to antigens of the infecting agent (Cruickshank and Wood, 1960; Kerbs *et al*., 1977). Delayed hypersensitivity responses appear to be important in the development and clearance of the lesions of dermatophytoses. Cox, (1989); Kerbs *et al*. (1977) found that in guinea pigs with experimental *Trichophyton mentagrophytes* infections, the degree of maximal erythema occurred when the animals developed cell-mediated immunity.

Lepper (1974) noted that in cattle infected with *T. verruccosum*, the development of inflammation in primary infections coincided with the development of delayed hypersensitivity to fungal antigens. Green *et al*., (1982) used *T. mentagrophyles* infections of guinea pig skin grafts on nude mice to demonstrate that cell- mediated immunity is required to sustain inflammation in the infected skin and to eliminate the infecting organisms. In the bovine model of dermatophytosis, delayed- hypersensitivity reactions appeared to promote the cleanup of the infection by increasing the rate of desquamation of the stratum corneum (Jones, 1974). This array of defense mechanisms thought to be active against dermatophytes consists of α2-macroglobulin keratinase inhibitor (Yu *et al.*, 1972), unsaturated transferrin (King *et al*., 1975), epidermal desquamation , and lymphocytes, macrophages, neutrophils, and mast cells (Calderon, 1989).

There are two major classes of dermatophyte antigens: glycopeptides and keratinases. The protein portion of the glycopeptides preferentially stimulates cell-mediated immunity (CMI), whereas the polysaccharide portion preferentially stimulates humoral immunity (Dahl, 1993; Woodfolk, 1998; 2005; Mignon, 2008). Keratinases are produced by the dermatophytes to enable skin invasion, elicit delayed-type hypersensitivity (DTH) responses when injected intradermally into the skin of animals (Grappel and Blank, 1972). Although the host develops a variety of antibodies to dermatophyte infection, i.e., immunoglobulin M (IgM), IgG, IgA, and IgE, they apparently do not help eliminate the infection since the highest level of antibodies is found in those patients with chronic infection (Dahl, 1987). IgE, which mediates immediate hypersensitivity, appears to play no role in the defense process (Dahl, 1993). Rather, the development of CMI which is correlated with DTH is usually associated with clinical cure and ridding the stratum corneum of the offending dermatophyte (Dahl, 1993, Jones, 1993). In contrast, the lack of CMI or defective CMI prevents an effective response and predisposes the host to chronic or recurrent dermatophyte infections (Jones *et al.,* 1974; Jones, 1986; Jones, 1993). Fungal metabolic products diffuse through the malphigian layer to cause erythema, vesicle or even pustule formation along with pruritus. The *in vivo* activity is restricted to the zone of differentiation, newly differentiated keratin and Adamson's fringe within the hair shaft (Chander, 1995). Acute dermatophytosis is associated with a DTH skin response against them, while persistent disease corresponds to HI responses, to high levels of IgE and IgG4 antibodies, and to the production of T_H2 cytokines by mononuclear leukocytes (Sandy, 2008).

2.6.4 Acquired resistance

The efficient and protective response against dermatophytosis is a cell-mediated response of the DTH, characterized namely by the action of macrophages as effector cells, interferon-α secretion from type 1 T-helper lymphocytes and by some key cytokines like interferon-γ (IFN-γ). Immune detection and chemotaxis occur via low-molecular weight chemotactic factors or alternative complement pathway activation. However, the immune response that is raised, and especially the degree of inflammation, varies according to the dermatophyte species, the host species and the pathophysiological status of the host (Jones, 1993; Verma and Heffenan, 2008; Sandy, 2008). In general, the zoophilic species cause more inflammatory infections, which may heal spontaneously and result in relative resistance to re-infection. The anthropophilic species usually cause more chronic, less circumscribed infections, which result in less resistance to re-infection (Grappel, 1974). Primary infection produces negative trichophytin test and minimal inflammation (mild erythema and scaling) due to increased keratinocyte turnover.

2.6.5 Antibodies

Antibody formation does not seem to be protective (Hay *et al*., 1984) The dermatophyte antigen is thought to be processed by epidermal Langerhans cells and presented in local lymph nodes to T lymphocytes which proliferate, migrate to the infected site, and produce inflammation. The epidermal barrier becomes permeable to transferring and migrating cells leading to spontaneous resolution of lesions. Trichophytin skin test is now positive and clearing of second infection will be more rapid (Verma and Heffenan, 2008). Rivalier (1929) showed that a dermatophytic infection in humans results in a relative resistance to subsequent infection seen mainly by the inflammatory forms (kerion), caused by zoophilic species, but not always follow the more chronic anthropophilic infections (Barlow and Chattaway, 1958; Grappel, 1974). Barlow and Chattaway (1958) pointed out that fungi which do not invade the hair follicle do not seem to give rise to an equivalent immunity when growing in the horny layer of the smooth skin. In contrast, such acquired immunity could not be demonstrated in experimental *T. rubrum* infection of smooth skin (Desai et al., 1963; Grappel, 1974).

2.6.6 Hypersensitivity ("Trichophytin" Reaction)

Dermatophytid reactions in (4–5% of patients) are inflammatory eczematous allergic skin reactions at sites distant from primary fungal infection (Gappel, 1974). When skin scappings are collected and subjected to direct examination in KOH, no hyphae and spores are visualized, therefore the result is interpreted to be negative. This skin reaction is associated with a delayed type hypersensitivity (DTH) response to trichophytin test and may involve a local DTH response to systemically absorbed fungal antigen (Gappel, 1974; Kaaman and Torssander, 1983).

2.6.7 Nonspecific resistance

Natural defenses against dermatophytes depend on immunological and nonimmunological mechanisms (Sohnle, 1989). Several host factors like number and activity of sebaceous glands (due to inhibitory effect of sebum on dermatophytes) in a particular body region, breaks in the skin barrier, increased hydration and macerated skin can encourage dermatophyte invasion (Rippon, 1988).

Host factors that help limiting the infection to keratinized tissue include their preference for cooler skin temperatures than the normal body temperature, serum inhibitory factors (beta-globulins, ferritin and other metal chelators) binding to iron essential for growth of dermatophytes (Mosher *et al*., 1936; Lorincz *et al*.,1958; Rippon, 1988; Dahl, 1994). Unsaturated transferrin inhibits the growth of dermatophytes by binding to the hyphae (King *et al.*, 1975). A growth modifying, α_2 macroglobulin keratin inhibitor, has also been identified in serum (Yu *et al*., 1972). The natural resistance of scalp to *T. capitis* in adults may be due to post pubertal, fungistatic and fungicidal, long chain saturated fatty acids.

Commensal Pityrosporum yeast aids lipolysis and increases pool of fatty acids available for inhibiting fungi (Lorincz *et al*., 1958). Humoral immunity has a minor role in acquired resistance to dermatophytoses (Gappel, 1974; Ahmed, 1983).

2.6.8 Invasive dermatophytosis

Invasion or dissemination of dermatophytes within the dermis is rare, but occurs mostly in the setting of a chronic dermatophyte infection (mostly *T. rubrum*) in an immunosuppressed individual (Nir-paz, 2003). Acute onset of ulcerating or draining dermal and subcutaneous nodes occurs after hematogenous spread. A more indolent process can occur, presenting most often as tender nodules over extremities (Gong *et al*., 2006).

A case of a fatal 'dermatophytic disease' due to *T. schönleinii* was documented in a family of three siblings with a familial immunological defect after eight years of evolution (Marill *et al*., 1975). This dermatophyte's arthrospores or conidia gained entry into the hosts bodies probably through injured skin, scars or burns.

2.7 Clinical Manifestations in Man

Traditionally, infections caused by dermatophytes (ringworm) have been named according to the anatomic locations involved by appending the Latin term designating the body site after the word tinea, e.g., tinea capitis for ringworm of the scalp, Tinea barbae (ringworm of the beard and mustache), tinea capitis (scalp, eyebrows, and eyelashes).

Several anatomic sites may be infected by a single dermatophyte species, and different species may produce clinically identical lesions. The major etiologic agents may be global, such as *T. rubrum*, while the distribution of others may vary geographically. The clinical conditions and their major etiologic agents are as follows:

2.7.1 Tinea barbae

Tinea barbae, an infection of the bearded area, may be mild and superficial or a severe inflammatory pustular folliculitis, the latter form more commonly caused by the zoophilic dermatophytes *Trichophyton verrucosum, T. mentagrophytes var. mentagrophytes*, and *T. mentagrophytes var. erinacei* (Kwon-Chung and Bennett, 1992).

2.7.2 Tinea capitis

Tinea capitis, an infection commonly involving the scalp, is usually caused by members of the Genera *Microsporum* and *Trichophyton*. The infection may range from mild, almost sub-clinical, with slight erythema and a few patchy areas of scaling with dull gray hair stumps to a highly inflammatory reaction with folliculitis, kerion formation, and extensive areas of scarring and alopecia, sometimes accompanied by fever, malaise, and regional lymphadenopathy (Nweze, 2010). Both the skin surface and hairs are involved. Infection of the hair may be described as ectothrix (sheath of arthroconidia formed on the outside of the hair shaft) or endothrix (arthroconidia formed within the hair shaft). The inflammatory type is mostly caused by *M. canis* and *M. gypseum* (Nweze, 2010). The cause of tinea capitis in most of North, Central, and South America is *T. tonsurans* (endothrix) replacing *M. audouinii* (ectothrix) (Rippon, 1985). In West Africa, the predominant species are: *M. audounii, M. canis, M. gypseum, T. verruccosum, T. violaceum, M. ferrugineum, T. schöenleinii, T. megnini* and *T. soudanense* (Weitzmann and Summerbell, 1995).

2.7.3 Tinea corporis

Ringworm of the body, usually involving the trunk, shoulders, or limbs, and occasionally the face (excluding the bearded area), may be caused by any dermatophyte. The infection may range from mild to severe, commonly appearing as annular, scaly patches with sharply marginated, raised erythematous vesicular borders (Weitzman and Summerbell, 1995).

2.7.4 Tinea cruris (‘‘jock itch’’)

Infection of the groin, perianal, and perineal areas, and occasionally the upper thighs, is usually seen in adult men in warm climatic regions (Macura, 1993). *T. rubrum* and *E. floccosum* are the most frequent etiologic agents. Lesions are erythematous to tawny brown and covered with thin, dry scales. They are usually bilateral and often asymmetric, extending down the sides of the inner thigh and exhibiting a raised, sharply marginated border that is frequently studded with small vesicles (Weitzman and Summerbell, 1995).

2.7.5 Tinea favosa

Tinea favosa, usually caused by *Trichophyton schöenleinii*, is severe and chronic, characterized by the presence on the scalp and glabrous skin of yellowish, cup-shaped crusts called scutula, which is composed of epithelial debris and dense masses of mycelium. The disease is most common in Eurasia and Africa (Weitzman and Summerbell, 1995).

2.7.6 Tinea imbricata

Tinea imbricata, the chronic infection which is a specialized manifestation of tinea corporis, is characterized by concentric rings of overlapping scales scattered throughout the body. It is geographically restricted to certain of the Pacific islands of Oceana, Southeast Asia, Mexico, and Central and South America (Rippon, 1985; 1988). *T. concentricum,* a strictly anthropophilic dermatophyte, is the only etiologic agent.

2.7.7 Tinea manuum

The palmar and interdigital areas of the hand are usually involved in tinea manuum, most frequently presenting as unilateral diffuse hyperkeratosis with accentuation of the flexural creases. Most infections are caused by *T. rubrum* (Weitzman and Summerbell, 1995).

2.7.8 Tinea pedis (‘‘athlete’s foot’’)

The feet, especially the soles and toe webs, are most frequently involved in tinea pedis. The most common clinical manifestation is the intertriginous form, which presents with maceration, peeling, and fissuring, mainly in the spaces between the fourth and fifth toes. Another common presentation is the chronic, squamous, hyperkeratotic type in which fine silvery scales cover pinkish skin of the soles, heels, and sides of the foot (moccasin foot). An acute inflammatory condition, characterized by the formation of vesicles, pustules, and sometimes bullae, is most frequently caused by *T. mentagrophytes.* The more chronic agents of tinea pedis *are T. rubrum, T. mentagrophytes var. interdigitale,* and *E. floccosum* (Weitzman and Summerbell, 1995)

2.7.9 Tinea unguium

Invasion of the nail plate by a dermatophyte is referred to as tinea unguium while infection of the nail by nondermatophytic fungi is called onychomycosis (Rippon, 1985). The latter word is often used as a general term for a nail infection. There are two main types of nail involvement: invasive subungual (distal and proximal) and superficial white mycotic infection (leukonychia trichophytica). *T. rubrum* and *T. mentagrophytes* are the most common dermatophytes causing this infection (Weitzman and Summerbell, 1995).

2.8 Dermatophytosis in Animals

Dermatophytoses occur frequently in pet animals and in livestock as well, sometimes also in wildlife. Contagiousness explains the high occurrence of ringworm in herds and animal collectivities. Clinical importance is usually mild except in heavily infected young animals such as calves and foals with an impact on general health condition and growth. In animals generally, clinical signs include mild to severe alopecia associated with erythema (Chermette *et al*., 2008). Fluorescence produced by some dermatophytes, such as *M. canis*, can appear on the fur within 7 days of exposure, and clinical signs can develop within 2 to 4 weeks. Because hair follicles are infected the most common clinical sign is hair loss (correctly termed alopecia) and the skin in affected areas often becomes dry and scaly (www.cfsph.iastste.edu). Prior to hair loss and scaling the first sign of infection may be erection of the hairs and slight elevation of the skin beneath them. Occasionally the centre of the lesions will heal leaving a ring of infected skin around it. The lesions are usually non-painful (some minor discomfort may be seen at the start of disease), are not itchy and are typically limited to the head and trunk; Lesions rarely occur in isolation (NADIS, 2015). Lesions can be single or multiple, and are localised on any part of the animal although the anterior part of the body and the head seem more frequently involved (Chermette *et al*., 2008). Usually, there is a centrifugal spread of lesions. Multiple lesions may coalesce, while a spontaneous healing at the centre with regrowth of hairs is generally observed.

Animals can be infected by a great variety of dermatophytes, mostly zoophilic but also geophilic species, and exceptionally anthropophilic dermatophytes. A wide variety of dermatophytes have been isolated from animals, but a few zoophilic species are responsible for the majority of the cases, viz. *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton equinum* and *Trichophyton verrucosum*, as also the geophilic species *Microsporum gypseum* (Chermette *et al.,* 2008).

Lesions due to *T. equinum* and *M.canis* are typically dry with thin powdery scales and hairs broken at their bases (Chermette *et al.,* 2008). Lesion are usually not pruriginous and kerion and military dermatitis may also occur which extend rapidly from the saddle and girth area throughout the body in horses (Chermette *et al*., 2008; Cafarchia *et al.,* 2013).

Birds are the only non mammals infected with dermatophytes. This mainly invades the combs amd wattle as white favic patches which can spread to other non feathered parts of the skin. *M. gypseum* and *M. gallinae* are the most commonly isolated dermatophytes in birds (Efuntoye and Fashanu, 2002). In a study carried by Nweze, (2011) skin scrapping from animals in seven states of Nigeria was examined mycologically for dermatophytes. The animals were cats, dogs, sheep, goats, cow, pigs, horses, rabbits, ducks and chickens. Various dermatophytes of the *Microsporum* and *Trichophyton* Genera were isolated from all the domestic animal species. *M. canis* was the predominant species affecting all the animals (37.4%), followed by *T. mentagrophytes* (22.9%) and *T. verrucosum* (15.9%). Chah *et al*., (2012) isolated various dermatophytes including *T. schöenleinii*, an antropophilic dermatophytes when he examined 4 species of domestic animals for dermatophytosis. *T. verrucosum* was also isolated from infected calves by Dalis *et al*., (2014) from Jos, Plateau state.

In horses, the main causative agents of ringworm are *T. equinum* in young and adult animals, and *M. canis* (syn. *M. equinum*) more often in young horses (Scott, 1988). Other species such as *T. mentagrophytes* or *M. gypseum* are also isolated from lesions. Anyawu *et al.,* (2013) isolated *T. mentagrophytes* and *T. verrucosum* from a mixed infected horse in Nsukka. Kerion is usually localised on the face and is due to *T. mentagrophytes*, *T. verrucosum*, or *M. gypseum*. The miliary and sandy aspect is due to multiple small crusty lesions that are particularly marked on the flanks. The usual causative agent of equine miliary dermatophytosis is *T. mentagrophytes* (Chermette *et al*., 2008).

2.9 Histopathology

Dermatophytosis tends to be restricted to the horny epidermal layers of the skin and to the nails and hair. Infection begins with hyphal penetration of the stratum corneum of the skin. Several weeks later, the fungus colonizes the base of hairs within the hair follicles and penetrates the medulla of the hair shaft (Klingman, 1952; Knignt, 1972). The newly keratinized material of the growing hair, extending through the aperture of the follicle, carries either within it (endothrix) or on and just beneath its cuticular surface (ectothrix) hyphae that round up and become converted into arthroconidia. In tinea favosa of the scalp, filaments, often empty or vacuolated, are seen within infected hairs, while the scalp bears conspicuous cup-shaped areas of densely interwoven mycelium, scales, and debris referred to as scutula. Inflammation with round-cell infiltrate is seen in the adjacent dermis (Rippon, 1985). Affected hair follicles tend to atrophy. In affected skin, peripherally raised and centrally depressed areas of scutula also tend to form. Development of a hypersensitive, kerion reaction on the scalp or similar tinea profunda lesions elsewhere is accompanied by extensive infiltration of lymphocytes, plasma cells, neutrophils, and eosinophils into the dermis (Kwon-Chung and Bennett, 1992). Other features may include penetration of hyphae into the dermis and perivascular and perifollicular inflammation.

2.10 Growth Requirement for Dermatophytes

Dermatophytes are moderately thermotolerant; most grow well at 37^0C *in vitro*. An exception is *M. persicolor*, a zoophile mainly associated with voles; this species grows poorly or not at all at 37^0C (Kane *et al.*, 1987). Growth optima for most dermatophytes are 25 to 35^0C, probably reflecting an external habitat with a temperature slightly below body temperature (Stockdale, 1953). Growth at temperatures over 40^0C is uncommon. Non-pathogenic congeners often do not grow at 37^0C: for example, members of the *T. terrestre* complex, ubiquitous on soil keratin, are unable to do so. Geophilic dermatophytes and congeners are moderately salt tolerant (Kane and Fisher, 1975) and therefore are likely moderately osmotolerant in general, as would be expected of organisms growing on easily desiccated keratin fragments. Anthropophilic dermatophytes in general have lower salt tolerance than do zoophilic and geophilic species, perhaps reflecting a lack of adaptation for growth on desiccated substrates (Kane and Fisher, 1975).

Some dermatophytes have distinctive nutritional requirements that set them apart from others (George and Camp, 1956). For example, *T. verrucosum* has a complete requirement for thiamine, and or inositol, *T. equinum* requires nicotinic acid, and *T. megninii* requires Lhistidine for growth. Other species such as *T. mentagrophytes* and *T. rubrum* appear to be completely autotrophic for the vitamins.

Vega *et al*. (2003) investigated the impact of nutrition on spore yields for various fungal entomopathogens in liquid culture. Jain (2001) studied the effect of four liquid media on keratinophilic and dermatophytic fungal growth and found that SDA medium showed maximum growth and sporulation of all fungi. This is in agreement with other investigation and findings. From investigations carried out by Singh, 1983 and Sharma, 1983, they compared different media and concluded that Sabouraud's dextrose agar was the best among natural media and glucose aspergin was the best among synthetic media for the growth of *T. equinum* and strains of *Nanizia fulva*. In an investigation by Sharma and Sharma, (2011) Sabouraud's dextrose agar was also found to be best for growth and sporulation of test fungi followed by Potato Dextrose medium.

2.11 Media and Diagnostic Technique for Dermatophytes

Many typical isolates of common dermatophytes can be identified directly from primary isolation media, particularly, Sabouraud glucose agar and potato glucose or potato flake agar (Weitzman and Summerbell, 1995). Identification characters include colony pigmentation, texture, and growth rate and distinctive morphological structures, such as microconidia, macroconidia, spirals, pectinate branches, pedicels, and nodular organs (Rebell and Taplin, 1970; Weitzman and Kane, 1991). The following dermatophytes are less common species and easily confused with common species, particularly, *Microsporum persicolor*, *Trichophyton equinum*, *T. violaceum*, *Trichophyton soudanense*, *Trichophyton megninii*, and *Microsporum praecox*, so that primary isolates compatible with these species should be recognized as unusual and studied under more exacting identification procedures(Weitzman and Summerbell, 1995). These species, although uncommon, are found with some regularity in North America and European clinical laboratories (Weitzman and Summerbell, 1995).

The development of microscopic structures may be enhanced by use of sporulation media such as lactrimel (Kaminski, 1985), pablum cereal, or oatmeal agars (Weitzman and Silva-Hutner, 1967). Conditions for inducing macroconidial formation vary from species to species: e.g., for *M. canis*, media such as rice grains (Rebell and Taplin, 1970); for *T. mentagrophytes* and *M. persicolor*, Sabouraud agar with 3 to 5% added sodium chloride (Kane and Fischer, 1973); and for *M. equinum*, niger seed medium 8 (Hironaga *et al*., 1980). A series of vitamin and amino acid test agars (Georg and Camp, 1957; Rippon, 1988) is available as the *Trichophyton* agars (Difco) and is used to confirm the identity of several species with distinctive responses to growth substance (Rebell and Taplin, 1970; Rippon, 1988; Weitzman *et al*., 1988; Weitzman and Kane, 1991). An unknown but characteristic nutrient requirement of *M. audouinii* is elucidated on autoclaved polished rice grains. The organism grows poorly on the grains and secretes a brownish pigment; *M. canis*, the main dermatophyte of differential diagnosis, grows well and usually secretes a yellow pigment (Rebell and Taplin, 1970). Urea agar or broth is used to facilitate recognition of the small number of urease-negative species, particularly, *T. rubrum* but also most isolates of *T. soudanense* (Rosenthal, 1965; Philpot, 1967; Weitzman and Rosenthal, 1984). This test must be used with caution given the prevalence of poorly visible, antibiotic-resistant bacteria in *T. rubrum* colonies which may cause false-positive reactions. Urease-positive isolates, formerly considered granular and African types of *T. rubrum* (English, 1980), are now placed in the segregate species *Trichophyton raubitschekii* by many mycologists (Kane *et al*., 1981). The urease test is not normally used for slow-growing, glabrous species such as *T. verrucosum*, *T. violaceum*, and *T. schöenleinii*, as results may be variable or slow to develop (Weitzman and summerbell, 1995). BCP-milk solids-glucose agar may be used to differentiate a number of dermatophytes, particularly, *T. rubrum*, *T. mentagrophytes* (Fischer and Kane, 1971; Summerbell *et al*., 1988), *M. persicolor* (Kane and Summerbell, 1987), *M. equinum* (Kane *et* *al*., 1982), *T. soudanense*, and *T. megninii* (Kane and Fischer, 1979), on the basis of their differences in the release of ammonium ion from casein and the catabolite repression of this process by glucose. The most common use of this medium is to differentiate the constitutively ammonifying *T. mentagrophytes* from *T. rubrum*; in which ammonification is suppressed and radial growth is restricted by glucose for approximately the first 10 days at growth at 25^0C. With the former fungus, the BCP indicator in the medium turns from its original sky blue colour to violet within 4 to 7 days, indicating a pH change to alkaline, whereas with the latter fungus, the sky blue indicating neutral pH is maintained until after 10 to 14 days.

A confirmatory test for atypical isolates is the *in vitro* hair perforation test of Ajello and Georg (1957). This test relies on the development by certain dermatophytes of specialized perforating organs invading detached hairs and engendering conspicuous conical pits at right angles to the long axis of the hair. The most common use of this test is to differentiate atypical isolates of *T. mentagrophytes* (perforation positive) from atypical *T. rubrum* (negative), but it is also useful for many other determinations, including differentiation of atypical *M. canis* (positive) from *M. audouinii* and *M. equinum* (negative) (Padhye *et al*., 1980).

2.12Treatment of Dermatophytosis

The strong biological variability of the dermatophytoses has so far prevented the emergence of a single agent or regimen effective against all manifestations of these diseases. The relative cost of different therapies has also been an important factor in bringing about therapeutic diversity (Weitzman and Summerbell, 1995). It was suggested by the same author that antifungal treatment should be systematically recommended in order to shorten the course of infection and reducuction in dissemination of arthroconidia to other animals and into the environment. Systemic antifungal drugs are used to speed up the resolution of the infection whereas topical antifungals are required to reduce the risk of transmission and environmental contamination. Treatment recommendations stemmed from both *in vivo* and *in vitro* studies concerning dermatophytes species commonly found in animals (Watson *et al*., 1995; Evans and Sigurgiersson, 1999; Roberts *et al*., 2003).

Environmental disinfection and the complete separation of infected animals from non-infected ones is important. As far as possible, therapeutic measures should include the combination of systemic and topical treatment (www.cfsph.iastste.edu). Conventional systemic treatment relies on oral antifungal drugs, principally griseofulvin. Newer antifungal drugs may be valuable even if their cost is usually a limiting factor for their administration to large animals (Weitzman and Summerbell, 1995). The general recommendation is to control the animal by culture of hair or skin samples once a month during treatment and to stop antifungal administration after two negative cultures (Chermette *et al*., 2008). In dogs and cats, combined systemic and topical treatment should be maintained for at least 10 weeks (Chermette *et al*., 2008). In large animals, the cost of antifungal drugs may lead to reduction in the duration of treatment.

A misdiagnosis or the combination of a dermatophyte with another pathogen may also be suspected in cases of treatment failure. The cost of antifungal drugs and the reluctance of the breeders to admit that their colony is infected usually account for the noncompliance with treatment recommendations (Weitzman and Summerbell, 1995). Cattle breeders usually consider dermatophytosis as a benign infection that does not deserve specific and expensive treatment. They are not aware of the economic importance of the disease and that healed skin lesions reappear after the tanning process (Chermette *et al*., 2008).

2.12.1 Systemic antifungal treatments

Griseofulvin has been the gold standard for the systemic treatment of animal dermatophytosis. This drug has been successfully used in pet carnivores, cattle, sheep, goats, horses, rodents and rabbits (Hays and Clayton, 1982). In dogs and cats, the micronised formulation of griseofulvin is administered orally at 25 mg/ kg twice daily and with fatty meals. In large animals, a dosage of 7.5–10 mg/kg once daily is usually recommended (Fischer and Kane, 1971). However, this dosage has not been validated and the use of griseofulvin is now prohibited in European countries for food animals. Griseofulvin is contraindicated in pregnant animals as it is a teratogenic drug, particularly during the first weeks of gestation. Adverse reactions include vomiting, diarrhoea and anorexia (Chermette *et al*., 2008).

