

**A contribution to the study
of tinea capitis in Lusaka,
Zambia**

By

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A dissertation submitted to the University of Zambia in
partial fulfilment of the requirements for the degree of
Master of Science in Microbiology.

University of Zambia

Lusaka

1988

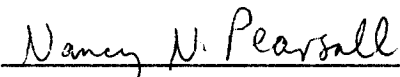
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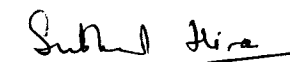
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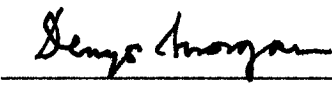
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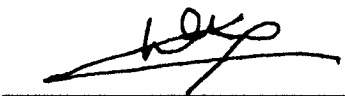
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Acknowledgements

I am indebted to my supervisor, Professor D. Morgan, for his guidance and for his continued interest in the work. Similarly, I am grateful to Mr. Miles Banda, Permanent Secretary, Ministry of General Education and Culture, for granting permission to visit a selection of primary schools chosen for this investigation. My other thanks go to the University of Zambia, Staff Development Office, for the funding of the research project and of my visit to the Prince Leopold Institute of Tropical Medicine, Medical and Veterinary Mycology Laboratory in Antwerp, Belgium.

My thanks are also to Professor Ch. De Vroey for inviting me to the Medical and Veterinary Mycology Department, Prince Leopold Institute of Tropical Medicine, Antwerp in order to experience specialised studies on the pathogenic fungi. Likewise, many thanks go to Dr. Danielle Swinne who introduced me to and helped me in the study of some aspects of Cryptococcus diagnostic techniques and to Paule De Vooght and Maria for their kind help in the course of my visit to Antwerp, Belgium. Chris Raes-Wuytack and Mr. J. Bosmans (Chief Technician) for their guidance in the various aspects of mycological techniques.

I extend my sincere thanks to Professor J.J. Moore for making it possible for me to learn the use of a computer and for his consistent assistance and timely advice during the course of my work and Professor C.E.A. Lovelace for her willingness to read through my script and for her very positive suggestions.

Further thanks go to the technical staff in the Biology Department, University of Zambia for all their help. I extend my sincere thanks to Messrs. S.Kapanji, S.Zeko, D.Chipokolo and all the other technical staff who assisted with my work during my research project.

To my late grandmother and grandfather for their
inspiration.

M.Y.S.R.I.P.

MUKOMA.

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1 . INTRODUCTION

1.1 HISTORICAL REVIEW.

The mycological basis of dermatophytic disease in man spans more than a century - about 149 years. The first mention of a fungus causing skin infection was published in 1839 by Schoenlein, but this was challenged by David Gruby in 1842, on the grounds of inadequate characterisation of the skin lesions by Schoenlein. Paul Remak, in 1845, cultivated and identified the aetiological agent during his work in Schoenlein's clinic. This was after he had failed to recognise the structures observed microscopically as being of a fungal nature. Remak also established by self-inoculation the pathogenicity of the fungus Achorion schoenleini. However, about the sametime, Gruby reported on several different types of dermatophytes causing skin lesions: tinea favosa, ectothrix and endothrix trichophytosis and microsporiasis. This observation on tinea favosa (1841/1842) leaves little doubt that he may have been the co-discoverer of Achorion schoenleini. Gruby was also amongst the first to describe "thrush" due to Candida albicans. Between 1841 and 1844, Gruby described the main pathogens to be found in ringworm. Microsporum, an infection of the hair with spores on the outside, known as ectothrix, was first characterised by microscopic observation by Gruby, together with the large spores inside the hair (=endothrix). The endothrix infective agent was later named by Malmsten as Trichophyton tonsurans in 1848.

However, Raymond Sabouraud, through the technique of pure cultures which he introduced into medical mycology, demonstrated the plurality of the "dermatophytes" (=superficial pathogenic mycota i.e. disease causing fungi, infecting integumentary tissues). In 1892, Sabouraud started issuing numerous large reports which resulted in a single volume, "Les teignes" (=ringworm). This volume may be referred to as the "bible of dermatological mycology" (Wilson and Orda, 1974).

Sabouraud's fundamental studies on the structure and taxonomy of dermatophytes and relationships to clinical manifestations of dermatophytosis formed the basis for subsequent studies on changes induced in the infected host.

Sabouraud had also realised the complex manner in which these fungi grow in culture, with successive cultures taken from the parent stock often showing wide variations. This capacity for variation, known as pleomorphism, has rendered classification of these organisms extremely difficult.

The following years however, saw incomplete and inaccurate reporting because the diagnosis was not based on sound mycological techniques and the natural history was unknown or ignored. Due to misleading reports, several hundred "new species" were described and named as human pathogens. This confusion which hindered clinicians from classifying human diseases based on species of fungi, forced clinicians to adopt the clinicoanatomic or topographical categorisation.

In the early 1920s, Hopkins and Benham organised a laboratory-orientated study towards dermatologic mycology at Columbia University, New York.

In 1934, Emmons outlined in extensive detail a strict botanical classification listing all "dermatophytes" under three genera, embracing sixteen species. This was enthusiastically accepted by clinical mycologists and technicians alike.

With the use of Vanbreuseghem's hair-baiting technique (1952), new and nonpathogenic species of superficial mycota have been isolated, notable among them being Keratinomyces ajelloi (Trichophyton ajelloi) and Trichophyton terrestre which are soil inhabiting dermatophytes.

1.1.1 Dermatophytes.

Definition.

These superficial pathogenic fungi are a group of morphologically and physiologically related fungi which can cause well-defined infections: dermatomycoses e.g. tinea.

These fungal pathogens possess two important properties: they are keratinophilic and keratinolytic (Vanbreuseghem et al., 1978). This means that they have in common the ability to digest keratin generally as saprophytes (in vitro) and to establish an infection by invading via the hair follicle by means of an extension of hyphae. Most of the known species can grow as parasites in the keratinised layers of the skin, hair and nails, causing clinically well-defined lesions.

1. Asexual reproduction occurs by different kinds of aleurospores: arthro-, micro- and macroconidia which are not produced from specialised conidigenous cells.
2. Sexual reproduction on the other hand, through the use of biological techniques, is the production of asci containing ascospores inside an ascocarp, (cleistothecium or gymnothecium as proposed by Novak and Galgoczy in 1966), composed of loosely interwoven, thin-walled, nearly uniform, light-coloured hyphae characteristic of the family Gymnoascaceae. The sexual forms are currently classified in two closely-related genera: Nannizzia and Arthroderma based on the morphology of the peridial hyphae (peridium). To date, all Nannizzia species are known to have a Microsporum asexual (conidial) stage, and all Arthroderma species have a Trichophyton asexual stage.

1.1.2 Ecological Grouping.

Probably, some of these fungal pathogens in evolving from their natural habitat in the soil, have developed host specificity, so that three ecological groups are presently recognised: anthropophilic, zoophilic and geophilic.

Anthropophilic species are restricted to man, rarely infecting animals.

Zoophilic species are primarily animal pathogens, although they may cause ringworm in man.

Geophilic species are soil inhabiting, only rarely encountered as agents of ringworm with the exception of M. gypseum.

1.1.3 Sources of infection.

The actual cause of infection is ultimately by contact with an infective inoculum, which can be by either of two ways:

1. Infective propagules (infective particles) originating from saprobic sources (i.e. soil contaminated with spores generated by these skin-infecting fungi), being transmitted directly or indirectly, and referred to as saprobic-parasitic (s-p) infection.
2. Infective propagules originating from parasitised skin, scales or hair are then transmitted to a person. This transmission can be either direct or else indirect through an intermediate item such as a cap, clothes, towels, bedding or even from contaminated furniture or commonly shared shower-rooms or dressing-rooms. This is referred to as parasitic - parasitic (p-p) infection.

1.1.4 Keratinolytic Activity.

Based on the keratinolytic properties of superficial mycotic agents, Vanbreuseghem (1952) and English (1963) experimentally demonstrated in vitro the stages by which detached hair is attacked by keratinophilic fungi:

1. The fungus gives rise to lateral branches which form branched fronds of mycelium, the "eroding mycelium" which is able to force its way beneath adjacent scales. This marks the start of cuticle lifting.
2. Here, there is a continuation of the fronds produced, to form new fronds below the original ones. When observed, these are seen on sharply-marked troughs much deeper than of the cuticle-lifting fronds.
3. At this stage, the perforating organ appears, which penetrates tangentially or radially in hair and often projects backwards. This organ is said to arise from one or more hyphae forming short, wide cells which arch over the perforating organ and lie along the hair at either side of it, giving the appearance of a handle ("anse" Vanbreuseghem, 1952).
4. The last phase, is the colonisation of the medulla observed by Vanbreuseghem (1952), which takes place regularly in hair. Growth is more rapid in medulla than through cortex, probably due to softer keratin in the medulla, with a less compact cellular structure.

In vitro studies so far indicate that mycotic invasion of the hair involves the use of perforating organs, eroding mycelium and enzymes. Mechanical pressure exerted by the perforating organs also plays a role in both radial and longitudinal penetration.

Raubitschek (1962), carried out an in vitro study which confirmed Vanbreuseghem's observations of the perforating organs of "dermatophytes" invading the nails and hairs and thereby obtaining their nourishment.

In the early researches considerable difficulty was experienced in isolating keratinolytic enzymes from fungal cultures grown on laboratory media. However, Yu et al. (1968) isolated a keratinase enzyme from Trichophyton mentagrophytes var. granulosum (T. granulosum Sabouraud, 1909). This enzyme was demonstrated to have keratinolytic activity on guinea pig hair. Sanyal et al. (1985), were also able to isolate an exocellular proteinase enzyme produced from T. rubrum. The proteinase enzyme was found to hydrolyse proteins, such as azoalbumin, casein, bovine serum albumin, and keratin.

Besides the mechanical wedging activity, the pathogenic process depends also on the action of specific keratin-attacking enzymes (Smith, 1982).

1.1.5 Morphology and Classification.

The classification of the superficial mycotic pathogens is based largely on the appearance of asexual spores: large multiseptate macroconidia, the single-celled microconidia with a typical arrangement on the hyphae, chlamydospores, arthrospores, spiraled and raquette-formed hyphae.

Many authors classify the "dermatophytes" into three genera, namely: Epidermophyton, Microsporum and Trichophyton.

However, according to the classification of Vanbreuseghem and De Vroey (1978), five genera can be distinguished: Epidermophyton, Keratinomyces, Microides, Microsporum and Trichophyton.

The classification of the five genera is as follows:

1. Epidermophyton

Macroconidia have only a few septa, 2-3 (7-12 x 20-40µm) and are typically clubshaped, with smooth and moderately thickened walls. They are often borne in clusters resembling bunches of bananas. There are no microconidia.

2. Keratinomyces

Microconidia are absent, while the macroconidia are smooth and rather thick walled. They are multiseptate (5-7) and spindle shaped.

3. Microides

Microconidia: spherical, 2-3 µm in diameter, very numerous, clustered "en grappe" (=grape-like) or "croix de Lorraine".

Macroconidia: multicellular, smooth and thin-walled, with very irregular outline; usually scarce. Spirals and antler-like hyphae present.

4. Microsporium

Macroconidia are spindle-shaped, multiseptate, 5-10 (8-15 x 30-150µm) with slightly tapering ends, symmetrical thick-walled and rough surface. Club-shaped (clavate) microconidia are found.

5. Trichophyton

Macroconidia vary in morphology. They may be cylindrical or club-shaped and multiseptate (2-10) cells with blunt ends. The wall are thin and smooth. Microconidia are as in Microsporium.

1.2 TYPES OF RINGWORM.

The types of tinea will be mentioned only briefly here, and later will be noted in more detail as microsporic and trichophytic infections.

1. Tinea corporis - ringworm of the glabrous skin. The clinical manifestations result from invasion and proliferation of the causal fungi in the stratum corneum. Terminal hair may be invaded.

- a) Herpes circinatus - initially appears as a small red squamous papules which enlarge centrifugally whilst healing at the centre. It is caused by Microsporum and Trichophyton species e.g. M.canis from cats and dogs, T.verrucosum from cattle. Of the anthropophilic species, T.rubrum may invade the buttocks and lower parts including distant sites of the trunk.
- b) Tinea barbae - ringworm of the beard and moustache areas of the face with invasion of coarse hairs. It is a disease of the adult male. Tinea of the chin and upper lip in females and children are considered as tinea faciei i.e. ringworm of the skin of the face. The zoophilic mycota: T.verrucosum and T.mentagrophytes are responsible for the majority of cases. Anthropophilic species; T.violaceum, T.schoenleini, T.megninii, and T.rubrum are recognised as occasional causes.

2. Ringworm of the intertriginous areas of the body.

- a) Eczema marginatum hebrae: this presents as erythemasquamous plaques situated in the groin.
- b) Tinea pedis - a mycotic infection of the foot which often begins between the fourth and fifth toes and is characterised by three clinical forms:

(i) Chronic infections may also affect the heels, soles and dorsum of the feet resulting in papulosquamous hyperkeratotic plaques. It may be accompanied by severe pruritus.

(ii) Subacute infections are characterised by soles being freed out, with the sides bleeding.

(iii) Acute lesions are characterised by the formation of vesicles on the soles extending to stratum granulosum of dermis. This is accompanied by superinfection with bacteria and swelling of regional lymph nodes.

3. Ringworm of nails (=tinea unguium) - is a mycotic infection of the finger- and toe-nails. The nail plate becomes very thickened, detached from the nail bed, and gradually falls off from the finger or toe nail.

1.2.1 Tinea capitis - Pathogenesis.

Definition:

Tinea capitis is a medical term, and by definition refers to infection caused by pathogenic mycota involving the scalp and hair shafts (Krowchuk et al., 1983; Roberts et al., 1979).

When an infective inoculum lands on a child's head, it grows radially on the stratum corneum penetrating surrounding hair follicles. "Dermatophytes" prefer actively growing hairs (anagen), hence, the resting hairs (telogen)

are relatively immune from infection (Krowchuk et al., 1983).

Tinea capitis is almost exclusively a disease of children and adolescents with only a small number occurring in adults (Krowchuk et al., 1983; Okoro, 1981). This disease of the scalp shows by hair loss and varying degrees of inflammation (scaling, redness and formation of pustules). Occasionally infected areas may be accompanied by pronounced swelling, developing into a deep boggy elevated ulcerative area, releasing pus. This is known as kerion.

In an article by Vanbreuseghem and De Vroey (1970), presenting the distribution of superficial mycota isolated from the different geographical regions of the world and collated as to the common body location of the isolates, tinea capitis is been shown to be of higher incidence than other ringworms of the body such as tinea corporis, tinea pedis etc.

1.2.2 Hair invasion and clinical types.

The type of hair invasion is differentiated mycologically by direct microscopy. The two main types of scalp infections are: ectothrix (=spores outside the hair) and endothrix (=spores inside the hair). The type of hair invasion depends on the dermatophyte.

a) Microsporic tinea - This occurs when the hair shaft is invaded in the mid follicle. The intra-pilary hyphae continue to grow inwards towards the bulb of the hair. Secondary extra-pilary hyphae burst out and grow in a tortuous manner over the surface of the hair shaft which is growing outwards continuously. The secondary extra-pilary hyphae segment to produce a mass of small arthrospores (2-3µm) which eventually round to become spherical and surround the hair shaft. The term "ectothrix" is used to

describe the presence of spores outside the hair. Parasitised hairs show under Wood's light (U.V. 360 nm of "Blacklight") a green fluorescence.

Microsporic tinea produces large circumscribed scaling plaques (=patches). The hairs are broken off a few millimetres from the follicle. This is caused by Microsporum species, e.g. M.audouinii, M.canis, M.ferrugineum etc.

b) Trichophytic tinea - In this infection, the intra-pilary hyphae fragment into arthrospores (>4µm) which are within the hair shaft (=endothrix). Affected hair is fragile and breaks close to the scalp. Formation of black dots as the hair breaks is classical in this condition. This condition is always caused by anthropophilic Trichophyton species, such as T.violaceum, T.soudanense, T.tonsurans, T.yaoundei etc.

This results in several little scaly patches commonly referred to as "black dot tineas". Infected hair is broken off very short in the follicle. Some are similar to microsporic tinea, and sometimes they are suppurative.

Favus - Cup-shaped yellow crusts are formed around the hair shaft at the opening of the hair follicle. The hair is not broken but disappears all together with the destruction of the follicle. The result is a permanent alopecia. This condition is caused by anthropophilic T.schoenleini. This is a tinea capitis where:

The hair follicle is irreversibly destroyed and without treatment, it is life long.

Kerion (celsi) - This is the most severe type of reaction. It is a painful inflammatory mass with temporary hair loss. Follicles may be seen discharging pus. It occurs frequently as solitary or multiple lesions. They are caused by zoophilic dermatophytes, such as T.verrucosum and M.mentagrophytes.

1.2.3 Host preferences.

An imposing number of at "least forty-two species" of fungi have been incriminated as agents of ringworm in man and other animals. Individual superficial mycota differ considerably in their host range and importance as agents of disease in man.

Ecologically, as indicated earlier, these organisms have been divided by Georg (1960) into three ecological groups: anthropophilic, zoophilic and geophilic.

1.2.4 Anthropophilic mycota.

Anthropophilic species are primarily adapted for parasitism of man, but some species occasionally cause ringworm in animals.

Four of the Microsporum species are distinguished from each other on clinical, epidemiologic and mycologic grounds: M.audouinii, M.langeroni, M.ferrugineum and M.rivalieri. M.langeroni Vanbreuseghem, has been separated from the classic M.audouinii by its geographic origin (isolates originate from Central Africa), and unlike M.audouinii can cause tinea corporis (ringworm of the glabrous skin), can be inoculated into guinea pigs and has peculiar macroconidia and microscopic mycological characteristics (Vanbreuseghem, 1963).

M.rivalieri Vanbreuseghem, is a separate species of African origin and is found less frequently. Reported cases outside of Africa, include those from Florida in the United States (Zaias et al., 1965) and from East Anglia in England (Whittle et al., 1970).

M.ferrugineum is known to be mainly an Afro-Asiatic species.

The anthropophilic Trichophyton species, are categorised in three groups, viz.,

- (a) T.rubrum and T.concentricum. T.rubrum is a common cause of tinea unguium and tinea cruris and tinea pedis. T.rubrum very rarely invades hair in vivo, while T.concentricum causes only skin lesions.
- (b) T.violaceum, T.tonsurans and T.soudanense are the main cause of endothrix type of tinea capitis. They are responsible for the "black dot tinea", T.violaceum being particularly common in Africa (De Vroey, 1985). T.tonsurans is relatively unknown in Africa but is an important cause of ringworm in the Americas (Sinski et al., 1984; Gugnani et al., 1985).

Other species causing human endothrix tinea capitis are T.yaoundei and T.kuryangei with a limited geographic distribution, reported only from Africa (Vanbreuseghem et al., 1961; Rosenthal et al., 1962). T.soudanense is another cause of tinea capitis in Africa, commonly reported in West African countries e.g. Nigeria, Ivory Coast, Ghana etc. (Verhagen, 1974; Gugnani et al., 1985).

- (c) T.schoenleini is the only one in this group which is an agent of favus, still largely endemic in North Africa.
- (d) Microides interdigitalis, a member of the Microides mentagrophytes complex, is essentially a cause of tinea pedis and never invades hair in vivo.

1.2.5 Zoophilic mycota.

Zoophilic species are basically animal pathogens, with either a single preferred-animal host or a very limited host range, outside which they are found in exceptional circumstances (English, 1972). Three main zoophilic mycota are:

M.canis, T.verrucosum and T.mentagrophytes are important agents of ringworm in man, although the remainder have at one time or the other been associated with human infection. M.canis commonly infects pet animals and especially cats, which can shed infective particles into the domestic environment and contact with this results in familial infections (De Vroey, 1985). T.verrucosum is a common cause of tinea in cattle. Close contact by man with infected animals and their fomites leads to contracting the fungus in man. T.verrucosum is mainly an agent of inflammatory skin and scalp lesions (kerion).

Microides (Trichophyton) mentagrophytes complex has been found to represent two perfect (=sexual) "dermatophyte" species, viz., Arthroderma vanbreuseghemii (Takashio, 1973), A.benhamiae (Ajello and Cheng, 1967) and M.(T.) interdigitales, an imperfect species which resembles morphologically the conidial state of A.vanbreuseghemii. It is therefore considered a member of the M.(T.) mentagrophytes complex. Members of the M.(T.) mentagrophytes complex (with the exception of M.(T.) interdigitalis) are

mainly transmitted from infected wild rodents, and 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. North America. The African hedgehog (Erinaceus albiventris) together with its European counterpart (E.europeans) are the reservoir of the var.erinacei (WHO report, 1986). Others like T.equinum cause tinea in horses.

1.2.6 Geophilic mycota.

Geophilic species exist as saprophytes in the soil with the ability to colonise keratinous substrates successfully. Their distribution appears to relate to the distribution of available keratin. A few geophilic species do have an additional capacity to cause ringworm in some species of animals including man.