Azole derivatives have been proposed as an alternative therapy of animal dermatophytosis. Ketoconazole is the first orally administered azole derivative that has been used in veterinary medicine. A dosage of 2.5–5 mg/kg twice daily is usually recommended in the treatment of ringworm (Chermette *et al*., 2008). In most countries, the drug is labelled for use in dogs, but not in cats that are more susceptible to adverse effects of the drug. Adverse effects of ketoconazole include anorexia, vomiting and diarrhoea. A dose dependent elevation in liver enzymes may also occur and it was recommended to monitor these enzymes at a monthly interval during therapy (Chermette *et al*., 2008). Ketoconazole is contraindicated in pregnant animals. It interferes with steroid hormones synthesis and may decrease serum concentrations of testosterone and cortisol. Itraconazole is a relatively new systemic active azole derivative available for veterinary use (Chermette *et al*., 2008). The drug was proved to have a very broad spectrum of activity and is labelled for use in cats. The recommended dosage may vary according to the animal species and the fungal diseases. For feline dermatophytosis, the efficacy of a protocol based on pulse administration of 5 mg/kg/day for one week, every two weeks, has been demonstrated (Hay *et al*., 1983). With this protocol, the total treatment period is 6 weeks. In dogs and cats, itraconazole seems to be much better tolerated than ketoconazole and anorexia is the only problem that could be occasionally detected. Although itraconazole is not teratogenic at 10 mg/kg, embryotoxicity and teratogenicity were observed at very high dosages (160 mg/kg) in rats (Hebert *et al*., 1985) and the use of this drug in pregnant animals is not suggested.

To date, terbinafine is not labelled for use in veterinary medicine (Chermette *et al*., 2008). However, a few investigations demonstrated the efficacy of this allylamine drug in cases of dermatophytosis in dogs and cats (Hejimanek, 1992). The dosage of 20–30 mg/kg daily is usually suggested (Weitzman and Summerbell, 1995).

Lufenuron is a chitin synthesis inhibitor commonly used for the prevention of flea infestations in dogs and cats. Several investigations aim at demonstrating the antifungal activity of lufenuron in animals. A first retrospective study was conducted in Israel by Hejimanek and Lenhart, (1970) and suggested that lufenuron treatment was strongly associated with recovery in many dogs and cats with a number of fungal infections, including dermatophytosis. In a large investigation conducted in France (Hejimanek and Lenhert, 1972), cats from two infected catteries were treated by weekly rinses with enilconazole (0.2%) for 4 weeks and by oral lufenuron (60 mg/kg) twice at day 0 and day 30. Due to the high safety margin of lufenuron, its easy use and its convenience, lufenuron was proved to be a viable alternative to griseofulvin in the management of dermatophytosis due to *M. canis* in these catteries. However, eradication of *M. canis* was not obtained and further investigations performed in dogs, cats and horse gave contradictory results (Hejimanek *et al.*, 1992). As a consequence, increasing scepticism about efficacy of lufenuron rapidly occurred and the use of lufenuron is not recommended anymore for the treatment of dermatophytosis in animals.

Another form of systemic treatment may be immunization using an anti-dermatophyte vaccine. The cellular wall of dermatophytes is composed mainly of chitin, glucans and glycopeptides, which are the main antigens of these fungi (Wagner and Sohnle, 1995). The most important antigens are the proteic portion of glycopeptides that stimulate the Humoral

Immunity (HI) response, and keratinases, which produce a delayed hypersensitivity response when inoculated intradermally (Dahl, 1993).

The development of effective immunoprophylactics offers an interesting alternative in the control of this disease, once the protective status of antigenic extracts is proven. A great variety of veterinary vaccines effective against fungal disease have been marketed in different countries, in some cases for many years. The inactivated vaccines stimulate the Cell Mediated Immunity (CMI), as demonstrated by skin tests and leukocyte migration inhibition tests. Vaccines containing *T. verrucosum* conidia inactivated with formalin have been described for use in cattle (Wawrzkievicz and Wawrzkievicz, 1992). An inactivated vaccine plus adjuvant containing conidia and mycelium of two *T. equinum* strains has been used in the immunization of horses (Pier and Zancanella, 1993). The vaccine does not prevent the disease, but the lesions are less severe in vaccinated animals when compared to non-vaccinated animals.

The most widely used inactivated vaccine is Insol Dermatophyton1®, developed in Switzerland by Boeh- ringer Ingelheim (Chermette *et al*., 2008). The manufacturers indicate that it is effective in horse, dog and cat, and that it can be used as treatment of the disease, improving the clinical outcome. It contains strains of *T. verrucosum, T. mentagrophytes, T. sarkisovii, T. equinum, M. canis, M. canis var. distortum, M. canis var. obesum,* and *M. gypseum*.

Another commercial vaccine, Feo-O-Vax MC-K1® was developed by Fort Dodge in USA. It is an inactivated vaccine containing the mycelium of *M. canis* and an adjuvant. This vaccine produces anti-dermatophyte antibody titres similar to those developed in the course of the natural infection, with a low CMI. All vaccinated cats developed the disease after a topical application of *M. canis* conidia; however, the lesions were smaller than those in the control animals. The fact that all of the animals vaccinated had lesions suggests that high titres of antibody against *M. canis* may not be enough for protection against the infection (DeBoer and Moriello, 1995).

The inactivated vaccine Dermatovac-IV® has been developed in guinea pigs. It contains an adjuvant and an optically standardized inactivated suspension of conidia and mycelium of the fungi *M. canis, T. equimun, M. gypseum* and *T. mentagrophytes* (Pier *et al*., 1995).

Without doubt, the most effective and widely used have been the live vaccines. The Ringvac bovis LTF- 1301® vaccine, marketed by Alpharma, and elaborated with the LTF-130 strain of *T. verrucosum*, which has a characteristic high level of immunogenicity, low virulence and great stability, fundamental requirements of a strain used in a vaccine. It has been used effectively in Russia and Norway, where its effectiveness was demonstrated with experimental animals as well as in field studies. It is administered intramuscularly, and it contains a residual virulence able to stimulate the appropriate immune response, producing a delayed hypersensitivity reaction, which is considered essential for the removal of ringworm lesions (Rybnikar *et al.,* 1998). The live vaccine Permavax-Tricho1®, marketed in the Czech Republic by Bioveta Ivanovice, contains an attenuated strain of *T. verrucosum*. This vaccine triggers a protective immunity status 28 days after the second inoculation, preventing the appearance of the clinical disease for 1 year after vaccination (Rybnikar *et al*., 1998, Mignon *et al.,* 1999).

2.12.2 Topical antifungal treatments

Many products have been proposed for the topical treatment of animal dermatophytosis. Azole derivatives and a polyene (natamycine) are frequently used (Chermette *et al*., 2008). A solution of enilconazole at 0.2% is approved for use in dogs, cats, horses and cattle in most countries. Enilconazole, first described in 1969, can be given orally, but its poor solubility limits its use to topical applications on the skin (or the upper respiratory tract including nasal cavities and sinuses for treatment of other mycoses such as aspergillosis). Local or general side effects are very seldom reported, even in cats. A combination of 2% miconazole and 2% chlorhexidine administered topically twice weekly was proved to be efficient for the treatment of dermatophytosis in cats (Hejimanek *et al.,* 1992) and horses (Helender, 1978). Lime sulphur is commonly used in the USA, but is not available in most European countries (Chermette *et al*, 2008). This drug may be used in dogs and cats in combination with griseofulvin or itraconazole (Hernandez-Perez, 1984), but it has an offensive odour and may stain light-coloured hair. As oral ulcerations have been sometimes reported in cats, an Elizabethan collar must be used to prevent cats from licking the solution. Sodium hypochlorite solution has been used as topical treatment of ringworm in cats. However, this product is not recommended because it dries and irritates the skin and bleaches the hair coat (Chermette *et al.,* 2008). The decision to use topical therapy is based upon the owner's or breeder's ability and willingness to pour or sponge the product over the entire surface of the body of the infected animals, whatever the distribution of the lesions could be. Spot treatment of lesions is not recommended. The frequency of topical treatment should be at least twice a week. The clipping of the hair coat is sometimes useful, especially in severely infected animals, long-haired cats or dogs or in multi-animal households. Clipping makes topical therapy application easier and allows for better penetration of the drug (Chermette *et al*., 2008). However, clipping must be performed carefully not to wound the skin that would promote extension of lesions, and it may require sedating. Moreover, the unavoidable contamination of the material such as clipper, tables, etc. with infected hairs requires a meticulous disinfection after clipping. In cattle, crusts should be removed, and further destroyed, before the application of topical antifungal drugs (www.cfsph.iastste.edu).

Environmental disinfection can also prevent the spread of the disease (www.cfsph.iastste.edu). Infective material is easily spread and can remain viable in the environment for up to several years under optimal conditions of temperature and humidity

(Sparkes *et al*., 1994). Infected animals, with or without clinical signs, and contaminated environments represent a long term exposure to other animals and humans. Decontaminating the environment involves thorough cleaning and regular disinfectant application. Studies by Ishii *et al.* (1983) demonstrated that undiluted bleach and 1% formalin were able to kill all dermatophytes arthroconidia in the environment. However, because of toxic properties, both drugs are not recommended for use in households. Bleach at 1:10 dilution and enilconazole solution (0.2%) was also proved to be active (Chermette *et al*., 2008). All other disinfectants demonstrated poor efficacy. An enilconazole smoke fumigant formulation is available in most European countries. All grooming kit (brushes and combs), pet or horse equipment, cages and rooms that have been used for grooming and housing infected animals should be carefully washed and if possible, treated with a solution of enilconazole or 1:10 dilution of household bleach (Rycroft and Mclay, 1991)). Vehicles used for transporting the animals should be also treated (www.cfsph.iastste.edu; Chermette *et al*., 2008).

2.13 Antifungal Susceptibility Testing Methodologies

Antifungal susceptibility tests are performed on those fungi causing disease especially if they belong to a species exhibiting resistance to commonly used antifungal agents. It is also important in resistance surveillance, epidemiological studies and in comparison of the *in vitro* activity of new and existing agents (EUCAST, 2008).

A standard method for susceptibility testing of dermatophytes is lacking, but good results of Minimun inhibitory concentration (MIC) using either broth macrodilution or broth microdilution tests have been obtained in several reports (Pfaller *et al*., 1990; Venupogal *et al*., 1994; Moore *et al*., 2000; Rambali *et al*., 2001; Rodriguez- Tedula *et al*., 2003).

Dermatophytes grow slowly, and therefore agar-based methods have often been employed (Macura, 1993; Venupogal *et al*., 1994; Butty *et al*., 1996). Most studies, however, have focused on drug comparisons and employed methods that are not standardized or correlated with clinical outcomes (Pfaller *et al*., 2001).Thus, the clinical relevance of in vitro testing for the dermatophytes remains unclear. However, multiple efforts are under way to develop standardized methods for these fungi (Norris *et al*., 1999; Jessup *et al*., 2000; Hazen *et al*., 2000). Much of the initial work has focused on the selection of medium, with particular attention to choosing a medium that supports conidial formation in *Trichophyton rubrum*.

Although the disk diffusion method has been standardized for yeasts by the CLSI (M44 document) (Pfaller, 2008; Canton *et al*., 2009), there is no ne for dematophytes. The method employs Mueller- Hinton agar supplemented with glucose (2%) and methylene blue (0.5 μg/ml). Qualitative results can be obtained after 24-hour incubation, but some slow-growing isolates of *C. krusei*, *C. parapsilosis* and *C. glabrata* may require 48-hour incubation. CLSI M44-A2 document supports an established procedure validated only for *Candida* spp., and M44-S3 provides zone interpretive criteria for caspofungin, fluconazole, voriconazole and quality control ranges for caspofungin, fluconazole, posaconazole and voriconazole (www. CLSI.org). this method has been used succesfully for the susceptibility testing of dermatophytes by Nweze *et al*., 2007 and Agarwal *et al*., 2015.

2.14 Prevention

Contact with infected animals or contaminated environments represent the major risk of infection (Chermette *et al*., 2008). As a consequence, the best way to avoid infection is to prevent this contact. This prophylactic strategy is very simple, but not always feasible because infected animals do not systematically express obvious clinical signs. Asymptomatic carriers are frequently observed in some animal populations.

To protect animals, the preventive use of antifungal drugs has been proposed (Chermette *et al*., 2008), but this is not in line with best practices in the bid to prevent the occurance of resistance to antimicrobial agents. Oral antifungal drugs however, were not proven to be appropriate. Carefully controlled studies in humans demonstrated that oral griseofulvin had no prophylactic action (Chermette *et al.*, 2008). Investigations in times past showed that oral lufenuron may delay the initial establishment or progression of dermatophytoses in cats reflecting some inhibitory effect, but lufenuron did not prevent the infection (Ishzaki, 1993). Topical treatments are much more useful. The general recommendation is to apply an antifungal shampoo or rinse on the entire body of any animal that has been in contact with an infected animal or a contaminated area. Efforts in developing fungal vaccines to prevent dermatophytosis in different animal species have started more than forty years ago, (Chermette *et al*., 2008) and many antifungal vaccines are in use today.

2.15 Dermatophytosis in Africa

A survey of superficial skin mycoses was carried out among miners and office workers in Nigeria (Ayabimpe *et al*., 1995). Out of 434 persons examined, 117 had lesions suggesting a fungal infection, which was proven in 45 persons. *Malassezia furfur* was found in 25 cases, and different dermatophyte species were isolated: *T. soudanense* from six patients (5.1%), *T. rubrum* and *T. mentagrophytes* each from two patients (1.7%) and *M. audouinii* from one patient. Only three persons (0.7%) had clinical lesions on the feet. Children aged from 4 to 16 years (total number of 2,193) were also screened for dermatophytosis (Nweze, 2001) in Maiduguri, North easthern Nigeria. Tinea capitis was the predominant diagnosis followed by tinea corporis, and dermatophyte isolates were distributed as follows: *T. schöenleinii* 32 (28.1% of all the isolates), *T. verrucosum* 23 (20.1%), *T. mentagrophytes* 19 (16.7%), *T. tonsurans* 12 (10.5%), *T. yaoundei* 5 (4.4%), *M. gallinae* 21 (18.4%) and *M. gypseum* 2 (1.7%). In addition, there was a significant difference in the incidence of dermatophytosis amongst children from urban or rural areas (Nweze, 2001).

In another survey encompassing 5,780 children from 13 schools in western Kenya, the prevalence of dermatomycosis was 10.1% (Schmeller *et al*., 1997). The clinical forms were:

tinea capitis 74%, Kerion Celsi 1%, tinea faciei 7%, tinea corporis 6%, tinea pedis 2% and pityriasis versicolor (a yeast infection) 10%. *Microsporum audouinii* ranked the first among the causative agents of tinea capitis (40%), *T. violaceum* and *M. canis* representing 18 and 12% of the isolates, respectively, the remaining isolates being non dermatophytic moulds and bacteria (30%) (Schmeller *et al*., 1997).

Oyeka and Ugwu (2002) also examined specifically the fungal biota of toe webs in young Nigerian adults. Clinical evaluation showed that only 10 of the 100 volunteers showed some scaling, fissuring and peeling of the toe webs. Besides yeasts and nondermatophytic moulds, *M. gypseum was* recovered from 10 patients, *T. mentagrophytes* from seven and *T. rubrum* from two. The authors concluded that the isolation of dermatophytes from apparently healthy toe webs was significant and should not be regarded as merely transient organims of human toe webs.

A further study describes the spectrum of dermatophytes in Malawi, Africa (Pönnighaus *et al*., 1996). In the course of a total population survey carried out between 1987 and 1989, in Karonga District, northern Malawi, about 1.5–2.5% of the population was diagnosed as having tinea faciei, tinea corporis, tinea inguinalis or tinea cruris. The distribution of the isolated dermatophytes was as follows: *M. audouinii* 56.7%, *E. floccosum* 16.9%, *T. mentagrophytes* 18.1%, *T. violaceum* 3.6%, *T. rubrum* 1.2%, *T. tonsurans* 1.2%, *T. terrestre* 1.2%, *and M. persicolor* 1.2%. A majority (53.4%) of the *M. audouinii* strains isolated were from children aged less than 9 years.

The epidemiology of tinea pedis and onychomycosis was also investigated in 1,300 patients in a hospital in Algiers, Algeria (Djeridani *et al*., 2006). Each patient received a complete dermatological examination and, for patients presenting signs of tinea pedis and/or onychomycosis, skin of the feet and toenails were sampled for mycological examination. Clinical signs compatible with tinea pedis and onychomycosis were seen in 249 (19%) and 72

(5%) patients, respectively. Tinea pedis was confirmed in 197 (15%) cases and onychomycosis in 60 (4.6%). Regarding the etiological agents of tinea pedis, the most common dermatophyte was *T. rubrum* (17%), followed by *T. interdigitale* (13%), *T. violaceum* (6%), *T. mentagrophytes* (2.4%) and *E. floccosum* (2.4%), while 59.2% of fungal isolates were yeasts (54%) and non-dermatophytic filamentous fungi (5.2%). For onychomycosis, there was also a clear predominance of *T. rubrum* 35%, followed by *T. violaceum* 8.3%, *T. interdigitale* 6.7% and *T. mentagrophytes* 5%. Yeasts, primarily *Candida parapsilosi*s (28.3%) and *Candida albicans* (6.7%), constituted the remaining isolates.

In various studies carried out in west Africa, *T. soudanense, M. audounii, T. rubrum, T. mentagrophytes, T. tonsurans, T. violaceum*, play the dominant role in human dermatophytoses (Nweze, 2001; Ellabib *et al*., 2002; Menan *et al*., 2002; Adou-Bryn *et al*., 2004; Adeleke *et al*., 2008). In Nigeria, Efuntoye and Fashanu (2002) isolated *M. gallinae* and *M. gypseum* from both birds and poultry houses in Oyo state. In a study carried out by Nweze (2011) in seven states in Nigeria, (Enugu, Anambra, Ebonyi, Abia, Imo, Kogi and Delta.) in different domestic animals, a large spectrum of dermatophytes consisting of 10 species was recovered in the study. They were mostly zoophilic species and include *M. canis, T. mentagrophytes, T. verrusosum, M. gypseum, M. gallinea, T. equinium, M. nanum, M. equinium, M. persicolor* and *T. gallinae*. Out of these, *M. canis* was the most predominant species consisting of 37.4% of all positive samples.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study area is Kaduna State which occupies an area of approximately 48,483.2km^2 in the northern guinea savannah zone (ABU, 2000). It has an annual rainfall of 1000 to 1250mm and temperature ranges of 17^{0}C and 33^{0}C. The state lies between longitude 30^{0}E and latitude11^{0}3^{0}N (www.kadunastate.gov.ng, 2011). There are two marked seasons in the state; dry windy season (Harmattan) and the wet rainy season (ABU, 2000)

The state has a predominant Hausa/Fulani population in the Kaduna North and Kaduna central senatorial district. The main livelihood of its people is agriculture. (ABU, 2000) and this includes both cash and food crops and animal husbandry. Horses are among the animals produced, although mostly used by the elite for polo games, horse racing and processions during festivities.

3.2 Sampling Method

Purposive sampling method was used. Samples were collected based on the availability of horses (with clinically suggestive lesions) from the four local governments where horses could be found. Sampling was from March to September 2014 from farms, homes, stables and polo clubs in Igabi, Kaduna North, Sabon Gari and Zaria local Government areas of the state.

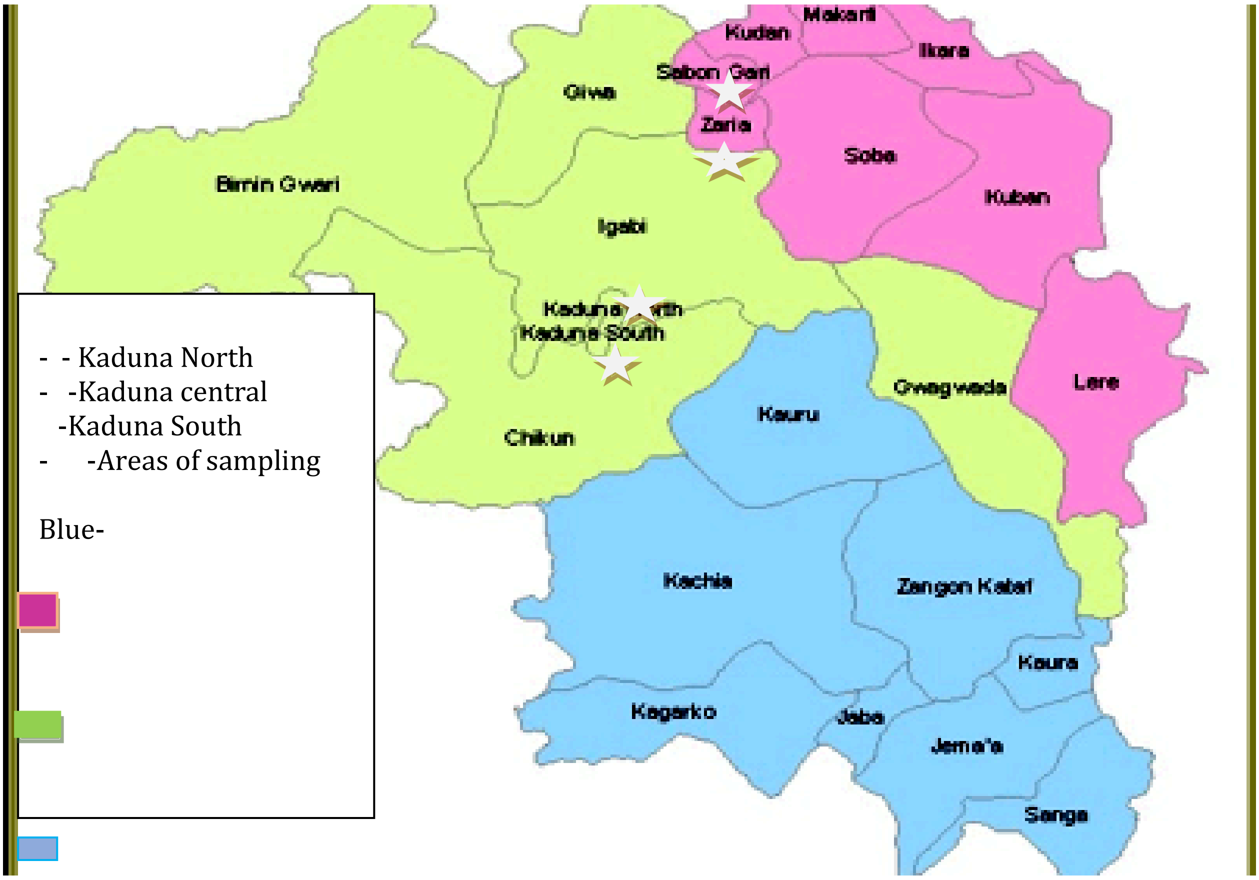

Figure1: Map of Kaduna State showing areas of sampling.

3.3 Sample Size

Samples from 102 horses having clinical signs suggestive of dermatophytoses were collected from March to September 2014. Biodata of the horses was recorded and photographs of lesions taken, the sites of the lesions on the horses was also recorded.

3.4 Sample Collection and Processing

Skin scrapings as well as hair samples were collected from the margins of the suspected lesions of dermatophytosis, according to the method of Elewski, (1995). Also sterile toothbrush was used to brush horses with small skin lesions and impressed on the surface of the culture medium. Samples were placed in coloured envelops in separate polyethene bags and transported as dry packet to the Microbiology laboratory of the Department of Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University.

3.5 Laboratory Examination of Samples

3.5.1 Direct examination

A small portion of each scrapping was digested by placing on a microscope slide and one to two drops of 10% potassium hydroxide added. A cover slipwas placed on top and the slide gently heated over a flame as described by Hainer, (2003). Each treated slide was carefully examined under low objective (×10) and high (×40) power objectives to observe for fungal elements.

3.5.2 Culture and isolation of dermatophytes

Sabouraud's dextrose agar (Oxiod, U.K.) with chloramphenicol 40mg/l (Fluca, U.K), cyclohexamide 500mg/l (Sigma, Germany) and nicotinic acid (100µg/ml) a selective media was used for primary isolation (Sharma and Sharma, 2011), using agar plates. A set of SDA (vitamin free) was seeded concurrently. Seeding was carried out with a portion of the scabs and hair collected incubated at room temperature for one to four weeks and checked for growth every other day.

3.5.3 Identification of isolates

Potato dextrose agar (Oxoid, U.K) plates were used. Growth from Pure SDA cultures were sub cultured on the PDA (Raymond and Piphet, 2008) and 5% NaCl SDA (Issa and Zangana, 2009) and incubated at room temperature for one to four weeks. Colony and microscopic characteristics were identified using the Fungal colour Atlas (Frey *et al.*, 1979; Evans and Richard; 1989; Baron *et al.*, 2003; Ellis *et al*., 2007). Lactophenol cotton Blue stain was used in the preparation of slides for microscopy (Bernado *et al*., 2005).

3.5.4. Slide culture preparations

Dermatophytes identification that were inconclusive due to lack of sporulation was subjected to slide cultures.This was essential to observe the precise arrangement of the conidiophores and the way in which spores were produced (conidial ontogeny). A simple modification of Riddel's method of slide culturing (1950) as described below was used.

Potato dextrose agar was used. In glass petri dish, cotton wool, two small sticks were arranged and a clean glass slide and cover slip were placed on them and sterilized in a hot air oven. The cotton wool was moistened with sterile water. Using a sterile blade agar blocks (1 x 1cm) were cut.The agar blocks were flipped up onto the surface of sterile glass slide and the four sides of the agar block were inoculated with spores or mycelia fragments of the fungus. Cover slips were placed centrally upon the agar blocks leaving space between the agar and the edge of the cover slip. The plates were covered and incubated at room temperature until growth and sporulation occurred. The cover slip were removed from the agar block and gently lowered onto a small drop of Lactophenol cotton blue on a clean glass slide and observed for conidia.

This technique is based on the fact that when a dermatophyte grows in the 1cm by 1cm agar provided even unto the glass slide cover, it meets an empty space. Fearing starvation, it starts to produce spores for survival. These spores are usually diagnostic for each species and can then be recorded.

3.5.5 Antifungal susceptibility test (broth microdilution method)

This was performed according to the method discribed by Nweze *et al*., 2007 and Araujo *et al*., 2009.

Antifungal agents

Five antifungal agents Fluconazole(Fl)-50mg (Pfizer), Ketoconazole(KE)-100mg (Oxoid,UK), Terbinafine(TER)-100mg (Novartis), Griseofulvin(GRI)- 5g, (Oxiod, UK) and Amphotericin B(AMP)-50mg (Neon Laboratories) were tested against the isolated dermatophytes. Amphotericin B, Terbinafine, and Griseofulvin were dissolved in Dimethyl sulfoxide (DMSO) (Oxoid) while Fluconazole and Ketoconazole were dissolved in sterile distilled water into working solutions of 256µg/ml (FLU), 64µg/ml (KE, TER and AMP) 32µg/ml (GRI).

Preparation of inocula

The isolates were subcultured on PDA slants and incubated at room temperature for four days. The colonies were covered with 5ml of sterile distilled water supplemented with 0.1% tween 20 as a wetting agent. The conidia were dislodged with a sterile loop and the suspension collected with a syringe into a sterile tube. The suspension was shaken to homogenize and left to settle for 15 to 20 minutes. The upper layer of the suspension was removed and adjusted to 0.5 Macfarland standards with sterile distilled water.