For example, M.nanum which causes ringworm in animals, especially pigs, is mainly associated with surroundings having pigs (Vanbreuseghem et al., 1979). M.ripariae is reported to be associated with birds (Hubalek et al., 1973). M.praecox can be found in large numbers in sites associated with horses. It is a saprophyte with weak pathogenicity. Other geophilic mycota include T.terrestre and Keratinomyces ajelloi (T.ajelloi), which are nonpathogens. K.ajelloi is commonly found in colder climates, but is sporadic in hot climates. However, the principal geophilic mycota are members of the M.gypseum complex. M.gypseum is found to represent three perfect (=sexual) "dermatophyte" species, viz., Nannizzia incurvata, N.gypsea (Nann.), and N.fulva, a perfect state of M.fulvum (Stockdale, 1963). M.gypseum has been documented as a pathogen in man and animals. Of the three mycotic species, pathogenicity studies with laboratory animals have shown N.fulva to be the least pathogenic, while no notable differences have been observed with N.gypsea and N.incurvata (Weitzman et al., 1967). The distribution of M.gypseum is world-wide.

1.2.7 Other factors.

Sex.

A higher number of males are infected with ringworm of the scalp than females. This is attributed in part to poorer personal hygiene amongst boys than girls and habitual sharing of combs by boys. The approximate ratio of males:females is 3:1.

Age.

Tinea capitis has been found more commonly to be a disease of children and adolescents rather than of adults. It is especially common during the school-going age. A number of reasons have been advanced to try to explain the high incidence of ringworm in the younger generation. These range from lack of enough sebum secretion to high rates of physical contact and sharing of fomites among children.

Occupation.

Some types of work predispose more to tinea infections than others. For example, zootechnicians, farm workers, hairdressers, barbers, manicurists, weavers, washing and/or bathing attendants, etc. are more prone to tinea infections than others.

Nutrition.

Protein malnutrition, according to Vanbreuseghem (1958), is a predisposing factor to ringworm infections.

Geographical Distribution.

"Dermatophyte" species can be categorised as being:

a) cosmopolitan or b) geographically limited (Philpot, 1978), e.g.

<u>M.audouinii</u>	(Cosmopolitan)
<u>M.ferrugineum</u>	(Asia, C.Africa, S.America, E.Europe).
<u>M.langeroni</u>	(C.Africa).
<u>T.soudanense</u>	(Africa - especially N.Central Africa).
<u>T.kuryangei</u>	(Rwanda and Burundi).

T.kuryangei is a human pathogen first discovered by Vanbreuseghem (1956), and is confined to a restricted geographical region. T.violaceum is indicated by Philpot (1979) as the commonest agent of tinea capitis in North Africa including Egypt. T.schoenleini, the agent of favus, is sporadic in Central Africa. Table 1 (De Vroey, 1985) shows the relative importance of the various common species of superficial mycotic infections and table 2, the importance of each species according to the 5 continents.

Table 1 -

**RELATIVE IMPORTANCE OF COMMON SUPERFICIAL MYCOTIC SPECIES
WORLDWIDE: ISOLATES OF CLINICAL ORIGIN UP TO 1980.**

Species	No. strains	%
T.rubrum	35 414	40.6
T.violaceum	18 768	21.5
T.mentagrophytes	12 557	14.4
M.canis	8 377	9.6
M.audouinii	3 303	3.8
T.tonsurans	2 153	2.5
E.floccosum	2 140	2.4
T.schoenleini	1 625	1.9
T.soudanense	1 162	1.3
T.verrucosum	831	0.9
T.megninii	242	0.3
M.gypseum	198	0.2
T.concentricum	196	0.2
M.ferrugineum	112	0.1
Others	39	<0.1
Total	87 117	

From De Vroey, Ch., 1985. Seminars in Dermatology. 4:190.

Table 2.Main agents of *Tinea capitis*.

Species	W.wide	Europe	North (America)	South	Africa	Asia
<i>T.violaceum</i>	54.6	51.1	+	+	42.2	92.0
<i>M.canis</i>	1.3	34.2	6.6	40.0	4.1	1.8
<i>M.audouinii</i>	9.1	2.4	70.4	29.3	18.6	+
<i>T.tonsurans</i>	4.7	4.1	20.4	20.7	+	1.7
<i>T.schoenleini</i>	4.7	6.3	+	6.8?	4.0	2.8?
<i>T.soudanense</i>	3.3	+	-	-	25.0	-
<i>T.mentagrophytes</i>	0.6	+	+	1.8	1.7	+
<i>T.verrucosum</i>	0.5	+	+	+	1.2	+
<i>M.ferrugineum</i>	0.3	+	-	-	1.7	+

* +: < 0.2% -: Absent or negligible.

De Vroey, Ch., 1985. Seminars in Dermatology. 4:190.

1.3 MYCOLOGICAL PROCEDURES.

There are two basic mycological procedures used in the laboratory for specimens suspected of tinea infection: direct microscopy and culture,

1.3.1 Direct Microscopy.

Direct microscopy can provide a first indication in suspected mycotic diseases. It allows for direct examination of fungal elements in the cutaneous scales, nail material and hair. To clear the keratin and expose the fungal elements, 10% or 20% KOH has traditionally been used. However, nowadays, chlorallactophenol is used in preference to KOH for clearing hair. Microscopical examination is made using a low intensity light source to ensure a good contrast between the fungus and the keratinous material. The microscopical examination, however, can only indicate the group of the mycotic species in question. Therefore, the use of culture methods to identify the mycotic species is necessary.

1.3.2 Culture.

Superficial mycota are normally grown on Sabouraud's dextrose agar, which provides a sufficient nutrient source for the superficial mycota. The medium is usually supplemented with cycloheximide (Actidione) which inhibits most contaminant mould fungi other than "dermatophytes", and chloramphenicol to suppress the growth of bacteria. The agar is poured into test tubes and made to form slopes. This is inoculated with fragments of tinea specimen and incubated at 25°-28°C for 7 to 21 days.

1.3.3 Identification.

Identification of superficial mycota by microscopic examination of the culture relies on the morphological characteristics such as the presence or absence and

characteristics of micro- and macroconidia and the appearance of the mycelium, to determine the aetiological agent.

After the primary culture has been isolated, it is subcultured onto a diluted Sabouraud's (=Takashio's) medium. This is a low nutrient medium which contains 10 times less sugar and nitrogen compared to normal Sabouraud medium. This medium is meant to stimulate and promote sporulation or to rejuvenate the culture for identification.

When confirmative identification of the "dermatophyte" species is not possible, supplementary tests, such as amino acid and vitamin requirement can be used (especially for the species of T.violaceum, T.roseum, T.tonsurans, T.ochraceum).

Similarly, the use of hair perforation technique for the species of T.rubrum and T.mentagrophytes can be used. With this test, sterile hair is put into a Petri dish and about 5ml sterile distilled water is used to "wash" the spores into suspension from the culture. This is then poured into the Petri dish containing the sterile hair, and is then incubated at 25°C for about 15 days before observing under the microscope for perforations in the hair.

1.3.4 Sexual Forms.

These are determined mainly by the use of biological techniques or their modifications. Sexual forms have been discovered repeatedly in some of the "dermatophytes" which are now classified into two genera: Nannizzia and Arthroderma. Mating of compatible strains of "dermatophytes" in vitro has made their identification more accurate and reliable. However, in some "dermatophytes" sexual forms are yet to be discovered, especially the anthropophilic species where there is no known sexual form.

1.4 GRISEOFULVIN.

This antibiotic is a product of some Penicillium species and was first isolated in 1939 by Oxford, Raistrick and Simonart from P.griseofulvum Dierckx.. The "curling factor" isolated in 1946 by Brian, Curtis and Heming was demonstrated in 1947 to be identical to griseofulvin. Its antifungal effect was therefore a matter of interest, which led to its use in agriculture.

However, it was not until 1959, that Gentles published experimental evidence of griseofulvin's fungistatic activity against ringworm in guinea pigs inoculated with M.canis. Blank, in the United States, was the first to use it by oral application in clinical trials in human medicine. It is of interest to note that the fungistatic activity of griseofulvin in vivo is related to its penetration of keratin: it inhibits the growth of "dermatophytes" and therefore, the mycotic species regress where the griseofulvin front advances (Gentles,1959).

2.1 PRESENT INVESTIGATION.

2.1.0 Aims of the study.

The objectives of this research were to:

1. Ascertain the degree of clinical prevalence of tinea capitis and
2. Ascertain the superficial mycota causing tinea capitis in schoolchildren, based on a survey of primary schools in Lusaka.
3. Determine the sensitivity of the isolated superficial mycotic species to griseofulvin.

2.1.1 Geographical Location.

Zambia is a sub-tropical country, lying within latitudes 8 and 18 degrees south, with a savannah climate, characterised by one rainy season and a dry season of seven months. The yearly weather pattern is mostly determined by the movement of the inter-tropical convergence zone, and the four seasons can be identified: the Rainy season (November-March); the Post-Rains warm season (April-June); the winter Dry season (June-August); and the Hot season (September-November) (Williams, 1980). Zambia is situated mainly on the interior African plateau, mostly developed on the pre-cambrian rocks (Hywel Davies, 1970).

Lusaka, in which this study was conducted, is the capital and largest city of Zambia. It is situated at an altitude of about 1,300m (4,200ft) above sea level on the mid-tertiary plateau surface.

The population of Lusaka was 538,469 at the time of the last census, in 1980; which is almost 10% of the total population of the country.

The sampling of children was conducted in the primary schools in Lusaka urban and rural areas. The use of primary schools was approved by the Ministry of General Education and Culture. The schools were chosen in terms of transport costs, time period in which to conduct the research accessibility of the schools, and whether they could give sufficient information for the study.