Susceptibility test procedure

Test was performed using plastic disposable microdilution plates in duplicates. To wells one to 12, 100µl of RPMI 1640 medium was dispensed. 100µl of drug solution was added to well 1 of each plate and a 2 fold serial dilution was carried out to well 11. 100µl of the leftover from the last well discarded. 100µl of the inoculum was then dispensed into well one to 10, with well 11 been the media control and well 12 the solvent control. The plates were incubated at room temperature and MIC read after 48 hours (Nweze, 2007).

Endpoint determination

End point determination was done according to the class of drug. Growth was compared to that of drug-free control and scored by visual inspection (Araujo *et al.*, 2009). The MIC was reported as the concentration in which 50% of the growth was inhibited for K, FL, TER, GRI, and complete growth inhibition was observed for AMB.

3.6. Data Presentation and Analysis

The results obtained were described using percentages and presented in tables, charts and pictures. Chi-square and Fischer's exact test were also employed where applicable.

CHAPTER FOUR

RESULTS

4.1: Distribution of Dermatophyte Isolates Based on Local Government Areas

Out of the 102 horses with skin lesions that were sampled in from different parts of Kaduna State, dermatophytes were isolated from 18 (17.6%) (Table4.1). Zaria LGA had the highest isolation rate of 35% (14 from 40), followed by Sabon Gari LGA, 10% (1 from 10), Igabi LGA, 5.9% (2 from 34) and Kaduna North 5.6% (1 from18). There was a significance association (P=0.0033) between the dermatophytes isolation rate and the Local Government Areas where samples were collected.

TABLE 4.1: Isolation Rate of Dermatophyte From Horses With Skin Lesion Sampled Between March And September, 2014 In Kaduna State.

LGAs	No. of samples collected	No. of samples positive for dermatophytes (%)
Zaria	40	14 (35)
Sabon Gari	10	1 (10)
Igabi	34	2(5.9)
Kaduna North	18	1(5.6)
Total	102	18(17.6)

(P=0.0033)

4.2: Occurrence of Dermatophytes in Horses with Skin Lesions

Out of the 18 dermatophyte isolates, nine (50%) were *Microsporum* species and nine (50%) were *Trichophyton species*. *Trichophyton verrucosum*, *T. vanbreuseghemii, T. mentagrophytes*, *T. soudanense* and *T. equinum* were the Trichophyton species isolated while *M. equinum*, *M. canis, M. gypseum, M. gallinea* and *M. fulvum* were the *Microsporum species* isolated. *Trichophyton verrucosum* was seen to be the most commonly occurring dermatophytes with a frequency of 4(22.22%), followed closely by *Microsporum equinum,* 3(16.67%) (Table 4.2).

TABLE 4.2: Distribution by Species of Dermatophytes Isolated from Horses in Kaduna State

Species Isolated	**Number**	**Percentage (%)**
T. verrucosum	4	22.22
M. equinum	3	16.67
M. gypseum	2	11.11
M. canis	2	11.11
T. vanbreuseghemii	2	11.11
M. gallinae	1	5.56
M. fulvum	1	5.56
T. mentagrophytes	1	5.56
T. soudanense	1	5.56
T. equinum	1	5.56
Total	18	100

4.3: Anatomical Distribution of Dermatophyte Isolates

Most dermatophytes lesions were found on the saddle area of the horses with 5(33.33%) dermatophytes isolation from 15 samples collected from the region, followed by the flanks of the horses with 3(12%) from 25 samples. The least was on the head with 1(8.3%) from 12 samples (Table 4.3, 4.4). Microsporum species caused lesions on the horses that were mostly areas of dry, flaky to crusty alopecia. Horses that were infected with Trichophyton species of dermatophytes had skin lesions that ranged from generalized areas of alopecia with extensive papular reaction (*T. vanbreuseghemii*, platexxxix, *T. soudanense*, plate xxx), to areas of descrete kerions (*T. verrucosum*, Plate xxvii). Lesion caused by *T. mentagrophytes* was observed to be a descrete area of crusty alopecia (plate xxxiv).

On SDA plates, both Trichophyton and Microsporum species colonies were observed to be pigmented from white (*T. vanbreuseghemmii*, plate xl), cream (*T. mentagrophytes*, plate xxxv), buff (*M. canis*, Plate xx) and pink (*M. gallinae*, plate xxiv) on the obverse and also on the reverse.

Table 4.3: Distribution of Dermatophytes Based on Anatomical Site of Lesion

Anatomical site of lesion	No. of samples	No. of samples positive for dermatophytes (%)*
Saddle	15	5(33.3)
Limbs	20	5((25)
Generalized	13	3(23.1)
Rump	5	1(20)
Flank and Girth	25	3(12)
Head	12	1 (8.3)
Neck	7	0 (0)
Whithers	5	0 (0)
Total	102	18

*percentage isolation of dermatophytes from different anatomical regions of horses

Table 4.4: Anatomical Locations of Different Dermatophyte Isolates

Sample	**Anatomical site of lesion**	**Species isolated**
11z	Limbs	*T. verrucosum*
26z	Limbs	*T. verrucosum*
29z	Rump	*T. verrucosum*
30z	Limbs	*T. verrucosum*
16z	Saddle area	*M. equinum*
27z	Flank and girth area	*M. equinum*
19z	Saddle area	*T. equinum*
4z	Saddle area	*M. gypseum*
22z	Limbs	*M. gypseum*
12z	Head	*M. fulvum*
23z	Limbs	*T. mentagrophytes*
9z	Generalized	*T. vanbreuseghemii*
13z	Generalized	*T. vanbreuseghemii*
21z	Flank and girth area	*T. soudanense*
30i	Flank and girth area	*M. gallinae*
33i	generalized	*M. canis*
5kn	Saddle area	*M. equinum*

5s	Saddle area	*M. canis*

PLATE I: kerion formation on the ventrum of an infected horse (a) falling off to leave alopecia (b)

PLATE II: white colony downy colony of *M. equinum* (17days) isolated from lesion on the flank of a horse (from Plate I)

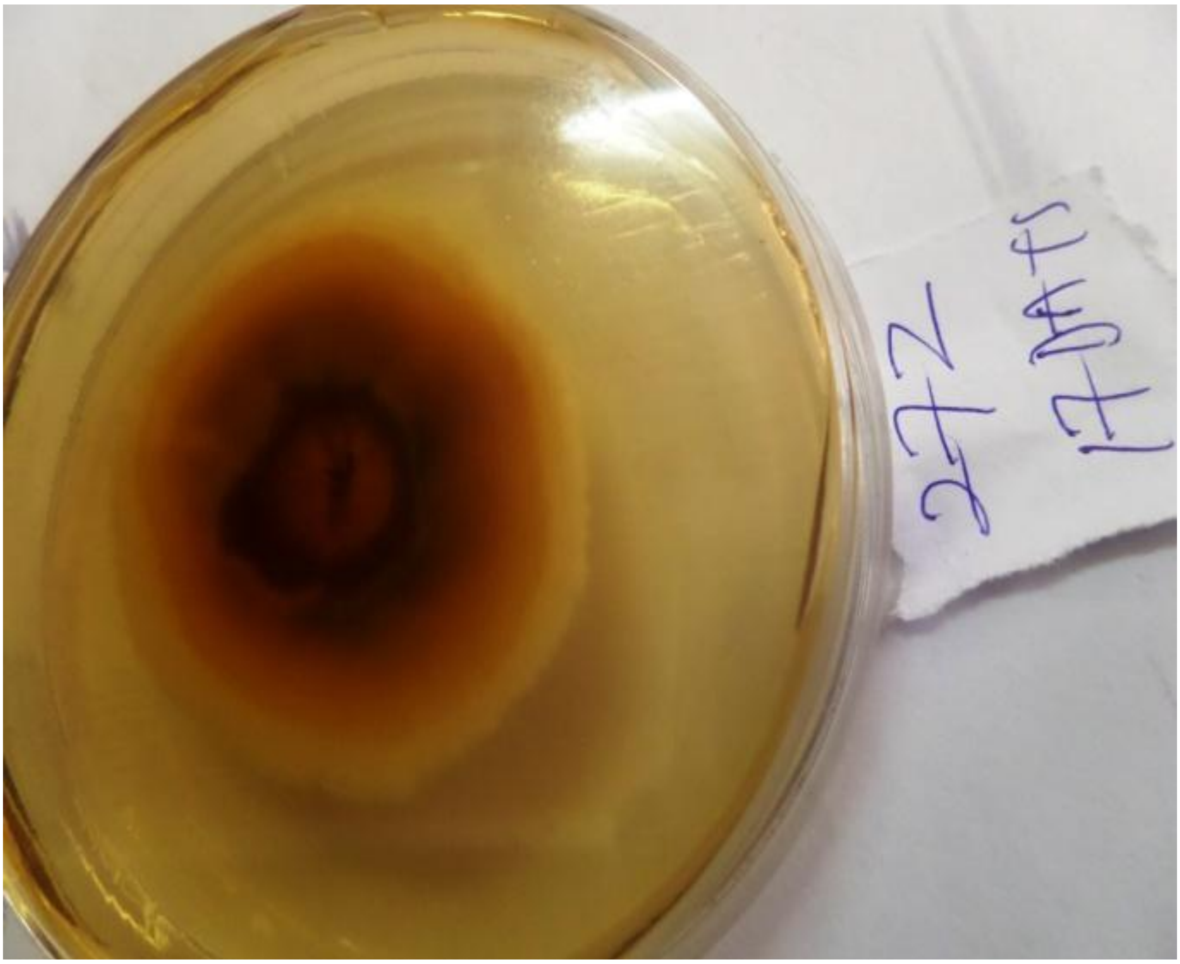

PLATE III: Reverse of colony of *M. equinum* in plate II. Note: Reddish –brown colour of reverse with a darker brown center.

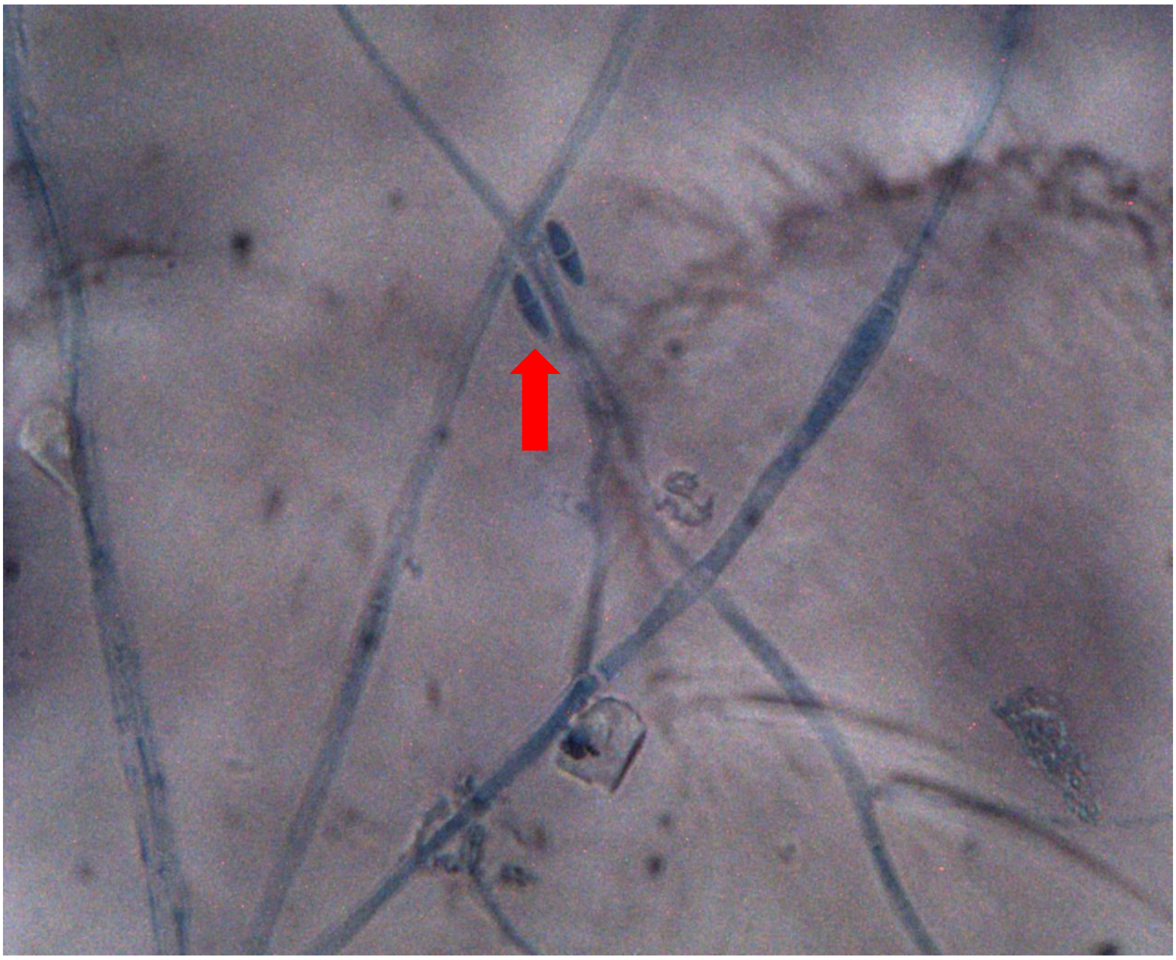

PLATE IV: *Microsporum equinum,* Note: spindle-shaped 2 celled thick-walled macroconidia (arrow). (LCB 400x)


PLATE V: Flaky area of alopecia on the back of a horse. Note the area of central healing (arrow).

PLATE VI: Colony (obverse) of T. *equinum* in vitamin free agar (17 days post inoculation).

Note the rudimentary growth showing a requirement for nicotinic acid.

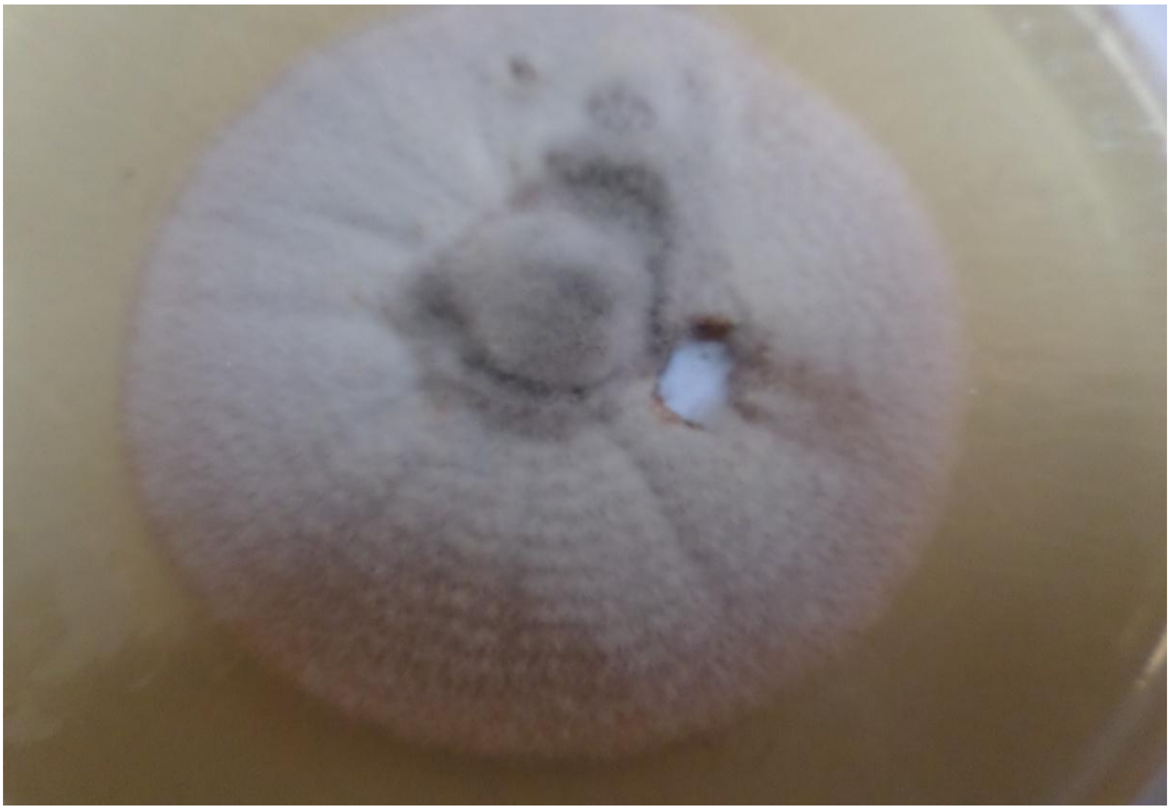

PLATE VII: colony (obverse) of *T. equinum* (17days) on nicotinic acid enriched SDA. Compare with plate VI. Note the spreading white colony with a central umbo and fringed edge.

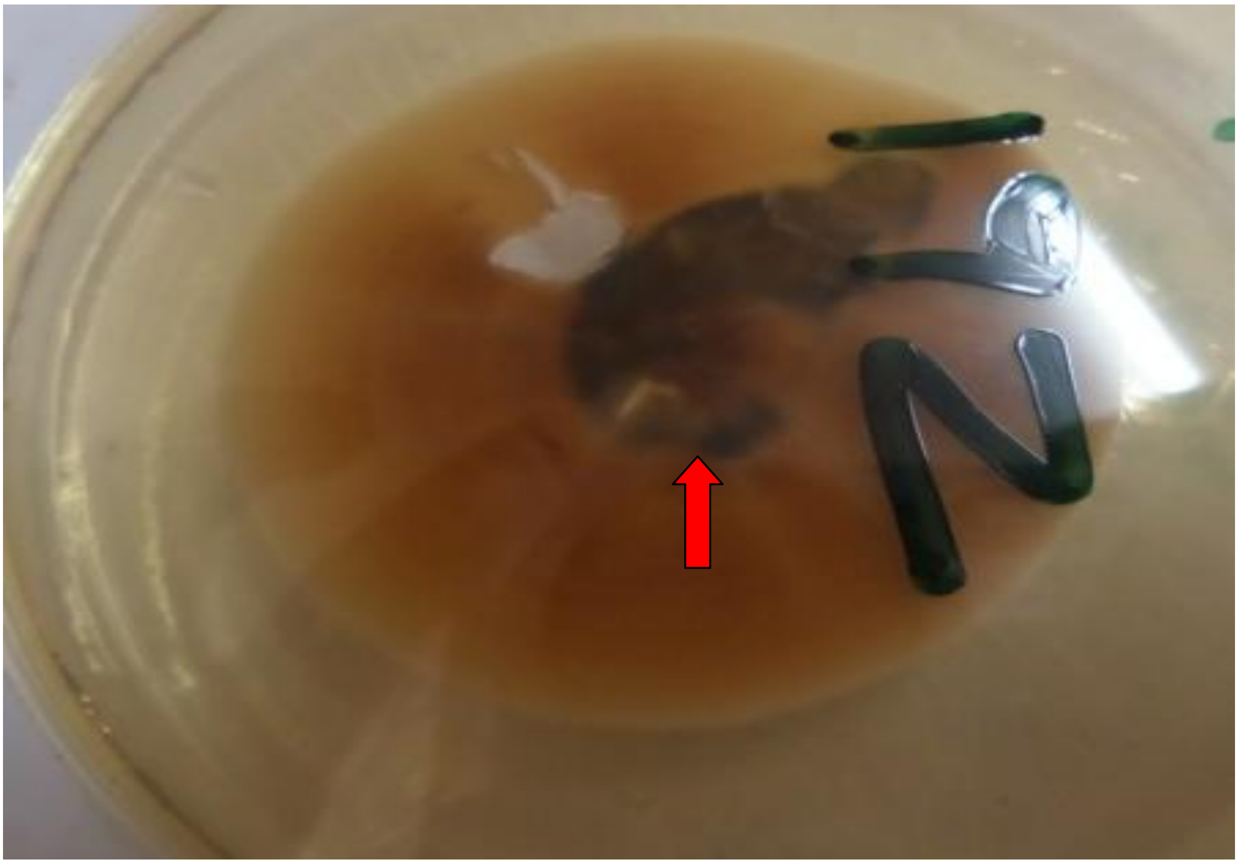

PLATE VIII: Reddish-brown reverse colony *T. equinum* with central reddish pigmentation (arrow).

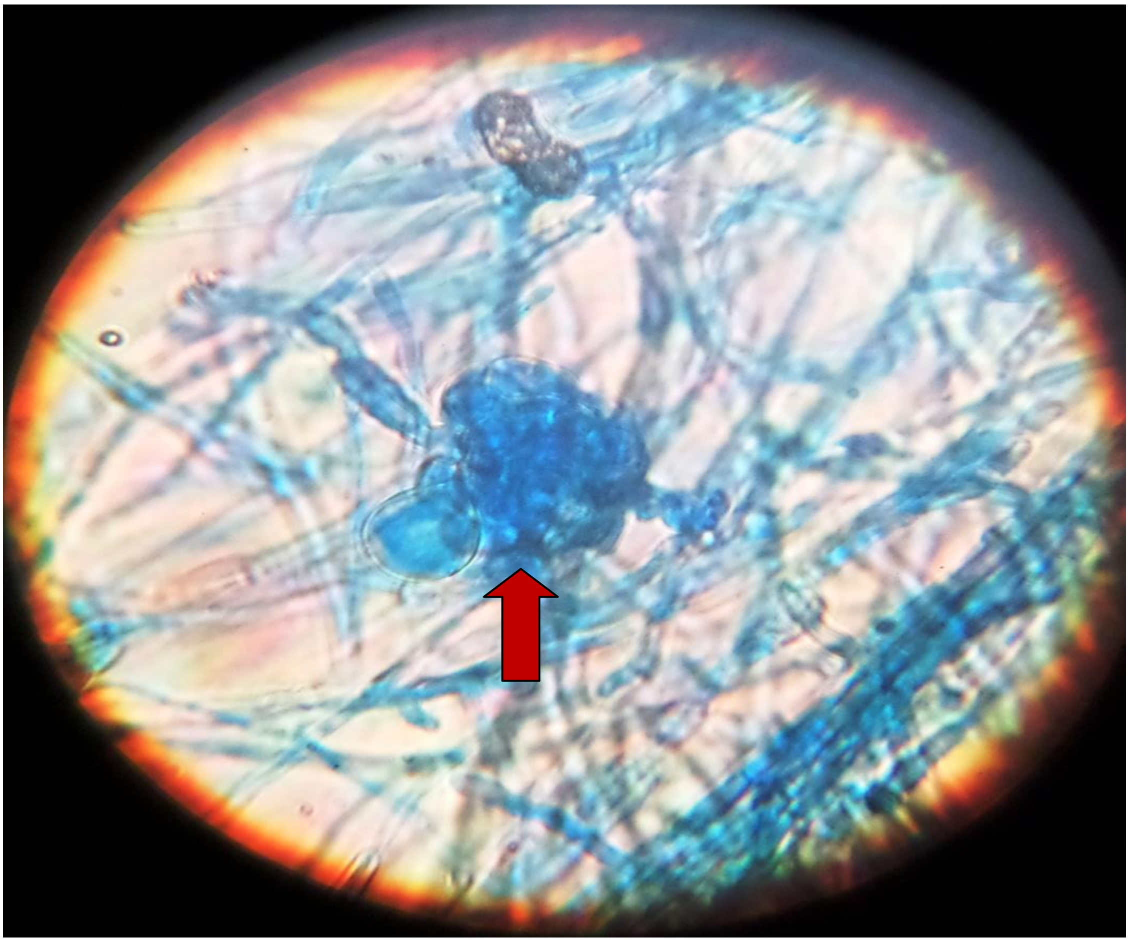

PLATE IX: Microscopy of *T. equinum* showing a nodular body (arrow) (LCB stain400×)

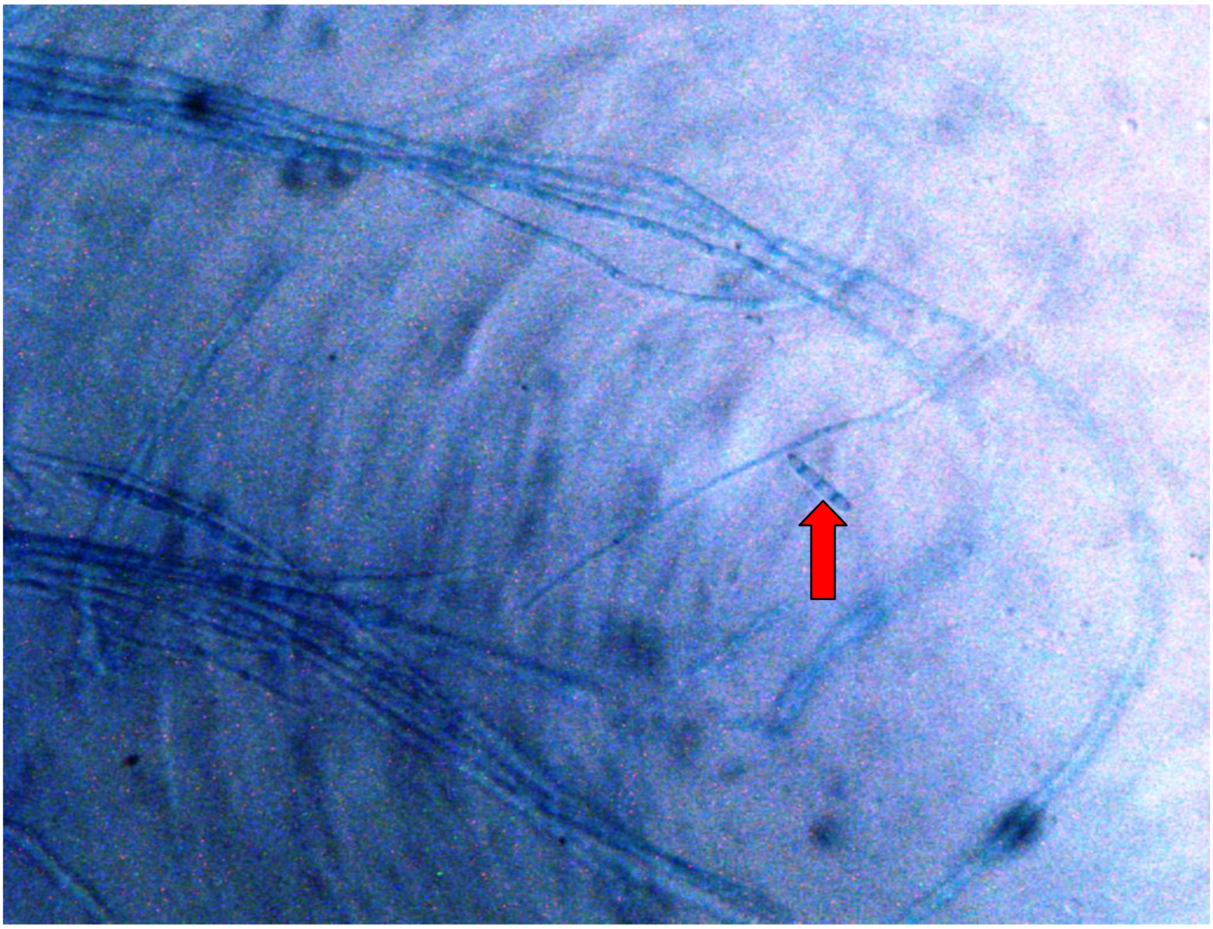

PLATE X: Small spindle shaped macroconidia (arrow) of *T. equinum* having four cells.


PLATE XI: Small circumscribed area of crusty alopecia on the back of a horse.

PLATE XII: Whitish colony of *M. gypseum,* note the central umbo (arrow) with radial grooves from the back of a horse (Plate XI).

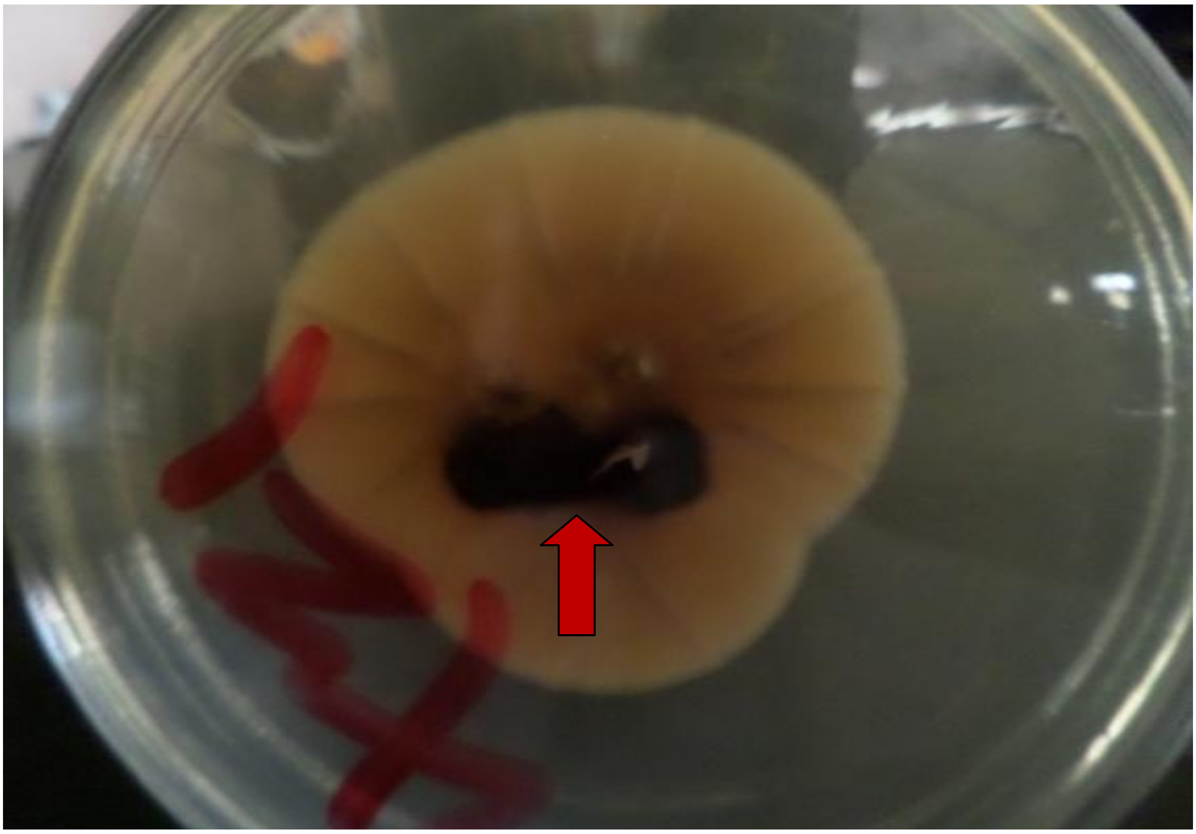

PLATE XIII: Reverse of colony of *M. gypseum* (Plate XII). Note: Tan reverse with a central darker brown spot (arrow).

PLATE XIV: Microscopy of *M. gypseum* (from sample of horse on plate XI). Note: numerous 4-6 celled thick walled macroconidia (arrows) (LCB stain 400×)

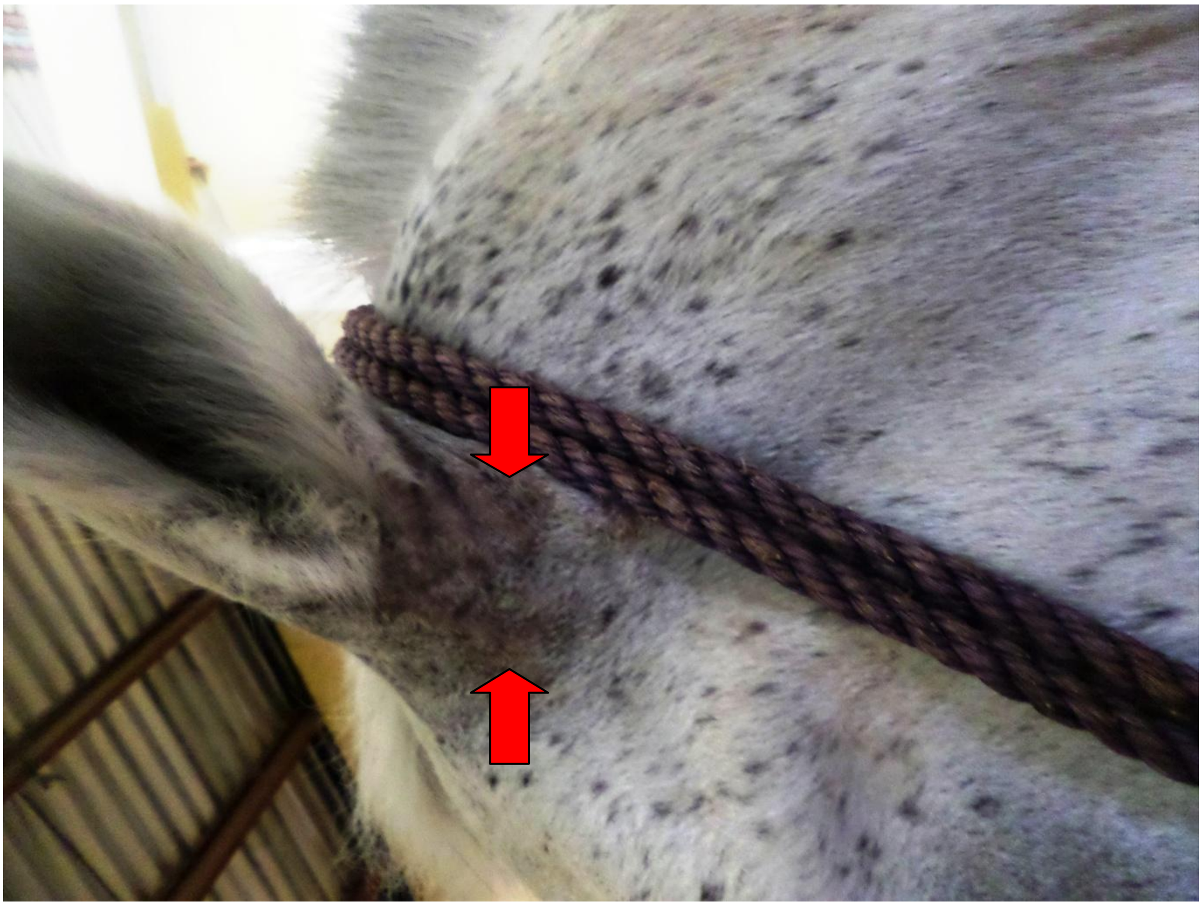

PLATE XV: Dermatophye lesion at the base of the ear of a foal (arrows).

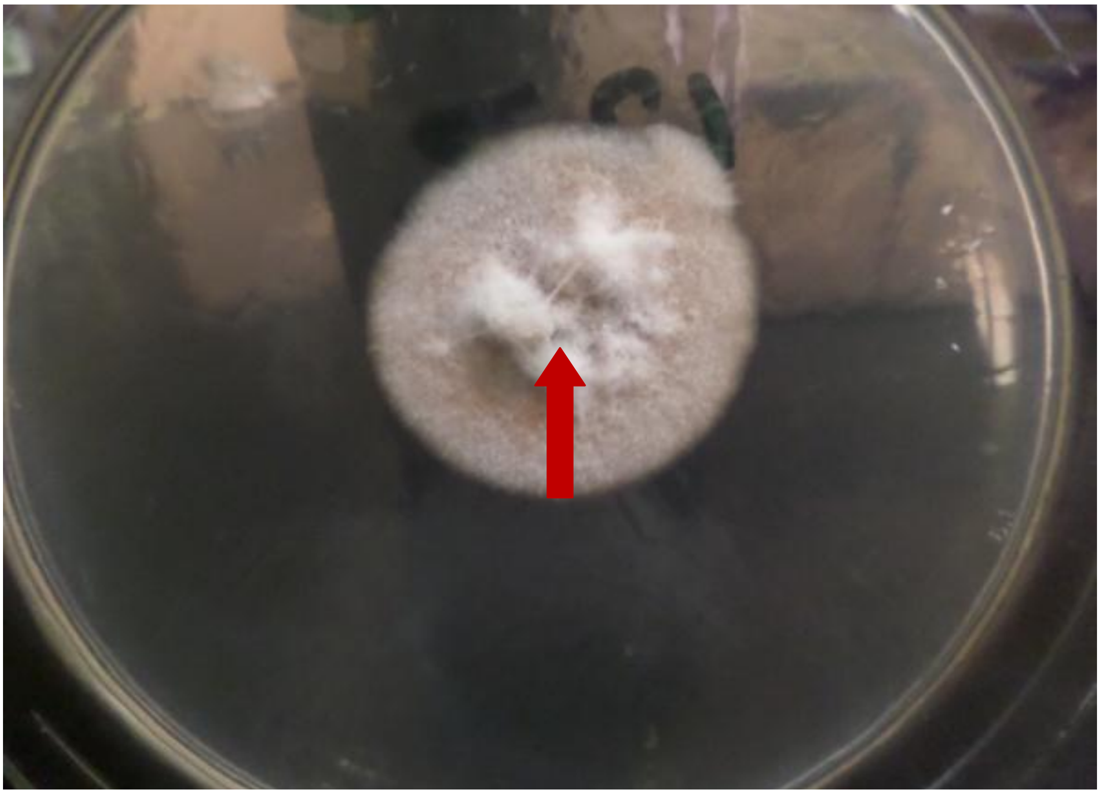

PLATE XVI: Colony (obverse) of *M. fulvum* (5days). Note: Pinkish-buff pigmentation with central white thuft of mycelia (arrow).

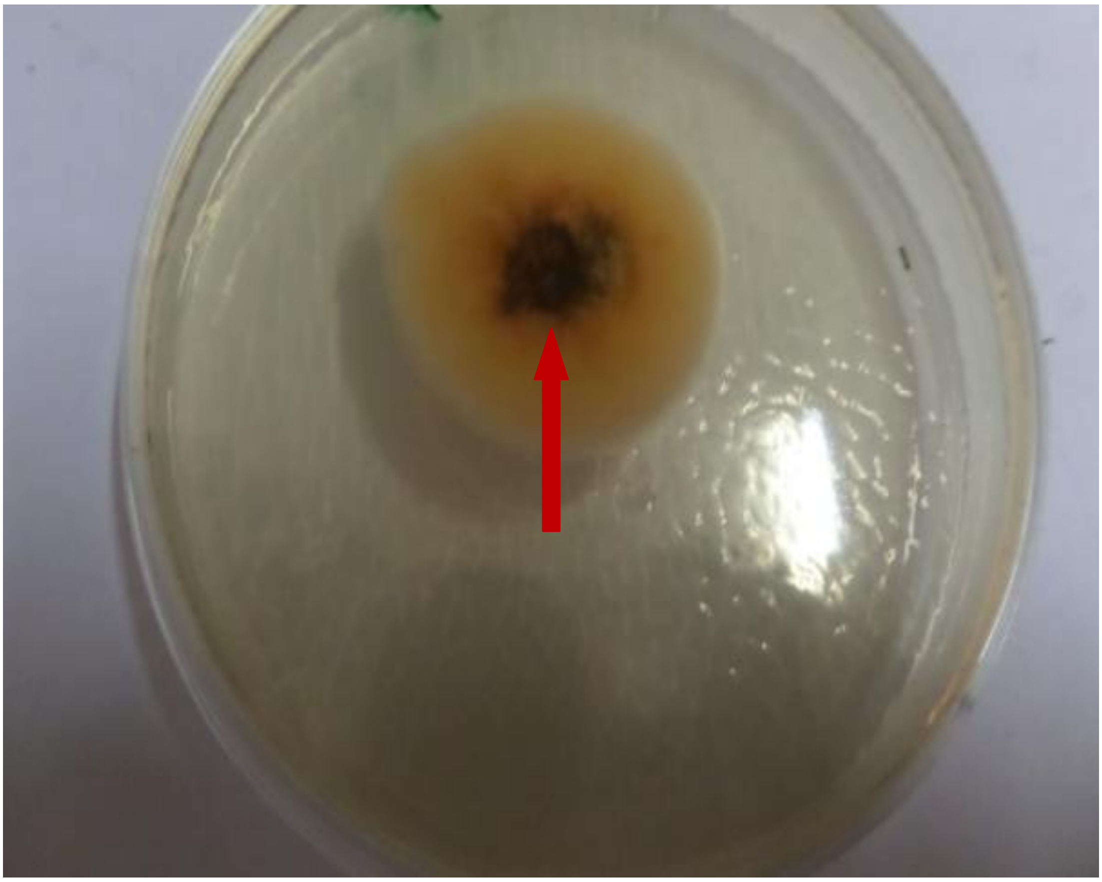

PLATE XVII: Yellow-brown reverse colony of *M. fulvum* with central darker brown pigmentation

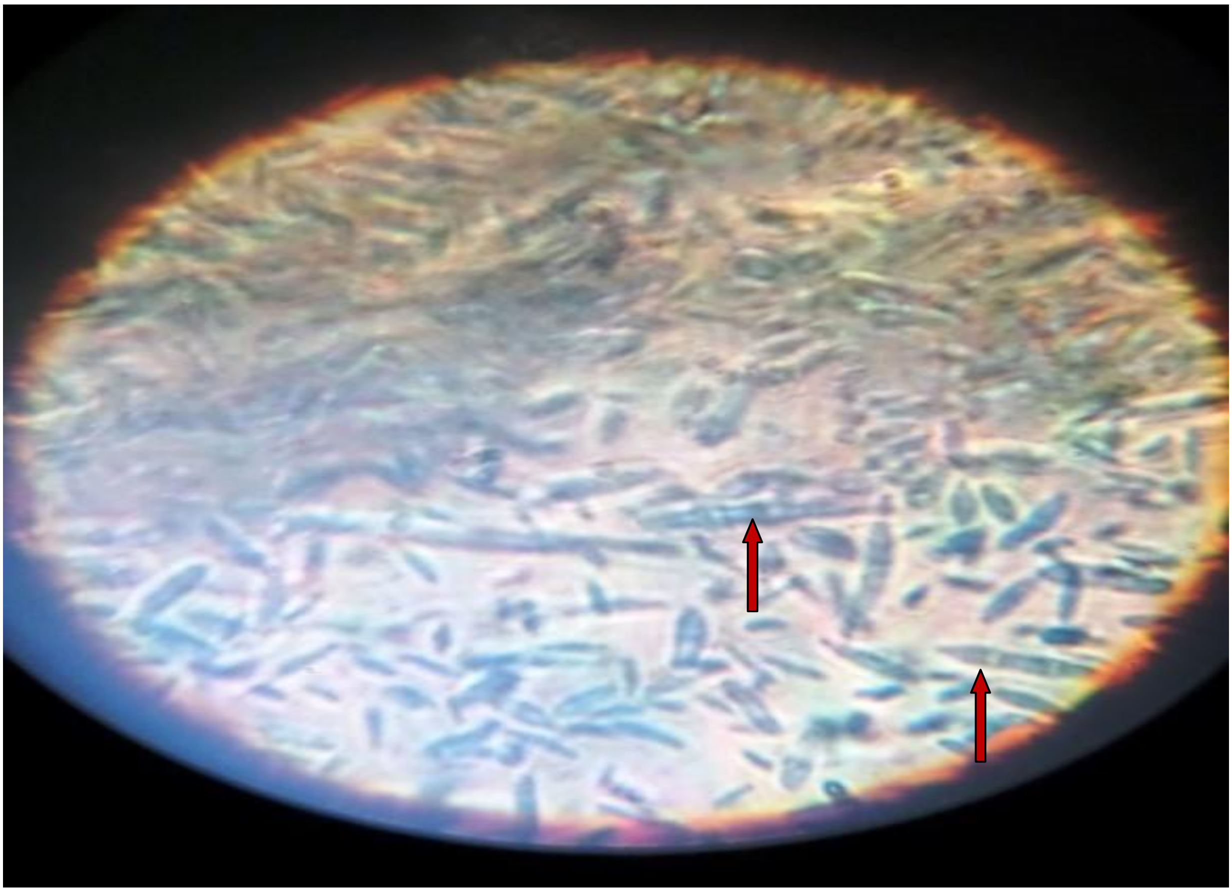

PLATE XVIII: Numerous slender 3-6 celled Macroaleurospores of *M. fulvum* (from Plate XVI) with tapered ends. (LCB stain 400×)

PLATE XIX: Circumscribed areas of scaling (a) and alopecia (b) on the limb of a horse.


PLATE XX: Whitish- buff coloured colony of *M. canis* (8 days) from the horse on Plate xix Note the granular surface and the fringed edge.

PLATE XXI: Reverse of colony of *M. canis* (Plate xx)

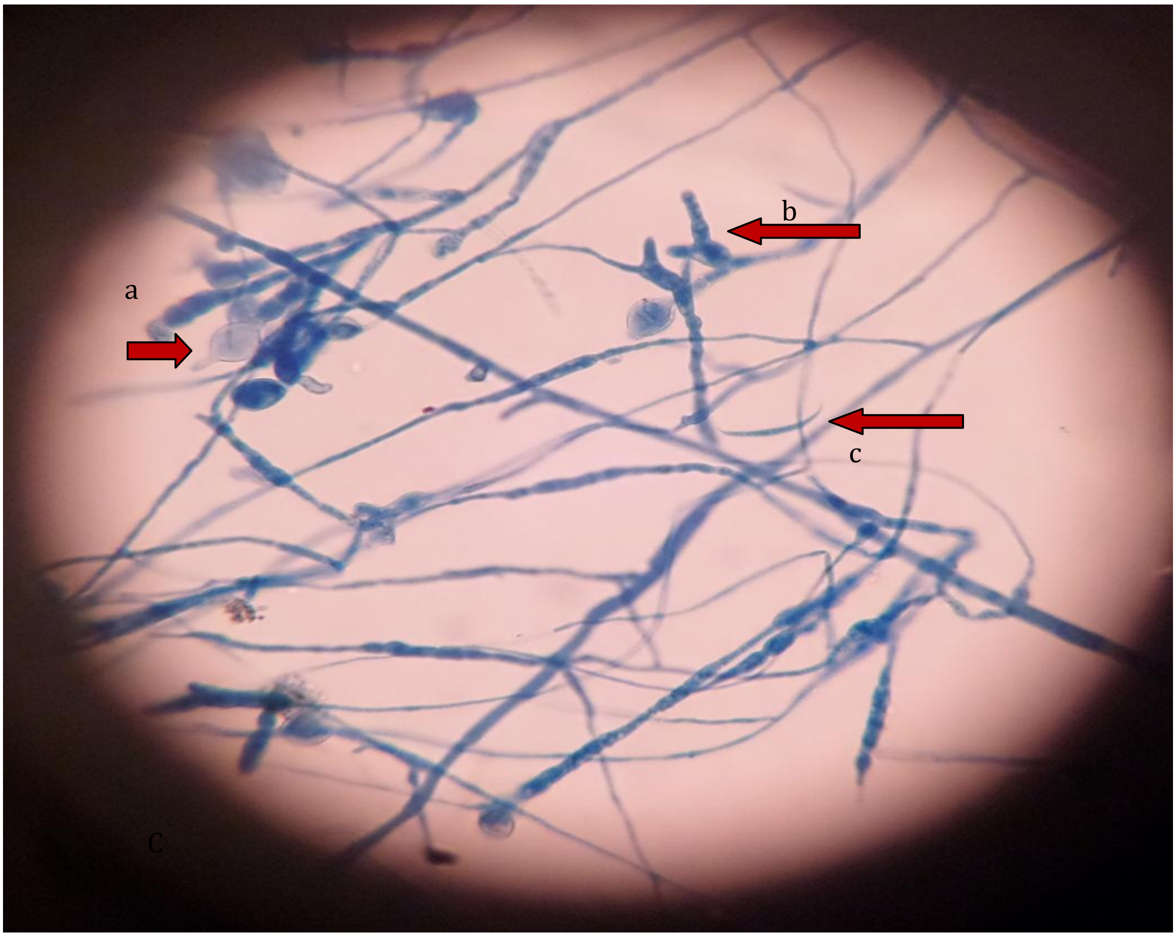

PLATE XXII: Microscopy of *M. canis* (LCB stain 400×)

a. Terminal chlamydospores of *M. canis*
b. Distorted macroconidia of *M. canis*
c. spindle shaped macroconidia with curved ends


PLATE XXIII: Descrete area of alopecia on the flank of a horse.


PLATE XXIV: Fringed, Pink-tinged, white to cream colony of *M.gallinae* isolated from horse on plate xxiii.

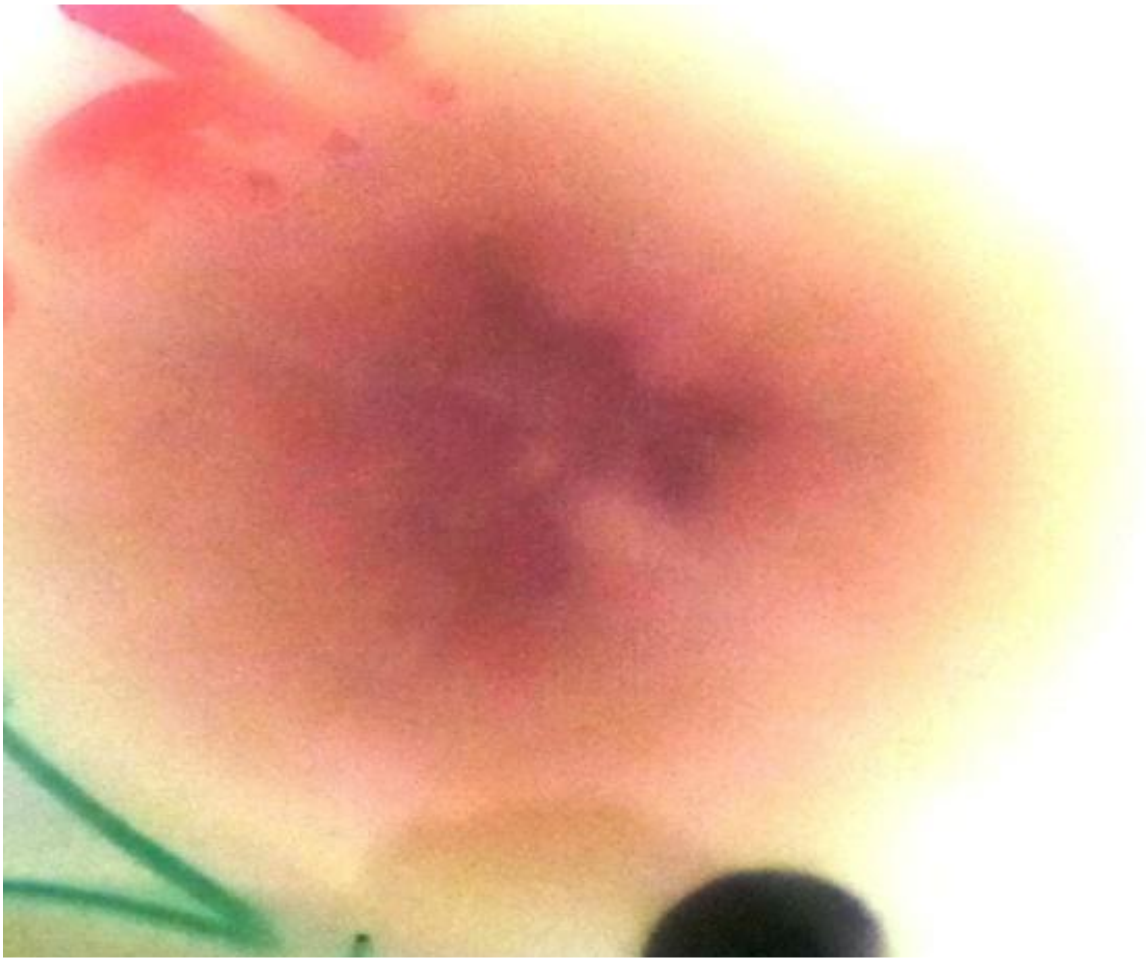

PLATE XXV: Diffusing Strawberry-pink reverse of colony (from plate xxiv)

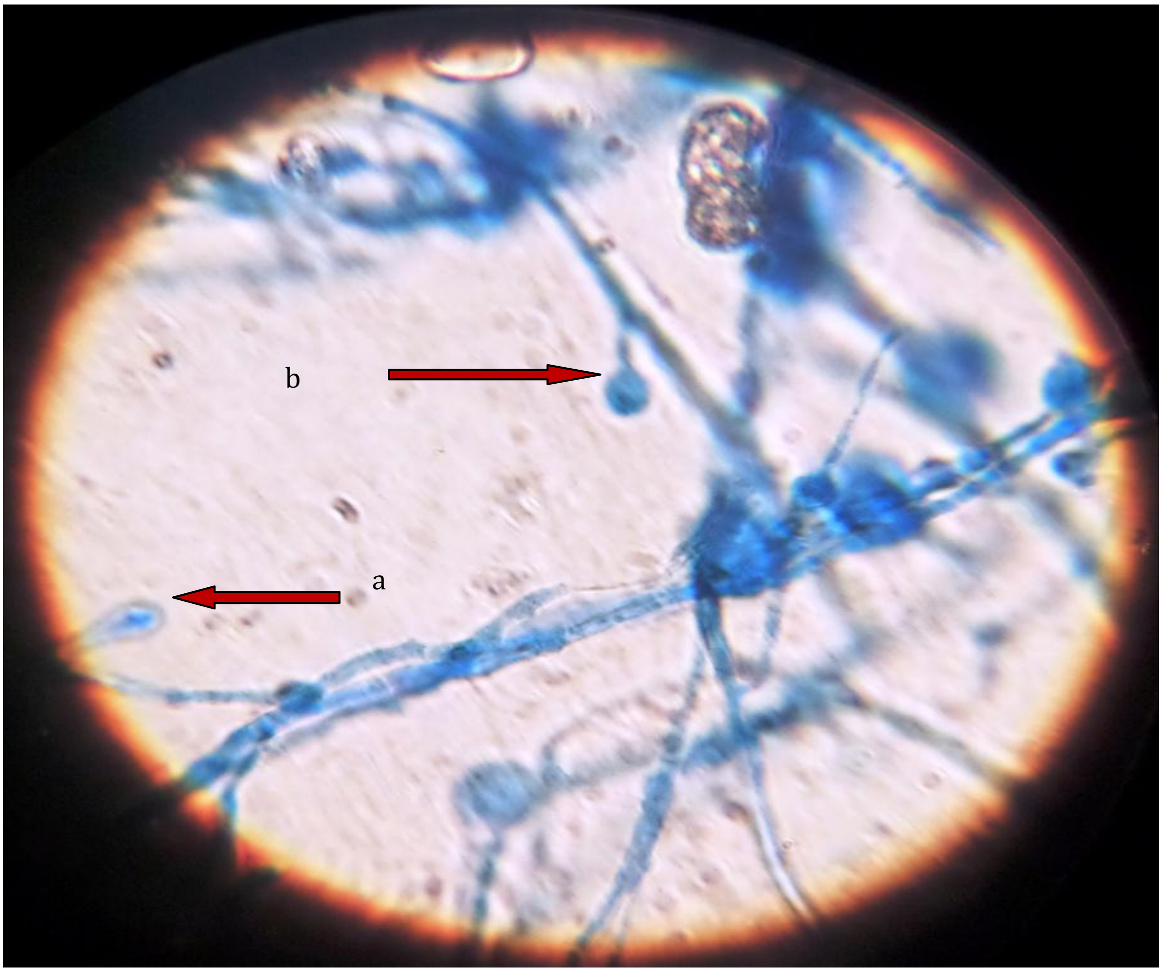

PLATE XXVI: Pyriform (a) and globuse (b) Microconidia of *M. gallinae* (LCB stain 400×)

PLATE XXVII: areas of alopecia (a) and kerion (b) on the limb of a horse


PLATE XXVIII: Button shaped colony (obverse) of *T. verrucosum* (8 days) from horse on plate xxvii.