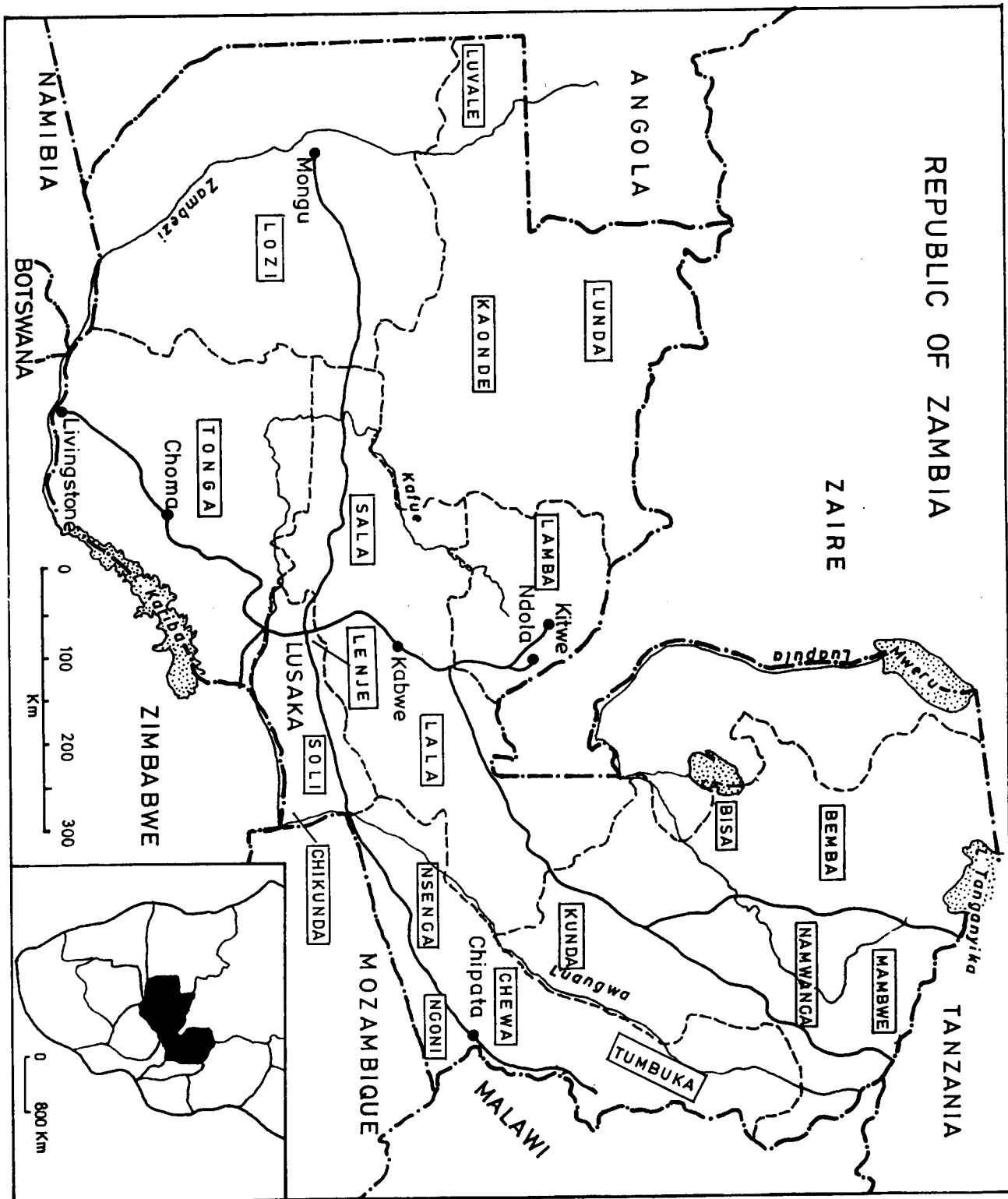


Fig.1. Map of Zambia.

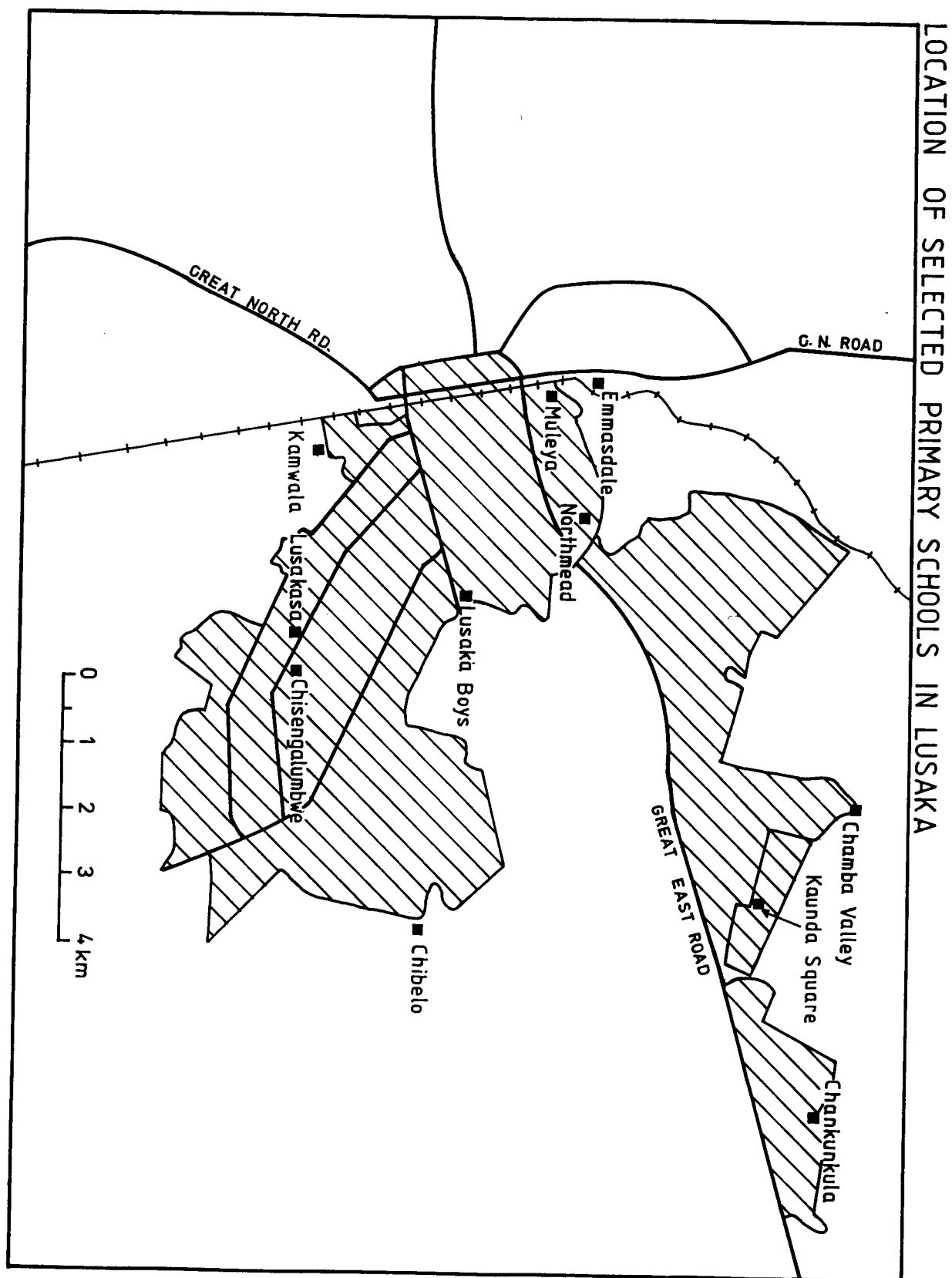


Fig.2. Location of Selected Primary Schools in Lusaka.

2 . METHODODOLOGY

2.2 MATERIALS and METHODS.

2.2.0 Clinical Examination.

In the course of the study, the visits were divided into two parts. The first part consisted of :

- i) Giving a short lecture to the classteachers on the causative agents and modes of transmission in ringworm infections.
- ii) Schoolchildren from grades one to seven in the 11 schools (Figure 2*) were given the Patient Consent Form (P.C.F.), to take home for parents to see and to be asked to sign. Parents were required to sign to indicate their willingness to have their child included in the study. The signed forms were then delivered back to the school by the pupils. The forms were collected and numbered. With the help of a table of random numbers, pupils from each school (as shown in appendix) were finally included in the study. These were checked for signs of infection. Children with any abnormal condition, such as alopecia, erythema, skin abrasion, folliculitis, scarring or small patches of minimal dry scaling were considered suspect of having ringworm.

*Three schools from rural Lusaka (Chilanga, Mt.Makulu and Musamba) are not shown on the map (see appendix).

2.2.1 Sampling.

When obtaining material for microscopic examination and culture, sterilised forceps were used. The forceps was sterilised by flaming to avoid cross-contamination among the children. Lesions with ointment were swabbed with cotton wool moistened with 70% v/v alcohol to remove any superficial bacteria and mould spores and the ointment in

order to improve the chances of isolating the fungi. Hairs from the lesion(s) were manually removed with epilating forceps. Broken lustreless hairs were selected from the margins of the lesion and transferred individually into a philatelic envelope. Only minimal scraping from lesion margins was done.

Besides obtaining information e.g. name, address, ethnic group, sex and grade, data were collected on the family contacts and animal exposure. Clinical information and mycological investigations were recorded on the case card form.

2.2.2 Laboratory - Handling.

Although ringworm specimens are not really dangerous to handle, some precautionary measures were taken as standard practice when handling the material. The working bench was always disinfected after processing the material and the hands were washed with disinfectant, and then soap and water.

The samples were stored at laboratory temperature (around 20-25°C), and the duplicate samples were posted within 3 days of having been collected to the Medical and Veterinary Mycology Laboratory, Institute of Tropical Medicine, Antwerp, Belgium. The samples in the laboratory were examined within 2 to 3 days of collection, by direct microscopy and then positive hairs cultured on Sabouraud's medium supplemented with antibiotics. For microscopic examination, recognition of the characteristic filaments (hyphae) and spores (arthrospores) confirmed the ringworm diagnosis. The positive specimens were cultured in order to establish the identity of the fungal species.

2.2.3 Direct Microscopy

In the Institute of Tropical Medicine, Mycology Laboratory, Antwerp, Belgium, short hairs (5 to 10mm) were placed in a drop of mounting fluid of chlorallactophenol on a clean slide and a coverslip was applied. The purpose of the chlorallactophenol (as for KOH) is to soften and wetten the specimen and render the hyphae and spores (arthrospores) visible. The cleared hair material was examined systemically at a magnification of 25x. The intensity of illumination was kept low because very bright light is not suitable for observing the hyphae and arthrospores.

In the Biology Laboratory (University of Zambia), 10% KOH was used as clearing fluid for keratin. The average time allowed for clearing the hair was 6 to 10 minutes. This was used due to lack of chlorallactophenol. Chlorallactophenol is better than KOH as it does not disintegrate the hair, and mounted slides can be kept for a long time without damaging the hair preparation. Chlorallactophenol can therefore, be used to make permanent slides.

2.2.4 Microscopic Measurements.

To measure the approximate size of the arthrospores in the infected hairs and conidia from culture, an ocular (or eyepiece) micrometer was used. The engraved scale has one hundred (100) accurately spaced divisions.

Objective	Value of one eyepiece division
x5	33.3 μm
x10	14.3 μm
x40	3.1 μm
x100	1.4 μm

A table of values for a calibrated eyepiece micrometer.