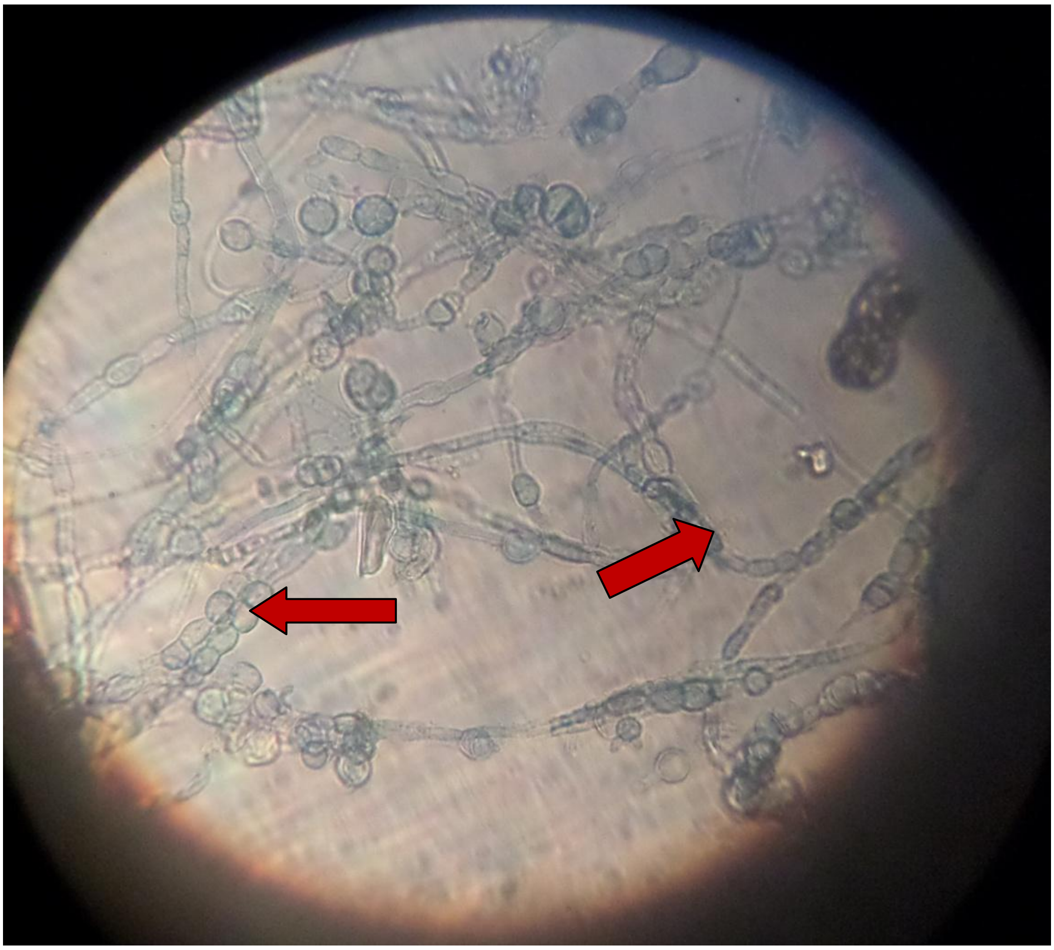

PLATE XXIX: Chains of Chlamydospores of *T. verrucosum* (LCB stain 400×)


Plate XXX: Generalized areas of crusting and Kerion on a horse .

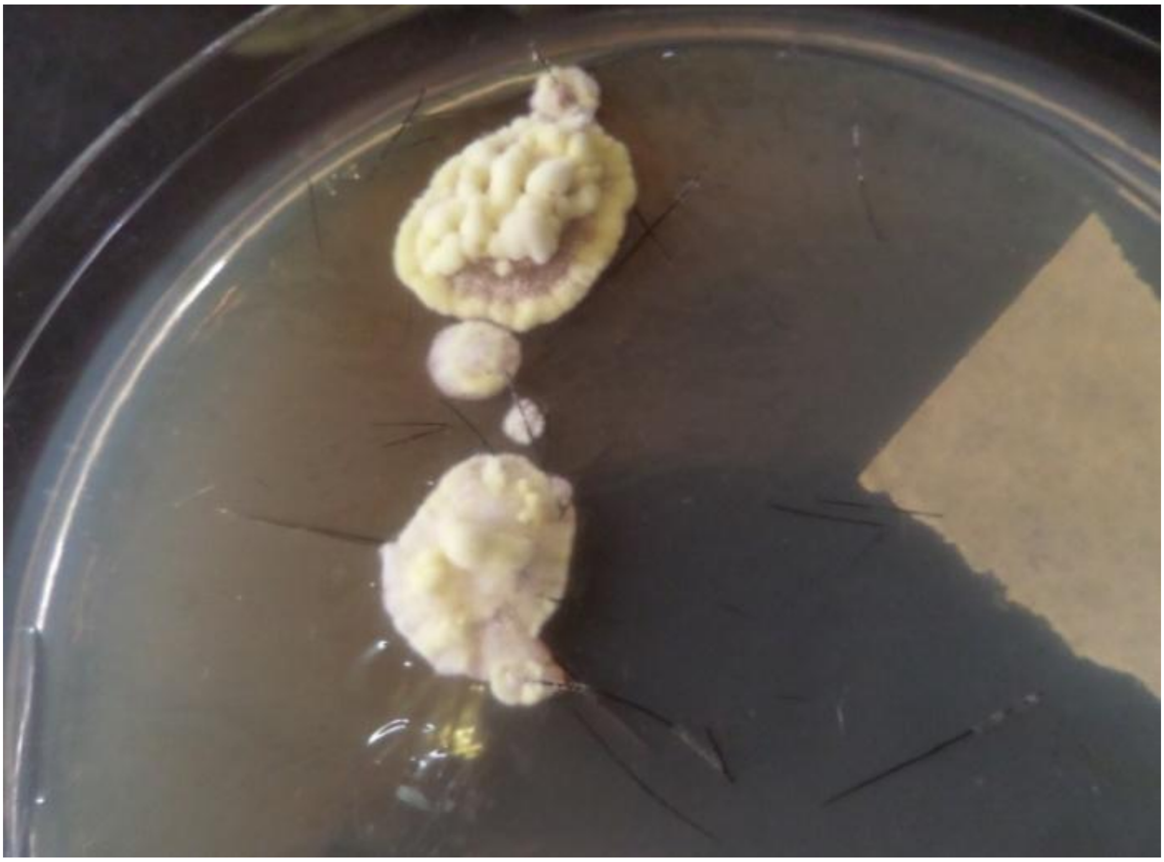

PLATE XXXI: Whitish-Yellow brown heaped and granular colony of *T. soudanense* (8 Days).
Note: This dermatophyte usually found on man is an unusual isolate from a horse.


PLATE XXXII: Burnt orange reverse of *T. soudanense*

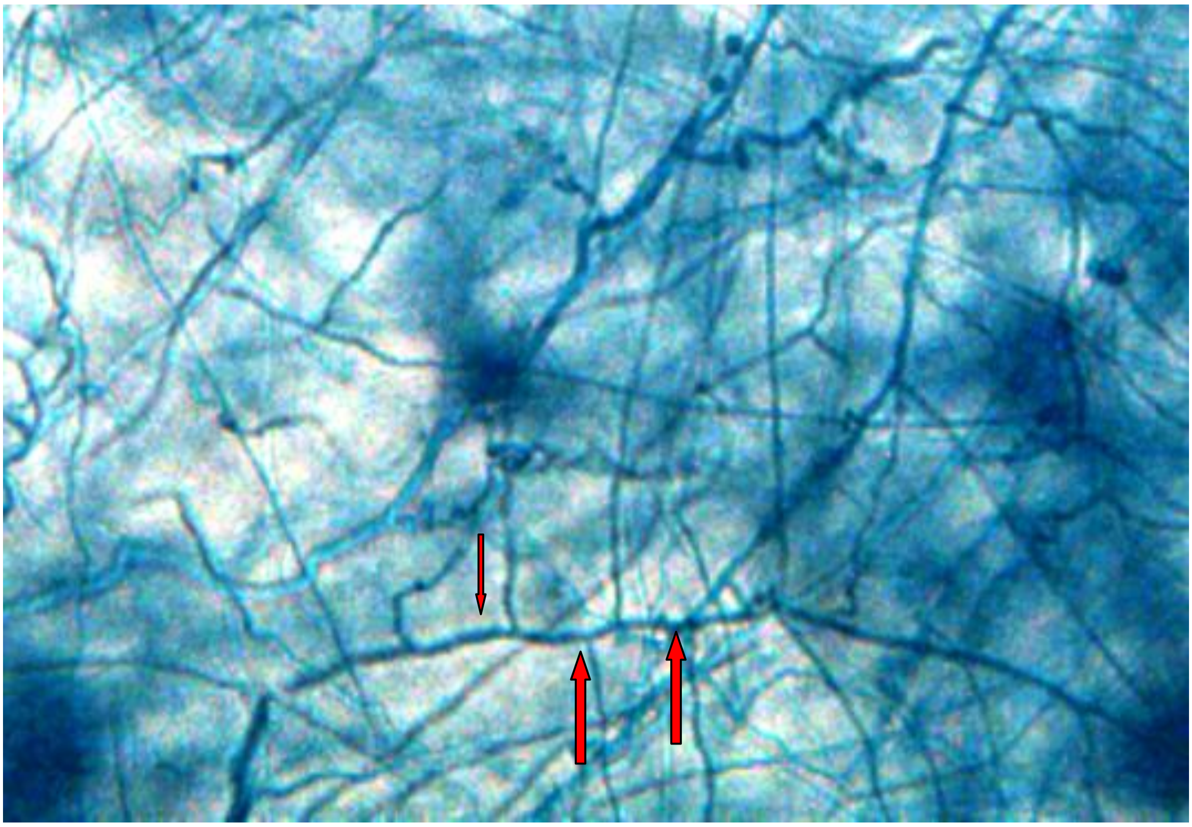

PLATE XXXIII: Right-angle branching of hyphea of *T. soudanense* (LCB stain 400×).

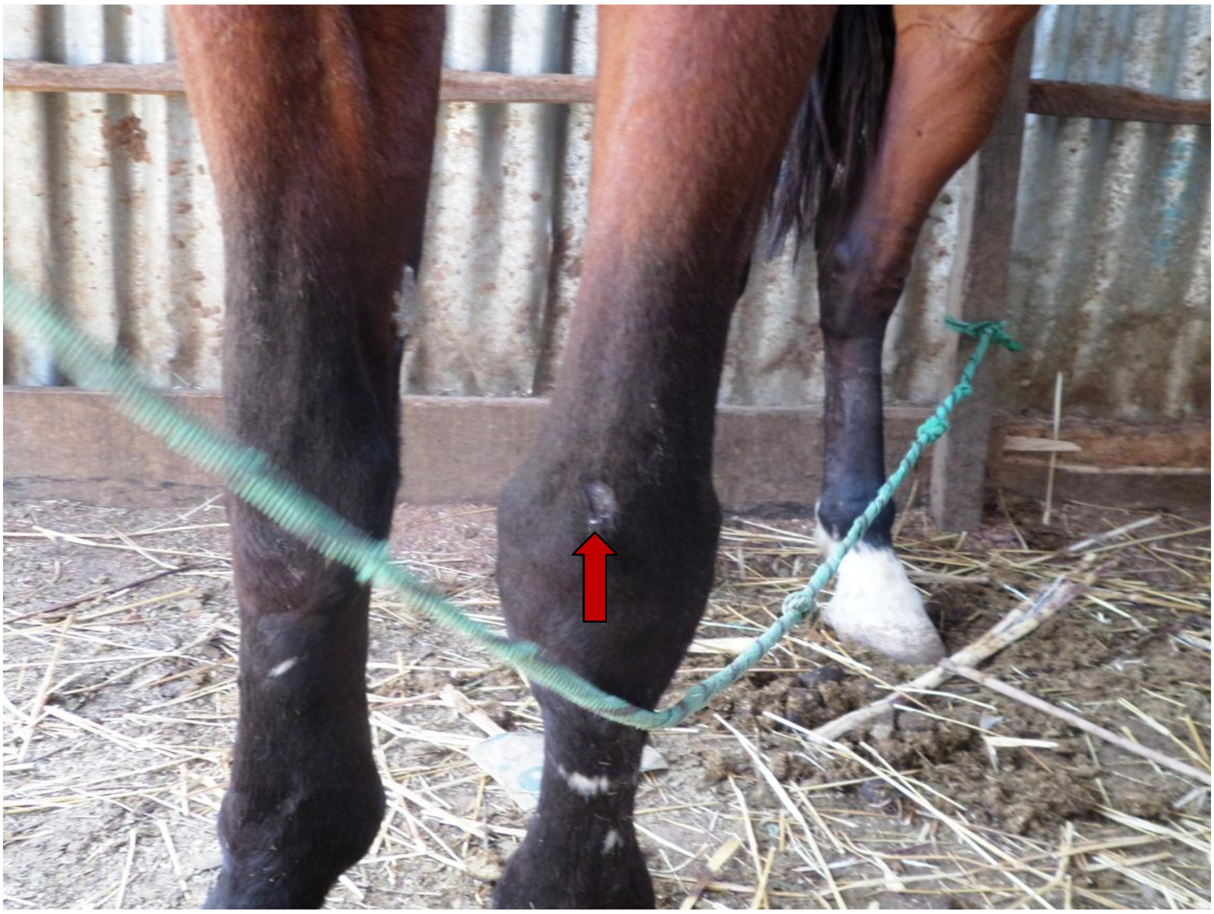
PLATE XXXIV: small circumscribed crusty area of alopecia on the limb of a horse

PLATE XXXV: Fringed whitish- cream colony of *T. mentagrophytes* (5 days) with a raised center and radial furrows.

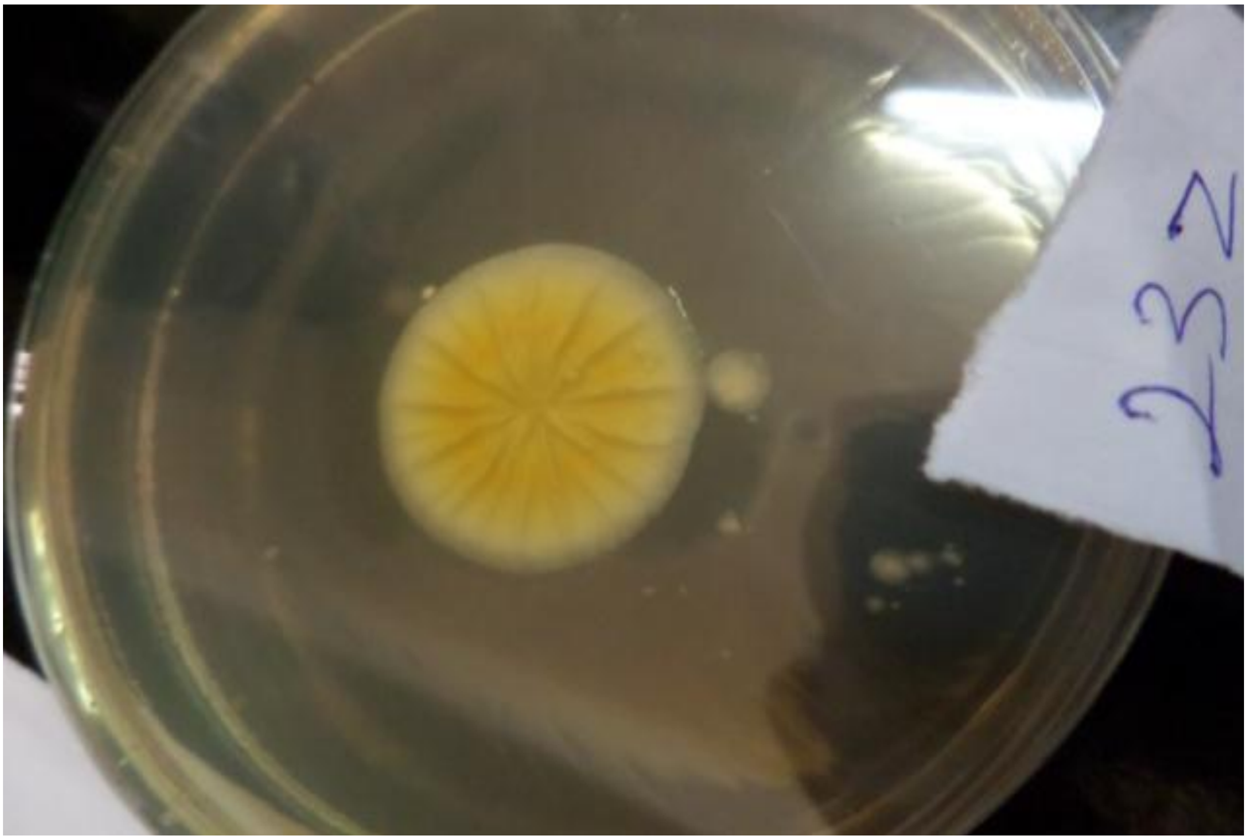

PLATE XXXVI: Yellowish reverse of colony of *T. mentagrophytes*

PLATE XXXVII: Granular white-cream colony (obverse) of *T. mentagrophytes* (17days)

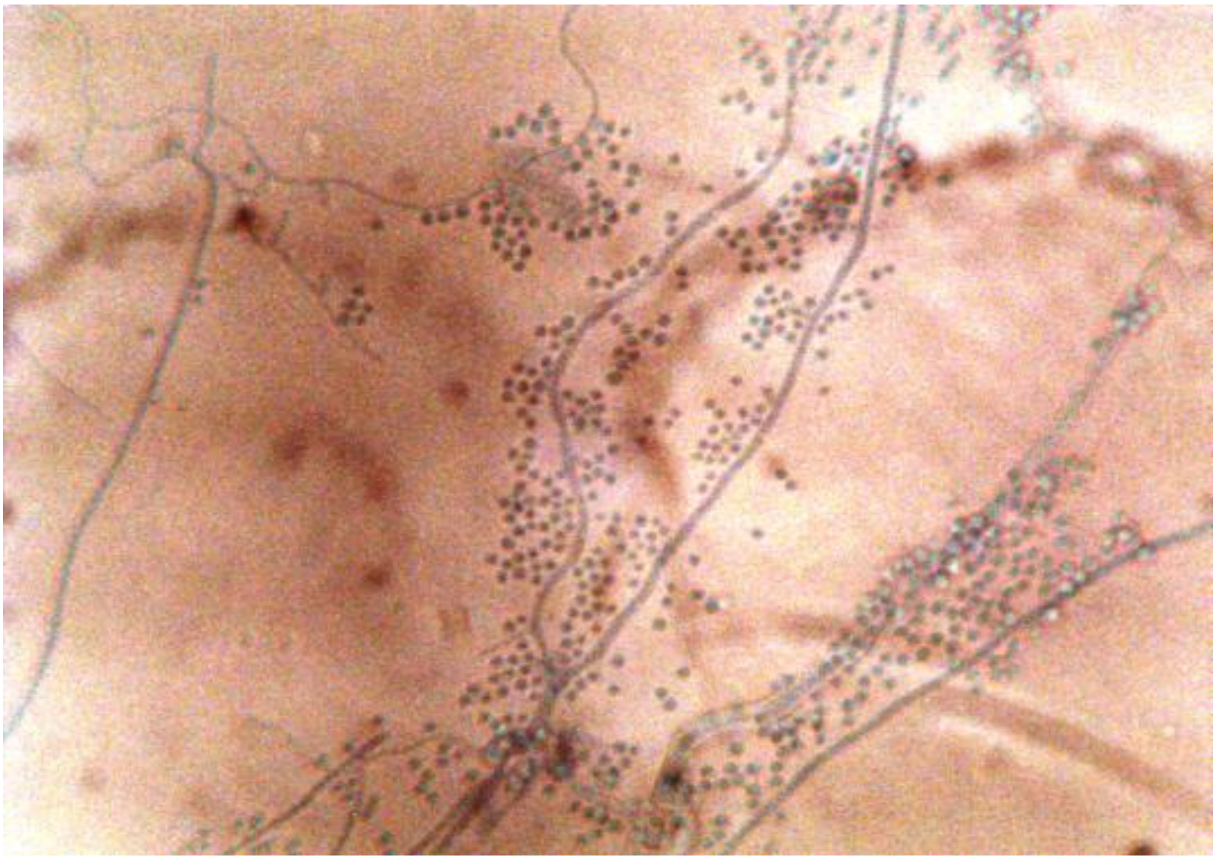

PLATE XXXIII: Numerous spherical microconidia in grape-like clusters (LCB stain 400×) stain fron colony of *T. mentagrophytes*.

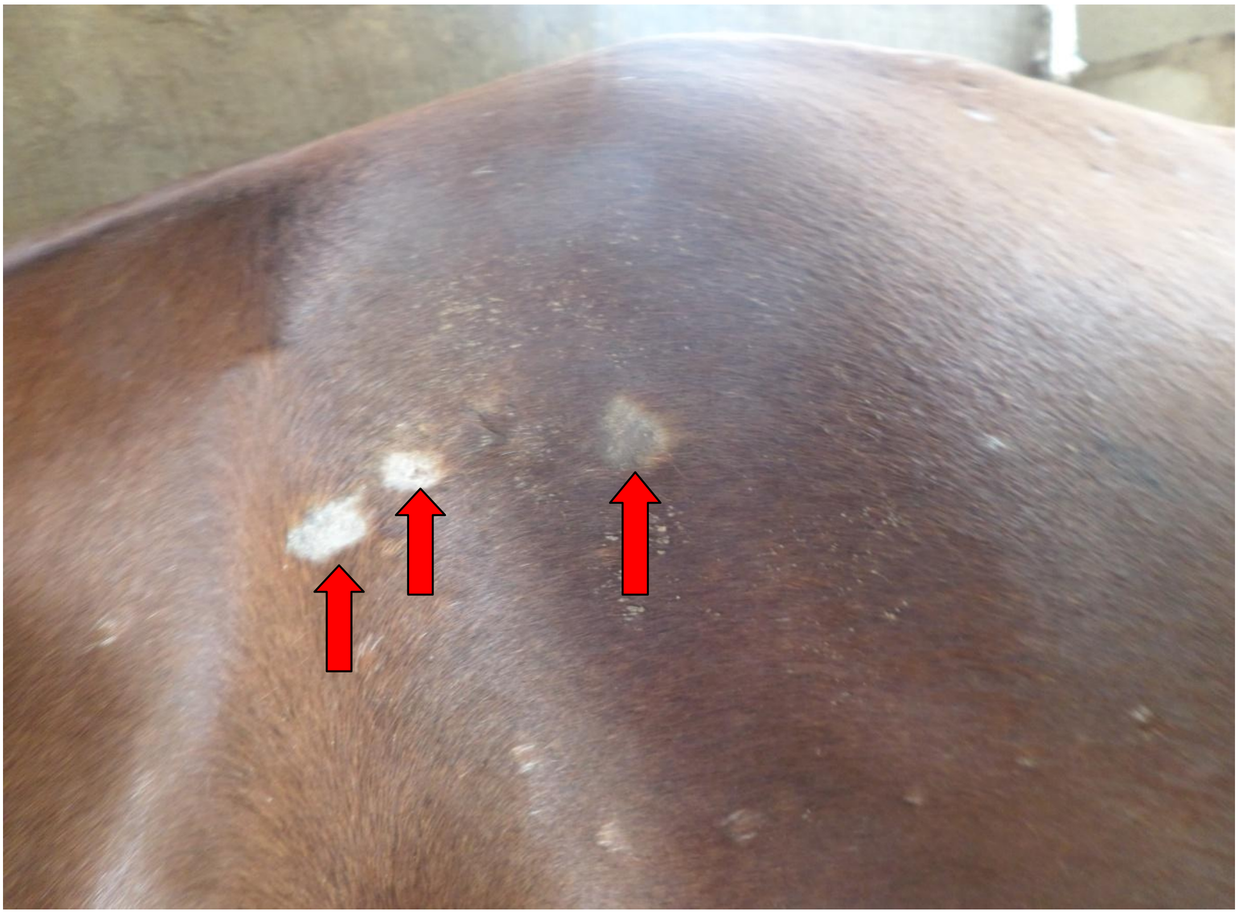

PLATE XXXIX: Generalized areas of alopecia on a horse (arrows).

PLATE XL: White downy colony of *T. vanbreuseghemii* (7 days)

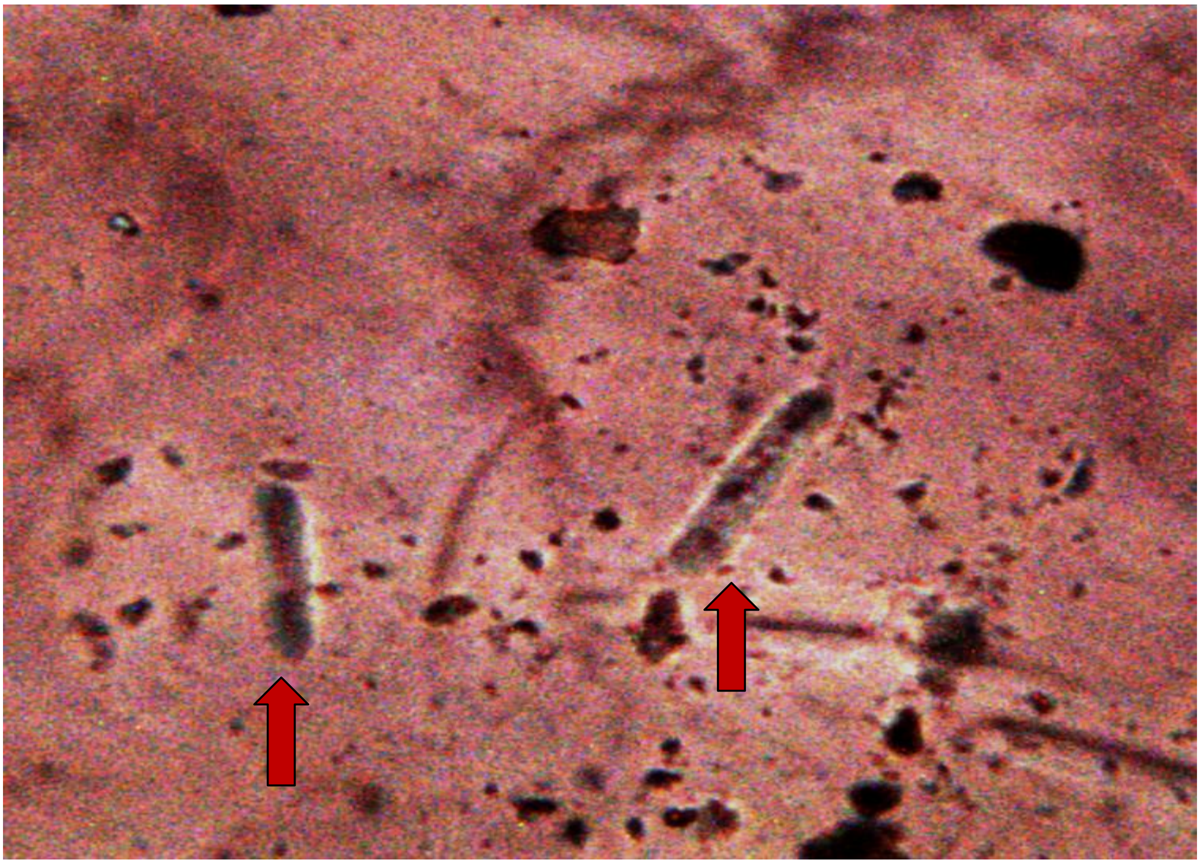

PLATE XLI: Club- shaped macroconidia of *T. vanbreuseghemii* 3-4 cells (LCB stain 400×).

4.4: Distribution of Dermatophyte Isolates Based on Sex of Horses

Male horses were found to have a higher rate of infection than females (Table 4.5), although there was no significant association between sex and isolation rate (p=0.1688).

TABLE 4.5: Distribution of Dermatophytes Based on Sex of Horses in Kaduna State

Sex	**No sampled**	**N (%)**
Male	53	12(22.6)
Female	49	6(12.24)
Total	102	18

P=0.1688 χ^2=1.894

4.5: Minimum Inhibitory Concentration of Antifungal Drugs Against Dermatophytes Isolated From Horses In Kaduna State.

The antifungal drugs tested were; ketoconazole (0.03-64 µg / ml) with MIC ranges for isolates (0.1->64 µg / ml), fluconazole (0.125-256) with MIC ranges (0.2- >256 µg / ml), griseofulvin (0.0125-32 µg / ml), MIC ranges(0.03-24 µg/ml), terbinafine (0.03-64 µg/ml), MIC ranges being (0.03-64 µg / ml), and amphotericin B. (0.03-64 µg / ml), MIC (0.03-2.0µg/ml). Most of the isolates were susceptible to the antifungal agents tested with 16 (88.89%) out of 18 but 2(11.11%) were not susceptible. These were for ketoconazole against *M. equinum* and fluconazole against *M. gypseum*. Terbinafine against *M. gypseum*, Fluconazole against *M. equinum* and griseofulvin against *M. gypseum* had the highest MIC of 64 µg / ml, 19.2 µg / ml and 24 µg / ml respectively while terbinafine against *T. verrucosum*, amphotericin B. against 6 isolates and griseofulvin against *M. canis* had the lowest MIC of 0.03 µg / ml (Table 4.6).

TABLE 4.6: Minimum Inhibitory Concentrations Of Antifungal Drugs Against Dermatophytes Isolated from Horses in Kaduna State.

SAMPLE (N)	KE	FL	GR	TER	AMB
M. gallinae (21z)	0.4	0.8	0.3	2	0.4
M. equinum(27z)	>64	9	0.4	0.6	0.1
M. canis (5s)	0.1	3.8	0.2	1.7	2
M. canis(33i)	0.6	0.2	0.03	0.5	0.03
M. equinum(16z)	3.2	6.8	0.2	0.3	0.6
M. eqinum(5kn)	0.2	19.2	0.6	0.2	0.03
T. equinum(19z)	3	1.6	9.6	1.6	0.2
M. gypseum(4z)	3	>256	24	64	0.03
M. gypseum(22z)	3	9	0.5	6.8	0.03
M. fulvum (12z)	0.6	0.2	0.5	2	0.5

T. soudanense(30i)	2	0.6	1.2	0.8	0.8
T. verrucosum(26z)	0.2	4.8	2.4	1.7	0.1
T. verrucosum(30z)	0.3	0.9	0.06	0.03	0.03
T. verrucosum(11z)	0.3	1	0.06	0.2	0.03
T. verrucosum(29z)	0.3	1.4	0.2	0.3	0.9
T. mentagrophytes(23z)	3	0.4	0.7	0.7	0.8
T. vanbreuseghemii(9z)	0.2	1	0.3	0.2	1
T. vanbreuseghemii(13z)	0.6	0.2	0.5	2	0.5

CHAPTER FIVE

DISCUSSION

The result of this study showed that a range of dermatophytes within two Genera *Microsporum* and *Trichophyton* cause infections in horses in the study area. This is in agreement with the result obtained by Adekeye *et al.* (1989) who got an isolation rate of 18.5% of dermatophytes from different domestic animals from Zaria, Kaduna State. The 17.6% recorded in the present study is lower than 44% isolation rate gotten by Nweze (2011) for horses in seven States in Nigeria which included Anambra, Imo, Enugu, Ebonyi, Abia, Kogi and Delta States. The lower isolation rate in this study can be attributed to the lower humidity (high humidity favours growth of dermatophytes) in this part of the country. *Trichophyton verrucosum* had the highest frequency of 4 (22.22%), probably because of the existence of large herds of cattle which co-exist with horses, often sharing grazing areas from where they can pick up infective spores.

Out of the 102 horses sampled, 53 were males and 12 dermatophyte species were isolated, while six horses had dermatophytes from 49 females sampled. This was probably because more male horses were sampled than females as there was no association between the sex and the prevalence of the disease (p=0.1688).

There was a high incidence of dermatophyte lesions on the saddle. Communicable disease center (CDC, 1960) and Pascoe (1979) also discovered that dermatophyte lesions occurred more in pressure areas like the saddle area .The high incidence of dermatophytoses from lesions on the saddle area can be attributed to the abrasions caused by the saddle on the back of the horse as well as the increased local humidity of the area.

Dermatophytes infections are known to be facilitated by breaks on the skin (Hainer, 2003; Weeks *et al*., 2003), from where they can spread to other parts of the body.

Four local Government areas were sampled including Zaria LGA, where 40 horses were sampled and 14 dermatophytes isolated with the prevalence of 35%, Sabon Gari, 10 samples with 1 positive (10%), Kaduna North 18 samples with one positive (5.6%) and Igabi LGA, 34 samples with two positive (5.9%). There was an association between local government area and dermatophytes (p=0.0033) .The isolation rate was higher in Zaria LGA probably because it is the focal point of most tournaments involving horses in the state. Horses have been known to be brought from even neighbouring countries such as Mali, Niger, e.t.c. with the attendant introduction of different infectious agents.

Three out of the eighteen isolates were *M. equinum.* This dermatophyte been reported in both human and equine infections in many countries like Algeria,Norway, Australia, Iran, Zaire and Nigeria (Ainswort *et al*., 1973; Aho, 1980; Al-Ani *et al*., 2002; Nweze, 2011), and has been the cause of large epizootics in stables. It is reported to be the dermatophytes most associated with horses (Yahyaraerat *et al*., 2009).

Trichophyton equinum was isolated from sample collected from the saddle region of an infected horse. Being fastidious was able to grow with the addition of nicotinic acid in the culture medium. Hesagawa and Usui (1975), Al- Ani *et al*., (2002), Chermette *et al*., (2008) and Nweze, (2011) are among numerous authors that have reported *T. equinum* as the most important dermatophytes affecting horses.

Two isolates were *M. canis, a* zoophilic dermatophytes presented with small areas of alopecia, thin powdery scales and broken hairs. Lesions were found on the saddle region, and had spread to other parts of the body. This is in agreement with Chermette *et al., (*2008). On KOH examination, spores in ectothrix formation were observed. Numerous other authors have documented *M. canis* as one of the most common dermatophytes isolated from horses (Hasegawa, and Usui, 1975; Nweze, 2011).

From this study, the isolates of *M. gypseum* were from samples taken from the saddle and limbs. The horses may have picked up infective spores from formites in the stables, rolling on the ground and even dust from the shoes of riders. A profuse growth in 5% NaCl SDA showed osmotolerance. Although this is a soil-inhabiting organism, there have been reports of isolation of *M. gypseum* from many symptomatic and asymptomatic animals (Cabanes, 2000; Chermette *et al*., 2008; Nweze, 2011).

Isolation of *T. mentagrophytes* in this study was from samples collected from infected sites on the limb and thus, the horse may have been infected from rodents that nestle in the hay or perhaps from formites in the housing or even infected equipment like grooming tools, saddles (Oyeka, 2000). Colony morphology was observed to be a cream to white, fringed grwth with radial furrows that became granular with age. The organism was able to grow on 5% salt SDA, in agreement with earlier work by Issa and Zangana, (2009). *Trichophyton mentagrophytes* is a zoophilic dermatophyte that has been isolated from all domestic animals (Al-Ani *et al.,* 1995; Chermette *et al*., 2008; Nweze, 2011).

Four samples were positive for *T. verrucosum*. The sites of infection were on the limbs, ventrum and pelvis. The lesions observed on the horses were mainly descrete kerion formation on the skin. Direct examination revealed ectothrix spore infestation of hair. Small and slow growing botton-shaped colonies were observed on SDA culture at room tempereature. Various authors (Shimozawa *et al*., 1997; Khrosran and Mahmoud, 2003; Nweze, 2011; and Anyawu *et al*., 2013) have isolated *T. verrucosum* from horses. The high incidence of these dermatophytes could be attributed to the high degree of contact with cattle in this part of the country. Although *T. verrucosum* mainly infects cattle, it has been isolated from all mammals including man (Chermette *et al*., 2008).

Two *T. vanbreuseghemii* isolates were obtained from this study. Samples were collected from horses with generalized areas of patchy alopecia. *T. vanbreuseghemii* is a geophilic fungus with very few reports of pathogenicity (Londero and Ramus, 1980; Lopes *et al*., 1994). The horses could have been infected from contaminated soil when rolling on the ground.

Trichophyton soudanense, reported to be human pathogen (Nweze, 2001) have been isolated in this study. It is an antropophilic dermatophyte endemic to Africa where it is the most common causes of onychomycosis, tinea capitis and tinea faciea in children and young adults (Rubben and Krause, 1996). In Nigeria, Apata *et al*., 1992; Nweze, 2001; 2010), have various reports of *T. soudanense* as the causal organism of different forms of dermatophytoses in man. Although there is no recorded case of *T. soudanense* in domestic animals, there are many cases of antropophilic dermatophytes infecting animals (Frey *et al*., 1979; Abdel-Gawad, 1989; Moriello and De Boer, 1991; Terreni *et al*., 2007). The European Confederation of Medical Mycology in a multicentric study, demonstrated a change in the clinical pattern of the lesions caused by dermatophytes and an increase in the number of unusual agents (Hay *et al*., 2001). Isolation of anthropophylic dermatophytes during mycological studies of animals could reflect the dermatophytic flora of man in contact with the animals. Cases of pathogenic *T. tonsurans*, *E. flucossum and T. rubrum* in domestic animals having close contact with their infected owners that had a predisposing factor like age, and presence of an immune compromising condition have been recorded (Kano *et al*., 2002; Brilhante *et al*., 2006). The isolate in this case was white to yellow to orange-brown and folded on the obverse with a burnt orange reverse.

One isolate of *M. gallinae* was obtained from the study. The sample was collected from a tick infested horse and many birds were observed to hover around it, picking them off. This was probably the source of infection. The horse could also have contracted the infection from its handlers. *M. gallinae* is a zoophilic dermatophytes that mostly affects birds.There are occassional reports of its occurance in other animal species (www.cfsph.iastste.edu), although

Nweze, (2001) reported it as being the most common dermatophytes isolated from young prepubescent boys in the north-easthern part of the country. There are cases of isolation from onychomycosis and tinea coporis (Straff *et al*., 2001; Miyasata *et al.*, 2011) in Europe and Asia.

The geophilic dermatophyte *M. fulvum* was isolated from sample collected from a single area of patchy alopecia on the face of a foal. There have been reports of pathogenicity of these dermatophytes, even human cases (Sadegh *et al*., 2013).

Terbinafine against *M. gypseum*, Griseofulvin against *M. gypseum* and fluconazole against *M. equinum* recorded the Highest MIC and this could be as a result of their abuse as there are many topical preparations that can be bought in any pharmacy without a prescription. Griseofulvin and fluconazole are also relatively cheaper.

Griseofulvin, terbinafine and amphotericin B. had the lowest MIC of 0.03 µg/ml and were the most potent drugs.This could be attributed to the fact that terbinafine is relatively new to the market, and is expensive; amphotericin B is expensive and is not usually the drug of choice for the disease. Many authors have cited terbinafine as the most potent antifungal agents in use (Santos *et al.*, 2005; Nweze *et al.*, 2007).

When tested against ketoconazole, *M. equinum* did not respond to the antifungal agent. The same was also observed in the assay of fluconazole against *M. gypseum*. This resistance can be attributed to misuse of the drugs as there have been reports of resistance of dermatophytes (Shah *et al.*, 1988).

Although there were 2 cases of resistance, the *in vitro* test carried out suggests that good clinical outcomes can be obtained from the use of these antifungal agents in the treatment of dermatophytosis as most of the M.I.Cs compares favourably with the ranges obtained by other authors (Nweze *et al*., 2007; Araujo *et al*., 2009).

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

In this study, the incidence of dermatophyte infection from horses in four local Government Areas (Zaria, Sabon Gari, Kaduna and Igabi) in Kaduna state was 17.6%. Zaria local Government had the highest number of dermatophyte infection and the highest number of positive cases (14, 77.8%) of all cases, followed by Igabi LGA (two, 11.11%), Kaduna north and Sabon Gari both had (one, 5.5%) each making them the LGAs with the lowest isolation rates (p=0.0033). More males (53) than female (49) had the ring-worm lesions (p=0.1688). The lesions were mostly found on the flank and girth area (3 out of 25), limbs (five out of 20) and saddle area (5 out of 15). Equal number (9) of *Microsporum* and *Trichophyton* Species were isolated. *T. verrucosum* was the species with the highest frequency (4 out of 18) followed by *M. equinum* (three out of 18). *T. mentagrophtes*, *T. soudanense* and *M. fulvum* all had a frequency of one out of 18 each. Most dermatophytes isolates were susceptible to the range of antifungal agent dilutions except for a few cases of resistance of ketoconazole, and fluconazole.

6.2 CONCLUSION

In conclusion, there is a broad range of dermatophytes causing skin infections of horses in Kaduna state. All the dermatophytes isolated have the potential of being transmitted to other animals and even human hosts which may cause cosmetic and even severe systemic infections. Most of the dermatophytes are susceptible to commonly available antifungal agents.

6.3 RECOMMENDATIONS

This study indicates that dermatophytes are prevalent in horses in Kaduna state. Horses are prized animals that are involved in sports both locally and internationally, and could potentiate the spread of infections from place to place. It is therefor important that;

1. Veterinarians do not overlook skin disease as being only superficial as the animals can serve as reserviours of infections to other animals and humans where the cause chronic and recurrent infections. Proper diagnosis and treatment should always be carried out.
2. Treatment of dermatophyte infections usually takes a long time before results are seen. Antifungal susceptibility tests should be carried out to find out the drug with the best potential for effectiveness.
3. Further studies are carried out on grooms and other stable hands to compare the pattern of spread of the disease in this part of the country.

REFERENCE

Abdel-Gawad, K.M. (1989) Fungi on the claws of buffalo and cow in Egypt. *Journal of Basic Microbiology*, **29**, 323–8

Adekeye, J., P. Addo, C.N. Kwanashie, Adeyanju,J. and Abdullah, S. (1989) Prevalence of Animal Dermatophytes in Zaria. *Zariya Veterinarian*, **4**, 83-86

Adeleke, S. I., Usman, B. and Ihesiulor ,G. (2008) Dermatophytes among Itinerant quaranic scholars in Kano (North-Westhern Nigeria). *Nigerian Medical Practitioner*, 53,33-35

Adou-Bryn, K. D., Assoumou, A., Haddad, R. N., Aka, B. R., and Ouhon, J. (2004) Epidermiology of Tinea Capitis in Abidjan, Cote D'Ivoire. *Medical Tropics*, 64,171-175

Agarwal, R.K., Gapta, S., Muttal, G., Khan, F., Roy, S., and Agarwal, A. (2015). Antifungal susceptibility testing of DermatophytesBy agar based Disk diffusion Method. *International Journal of Current Microbiology and Applied Science*, 4(3), 430-436

Ahmadu Bello University (ABU, 2000) Zaria Master plan, Department of Urban and Regional Planning, ABU, Zaria, Nigeria.

Ahmed, A.R. (1983) Immunology of human dermatophyte infections. *Archives of Dermatology*, 118,521–5

Aho, R. (1980) Studies of fungal flora in hair from domestic and laboratory animals suspected of dermatophytes. *Achives of Pathology and Microbiology in Scandinavia*, 88,79-83.

Ainsworth, G. C., and Austwick, P. K. C. (1973) Fungal diseases of animals, Commonwealth Agricultural Bureaux, Farnham Royal, Slough, England. p. 10-36.

Ajello, L. (1959) A new *Microsporum* and its occurrence in soil and on animals. *Mycologia*, 51,69-76

Ajello, L. (1962) Present day concepts in the dermatophytes. *Mycopathology and Mycological Applications*, 17,315–324.

Ajello, L. (1968) A taxonomic review of the dermatophytes and related species. *Sabouraudia*, 6,147–159.

Ajello, L. (1974) Natural history of the dermatophytes and related fungi. *Mycopathology and Mycological Applications*, 53, 93–110.

Ajello, L. (1977) Taxonomy of the dermatophytes: a review of their imperfect and perfect states, In K. Iwata (ed.*), Recent Advances in Medical and Veterinary Mycology*. University of Tokyo Press, Tokyo. Pp. 289–297

Ajello, L., Georg, L.K., Kaplan, W. and Kaufman, L. (1966) Laboratory Manual for Medical Mycology; U.S. Department of Health, Education and Welfare, Public Health Service, Communicable Disease Centre, Antlanta, Georgia

Ajello, L. and Georg, L. K. (1957) *In vitro* hair cultures for differentiating between atypical isolates of *Trichophyton mentagrophytes* and *Trichophyton rubrum*. *Mycopathology and Mycological Applications,* 8, 3–17.

Akpata, L.E., Gugnani, H. C., Srivastava, R. and Utsalo, S. J. (1992) Dermatomycoses among industrial workers in Cross Rivers State, Nigeria. *Mycoses*, 35,371-374

Al-Ani, F.K., Al-Bassam, L.S. and Al-Salaha, K. A. (1995) Epidemiological study of Dermatomycosis due to *T. schöenleinii* in Camels in Iraq. *Bulletin of Animal Health and Production in Africa*, 43, 87-92

Al-Ani, F.K., Younes, F.A. and Al-Rawashdeh, F.O. (2002) Ringworm Infection in Cattle and Horses in Jordan. *Acta Veterinaria Bruneo*, 71, 55-60

Al-Ani, F.K., Al-Bassam, L.S. and Al-Salahi, K.A. (1995). Epidemiological study of dermatomycosis due to *T. Schöenleinii* in camels in Iraq. *Bulletin of Animal Health and Production in Africa*, 43, 87-92

Aljabre, S.H., Richardson, M.D., Scott, E.M., Rashid, A. and Shankland, G.S. (1993) Adherence of arthroconidia and germlines of antropophilic and zoophilic varieties of *T. mentagrophytes* to human corneocytes as an early event in the pathogenesis of dermatophytosis. *Clinical Experiments in Dermatology*, 18,231–5.

Alsop, J. and Prior, P.P. (1961) Ringworm infection in a cucumber green house. *British Medical Journal*, 1, 1081-1083.

Alteras, I., Feuerman, E. J., David, M. and Segal, R. (1986) The increasing role of *Microsporum canis* and the variety of dermatophytic manifestations in Israel. *Mycopathologia*, 15, 105–107.

Anyawu, M. U., Onoja, R. I. and Noel-Uneke, O. A. (2013) Mixed Infection of Trichophyton species in a Nigerian Part Arab horse with Dermatophytoses. *Journal of Veterinary Medicine and Animal Health*, 5(5), 124-128

Apprich, V., Sperger, J., Rosengarten, R. and Stanek C. (2006) *In vitro* degradation of equine keratin by dermatophytes and other keratinophylic fungi. *Veterinary Microbiology*, 14,352-8

Araújo, C., Karla, C. M., Orionalda, D., Lisboa, F., Ailton, J., and Maria Do Rosário, R. S. (2009) *In vitro* susceptibility testing of dermatophytes isolated in Goiania, Brazil, against five antifungal agents by broth microdilution method. *Revista Instuto De Medicina Tropical De Sao Paulo*, 51(1):9-12

Arnow, P. M., Houchins, S. G. and G. Pugliese (1991) An outbreak of tinea corporis in hospital personnel caused by a patient with *Trichophyton tonsurans* infection. *Pediatrics Infectious Disease Journal*, 10,355–359.

Ates, A., Likit, M., Ozdermir, R., Ozdean, K. (2008) Dermatophytes isolated from asymptomatic dogs in Adana, Turkey: a preliminary study. *Journal of medical Mycology*, 18,154-7

Auger, P., G. Marquis, Joly, J., A. Attye (1993) Epidemiology of tinea pedis in marathon runners: prevalence of occult athlete's foot. *Mycoses*, 36,35–41.

Ayanbimpe, G.H., Bello, C.S.S., Gugnani, H.C. (1995) The aetiological agents of superficial cutaneous mycoses in Jos, Plateau State of Nigeria. *Mycoses*, 38, 235–7.

Badillet, G. (1988) Dermatophytes et immigration. *Annals of Biology Clinics*, 46, 37–43.

Bakerspigel, A. (1974) The Keratinophilic Fungi of Ontario, Canada. *Mycopathology and Mycology Application*, 53,1-12

Barlow, A.J., Chattaway, F.W. (1958) The parasitism of ringworm group of fungi. *Association of Medical Academy Archives of Dermatology*, 77,,399–405.

Baron, E.J., Murray, P.R., Jorgensen, J.H., Pfaller, M.A.and Yolken, R.H. (2003) *Manual of Clinical Microbiology* 8th edition, SM Press, Washington. Pp 1798-1817

Barry, A. L., and Brown, S. D. (1996). Fluconazole disk diffusion procedure for determining susceptibility of *Candida* species. *Journal of Clinical Microbiology*, 34,2154–2157.

Baxter M, Pearson R.D. (1969). The occurence of *Microsporum nanum* as a human pathogen and animal pathogen in New Zealand. *New Zealand Journal of Medical Laboratory Techniques*, 23, 87-90.

Baxter, M. (1966). Isolation of *Trichophyton mentagrophytes* from British soil. *Sabouraudia*, 4, 207-209.

Baxter, M. (1973) Ringworm due to *M. canis* in cats and dogs in New Zealand. *New Zealand Veterinary Journal*, 21, 33-37

Beneke, E. S. and Rogers, A. L. (1980) Medical Mycology Manual. Burgess, Minneapolis

Benham, R. W. (1953). Nutritional studies of the dermatophytes: effect on growth and morphology, with special reference to the production of macroconidia. *Transcrits of New York Academy of Science,* 15,102–106.

Bernado, F., Lanca, A. M., Guerra, M., Martins, H.M. (2005) Dermatophytes isolated from pet dogs and cats in Lisbon, Purtugal (2000-2004) *Republic of Purtugal College of Veterinary,* 100(553-554),85-88

Blank, F., Craig, C.E. (1977) Family epidermics of ringworm contracted from cattle. *Canadian Medical Association Journal*, 71,234

Böhme H. (1972) Über die Variabilität physiologischer Eigenschaften in der Gattung *Microsporum Gruby*. I. Verwertung von Oleum pedum tauri durch *M. audouinii* und *M. gypseum*. *Zoonoses, Allegies Mikrobiologie*, 12, 443-450.

Borelli, D. (1965) *Microsporum racemosum* nova species. *Acta Medicano Venerology,* 12: 148-151.

Brilhante, R.S.N., Cordeiro, R.A., Gomes, J.M.F., Sidrim, J.J.C. and Rocha, M.F.G. (2006) Canine dermatophytosis caused by an anthropophilic species: molecular and phenotypical characterization of *Trichophyton tonsurans*. *Journal of Medical Microbiology,* 55:1583–6.

Bronson, D. M., Desai, D. R., Barskey, S. and McMillen, F. S. (1983) An epidemic of infection with *Trichophyton tonsurans* revealed in a 20 year survey of fungal infections in Chicago. *Journal of American Academy of Dermatology,* 8:322–330.

Butty, P., J., C. Lebecq, M. Mallie, and J. M. Bastide.(1996). Evaluation of the susceptibility of dermatophytes to antifungal drugs: a new technique.*Journal Medical and Veterinary Mycolology*, 33:403–409.

Cabanes, F.J. (2000) Dermatophytes in Domestic Animals. *Revista Iberoamericana de Micologia*, Apdo.699, E-480480 Bilbao, Spain.pp 104-108

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

Cafarchia, C. *et al.,* (2013*)* Fungal diseases of horses.*Veterinary Microbiology,* (Article in Press).

Calderon, R. A. (1989) Immunoregulation of dermatophytosis. *Critical Reviews in Microbiology,* 16:338–368.