2.3 ISOLATION.

2.3.1 Isolation Media.

Fungi can be cultured on a variety of media. Such media can either be liquid or can be made solid by addition of agar. Most fungi are known to grow well in media high in carbohydrates, with a pH range between 5 and 6 (Alexopoulos, 1969).

2.3.2 Selective Medium.

By addition of various dyes, antibiotics or chemical compounds, the fungal media can be made to inhibit selectively the growth of bacterial and fungal contaminants but permit the growth of pathogenic fungi, and especially in this case, the superficial mycota.

In the Biology Laboratory (University of Zambia), the principal selective medium used was Sabouraud's medium containing chloramphenicol, while in the Laboratory of Medical and Veterinary Mycology, Antwerp, Belgium, Sabouraud's medium containing both chloramphenicol and cycloheximide (Actidione) was used as the primary isolating medium.

Dermatophyte Test Medium (D.T.M) containing penicillin and streptomycin besides chloramphenicol to prevent the growth of bacterial contaminants was used in the Biology Laboratory. D.T.M. was used to differentiate the "dermatophytes" from other fungi. However, the disadvantage of this medium is the possibility of false-positive reactions showing, due to contaminants.

2.3.3 Subculture.

Diluted Sabouraud's or Takashio's Medium.

To improve sporulation or rejuvenate cultures of fungi, Takashio's medium (or diluted Sabouraud's medium) developed in the Laboratory of Medical and Veterinary Mycology, Antwerp, Belgium, was used. This medium is 10 times less concentrated in dextrose and peptone than ordinary Sabouraud's medium. On this poor medium most species of superficial fungal pathogens and even other moulds sporulate much better and can be kept in a "good state" for a long time. Whereas, when subcultured on Sabouraud the fungi more or less degenerate rapidly and produce less spores.

This medium is named after Takashio, the late Japanese mycologist. The medium used in Lusaka was as developed in the Mycological Laboratory of Antwerp, except that mycological peptone (Oxoid) was used instead of neopeptone (Difco).

2.3.4 Slopes.

The prepared medium was poured into test tubes of 20 cm in length using aseptic technique. After solidification of the medium, the hairs were inoculated using a bent loop onto Sabouraud's medium supplemented with antibiotics. The hairs were partly pushed into the medium. Smaller hair sizes, approximately less than 5mm in length were selected for culture. Hair of smaller size in enough quantity increases the chances of fungi growing on the medium. This was incubated at 25°C and checked every day for growth, growth rate, topography and colour pigmentation. The cultures were normally left to incubate for 7 days, except for the slow growing cultures, which were left for 14 to 21 days. Agar that showed no growth or where contaminants only grew even after repeat inoculation were regarded as negative.

2.3.5 Sabouraud's Medium.

The composition of this medium is the same as the one containing antibiotics, but in this medium antibiotics are not incorporated. This is used for subculturing since these superficial fungi produce better macroscopic appearance for identification on this medium than on diluted Sabouraud's medium.

2.3.6 Needle Mounts.

Needle mounting forms an important part of culture examination. A needle mount preparation is meant to give sufficient information on the type and arrangement of conidia together with the accessory structures to enable identification to be made.

A small portion of the fungal growth is collected with a sterile sharp needle, transferred to the slide and mounted with the minimum disturbance to the fungal structures. A straight nichrome wire with the distal 1.5cm bent at an angle of 90°C is used. This allowed the transfer of fungal material from surface growth on the Takashio medium.

The fungus fragment is introduced into a drop of lactophenol and a coverslip superimposed, excess lactophenol being removed by blotting paper.

2.3.7 In vitro Testing with Griseofulvin.

125 mg of griseofulvin was dissolved in 12.5ml acetone, to which 50ml of liquid Sabouraud was added to make a final concentration of 2mg/ml. This served as the stock solution. 10ml of the stock solution was diluted to a concentration of 294µg/ml by adding 68ml of liquid Sabouraud medium. 40ml was transferred to another flask to which 280ml of liquid Sabouraud medium was added, to give a final concentration of 42µg/ml. 42µg/ml was the highest concentration for the purposes of the sensitivity tests. The 320ml containing 42µg/ml was then serially diluted into 6 flasks, by taking half volume of the griseofulvin solution and adding to them an equal amount of liquid Sabouraud's medium. This was dispersed into 7 test tubes to a volume of 4ml. The control had only liquid Sabouraud medium without griseofulvin. These were sterilised by reautoclaving, as griseofulvin is thermostable.

The test tubes containing different concentrations were inoculated with small pieces of 2 to 3 week old cultures of Trichophyton violaceum and Microsporum langeroni. This was incubated for 7 days at 25°C.

3. RESULTS

3.1 RESULTS.

3.1.0 Direct Microscopy.

The types of hair parasitism observed using direct microscopy are summarised in table 3.

-Endothrix type represented 78% of the 67 positive infections.

-Ectothrix (microsporic) type was 10% of the 67 positive cases.

In some samples no parasitised hair could be observed, but only mycelium in skin scales. This accounted for the other 12% of the 67 positive cases.

Table 3

Type of hair invasion.

Endothrix	Ectothrix	Positive(mycelium)
52 (78%)	7 (10%)	8 (12%)

3.1.1 "Dermatophyte" species isolated.

Of the 150 suspected cases of tinea capitis, 67 (44.7%) of the clinical specimens were proved microscopically positive. From these, 35 Trichophyton violaceum and 12 Microsporum langeroni were isolated and 20 were culture-negative. The fungi were of 2 species only (table 4).

Table 4

Superficial fungal species.

	No.	%
T.violaceum	35	74.5
M.langeroni	12	25.5
Total	47	100.0

When the results of direct microscopy (DM) and culture are combined, the summarised table is thus:

Table 5

Direct Microscopy and Culture.

species	Endothrix	Ectothrix	Positive(mycelium)
T.violaceum	30 (57.7%)	1 (14.3%)	4 (50.0%)
M.langeroni	6 (11.5%)	6 (85.7%)	0 (0.0%)
Culture/-/	16 (30.0%)	0 (0.0%)	4 (50.0%)
Total:	52/67(78%)	7/67(10%)	8/67(12%)

The endothrix type on culture produced 57.7% Trichophyton, 11.5% Microsporum and 30.8% negative cultures.

The ectothrix type on culture produced 85.7% Microsporum, 4.3% Trichophyton and no negative cultures.

The 4 samples with only positive mycelium on culture produced 2 Trichophyton, no Microsporum, and 2 negative cultures.

3.1.2 Mycological Aspects.

The following are characteristics of the two aetiological agents of tinea capitis:

M.langeroni.

Macroscopic appearance

Growth - normal growing.

colony - flat,velvety, with whitish tan.

Colour - beige to salmon.

Texture - velvety.

Microscopical characteristics.

-Characteristics similar to M.audouinii

-Chlamydospores 30 - 40 μ m

-Macroconidia often with slight constrictions in centre and verrucous (warty) walls.

Long 50 - 80 μ m

width 10 - 12 μ m

Septation 3 or 5 cells

Microconidia piriform (clavate) - more or less numerous.

T.violaceum.

Macroscopic appearance.

Growth - very slow.

Colony - heaped and verrucose.

Colour - deep violet (=violaceous).

Texture - Waxy and glabrous.

Microscopical characteristics.

1. Macroconidia and microconidia absent.
2. Irregular mycelium with chlamydospore-like cells joined together with septation.
3. Brownish appearance under microscopy.

3.1.3 Prevalence.

Of the 400 schoolchildren examined in the 14 schools, 150 were suspected of having tinea capitis infection. Of these 67 were confirmed of the infection using direct microscopy.

No. of children examined:	400
Clinical suspects	150
Direct Microscopy	67
%	16.8(+/- 3.6)%

Table 6. Prevalence based on Direct Microscopy (DM).

Table 7Prevalence According to Age.

Age(years)	Total examined	DM(+)	%
6-8	151	17	11.3
9-11	181	39	21.6
12-14	68	11	16.2
Total:	400	67	16.8

shows the grouping of schoolchildren according to the following ages: 6-8 years, 9-11 years and 12-14 years. The mean age was 9.4(+/-2.3).

The rate of infection shows a peak at ages 9-11 years while it starts to decrease at the ages of 12-14 years.

3.1.4 Sex of children.

The frequency of tinea capitis was shown to be higher amongst the boys than girls.

Table 8Grouping by sex.

Sex	Total examined	DM(+)	%
M	244	44	18.0
F	156	23	14.7
Total:	400	67	16.8

(A chi-square showed no significant ($P > 0.05$) differences between the numbers of infected males and females)

3.1.5 Age and Sex.

Grouping the children by age and sex; the following picture emerges: with increase in age, generally, they are more boys than girls infected, and there is a general reduction in the number of infected children as shown in Table 9.

Table 9

Children grouped by age and sex.

Age(years)	Boys	DM(+)	%	Girls	DM(+)	%
6-8	94	12	12.8	59	5	8.5
9-11	112	24	21.4	67	15	22.4
12-14	38	8	21.0	30	3	10.0
Total:	244	44	18.0	156	23	14.7

(A chi-squared test proved very highly significant for the boys, but was not significant for the girls. The values of chi-squared were $P \leq 0.01$ and $P > 0.05$ respectively).

3.1.6 Inflammatory versus Non-inflammatory tinea.

T.violaceum and M.langeroni being anthropophilic species are mainly responsible for noninflammatory ringworm infections, with some inflammatory cases due to T.violaceum.

Noninflammatory ringworm in children was defined in terms of "black dot", grey patch and diffuse erythema and

scaling. Some illustrative examples of these are shown in figures 3,4,5,6 and 7. These were due either to T.violaceum or M.langeroni.

As can be seen from the results (Table 10), about 1 out of 5 cases of endothrix trichophytoses showed inflammation, or about 20%, whereas 1/7 ectothrix cases were inflammatory (close to 15%). This was mostly due to T.violaceum which also produces the "black dot" type of tinea. Figure 8 is an illustrative example of pustular inflammation or even kerion-like inflammation of the ringworm. Of course the figures are small to indicate whether or not there is a difference in the percentages of inflammatory cases in endothrix versus ectothrix trichophytoses.

However, similar clinical infections were observed in Zaire by Vanbreuseghem (1963) and more recently by Mamba Kwete (1987).

Table 10

The occurrence of Inflammatory versus Noninflammatory Tinea.