Calderon, R.A., Hay, R.J. and Shennan, G.I. (1987) Circulating antigens and antibodies in human and mouse dermatophytosis: use of monoclonal antibody reactive to phosphorylcholinc-likc epitopes. *Journal of Geneneral Microbiology,* 133 (9) (12): 2699

Cantón, E., Espinel-Ingroff, A. and Pemán, J. (2009) Trends in antifungal susceptibility testing using CLSI reference and commercial methods. *Expert Review And Infectious Therapeutics,* 7, 107-119.

Caprilli, F., Mercantini, R., Marsella, R. and Farotti, E. (1980) Aetiology of ringworm of the scalp, beard, and body in Rome, Italy. *Sabouraudia*, 18, 129–135.

CDC, (1960) Animal ringworm In Public health, Diagnosis and nature. U.S. Department of health, education and welfare public health service, Bureau of State Services Communicable D is e a s e Center, A tla n ta , Georgia Public Health Service Publication No. 727

Chah, K.F., Majiagbe, K.A., Kazeem, H.M., Ezeanyika, O. and Agbo, I.C. *(2012) Dermatophytes* from skin lesions of domestic animals in Nsukka, Enugu State, Nigeria. *Veterinary Dermatology*, 23(6):522-e104.

Chahota, R., Katoch, R., Mahajan, A., Verma, S. (2001) Clinical Bovine Mastitis caused by *Geotrichum candidum*. *Veterinary Archives*, 71:197-201

Chander J. (1995) Dermatophytoses, textbook of medical mycology. 11:91–112.

Chermette, R., Ferreiro, L., Guillot J., (2008) Dermatophytoses in animals. *Mycopathologia, 166:385-405*

Chineme, C.N., Adekeye, J.O., Bida. S.A. (1980) *Trichophyton verrucosum* in young goats. *Bulletin of Animal Health and Production*, 29: 75-78.

Chmel, L. (1980) Zoophilic dermatophytes and infections in man. *Medical Mycology,* 8 (Suppl.):61–66.

Clinical and Laboratory Standards Institute (2008) M38-A2. CLSI Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi Approved Standard.

Connole, M. D. (1977) Current status of the ecology and epidemiology of animal mycoses with special reference to Queensland, Australia, p. 123-128. In K. Iwata (ed.), *Recent Advances in Medical and Veterinary Mycology.* University Park Press, Baltimore, Md.

Connole, M.D. (1990) Review of Animal mycoses in Australia. *Mycopathologia*, 111,133-164

Conti Diaz, L.A., Vargas, R., Apolo, A., Morana, J.A., Pedrana, G., Cardoza, E. and Almeida, E. (2003) Mycotic Bovine Nasal Granuloma. *Revista instuto de medicina Tropical de Sao Paulo,* 45,163-6

Conti-Diaz, I. A., Civila, E. and Asconegui, F. (1984) Treatment of superficial and deep-seated mycoses with oral ketoconazole. *International Journal of Dermatology,* 23,207–210.

Cox, R.A. (1989) Immunology of the Fungal Diseases. eRe Press Inc. I-20,

Cruickshank, C.N.D, Wood, S.R. (1960) Studies on lrichophytinsensitivity. *Investigative Dermatology*, 35, 219.

Currah, R.S. (1985) Taxonomy of the Onygenales: Arthrodermataceae, Gymnoascaceae, Myxotrichaceae and Onygenaceae. *Mycotaxon*, 24, 1-216.

Dahl, M. V. (1987) Immunological resistance to dermatophyte infections. *Advances in Dermatology,* 2, 305–320.

Dahl, M. V. (1993) Suppression of immunity and inflammation by products produced by dermatophytes. *Journal of American Academy of Dermatology,* 28, S19–23.

Dahl, M.V. (1994) Dermatophytosis and the immune response. *Journal of American Academy of Dermatology,* 31, 34–41.

Dalis, J. S., Kazeem, H. M., Kwaga, J. K. P. and Kwanashie, C. N. (2014) An outbreak of ringworm caused by *Trichophyton verrucosum* in a group of calves in Vom, Nigeria. *African Journal of Microbiology Research*, **8**(8), 783-787

Dawson, C. O. and. Gentles, J. C. (1961). The perfect states of *Keratinomyces ajelloi* Vanbreuseghem, *Trichophyton terrestre* Durie & Grey and *Microsporum nanum* Fuentes. *Sabouraudia* 1, 49–57.

De Vroey, C. (1984) Ecological and epidemiological aspects in dermatophytoses. *Zeoble Bakteria Hygieneous*, 257,234-239.

De Vroey, C. (1985) Epidemiology of ringworm (dermatophytosis). *Dermatology*, 4, 185–200.

De Vroy, C. (1988) Dermatophytes, a "*nomem dobium*"? in Tumbay E. (Ed) FEMS-Symposium of Dermatophytosis in man and animals . *Izmir*, Turkey.16-23

DeBoer, D.J. and Moriello, K. A. (1995) Investigations of a killed dermatophyte cell-wall vaccine against infection with *Microsporum canis* in cats. *Researches in Veterinary Sciences,* 59, 110–113.

Deepika, T. L. and Krishnan, K. (2010) Review on dermatomycosis: pathogenesis and treatment *Natural Science*, 2 (7), 726-731

Dekeyser, J., L. Delcambe, D. Thienpont (1960) Activite therapeutique de l'iturine et du chinsol sur le teigne du cheval a *Microsporurn equitilitnn*. *Bulletin of Epizootic Diseases in Africa,* 8, 279-288.

Desai, S.C., Bhat, M.L. and Modi, P.J. (1963) Biology of *T. rubrum* infections. *Indian Journal of Medical Research,* 51, 233–43.

Detandt, M., and. Nolard, N.(1988) Dermatophytes and swimming pools: seasonal fluctuations. *Mycoses,* 31, 495–500.

Djeridane, A., Djeridane, Y. and Ammar-Khodja, A. (2006) Epidemiological study on tinea pedis and onychomycosis in Algeria. *Mycoses,* 49, 190–6.

Durie, E.B. and Frey, D. (1962) The presence of dermatophytes and other keratinophilic fungi in soil. *Australian Journal of Dermatology*, 6, 167- 171.

Efuntoye, M. O., and Fashanu, S.O. (2002) Occurrence of Keratinophyllic fungi and dermatophytes on domestic birds in Nigeria. *Mycopathologia*, 153, 87-89

Efuntoye, M.O., and Fashanu, S.O. (2002) Fungi isolated from skins and pens of healthy animals in Nigeria. *Mycopathologia*, 153, 21-23

Efuntoye, M.O., Sobowale, A.A., Mabekoje, O.O. and Agu, G. C. (2011) Onychomychosis among rural farmers in southwestern part of Nigeria. *Egyptian Dermatology online Journal,* 7 *(*1), 4. Retrieved from http://www.edoj.org.eg

Elewski B E. (1995) Clinical pearl: diagnosis of onychomycosis. *Journal of American Academy of Dermatology,* 32,500–501

Elewski, B.E. (1995) Onychomycosis; Pathogenesis, Diagnosis and management. *Clinical Microbiology Reviews,* 11, 415-425.

Ellabib, M. S., Khalifa, Z. and. Kavanagh, K. (2002) Dermatophytes and other fungi associated with skin Mycosis in Tripoli, Libya. *Mycoses*, 45, 101-104

Ellis, D., Stephen, D., Helen, A., Rosemary, H., Robyn, B. (2007) Descriptions Of Medical Fungi. Second Edition Mycology Unit Women's And Children's Hospital School Of Molecular & Biomedical Science University Of Adelaide ,Australia

Emmons, C. W. (1934). Dermatophytes: natural groupings based on the form of the spores and accessory organs. *Archives of Dermatology and Syphilology*, 30, 337–362.

English, M. P. (1972) The Epidemiology of animal ringworm in man. *British Journal of Dermatology*, 86 (suppl), 78-87

English, M. P. (1980) Ecological aspects of dermatophytes regarded essentially as anthropophilic. *Medical Mycology*, 8, 53–59.

ESCCAP (European Scientific Counsel Companion Animal Parasites.) Guidelines, (2011) Superficial Mycosis in Dogs and Cats, 2, 4-5

Evans, E.G., Richardson, M.D. (1989). Medical Mycology, a Practical Approach. IRL press Oxford, Newyork, Pp 65-95.

Evans, E.G.V. and Sigurgeirsson, B. (1999) Double blind, randomised study of continuous terbinafine compared with intermittent itraconazole in the treatment of toenail onychomycosis. *British Medical Journal*, 318, 1031–5.

Feuerman, E., Alteras, I., Honig, E. and Lehrer, N. (1975) Saprophytic occurrence of *Trichophyton mentagrophytes* and *Microsporum gypseum* in the coats of healthy laboratory animals. *Mycopathologia*, 55,13–15.

Fischer, J. B. and Kane, J. (1971). The detection of contamination in *Trichophyton rubrum* and *Trichophyton mentagrophytes. Mycopathology and Mycological Applications*, 43,169–180.

Florian, E. and Galgoczy, J. (1964) *Keratinomyces longifusus* sp. nov. from Hungary. *Mycopathologia*, 24,73–80.

Frey, D. (1971) Isolation of *Microsporum cookie* from a human case. *Sabouraudia*, 9,146-148.

Frey, D., Oldfield, R.J. and Bridger, R.C. (1979) A Colour Atlas of Pathogenic Fungi. Wolfe Medical Publications Ltd.

Fuentes, C. A. (1956) A new species of *Microsporum*. *Mycologia*, 48, 613–614.

Fulton, J. E. (1975). Miconazole therapy for endemic fungal diseases. *Archives of Dermatology*, 111, 596–598.

Gambale, W., Correa, B., Rodriguez, P.C., Purchio, A. and Larsson, C.E. (1987) Occurencia de fungus em Lasoes Superficiales de caes na cidade de Sao Paulo, Brazil. *Review de faculty de Medicine, Veterinaire Zootec University of Sao Paulo,* 24,187-191

Gentles, J.C. and O'Sullivan, J.G.(1957) Correlation of human and animal ringworm in West of Scotland. *British Medical Journal*, 2 (5046),678-682.

Gentles, J. C. (1958) Experimental ringworm in guinea pigs: oral treatment with griseofulvin. *Nature*, (London) 182, 476.

Gentles, J.C. (1962). Keratinophilic fungi and diseases caused by them. In: Dalldorf, G. (Ed.) Fungi and fungous diseases. Charles C. Thomas, Illinois: 11-21.

Georg, L. K. (1952) *Trichophyton tonsurans* ringworm—a new public health problem. *Public Health Reports*, 67, 53–56.

Georg, L. K. (1952) Cultural and nutritional studies of *Trichophyton gallinae* and *Trichophyton megninii*. *Mycologia*, 44, 470–492.

Georg, L. K. (1960) Epidemiology of the dermatophytoses: sources of infection, modes of transmission and epidemicity. *Annals of New York Academy of Science*, 89, 69– 77.

Georg, L. K., and. Camp, L. B. (1957) Routine nutritional tests for the identification of dermatophytes. *Journal of Bacteriology*, 74, 113–121.

Georg, L. K., Ajello, L., Friedman, L., and Brinkman, S.A. (1962) A new species of *Microsporum* pathogenic to man and animals. *Sabouraudia,* 1,189–196.

Georg, L.K. (1956) The role of animals as vectors of human fungus diseases. *Transcripts of NewYork Academy of Science*, 18, 639-647.

Gong, J.Q., Liu, X.Q., Xu, H.B., Zeng, X.S., Chen, W. and Li, X.F. (2006) Deep dermatophytosis caused by *Trichophyton rubrum*: Report of two cases. *Mycoses,* 50,102–8.

Grappel, S. F. and Blank, F. (1972) Role of keratinases in dermatophytosis. I. Immune responses of guinea pigs infected with *Trichophyton mentagrophytes* and guinea pigs immunized with keratinases. *Dermatologica,* 145, 245–255.

Grappel, S.F., Bishop, C.T. and Blank, F. (1974) Immunology of dermatophytes and dermatophytosis. *Bacteriology Reviews,* 38,222–50

Green F., Lee, K.W. and Balish, E. (1982) Chronic *T. mentagrophytes* dermatophytosis of guinea pig skin grafts on nude mice. *Journal of Investigative Dermatology,* 79, 125

Griffin, D. N. (1960) The re-discovery of *Gymnoascus gypseum*, the perfect state of *Microsporum gypseum*, and a note on *Trichophyton terrestre*. *Transcripts of British Journal of Dermatology,* 43,637–641

Griffin, D. N. (1960) The re-discovery of *Gymnoascus gypseum*, the perfect state of *Microsporum gypseum*, and a note on *Trichophyton terrestre*. *Transcripts of British Mycology Society,* 43,637–641.

Gruby, D. (1841). Me´moire sur une ve´ge´tation qui constituent la vraie teigne. *Archives de parasitilogie* 13, 72–75.

Gruby, D. (1841). Sur les mycodermes qui constituent la teigne faveuse. *Archives de parasitilogie* 13,309–312.

Gruby, D. (1843). Recherches sur la nature, le sie´ge et le developpement du porrigo decalvans ou phytoalpe´cie. *Archives de parasitilogie,* 17,301–302.

Gruby, D. (1844) Recherches sur les cryptogames qui constituent la maladie contagieuse du cuir chevelu de´crite sous le nom de Teigne tondante (Mahon), *Herpes tonsurans* (Cazenave).*Archives de parasitilogie*,18,583–585..

Gugnani, H.C. (1982) Mycosis as a Public Health Problem in Nigeria. *Nigerian Journal of Microbiology,* 2, 47

Hainer, B.L. (2003) Dermatophyte infections. *American Family Physician*, 67, 101-108, 20

Hasegawa, A., and Usui, K. (1975) Isolation of *Trichophyton equinum* and *Microsporum canis* from equine dermatophytosis. *Japan Journal of Medical Microbiology*, 19, 11-13.

Hay, R. J.,. Clayton, M., Howell, S.A. and Noble, W. C. (1988) Management of combined bacterial and fungal foot infection in coal miners. *Mycoses*, 31, 316–319.

Hay, R. J., Reid, S., Talwat, E. and K. Macnamara (1983). Immune responses of patients with tinea imbricata. *British Journal of Dermatology*, 108, 581–586.

Hay, R. J. and Clayton, Y.M. (1982) Treatment of chronic dermatophyte infections. The use of ketoconazole in griseofulvin treatment failures. *Clinical and Experimental Dermatology,* 7, 611–617.

Hay, R.J. and Moore, M.K. (2004) ‘Mycology’ in textbook of Dermatology. In: Burns T, Breathnach S, Cox N, Griffiths C, editors. 7th ed. Vol. 2. United States: Blackwell Oxford

Hay, R.J., Robles, W., Midgley, G. and Moore, M.K. (2001) Tinea capitis in Europe: new perspective on an old problem. *Journal of European Academy of Dermatology and Veneorology,* 15, 229-233.

Hazen ,K. C.,. Moon, A. E. and Atillasoy, E. S. (2000) Program Abstr. 40th Intersci. Conf. *Antimicrobial Agents Chemotherapy*. abstr. 204,

Hebert, A. A., Head, E. S. and Macdonald, E. M. (1985) Tinea capitis caused by *Trichophyton tonsurans. Pediatric Dermatology,* 2, 219–223.

Hejtma´nek, M., and Lenhart, K. (1972) Genetic complementation of virulence in avirulent mutants of *Microsporum gypseum*. *Folia Biologica,* (Prague) 18, 225–230.

Hejtma´nek, M., and K. Lenhart,(1970) The genetic basis of virulence in dermatophytes. *Folia Biologica*, (Prague) 16, 363–366.

Hejtma´nek, M., Hejtma´nkova, N., Kunert, J., Lenhart, K. and. Weigl, E. (1992) Genetics of dermatophytes. *Acta Universiti Palacki. Olomuc. Faculty Medica Supplimenta,* 19,5–611.

Helander, I. (1978) The lymphocyte transformation test in dermatophytosis. *Mykosen,* 21, 71–80.

Henrich, T.J., Marty, F.M., Milner, Jr. D.A., Thorner, A.R. (2009). In Disseminated *Geotrichum candidum* infection in a patient with Relapsed acute Myelogous Leukamia following Allogeneic stem cell Transplantation and review of the Literature. *Transplantation Infectious Diseases;* 11,458-462

Hernandez-Perez, E. (1984) Bifonazole cream: once-a-day application every second day in tinea cruris and tinea corporis. *Dermatologica,* 169(1), 93–98.

Hironaga, M., Nozaki, K. and Watanabe, S.. (1980). Ascocarp production by *Nannizzia otae* on keratinous and non-keratinous agar media and mating behaviour of *N. otae* and 123 Japanese isolates of *M. canis*. *Mycopathologia*, 72, 135–141

Howard, D. H. (1983) Ascomycetes: the dermatophytes. In: Howard, D. H. (Ed) Fungi Pathogenic for Humans and Animals. Part A (*Biology*). Marcel Dekker, inc, NY, 113-147

Ishii, M., T. Hamada, Y. Asai (1983) Treatment of onychomycosis by ODT therapy with 20% urea ointment and 2% tolnaftate ointment. *Dermatologica*, 167, 273–279.

Ishizaki, H. (1993) Fungal taxonomy based on mitochondria DNA analysis. *Japan Journal of Medical Mycology*, 34, 243–251.

Issa, N.A. and Zangana, I. K. (2009) Isolation of *T. mentagrophytes* from naturally infected laboratory albino rats: experimental infection and treatment of rabbits. *Iraqi Journal of Veterinary Science*, 23(2), 29-34

Jagdish, C. (1995) Dermatophytoses. *Medical Mycology*, 1st Etdition, 106-107.

Jessup, C. J., Warner, J. , Isham, N. , Hasan, I. and Ghannoum, M. A. (2000) Antifungal susceptibility testing of dermatophytes: establishing a medium for inducing conidial growth and evaluation of susceptibility of clinical isolates. *Journal of Clinical Microbiology*, 38, 341–344.

Jones, H. E., Reinhardt, J. H. and Rinaldi, M. G. (1974) Model dermatophytosis in naturally-infected subjects. *Archives of Dermatology*, 11, 369-374

Jones, H.E. (1993) Immune response and host resistance of humans to dermatophyte infection. *Journal of American Academy of Dermatology*, 28, S12–18.

Jones, H.E., Reinhardt J.H. and Rinaldi, M.G. (1974) Acquired immunity to dermatophytosis. *Archives of Dermatology*, 109, 840 - 8.

Kaaman, T. and Torssander, J. (1983) Dermatophytid – A misdiagnosed entity? *Acta Dermatology et Venereol*ogy, 63, 404–8

Kaben, U. (1967) Verbreitung und epidemiologie der dermatomykosen tierischen ursprung. *Mykosen*, 10, 47-60.

Kaduna State Government (KDSG) Retrieved Febuary 23, 2014 from http://www.kadunastate.gov.ng

Kaminski, G. W. (1985) The routine use of modified Borelli's lactritmel between *Microsporum ferrugineum* Ota and *Trichophyton soudanense* Joyeux.

Kane J. A., Padhye, A., and Ajello, L. (1982) *Microsporum equinum* in North America. *Journal of Clinical Microbiology,* 943-947

Kane, J., and Smitka, C. M. (1980). A practical approach to the isolation and identification of members of the *Trichophyton rubrum* group. *Pan American Health Organization of Science Publication,* 396, 121–134.

Kane, J., and Fischer, J. B. (1975) Occurrence of *Trichophyton megninii* in Ontario: identification with a simple cultural procedure. *Journal of Clinical Microbiology,* 2, 111–114.

Kane, J., and. Fischer, J. B. (1975) The effect of sodium chloride on the growth and morphology of dermatophytes and some other keratolytic fungi. *Canadian Journal of Microbiology*, 21, 742–749.

Kane, J., Leavitt, E., Summerbell, R. C. , Krajden, S. S. and Kasatiya, S.S. (1988) An outbreak of *Trichophyton tonsurans* dermatophytosis in a chronic care institution for the elderly. *European Journal of Epidemiology,* 4, 144–149.

Kane, J., Salkin, I.F., Weitzman, I. and Smitka, C.M. (1981). *Trichophyton raubitschekii* sp. nov. *Mycotaxon,* 13, 259–266.

Kane, J., J. B. Fischer (1973) The influence of sodium chloride on the growth and production of macroconidia of *Trichophyton mentagrophytes. Mycopathology and Mycology Applications,* 50, 127–143.

Kane, J., Sigler, L. and Summerbell, R. C. (1987) Improved procedures for differentiating *Microsporum persicolor* from *Trichophyton mentagrophytes*. *Journal of Clinical Microbiology,* 25, 2449–2452.

Kane, J., Krajden, S., Summerbell, R. C. and Sibbald, G. (1990) Infections caused by *Trichophyton raubitschekii*: clinical and epidemiological features. *Mycoses*, 33, 499–506.

Kano, R., Hirai, A., Yoshiike, M., Nagata, M., Nakamura, Y., Watanabe, S. and Hasegawa, A. (2002) Molecular identification of *Trichophyton rubrum* isolate from a dog by chitin synthase1 (CHS1) gene analysis. *Medical Mycology*, 40, 439–42.

Kaplan, W., Gump, R. H. (1958) Ringworm in Dogs caused by *T. rubrum. Veterinary Medicine*, 53,139-142

Katarzyna, W., Krzysztof. K., and Iwona, T. (2013) Specific cell-mediated immune response in rabbits immunised and infected with *Trichophyton mentagrophytes*. *Bulletins of Veterinary Institute Pulaway*, 57, 329-333.

Kaul, S. and Sumbali, G. (1997) Keratinolysis by poultry farm soil fungi. *Mycopathologia*, 139, 137-140.

Keller, M., Krehon, S., Stanek, C., and Rosengarten, R. (2000) Keratinopathogenic mould fungi and dermatophytosis in healthy and diseased hooves of horses. *Veterinary Records*, 147, 619-622

Kerbs S., Greenberg, J. and Jesrani, K. (1977) "Temporal correlationof lymphocyte blastogenesis, skin test responses and erythema during dermatophyte infections," *Clinical and Experimental Immunology*, 27(3), 526–530.

Khosravi ,A.R., and Mahmoudi, M. (2003). Dermatophytes isolated from domestic animals in Iran. *Mycoses*, 46(5-6), 222-225.

King, R.D., Khan, H.A., Foye, J.C., Greenberg, J.H., Jones, H.E. (1975) Transferrin, iron and dermatophytes.Serum dermatophyste inhibitory component definitely identified as unsaturated transferrin. *Journal of Laboratory Clinical Medicine*, 86, 204–12

Kligman A.M. (1952) The pathogenesis of Tinea capitis due to *Microsporum audouini* and *Microsporum canis*. I. Gross observations following the inoculation of humans. *Journal of Investigative Dermatology*, 18(3), 231–246

Kligman, A. M. (1955) Tinea capitis due to *M. audouinii* and *M. canis*. Archives of Dermatology, 71, 313–348.

Kligman, A. M. (1956) Pathophysiology of ringworm infections in animals with skin cycles. *Journal of Investigative Dermatology*, 27, 171–185.

Kligman, A. M., and E. R. Constant (1951) Family epidemic of tinea capitis due to *Trichophyton tonsurans* (variety sulfureum) *Archives of Dermatology*, 63,493– 499.

Klokke, A. H., Purushotham, A. G. and Sundaraju, D. (1966) Mass treatment of non-fluorescent tinea capitis in an orphanage in India. Trial with a low griseofulvin dose using a modified brush-sampling technique. *Tropical Geography and Medicine,* 18,305–309.

Knight, A. G. (1972) A review of experimental human fungus infections. *Journal of Investigative Dermatology*, 59, 354–358.

Kuwano, A., Oikawa, M., and Takatori, K. (1996) Pathomorphological findings in a case of onychomycosis in a Race horse. *Journal of Veterinary Medical Science,* 58, 1117-1120

Kwon-Chung, K. J. (1967) Genetic study on the incompatibility system in Arthroderma simii. Sabouraudia 10:74–78. 152. Kwon-Chung, K. J. 1974. Genetics of fungi pathogenic for man. *Critical Reviews in Microbiology*, 3,115–133.

Kwon-Chung, K. J. and Bennett, J. E. (1992) Medical mycology. Lea and Febiger, Philadelphia.

La Touch, C.J. (1955) The importance of the animal reservoir in the epidemiology of animal-type ringworms in man. *Veterinary Record*, 67,666

Larone, D.H. (1995) Medically important fungi: A guide to identification, 3rd Edition, American Society for Microbiology

Lehenkari, E. and Silvennoinen-Kassinen, S. (1995) Dermatophytes in northern Finland in 1982 to 1990. *Mycoses*, 38(9-10), 411-14

Lenhart, K. (1970) Griseofulvin resistence in dermatophytes. *Experientia,* 26, 109–110. 161.

Lepper, A. W. D. (1974) Experimental bovine *Trichophyton verrucosum* infection. The cellular responses in primary lesions of the skin resulting from surface or intradermal inoculation. *Researches in Veterinary Science*, 16, 287–298.

Londero, A.T., and Ramus, C.D. (1980) A twenty Year Survey of Dermatophytosis in the state of Reo Grande Do Sul, Brazil. In International Conference on the Mycosis, 5^0. Washington D.C.PAHO, *Scientific Bulletin*, 396

Lopes, J.O., Salebain, A., Alves, S.H. and Benevenga, P.J. (1994) First case of Human Infection By *T. vanbreuseghemii* in Brazil. *Revista instuto de medicina Tropical de Sao Paulo*, **36**(4), 379-380

Lorincz, A.L., Priestley, J.O. and Jacobs, P.H. (1958) Evidence of a humoral mechanism which prevents growth of dermatophytes. *Journal of Investigative Dermatology*, 31, 15–7

Lundell E. (1965) *Microsporum cookei Ajello*, An eczematous skin lesion. *Mykosen*, 12, 123-126.

Mackenzie D.W.R. (1963) “Hairbrush diagnosis” in detection and eradication of non-fluorescent scalp ringworm. *British Medical Journal*, 2, 363-365.

Mackenzie, D. W. R. (1961) The extra-human occurrence of *Trichophyton tonsurans var. sulfureum* in a residential school. *Sabouraudia*, 1, 58–64.

Macura, A. B. (1993). *In vitro* susceptibility of dermatophytes to antifungal drugs: a comparison of two methods. *International Journal of Dermatology*, 32, 533–536.

Macura, A.B. (1993) Dermatophyte Infections. *International Journal of Dermatology*, 32, 313

Mantovani, A. (1978) The role of animals in the epidemiology of mycoses. *Mycopathologia*, 65, 61-66

Marill, F.G., Liautaud, B., Hamra-Krouha, M.S. (1975) Fatal evolution of a dermatophytic disease due to *Trichophyton schönleini*. *Bulletins of Sociology Pathology and Exotic Filiales*, 68, 450–6.

Marples, M.J. and Smith, J.M.B. (1960) The hedgehog as a source of human ringworm. *Nature*, 188, 867

Marples, M. J. (1965) The distribution of keratinophilic fungi in soils from New Zealand and from two Polynesian Islands. *Mycopathology Mycology Applications*, 25, 361-372.

Matsumoto, T. and L. Ajello (1987) Current taxonomic concepts pertaining to the dermatophytes and related fungi. *International Journal of Dermatology*, 26, 491–499.

Mayr, A. (1989). Infections which humans in the household transmit to dogs and cats. *Zentralbl Bakteriol Mikrobiol Hygeous*, 187, 508–526.

Mbata, T. I. (2009) Dermatophytes and other skin mycoses found in featherless broiler toe webs. *Sudanese Journal of Public Health*, **4**(4), 309-402

Medleau, L. and Rakich, P.M., (1994) *Microsporum canis* pseudomycetomas in a cat. *Journal of American Animal Hospitals Association*, 30, 573-576.

Menan, E. I., O. Zong-Bonou, F. Rouet, P. C. Keke-Barro, W. Yaro, F. N. N'Guessan and M. Kono, (2002) Tinea Capitis in School Children From Ivory Coast (Western Africa). A 1998-1999 cross-sectional study. *International Journal of Dermatology*, 41, 204-207

Mignon B, Tabart J, Baldo A, Mathy A, Losson B, Vermout S.(2008) Immunization and dermatophytes. *Current Opinions in Infectious Diseases*, 21, 134-40.

Mignon, B.R., Leclipteux, T., Focant, C.H., Nikkels, A.J., Pierard, G.E. and Losson, B.J. (1999). Humoral and cellular immune response to a crude exoantigen and purified keratinase of *Microsporum canis* in experimentally infected guinea pigs. *Medical Mycology*, 37, 123–129.

Miyasata, H., Yamaguchi, S., Taira, K., Kosokawa, A., Kayo, S., Sano, A., Uezato, H., Takahashi, K. (2011) Tinea coporis caused by *M. gallinae*, first clinical case in Japan. *Journal of Dermatology,* 38, 473-478

Moore, C.B., Sayers, N., Mosquera, J., Slaven, J., Denning, D.W. (2000) Antifungal drug resistance in *Aspergillus*. *Journal of Infection,* 41, 203-220.

Moriello, K. A. and DeBoer D.J. (1991) Fungal flora of the coat of pet cats. *American Journal of Veterinary Research*, 52, 602–6.

Mosher W.A., Saunders D.H., Kingery L.B., Williams, R.J. (1936) Nutritional requirements of the pathogenic mold *Trichophyton interdigitale. Plant Physiology*, 11,795–806

Mukesh, S. and Meenakshi, S. (2011) Influence of culture media on mycelial growth and sporulation of some soil dermatophytes compared to their clinical isolates. *Journal of Microbiology and Antimicrobials,* 3(8), 196-200

National Committee for Clinical Laboratory Standards (1997). Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard NCCLS document M27-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.

Ndako, J.A, Osemwegie, O.O, Spencer T.H.I., Olopade, B.K., Yunusa, G.A., and Banda, J . (2012) Prevalence of dermatophytes and other associated Fungi among school children *Global Advanced Research Journal of Medicine and Medical Sciences*, 1(3)49-56

Nir-Paz, R., Elinav, H., Pierard, G.E., Walker, D., Maly. A., Shapiro, M. (2003) Deep infection by *T. rubrum* in an immunocompromised patient. *Journal of Clinical Micrbiology*, 41:5298–301

Norris, H. A., Elewski, B . E. and Ghannoum, M. A. (1999). Optimal growth conditions for the determination of the antifungal susceptibility of three species of dermatophytes with the use of a microdilution method. *Journal of American Academy of Dermatology*, 40:S9–S13.

Nweze, E. I. (2001) Aetiology of Dermatophytosis among school children in north eastern Nigeria. *Medical Mycology,* 39,181-184

Nweze, E.I. (2010) Dermatophytosis in Western Africa: A Review. *Pakistan Journal of biological sciences,* 13,496-656.

Nweze, E.I. (2011) Dermatophytosis in domesticated animals. *Revista instuto de medicina Tropical de Sao Paulo,* 53(2), 95-99

Nweze, E.I.(2001) Aetiology of dermatophytoses amongst children in northeastern Nigeria. *Medical Mycology*, 39, 181–4.

Nweze, E.I., Ogbonna, C.C., and Okafor, J.I. (2007) *In vitro* susceptibility testing of dermatophytes isolated from pedriatic cases in Nigeria against five antifungals. *Revista instuto de medicina tropica deSao Paulo,* 49(5),293-295

Odds, F. C. (1991) Sabouraud's agar. *Journal of Medical and Veterinary Mycolology*, 29, 355–359.

Oyeka, C.A. (2000) *T. mentagrophytes* a keratinophylic Fungus . *Revista Iberoamericana de Micología, Apdo.* 699, E-48080 Bilbao (Spain)

Oyeka, C.A., Ugwu, L.O. (2002) Fungal flora of human toe webs. *Mycoses*, 45, 488–91.

Ozegovic, L. (1980) Wild animals as reservoirs of human pathogenic dermatophytes. *Zentralbl Bakteriol Mikrobiol Hygeous*, 8 ,369–380.

Padhye A.A., Connole M.D. (1994) Advances in Veterinary Mycology. *Journal of Medical and Veterinary Mycology*, 32, 169–187.

Padhye, A. A., and Weitzman, I. (1994) An unusual variant of *Trichophyton tonsurans var*. *sulfureum*. *Journal ofMedical and Veterinary Mycology*, 32, 147–150.

Padhye, A. and Carmicheal A. (1968) epidemic of infection with *Trichophyton tonsurans* revealed in a 20 year survey of fungal infections in Chicago. *Journal of American Academy of Dermatology*, 8, 322–330.

Pascoe, R.R. (1979) The epidemiology of ringworm in racehorses caused by *Trichophyton equinum var autotrophicum*. *Australian Veterinary Journal*, 55(9), 403-407

Pfaller M.A. (2008) *New developments in the antifungal susceptibility testing of Candida*. *Current Fungal Infections Reports*, 2, 125-133

Pfaller, M.A., Rinaldi, M.G. and Galgiani, J.N., (1990) Collaborative investigation of variables in susceptibility testing of yeasts. *Antimicrobial Agents Chemotherapy,* 34, 1648-1654.

Philpot, C. M. (1967) The differentiation of *Trichophyton mentagrophytes* from *Trichophyton rubrum* by a simple urease test. *Sabouraudia*, 5,189–193.

Philpot, C.M. (1977) Use of nutritional tests for the differentiation of dermatophytes. *Sabouraudia*, 5(2), 141- 150.

Pier, A.C., Hodges, A.B., Lauze, J.M., Raisbeck, M. (1995) Experimental immunity to *Microsporum canis* and cross reactions with other dermatophytes of veterinary importance. *Journal of Medical and Veterinary Mycology*, 33, 93–97.

Pier, A.C., Smith, J.M.B., Alexiou, H., Ellis, D.H., Lund, A. and Pritchart, R.C. (1994) Animal Ringworm- its aetiology, Public Health significance and control. *Journal of Medical and Veterinary Mycology,* 32 (1), 133-150

Pier, A.C. and Zancanella, P.J. (1993) Immunization of horses against dermatophytosis caused by *Trichophyton equinum*. *Equine Practice*, 15, 23–27.

Pipkin, J. L. (1952) Tinea capitis in the adult and adolescent. *Archives of Dermatology*, 66:9–40. 191.

Po¨nnighaus, J.M., Clayton, Y. and Warndorff, D. (1996). The spectrum of dermatophytes in northern Malawi (Africa). *Mycoses,* 39:293–7.

Quinn ,P.J. and Markey, R.K. (2003) Concise Review of Veterinary Microbiology. Blackwell Publishing Oxford, United Kingdom. Pp. 74-75.

Radovic-Kovacevic, V., Ratkovic, R., and Milenkovic, A. (1990) Obytin in the treatment of superficial skin mycoses. *Medianski Pregled*, 43, 319–331.

Rambali, B., Fernandez, J.A., and Van Nuffel, L., (2001). Susceptibility testing of pathogenic fungi with itraconazole: a process analysis of test variables. *Journal of Antimicrobial Chemotherapy*, 48, 163-177.

Raymond, R. and Pihet, M.(2008). Conventional methods for the diagnosis of Dermatophytes *Mycopathologia*, 166, 295-306

Rebell, G., and D. Taplin. (1970) Dermatophytes, their recognition and identification. University of Miami Press, Coral Gables, Fla.

Remak, R. (1845). Diagnostische und pathogenetische Unterschungen in der Klinik des Herrn Geh. Raths Dr. Schoe¨nlein auf dessen Veranlassung porrigo decalvans ou phytoalpe´cie. *Archives de parasitilogie*, 17, 301–302.

Reppas, G.P. and Snoeck, T.D. (1999) Cutaneous geotrichosis in a dog. *Australian Veterinary Journal*, 77,513

Rhyan, J.C., Stackhouse, L.L. and Davis, E.G. (1990) Disseminated Geotrichosis in two dogs. *Journal of American Veterinary Association,* 197,358-360

Richard J.L., Bebey M.C., Chermette R., Pier A.C., Hasegawa A., Lund A. and Bratberg A.M. (1994) The cellular responses in primary lesions of the skin resulting from surface or intradermal inoculation. *Researches in Veterinary Science, 16, 287*–298.

Riddell, R. W. (1950) Permanent stained mycological preparation obtained by slide culture. *Mycologia*, 42, 265-270.

Rippon, J. W. (1982) Medical mycology: the pathogenic fungi and the pathogenic actinomycetes, 2nd ed.,P p. 154–248. W. B. Saunders Co., Philadelphia.

Rippon, J. W. (1985) The changing epidemiology and emerging patterns of dermatophyte species. *Current Topics in Medical Mycology,* 1,209–234.

Rippon, J. W. (1988) Medical Mycology. The pathogenic fungi and the pathogenic actinomycetes, 3rd ed. W. B. Saunders, Philadelphia.

Rippon, J. W. (1992). Forty-four years of dermatophytes in the Chicago clinic (1944–1988). *Mycopathologia* , 119, 5–28.

Rippon, J.W. and McGinnis, M.R. (1995) Current Topics in Medical Mycology. New York: *Springer-Verlag*. The changing epidemiology and emerging patterns of dermatophyte species; Pp. 209–34.

Rippon, J.W. and Scherr, G.H. (1959) Induced dimorphism in dermatophytes. *Mycologia*, 51, 902-914.

Robert, B.J. and Friedlander, S.F. (2005) Tinea capitis: a treatment update. *Pediatrics Annals*, 34, 191-200.

Roberts, D.T., Taylor, W.D. and Boyle, J. (2003) Guidelines for treatment of onychomycosis. *British Journal of Dermatology,* 148, 402–10.

Rodriguez-Tudela, J.L., Chryssanthou, E., Petrikkou, E., Mosquera, J., Denning, D.W. and Cuenca-Estrella, M. (2003) Interlaboratory evaluation of hematocytometer method of inoculum preparation for testing antifungal susceptibilities of filamentous fungi. *Jounal of Clinical Microbiology,* 41, 5236-5237.

Rosenthal, S. A., and Sokolsky, H. (1965) Enzymatic studies with pathogenic fungi. *Dermatology Interests*, 4,72–79.

Rubben, A. and Krause, H. (1996) Tines superficialis due to *Trichophyton soudanense* in African immigrants. *Mycoses*, 39, 397-398

Rybnikar, A., Vrzal, V. and Chumela, J. (1998) Protective efficacy of vaccines against bovine dermatophytosis after double and single vaccination. *Mycoses*, 41, 83–86.

Rycroft, A.X. and Mclay, C. (1991) Disinfectants in the control of small animal ringworm due to *Microsporum canis*. *Veterinary Records*, 129, 239–41.

Sadegh, N., Sisakht, A. R., Matehkolaei, M. A., Mohammad, J.N., Kazou, S., Bahram, A. and Hosseinpour, L. (2013) *M. fulvum*, an Ignored Pathogenic Dermatophyte; A new Clinical Isolation from Iran. *Mycopathologia,* **176**, 157-160

Samdani, A. J. (2005) Dermatophyte growth and degradation of human stratum corneum *in vitro* (pathogenesis of dermatophytosis) *Journal of Ayub Medical College Abbottabad,* 17,19–21.

Samson, S.Y.W., Antonio, H.Y.N., Christopher, M.R., Jade, L.L.T., Garnet, K.Y.C., Rosana, W.S.P., Janet, J.Y.H., Frank, J L., Alex, L. and Kwok-Yung, K. (2012) Brittle Tail Syndrome is an emerging infection in horses caused by the fungus *Equicapillimyces hongkongensis*. *Veterinary Microbiology*, 155,399-408

Sandy, V. (2008) Pathogenesis of dermatophytosis. *Mycopathologia*, 166, 267–75.

Santos, D.A., Hamada J. S. (2005) Evaluation of broth microdilution antifungal susceptibility testing condition for *T. rubrum*. *Journal of clinical Microbiology*, **43**, 1917-1920

Schmeller, W., Baumgartner, S., Dzikus, A. (1997) Dermatophytomycoses in children in rural Kenya: the impact of primary health care. *Mycoses*, **40**, 55–63.

Schoenlein, J. L. (1839). Zur Pathogenie der Impetigines. *Archives of Anatomie Physiology*, Pp. 82.

Scott, D. B. and Scott, F. P. (1972) Dermatophytoses in South Africa. *Sabouraudia*, **11**, 279–282.

Scott, D. W. (1988) Superficial Mycosis in: Pedeson D.(ed). *Large Animal Dermatology*, W.B.Saunders Campany, Harcourt Brace Javanich Inc.pp, 172-185

Scott, D. W., Miller, W.H., Griffin, C.E. (2001) Fungal skin diseases in; Miller G.H., Kirk R.W. (eds), *Small Animal Dermatology, 6th ed.* Saunders, Philadelphia, Pp339-361

Seeliger, H. P. R. (1985) The discovery of *Achorion schöenleinii*: facts and ''stories.'' *Mykosen,* 28, 161–182.

Sequeira, H., Cabrita, J., De Vroey, C. and Wuytack-Raes, C. (1991) Contributions to our knowledge of *Trichophyton megnini. Journal of Veterinary Mycology,* 29,417-8

Shah, P. C., Krajden, S. , Kane, J. and. Summerbell, R. C (1988) Tinea corporis caused by *Microsporum* canis: report of a nosocomial outbreak. *European Journal of Epidemiology,* 4, 33–38.

Sharma, M. (1983) Taxonomical, Physiological and Para-clinical studies of fungi causing skin and other infections in human beings. Ph. D. Thesis, Department of Botany, University of Rajasthan, Jaipur, India.

Sharma, M., M. Sharma, (2011) Influence of culture media on mycelial growth and sporulation of some soil dermatophytes compared to their clinical isolates. *Journal of Microbiology and Antimicrobials,* 3(8), Pp. 196-200.

Shathele, M. S. (2014) Mycological Studies of *Trichophyton mentagrophyes* var. *mentagrophyes* causing ringworm in horses in Al Ahsa Province, Kingdom of Saudi Arabia. *Journal of Medicine and Medical Sciences*, 5(8) Pp. 157-161.

Shimmura, Y. (1985) Isolation of dermatophytes from human cases of dermatophytosis and from house dust. *Japan Journal of Medical Mycology*, 26, 74–80.

Shimozawa, K., Anzai, T., Kamada, M. and Takatori, K. (1997) Fungal and Bacterial Isolation from Racehorses with Infectious dermatosis. *Journal of Equine Science*, 8(4), 89-93.

Sidhu, R.K., Singh, K.B. J. and Joshi, D.V. (1993) Cutaneous geotrichosis in a dog and its handlers- a case report. *Indian Journal of Animal Health*, *32*, 75

Siesenop, U. and Bohm, H. (1995) Comperative studies on keratinase production of *Trichophyton mentagrophytes* strains of animal origin. *Mycoses*, 38(5-6), 205-209

Silva, M., R. W. Benham (1952) Nutritional studies of the dermatophytes with special reference to *Trichophyton megninii*. Blanchard 1890 and *Trichophyton gallinae* (Megnin. 1881) comb nov. *Journal of Investigative Dermatology*, 18, 453–472.

Simpanya, M.F.,and Baxter, M. (1996) Isolation of fungi from soil using the keratin baiting technique. *Mycopathologia*, 136, 85-89

Simpanyan, M.F., and Baxter, M. (1996) isolation of Fungi from the pelage of cats and dogs using hairbrush technique. *Mycopathologia*, 134, 129-133

Singh, K. V. (1983) Radial growth of soil inhabiting keratinophilic fungi and related dermatophytes on various media and at different temperatures. *Geobios*, 10(6), 284-286.

Sinski, J. T., Flouras, K. (1984). A survey of dermatophytes isolated from human patients in the United States from 1979 to 1981 with chronological listings of worldwide incidence of five dermatophytes often isolated in the United States. *Mycopathologia*, 85, 97–120.

Snider, R., Landers, S. and Levy, M. L. (1993) The ringworm riddle: an outbreak of *Microsporum canis* in the nursery. *Pediatrics Infectious Disease Journal,* 12, 145–148.

Sohnle P.G. (1989) Dermatophytosis. In: Cox RA, editor. Immunology of fungal diseases. Florida: CRC press Pp 1.

Sparkes, A.H., Werrett, G., Stokes, C.R., Gruffyd-Jones, T. J .(1994). Microsporum canis: inapparent carriage by cats and the viability of arthrospores. *Journal of Small Animal Practice,* 35, 397-410

Sparkes, A.H., Werrett, G., Stokes, C.R. and Gruffyd-Jones, T.J. (1994). *Microsporum canis:* inapparent carriage by cats and the viability of arthrospores. *Journal of Small Animal Practice,* 3, 5:397-401.

St-Getmain, G. and Summerbell, R. (1996) Identifying filamentous fungi: A clinical laboratory handbook. Star Publishing, Belmont.

Stiller, M. J., W. P. Klein, R. I. Dorman, and S. A. Rosenthal. (1992) Tinea corporis gladiatorum: an epidemic of *Trichophyton tonsurans* in student wrestlers. *Journal of American Academy of Dermatology*, 27, 632–633.

Stockdale, P. M. (1953) Nutritional requirements of dermatophytes. *Biology Reviews*, 28, 84–104.

Stockdale, P. M. (1961) *Nannizzia incurvata* gen. nov., sp. nov., a perfect state of *Microsporum gypseum* (Bodin) Guiart et Grigorakis. *Sabouraudia,* 1, 41– 48.

Stockdale, P. M. (1963). The *Microsporum* gypseum complex (Nannizzia in- curvata Stockd., *N. gypsea* (Nann) comb. nov., *N. fulva sp.* nov.) *Sabouraudia,* 3, 114–126.

Stockdale, P. M. (1968) Sexual stimulation between *Arthroderma simii* Stockdale, Mackenzie and Austwick and related species. *Sabouraudia*, 6, 176–181.

Straff, W., Spiewak, R., Poblete, P. and Frank, J. (2001) Zoophile Onychomykose durch *Trichophyton gallinae*. *Zoologia Hautkri*l, 76 (12), 749-750.

Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST) (2008). EUCAST Technical Note on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia-forming moulds. *Clinical Microbiology Infections*, 2008, 14, 982-984.

Summerbell, R. C. (1987). *Trichophyton kanei*, sp. nov., a new anthropophilic dermatophyte. *Mycotaxon*, 28, 509–523

Summerbell, R. C., Kane, J., Krajden, S. (1989) Onychomycosis, tinea pedis, and tinea manuum caused by non-dermatophytic filamentous fungi. *Mycoses*, 32, 609–619.

Summerbell, R. C., Rosenthal, S. A. and Kane, J. (1988). Rapid method of differentiation of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and related dermatophyte species. *Journal of Clinical Microbiology*, 26, 2279–2282.

Swartz, H. E., and L. K. Georg (1955) The nutrition of *Trichophyton tonsurans*.*Mycologia* 47, 475–493

Tanaka, S., R. C., Summerbell, R., Tsuboi, T., Kaaman, P. G., Sohnle, T. Matsumoto, and Ray, T. L. (1992) Advances in dermatophytes and dermatophytosis. *Journal of Medical and Veterinary Mycology*, 30(1), 29–39.

Terreni, A.A., Gregg, W.B. Jr, Morris, P.R. and DiSalvo, A.F. (2007) *Epidermophyton floccosum* infection in a dog from the United States. *Sabouraudia*, 23, 141–2.

Theodore, C.W., Brian, G.O., Yvonne, G. and Matthew, R.H. (2008) Generating and testing molecular hypotheses in the dermatophytes. *Eukaryotic Cell*, 7(8), 1238-1245.

Ural, K., Yağc, B. and Ocal, N. (2009) Cellular enzyme values in hunter/jumper and dressage horses with dermatophytosis. *Archives of Brasil Medical and Veterinary Zootechnology,* 61, 1233-1237

Vanbreuseghem, R. (1950) Contribution à l'étude des dermatophytes du Congo Belge: Le Sabouraudites (*Microsporum) langeroni* nov. sp. *Annals of Parasitology and Human Companions,* 25, 509-517.

Vanbreuseghem, R. (1952) Technique biologique pour l'isolement des dermatophytes du sol. *Annals et Societe de Belge Médicines et Tropicales*, 32,173-177.

Vanbreuseghem, R. (1963) Dermatomycosis; fait-il distinguer *M. langeroni* et *M. audounii*? *Bull sociology Fr Syphilis*; 70:34-37, J. W. Mating reactions of *T. simii* and *T. mentagrophytes* strains from poultry farm soil in India. *Sabouraudia*, 2, 100-107

Vanbreuseghem, R. (1963) Dermatophytes from the republic of the Congo and Ruanda-Burundi. New description of *M. rivalieri*. *Sabouraudia*, 2, 215-224

Vanbreuseghem, R., De Vroey, C., Takashio, M. (1978) A Practical guide to medical and veterinary mycology. Masson.

Varsavsky, E., Weitzman, I. and Reca, M. E. (1966). A smooth-walled mutant of *Nannizzia gypsea* (Nann.) Stockd. isolated from soil. *Sabouraudia*, 4, 242–243.

Vega, F.E., Jackson, M.A., Mercadier, G. and Poprawski, T.J. (2003). The impact of nutrition on spore yields for various fungal entomopathogens in liquid culture. *World Journal of Microbiology and Biotechnology,* 19(4), 363-368.

Venkatesan, R., Singh, A.J.A., Murugesan, A.G., Janaki, C. and Gokul, S. S. (2007) *T. rubrum* – the predominant etiological agent in human dermatophytoses in Chennai, India. *African Journal of Microbiology Research***,** 52**, 09**–12

Venugopal, P. V. and Venugopal, T. V. (1994). Disk diffusion susceptibility testing of dermatophytes with allylamines. *International Journal of Dermatology,* 33, 730–732.

Verma, S., Heffernan, M. (2008) In superficial fungal infections chapter 188 of Dermatology in general medicine. In: Wolff K, Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, editors. 7th ed. Vol. 2. New York: McGraw Hill.

Vidotto, V., A. Moiraghi, R., O. Cervetti (1982) Epidemiology of dermatophytosis in the metropolitan area of Turin. *Mycopathologia*, 80, 21– 26.

Viguie, C., Ancelle,T., Savaglio,N., Dupouy-Camet, J. and Tourte- Schaefer, C. (1992) Enquˆete ´epid´emiologique sur les teignes `a *Trichophyton soudanense* en milieu scolaire. *Journal of Mycology and Medicine,* 2, 160–163

Wagner, D.K. and Sohnle, P.G. (1995) cutaneous defences against dermatophytes and yeasts. *Clinical Microbiolology Reviews*, 8, 317–55.

Watson, A., Marley, J., Ellis, D. and Williams, T. (1995) Terbinafine in onychomycosis of the toenail: a novel treatment protocol. *Journal of American Academy of Dermatology,* 33, 775–9.

Wawrzkievicz, K. and Wawrzkievicz, J. (1992) An inactivated vaccine against ringworm. *Compositions of Immunology and Microbiology of Infectious Disease*, 15, 31– 40

Weeks, J., Mosser, S.A. and Elewski, B.E. (2003) Superficial cutaneous fungal infections. In: Dismukes W.E, Pappas, GE, Sobel JD (eds), *Clinical Mycology*. Oxford University Press Inc., New York. Pp. 370-373.

Weitzman, I. (1964) Incompatibility in the *Microsporum gypseum* complex. *Mycologia*, 56, 425-435.

Weitzman, I. (1964) Variation in *Microsporum gypseum* . A genetic study of pleomorphism. *Sabouraudia*, 3, 195-204.

Weitzman, I. (1964). Incompatibility in the *Microsporum gypseum* complex. *Mycologia*, 56, 425–435.

Weitzman, I. (1965). Variation in *Microsporum gypseum*. I. A genetic study of pleomorphism. *Sabouraudia,* 3, 195–204.

Weitzman, I., and Padhye, A. A. (1978). Mating behavior of *Nannizzia otae* (*Microsporum canis*.) *Mycopathologia*, 64, 17–22.

Weitzman, I., and Kane, J. (1991). Dermatophytes and agents of superficial mycoses,Pp. 601- 616. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology,5th ed. American Society for Microbiology, Washington, D.C.

Weitzman, I., and Silva-Hutner, M. (1967). Non-keratinous agar media as substrates for the ascigerous state in certain members of the Gymnoscaceae pathogenic for man and animals. *Sabouraudia*, 5, 335–340.

Weitzman, I., and Rosenthal, S. A. (1984). Studies in the differentiation *Mycopathologia,* 84, 95–101.

Weitzman, I., McGinnis, M. R. , Padhye, A. A. and Ajello, L (1986) The Genus Arthroderma and its later synonym Nannizzia. *Mycotaxon,* 25, 505–518

Weitzman, I., Rosenthal, S. A. and Silva-Hutner, M. (1988). Superficial and cutaneous infections caused by molds: dermatophytoses, Pp. 33–97. *In* B. B. Wentworth (ed.), Diagnostic procedures for mycotic and parasitic infections. American Public Health Association, Washington, D.C.

Weitzman, I. and Summerbell, R.C. (1995) The Dermatophytes. *Clinical Microbiology Reviews,* 8, 240-59.

Woodfolk, J. A. (2005) Allergy and dermatophytes. *Clinical Microbiology Reviews*, 18(1)30-43.

Woodfolk, J. A., Wheatley, L. M., Piyasena, R. V, Benjamin, D. C. and T. A. E. Platts-Mills. (1998). *Trichophyton* antigens associated with IgE antibodies and delayed type hypersensitivity. *Journal of Biology and Chemistry*, 273, 29489–29496

Woodfolk, J.A. and Platts-Mills, T.A. (2008) The immune response to dermatophytes. *Researches in Immunology*, 149, 436-45.

www.cfsph.iastate.edu

www.kadunastate.gov.ng

Yahyaraey, R., Shokri, A.C., Khosravi, A. R., Soltani, M., Erfanmanesh, A., Nikaein, D. (2009) Occurance of Animal Dermatophytes in Tehran, Iran. *World Journal of Zoology*, 4(3), 200-204.

Young, C.N. (1972) Range of variation among isolates of *Trichophyton rubrum. Sabouraudia*, 10(2), 164–170

Yu, R. J., Grappel, S. F. and Blank, F. (1972) Inhibition of keratinases by a2-macroglobulin. *Experientia*, 28, 886.