Hair invasion	DM(+)	inflam.	non-inflam.
Endothrix	52	11	41
Ectothrix	7	1	6
Total	59	12	47

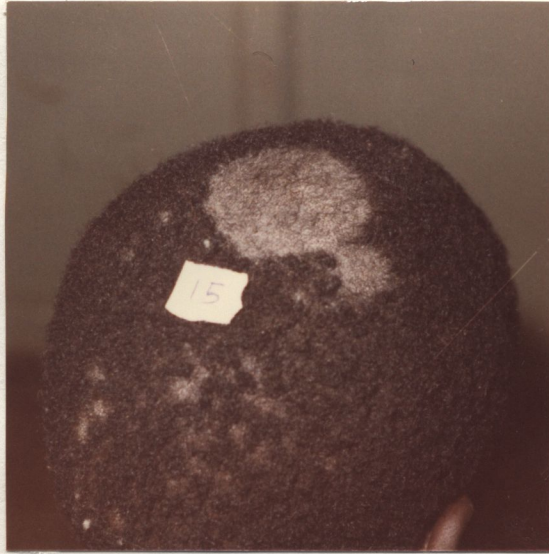


Fig.3. Large microsporic patches, grey scaling with broken hair a few mm from the follicle (= M.langeroni)



Fig.4. Microsporic plaques, with mild inflammation (= M.langeroni)

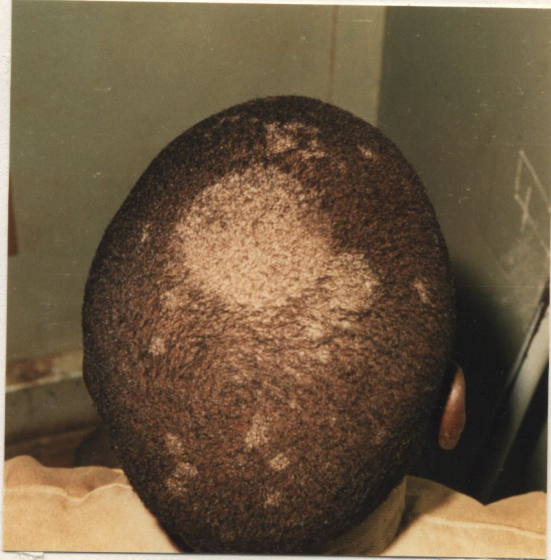


Fig.5. Large with small patches, with scaling and very short hair (= M.langeroni)

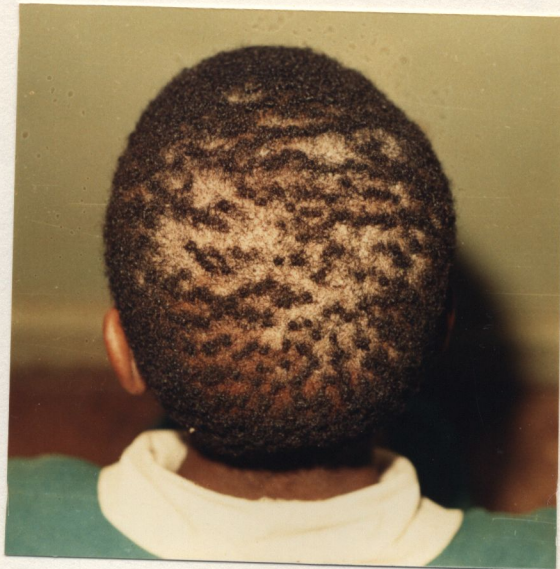


Fig.6. Several small scaly patches ("Black-dot" tinea
(= T.violaceum)

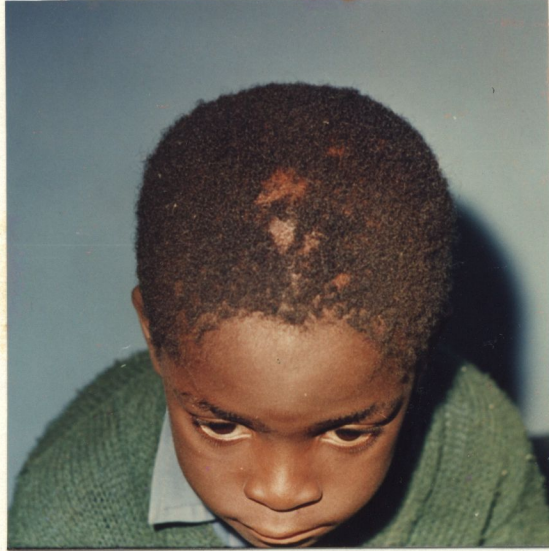


Fig.7. Small bald patches, with hair broken off very short in the follicle (= T.violaceum)

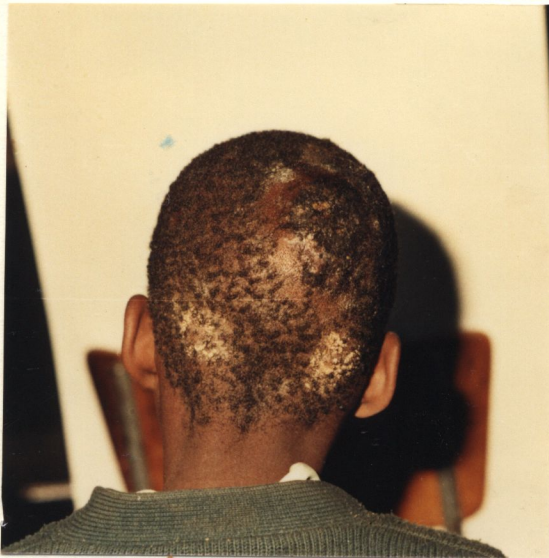


Fig.8. Several patches, with inflammation and a large kerion-like lesion (= T.violaceum)

3.1.7 Residential areas.

Using the classification of residential areas as adopted by the Central Statistics Office in the "Household Budget Survey 1974/1975", the housing areas are stratified into 3 categories, namely: squatter, low-cost and high-cost areas. The distinction between the residential areas is that squatter comprise informal or shanty-type housing, which are generally overcrowded housing developments. The low-cost housing includes site and service schemes, and domestic servant quarters. The high-cost residential areas enjoy a reasonably good sanitation and are more spaced.

Table 11

Number of schoolchildren from different residential areas.

Residential area	Total	DM(+)	%
Squatter	166	19	11.4
Low-cost	185	46	24.9
High-cost	49	2	4.1
Total:	400	67	16.8

(The distribution is significantly different between the three residential areas as shown by a chi-square of $P \leq 0.01$)

Generally there are more cases of infection among children from squatter and low-cost areas than high-cost housing areas. There is no simple explanation for the larger number of cases of tinea capitis among schoolchildren from low-cost housing areas as compared with from squatter compounds (bidon villes). Maybe a statistically larger population would provide an insight into this rather unexpected result.

3.1.8 Urban and Peri-urban (= Rural).

The city of Lusaka is divided into 2 areas, viz., urban Lusaka with a radius of about 15 - 18 km, from 18 to about 25 km from the city centre is rural Lusaka (=peri-urban). The examination of hair samples by direct microscopy showed 16.5% of the infections from urban Lusaka while 17.9% were from rural Lusaka.

Table 12

Urban and rural areas of Lusaka.

	Total examined	DM(+)	%
Urban	316	52	16.5
Rural	84	15	17.9
Total:	400	67	16.8

(there is no significant difference ($P > 0.05$) between the number of infected children in rural Lusaka compared to urban Lusaka)

3.1.9 Ethnic Groups.

The population of Zambia is made up of 73 tribes officially recognised by the Zambian Government, speaking different languages and/or dialects. Most people move to Lusaka from other parts of Zambia in search of employment. The population is therefore, heterogenous.

Table 13

Children grouped according to tribes

Tribes	Total examined	DM(+)	%
Bemba	78	14	18.0
Tonga	39	9	23.1
Nsenga	66	7	10.6
Chewa	22	10	45.4
Lozi	50	6	12.0
Others*	145	21	14.5
Total:	400	67	16.8

* "other tribes" represent a smaller proportion of the study and are indicated on the map. fig.1.

3.2 SENSITIVITY of the ISOLATES to GRISEOFULVIN.

The results after 7 days with in the in vitro sensitivity test with griseofulvin are given in table 14.

The growth of the control was assigned a score of 4 and degree of inhibition determined visually on a scale of 3, 2 and 1.

Isolates from Lusaka, Zambia were compared with recently isolated fungal pathogens from tinea capitis from Cameroun.

Table 14

In vitro Griseofulvin Sensitivity Tests.

Concn of griseofulvin ($\mu\text{g/ml}$)	0	0.6	1.3	2.6	5.2	10.5	21.0	42.0.
<u>Zambian T.violaceum.</u>								
RV65949	4	3	3	2	1	-	-	-
" 65950	4	3	2	1	-	-	-	-
" 65951	4	3	2	1	-	-	-	-
" 65952	4	3	2	1	-	-	-	-
" 65953	4	4	3	2	-	-	-	-
" 65954	4	3	3	1	1	-	-	-
" 65955	4	3	2	2	1	-	-	-
<u>Camerounian T.violaceum.</u>								
" 66147	4	3	2	1	-	-	-	-
" 66148	4	3	2	1	-	-	-	-
" 66152	4	3	2	1	-	-	-	-
" 66158	4	3	3	1	-	-	-	-
" 66159	4	2	1	3	-	-	-	-
" 66168	4	3	2	1	-	-	-	-

--no growth, 4=control, 3,2,1=decreasing amounts of growth

13 T.violaceum were tested - 7 from Zambia, 6 from Cameroun. For the 13 T.violaceum tested, the m.i.c. was mostly 4µg/ml.

Only 7 M.langeroni were tested, 3 from Zambia and 4 from Cameroun. With this species, there were some technical problems in interpreting the results. 4 isolates were very sensitive with an m.i.c. of 0.5-1.0µg/ml, 3 other isolates apparently grew at higher concentrations. These 3 were probably contaminated by bacteria and therefore, the m.i.c. could not be accurately determined.

4. DISCUSSION

4.1 DISCUSSION.

4.1.0 Clinical Type.

The results presented are based on direct microscopy for the confirmation of the clinical infection of the schoolchildren. Cultures were only made from the hair specimens which were found positive using direct microscopy. The negative hair specimens were not cultured because of the problem of "healthy carriers". This is the carriage of spores of pathogenic "dermatophytes" on human scalp and healthy animals, which has been repeatedly demonstrated for tinea capitis in a number of studies (De Vroey, 1985). The spread of spores is much more so for microsporic type of infections.

This is why the diagnosis of tinea capitis was based primarily on direct microscopy followed by culture. However, some of the negative results by direct microscopy may not necessarily mean that the children were not infected, but that the collected specimens were not sufficient in quantity and quality.

The results of direct microscopy and culture (table 5) show that six endothrix infections gave rise to M.langeroni. Usually, endothrix infections are caused by Trichophyton species so that these results may have been due to the "contamination" of the hair isolates with the spores of Microsporum. Furthermore, M.langeroni is a faster growing dermatophyte than T.violaceum, M.langeroni may therefore, have prevented T.violaceum from growing. The other possible explanation may be that the hairs used for direct microscopy were the only infected hairs and the rest were not infected. So, in the presence of only "contaminating" spores, they gave rise to the cultures. The fact that the endothrix hairs did not give rise to Trichophyton species but Microsporum species is also evidence for the problem of "healthy carriers" for the fungi causing ringworm of the scalp.

4.1.1 Aetiology.

Lusaka is at an altitude of about 1,300m (4,200ft) above sea level. The main agent of tinea capitis in the schoolchildren studied is T.violaceum accounting for 74.5% and M.langeroni 25.5%.

T.violaceum is therefore, undoubtedly the common agent of tinea capitis in Lusaka. This is in accordance with results from neighbouring countries, for example, Zimbabwe (Robertson, 1986), Mozambique, and in South Africa, Pretoria (Brede, 1972), Bloemfontein (Scott and Scott, 1973), and Transkei (Young, 1976). In Kinshasa, Zaire the dominant species are M.langeroni and T.soudanense (Mamba Kwete, 1987).

The results show that only one ectothrix type out of six was found to produce an inflammatory ringworm. This however, is a rare occurrence, although in a study by Dockx (1969), found that microspories due to M.langeroni were more inflammatory than tinea due to T.violaceum. Dockx, was however, uncertain of whether it was host response to M.langeroni or bacterial superinfection that caused the inflammation. The inflammation in this study, was probably due to a mixed infection with a Trichophyton and Microsporum species. The rest of the inflammatory infections were due to T.violaceum. The presence of 20-25% inflammatory ringworm in the tropics due to T.violaceum has also been reported from Kivu, Zaire by Vanbreuseghem (1963) and more recently from Kinshasa by Mamba Kwete (1987).

4.1.2 Prevalence.

Tinea capitis is a cosmopolitan infection. The prevalence of this disease being influenced by a number of factors, like socioeconomic, public health concern, way of life of

the people etc. Verhagen (1974), in his review of dermatophytes causing tinea capitis in Africa estimates the prevalence to be between 10% to 30% among schoolchildren in Africa.

This study shows that the prevalence is about 16.8% among the schoolchildren in Lusaka, affecting more boys than girls. A recent study by Mamba Kwete (1987) in Kinshasa found a prevalence of 10.22%. Studies carried out in Nigeria found the prevalence to be 6.0% in Lagos, 13.7% in Ibadan, Ife-Ife 55.1%, (Soyinka, 1978). Fekete (1978), in Guinea savanna, found a prevalence of 15.52%, tinea capitis being more common in schoolchildren than in adults. Ajao (1985), in Ile-Ife found 14.02% in schoolchildren below the age of 12 years. In Sudan Mahgoub (1968) found a prevalence of 4.2% rising to 17.0% in certain schools. In Kenya, in the highlands the prevalence is 5-10% (Verhagen et al., 1969) and Diallo (1985), in Senegal found a prevalence of 8.1%. All these studies, except for Ife-Ife (55.1%), show that the prevalence is around 30% as estimated by Verhagen (1974).

The statistics presented here may not reflect the true natural prevalence of "dermatophyte" infections in the Zambian population at large, as the schoolchildren in this study represented a selected sample of children from primary schools with different backgrounds.

4.1.3 School/Family Contacts.

In this study, 40.3% (27/67) of the 67 children confirmed infected indicated staying with an infected sibling(s) at home. Infections among siblings have been reported in some other studies as well, such as the Nigerian study in Ife-Ife (Ajao et al., 1985) and by Raubitschek (1959). Infections among siblings may be due to sharing of combs, beds, sleeping mats, and physical contact during play,

directly or indirectly leading to the general diffusion of the disease in the family (Ajao et al., 1985). The number of boys in a home has been found to be an important factor in the rapid spread of tinea capitis among siblings, as males are generally more prone to infection. The poor hygiene and the habit of sharing headgears is a contributing factor to high infection rates among boys. According to Philpot (1977), contact in school is probably the most important factor in rapid spread of tinea capitis infections.

Although some children had cats or other pet animals at home, this did not contribute to the tinea capitis infections in schoolchildren, as the two species isolated from such cases were anthropophilic.

In classrooms, in Lusaka, children are usually overcrowded, with a class meant for 35 pupils taking as many as 50 to 60 pupils. Such overcrowding increases the chances of physical contact amongst children and therefore, spread of infection. This may be the reason for an increased infection rates during school-going age, from age 7 to age 14 years, during grades I-VII of compulsory schooling in Zambia. The study by Mamba Kwete (1987) demonstrated a significant correlation, that is the higher the number of pupils in a class the higher the prevalence of tinea capitis.

4.1.4 Age.

Age has always been considered as an important factor in dermatomycoses of the scalp in children.

The mean age of the children was 9.4(+/-2.3), with an age range of 6-15 years. The highest frequency of infection occurred between 9-11 years of age. Mamba Kwete (1987), in Zaire (Kinshasa), found the age range 8-9 years with the highest rate of infection.

In studies conducted in other countries, such as the one in West Cameroun, the age range from 5-9 years was the most affected (Ekobo et al., 1984). Another study by Khosa et al. (1981), in India found that the age range 6-10 had a high rate of infection for tinea capitis as a single infection. These studies represent approximately 2-5 years in a child's life when s/he is most prone to infection with tinea capitis. Reid et al. (1968), found a reduction among girls from the age of 9 years in a study in Philadelphia.

In Sudan, children in the age range of 7-10 years had a higher prevalence of ringworm infection than the 11-12 year group (Mahgoub et al., 1968). Germeraad et al. (1962), found in Uganda that scalp ringworm was more common among the younger generation of 6-17 years accounting for 87.0%. Sehgal (1985), in India (New Delhi) found 92.0% of the infected children were younger than 15 years. Similarly, Hajini et al. (1970), in their survey in North India found children between 0-15 years as the most affected. These and other studies have demonstrated consistently the higher infection rates in children below 15 years of age.

However, there is no definite explanation for increased susceptibility to ringworm in the teenage years before puberty. Some authors have suggested the children's skin pH of about 6.5 as probably a major contributing factor. The pH dependence of these superficial fungal pathogens may therefore, lead to higher susceptibility of children before puberty. The high level of hyaluronic acid in the outer root sheath has been implicated as a source of support for the growth of the superficial fungal pathogens on children which leads to high infection rates (Graham et al., 1964). Children's own increasing involvement in recreational and/or extramural activities combined with the "favourable" skin pH, may be considered as the cause of high rates of infection in the school-children in Lusaka during school-going age. Below the age of 4 years, children have less

contact with other children outside the home so the chances of contracting tinea capitis depend on contact with an infected sibling.

The decrease in number of the infected children of both sexes and the disappearance of ringworm at about puberty and postpuberty age is attributed to hormonal changes, which modify the sebaceous secretion of sebum lipids with a fungistatic effect on dermatophytes.

Rothman, Smiljanic and Weitkamp (1947), demonstrated in vitro the fungistatic effect of long chain, odd-numbered, fatty acids which increase in postpubertal sebaceous glands, and so established that adult immunity to dermatophyte infection is due to increased concentration of about five times of fungistatic lipids in postpubertal sebum.

The decrease in pH is probably another additional deterrent factor in preventing "dermatophyte" growth on the skin after puberty. This drop in pH from 6.5 to 4.0 is attributed to the secretion of fatty acids. Sweat has also been suggested to have a definite influence on the pH of the skin, in turn, affects the fungistatic action of fatty acids (Prevost, 1979).

In contrast to the general observations that tinea capitis is more common in the younger generation, Pipkin (1952), indicates that in the United States the incidence of tinea capitis in the postpubertal person is higher than generally realised. This is certainly the case in endemic regions (Vanbreuseghem, 1963).

4.1.5 Sex.

The higher prevalence of tinea capitis caused by anthropophilic species in boys compared with girls and its decrease with age has been repeatedly demonstrated in a

number of studies (Hajini et al.,1970; Nsanzumuhire et al.,1979,1978; Sonyika,1978; Malhotra et al.,1979; Gugnani et al.,1985; Sehgal,1985). In Lusaka, of the children confirmed infected by direct microscopy 18.0% were boys as against 14.7% girls (44 boys and 23 girls). The male:female ratio being 1.8 to 1.

Some authors have attributed the high infection rates among boys as due to sharing of brushes, combs and caps (Sehgal,1985), and probably, the keeping of shorter hair in our Zambian boys and more physical contact during play is a contributing factor for the higher infection rate among boys than girls.

A study in Jerusalem of 6 390 schoolchildren of age range 1 to over 16 years, the highest prevalence was in children between 5 and 11 years, with a preponderance of males (Dostrovsky et al.,1955). Blank et al.(1974), in schoolchildren of age 0-16 years, found more males with tinea capitis than females.

However, a study conducted in Pondicherry (South India) found more females with tinea capitis than males (DasGupta et al.,1975). Another study in the Philadelphia area, where from the early 1960s, there were more cases in boys than girls, but in the late 1970s the incidence was similar in boys and girls. Urbach and Shockman (1983), suggested that the long hair styles ("Afro" or "Beatle") prevented the spores from reaching the scalp. Others have suggested universal schooling, leading to more females in schools than males (Diallo et al.,1985).

4.1.6 Age and Sex.

Generally, in most studies males have been found to predominate compared to the females, and the difference between boys and girls increases with age . In our study, the ratio girl/boy is: 1:1.5 at ages 6-8 years, 1:1 at ages

9-11 years and 1:2.1 at ages 12-14 years, this is in accordance with other reports.

4.1.7 Socioeconomic Factors.

Of the three residential areas, it is apparent that more children with tinea capitis come from the squatter and low cost housing areas, and only an insignificant number are from the high-cost housing areas. By implication, people living in squatter and low-cost housing areas are usually from the low-income group, although it may not be true in all cases, but the majority.

Some of the factors which may favour the spread of tinea capitis in squatter and low-cost areas are generally poor hygienic conditions, overcrowding due to small sized houses, especially in squatter areas, and perhaps non-refuse collection. These probably are the contributing factors to high infection rates of children from these areas. A study in Inkster, Michigan by Bocobo et al. (1965), showed that in certain areas with a concentration of cases of tinea capitis families were living in overcrowded housing developments with low sanitation facilities. In another study, Hajini et al. (1970), found all their 19 patients with tinea capitis belonged to a lower socioeconomic group, and had poor personal hygiene. Philpot (1977), explored the socioeconomic factors and concluded that contacts at home and school, poor hygiene and the number of children in the family do influence spread of tinea capitis. A study in Khartoum, found a difference in infection rates between high-income areas (0.7%) and low-income areas (6.0%) (Mahgoub, 1968).

A study by Reid, Shimkin and Blank (1968), in Philadelphia countered an earlier observation that infection rates with tinea capitis in Negroids in the USA was due to the low socioeconomic status, but showed that it was more associated with overcrowding living conditions and poor hygiene than income per se. In some parts of India where

some people have a low socioeconomic status, ringworm of the scalp is relatively low, some authors quoting 1 or 2%. Some investigators have suspected the use of certain oils with a prophylactic effect. This theory is supported by in vitro studies that such oils do exert an antifungal effect.

About a similar number of children from rural schools were infected with tinea capitis as their counterparts in the urban schools.

However, a study in Khartoum found a higher prevalence of tinea capitis in rural areas than in towns (Mahgoub, 1968).

4.1.8 Dermatophytes.

T.violaceum has been reported often in highland areas. According to Vanbreuseghem (1958), Trichophyton species seem to feel better at home in the mountains. Microsporum species in contrast, have been frequently isolated in low lying areas. Dockx (1969), in Rwanda and Burundi, found a decrease of M.langeroni as the country became mountainous while trichophytic tinea by T.violaceum increased.

Ringworm of the scalp due to anthropophilic species is characterised by 2 types of infections, that is, ectothrix microsporiasis and endothrix trichophytosis. In this survey, 74.5% of the tinea capitis were of the endothrix trichophytosis caused by T.violaceum, while 25.5% were of ectothrix microsporiasis (included are the six endothrix which gave rise to M.langeroni). The negative cultures were of the endothrix infections, whilst all the ectothrix infections gave rise to positive cultures.

The causative "dermatophytes" were T.violaceum for trichophytosis and only one M.langeroni for inflammation.

The only published report on tinea capitis in Zambia to my knowledge, is by Brede (1972), who received 102 hair samples sent to the reference laboratory from Nyanje on the

Great East Road (G.E.R.), Indola? and Ndola. Brede (1972), reports having isolated the following "dermatophyte" species: M.audouinii (probably M.langeroni) 43%, T.schoenleini 16%, T.violaceum 11%, T.mentagrophytes 8%, T.tonsurans 6%, and T.rubrum, M.gypseum, M.canis, T.verrucosum, each 4%. This study confirms the presence of M.langeroni (M.audouinii?) and T.violaceum as cause of ringworm in schoolchildren. The sources of Brede's samples as to sex, age and type of population is not indicated. T.schoenleini, T.rubrum, T.tonsurans together with the three zoophilic species and one geophilic species were not isolated in the course of this study. Other studies in Central Africa indicate a general absence of T.schoenleini as a causative agent of tinea capitis (Vanbreusegem, 1958,1963). T.rubrum is not known to cause tinea capitis, but is responsible for foot and body ringworm, while T.tonsurans to my knowledge is not been reported in Central Africa. Although, it has been reported in East and West Africa on a very small scale (Gugnani et al.,1985).

The remaining three, with the exception of M.gypseum; a geophilic species, are zoophilic fungi: T.mentagrophytes, M.canis and T.verrucosum, can occasionally cause ringworm in man. However, the presence of pets and cats in some children homes, at least in Lusaka, doesn't seem to contribute to tinea capitis infections in children, to judge from the present data.

A recent study by Mamba Kwete (1987), conducted in Zaire (Kinshasa) shows tinea capitis in schoolchildren is caused by M.langeroni (72.26%) and T.soudanense (24.29%).

Earlier work by Vanbreuseghem (1963), in Zaire (Lubumbashi and Kinshasa) found M.langeroni as the dominant species while T.violaceum was less common. T.violaceum has been taken over by T.soudanense, a species also responsible for trichophytosis but which was absent in our study in Lusaka. However, T.violaceum as a cause of tinea capitis has also

been reported in Zimbabwe (Harare), (Robertson, 1986), Mozambique and South Africa (Cape coastal) (Brede, 1972). Other parts of Africa with predominately T.violaceum as an agent of tinea capitis, are: Morocco and Somali (Vanbreuseghem, 1966), Egypt (Taylor and Bassaly, 1964), Uganda (Germeraad and Klokke, 1962), Ethiopia (Brinkmann, 1971), and Kenya (Verhagen et al., 1969), all countries that are found in North and East Africa.

T.violaceum has been commonly isolated from altitudes above 1,200m. Verhagen et al. (1969), have suggested that probably climate has an influence on the high incidence of T.violaceum in mountainous areas. Lusaka stands at an altitude of 1,300m (4,200ft), similar in altitude to the other areas where T.violaceum has been reported, such as Kenya, Rwanda and Burundi.

In Lusaka, T.violaceum was the only species isolated from the rural areas, while in the urban areas both T.violaceum and M.langeroni were isolated. However, T.violaceum was predominant over M.langeroni. Although the size of our sample is somewhat limited the above observations can be made.

4.1.9 Ethnic Groups.

The tribes with more than 5 patients were Bemba, Tonga, Nsenga, Chewa and Lozi, the other tribes had smaller numbers of patients. In this study comparisons of ethnic differences to tinea capitis susceptibility will not be discussed due to insufficient data.

However, differences between races in susceptibility to bacteria and viral infections are well established. It is also been found that this is so for some fungal infections as well, notably for coccidioidomycoses. A number of surveys on the epidemiology of ringworm infections have indicated racial differences may be a contributing factor

in the distribution of such infections (Vanbreuseghem, 1957; Scott et al., 1973).

Vanbreuseghem (1957), has suggested protein deficiency related to A avitaminosis as a plausible reason of racial predisposition to tinea capitis in Negroids. Scott et al. (1973), indicated that racial distribution of anthropophilic fungi are less readily explained.

It was this in mind that the data on ethnic groups was collected. However, further work needs to be done to provide a statistically larger population for definite conclusions to be drawn regarding susceptibility of different ethnic groups to superficial mycotic infections. Probably, factors like differences in accessibility to Lusaka from different parts of Zambia may affect the present ethnic composition, or the anticipated job opportunities to be found in Lusaka as opposed to other towns.

Importance of the isolated species
according to the Literature.

Species	Country	%	Author
<u>T. violaceum</u>			
"	Uganda(Southern)	54.43	(Germeraad et al. 1962)
"	Somalia(south)	75.0	(Vanbreuseghem,1968)
"	Sudan	42.2	(Mahgoub,1968)
"	Kenya	70.16	(Verhagen et al. 1969)
"	Ethiopia(Harar)	91.6	(Szczepanski,1972)
"	S.Africa	55.0	(Brede,1972)
"	Tanzania	25.4	(Nsanzumuhire et al. 1978)
"	Tanzania(DES)	16.7	"
"	Zimbabwe	88.0	(Wright et al.1986)
"	Zaire(Kinshansa)	0.5	(Kwete,1987)
"	Zambia(Lusaka)	74.5	(Simpanya,1987)
<u>M. langeroni (M. audouinii?)</u>			
"	Nigeria (West)	63.0	(Clarke,1953)
"	Nigeria (North)	31.0	"
"	Abidjan	62.71	(Adrieu et al.1959)
"	Egypt (Red Sea.C)	53.0	(EL Mofty,1962)
"	Ruanda-Burundi	41.8	(Vanbreuseghem,1963)
"	Sudan	45.6	(Mehgoub,1968)
"	Ghana	87.1	(Szczepanski,1972)
"	Tanzania	26.9	(Nsanzumuhire et al. 1978)
"	Tanzania (DES)	58.3	"
"	Libya (Benghazi)	23.8	(Malhotra et al. 1979)
"	Zaire (Kinshansa)	72.3	(Kwete,1987)
"	Zambia (Lusaka)	25.5	(Simpanya,1987)

4.2 GRISEOFULVIN.

All the T.violaceum tested were sensitive to griseofulvin including M.langeroni, which was sensitive at lower concentrations compared with T.violaceum. The minimum inhibitory concentration (m.i.c.) for T.violaceum was 4µg/ml, except for three species (RV 65949, 65954 and 65955) from Zambia which were inhibited at 8µg/ml. M.langeroni was sensitive at a lower concentration of 0.5 to 1.0µg/ml.

The m.i.c. found by in vitro sensitivity tests are similar to other reported results. Rosenthal et al.(1960), found m.i.c. of 3-6µg/ml for T.tonsurans and 4-6µg/ml for M.canis. Vanbreuseghem et al.(1966), found the African T.violaceum from Morocco and the Republic of Somalia were inhibited by 8-20µg/ml.

All the isolates were sensitive to griseofulvin. This is similar to the findings of Rosenthal and Wise (1960), that isolates from human subjects receiving treatment showed no increased in vitro resistance to the antibiotic.

It can therefore, be stated that pathogenic "dermatophyte" species are almost always sensitive to this griseofulvin in vitro. Poor or negative clinical results of treatment in certain patients are understood to be due to intestinal absorption, i.e. poor resorption (enhanced by concomitant intake of fatty substances). Some individuals are known to be "poor absorbers" and therefore attain lower blood levels of the drug at all times, a factor that may explain unsatisfactory therapeutic results in some patients (Drouhet and Dupont, 1987).

5. CONCLUSION
and
RECOMMENDATIONS

5.1 CONCLUSION and RECOMMENDATIONS

Of the 67 positive specimens examined by direct microscopy, 78% were endothrix, 10% ectothrix and 12% mycelial. Of the 2 "dermatophyte" species isolated, T.violaceum accounted for 74.5% and M.langeroni for 25.5%. The prevalence of tinea capitis among schoolchildren in Lusaka is 16.8%, with a preponderance of boys (18.0%) over girls (14.7%). With an increase in age more boys than girls become infected.

Clinically, endothrix caused by T.violaceum produced more inflammation compared to only one example of inflammation due to M.langeroni.

A higher number of children from squatter (11.4%) and low-cost (24.9%) housing areas were infected with tinea capitis than children from high-cost (4.1%) areas.

A similar percentage of children from rural schools (17.9%) were infected with tinea capitis as children from urban schools (16.5%).

Of the 67 infected children, only 1/3 had visited a hospital and/or clinic to seek medical treatment, as Verhagen et al.(1969), put it, "that in Africa tinea capitis is hardly considered a serious disease to need medical treatment". This is probably due to its non-life threatening nature.

Tinea capitis, although it has received scanty attention, does constitute a health problem in schoolchildren. This being the first conducted study on superficial mycoses, the extent of the problem may not be fully revealed.

5.1.1 Control.

Some control measures which can be especially useful in the long term, are those directed toward educating adults and children in particular on the preventive aspects of ringworm infections. This can mean involvement of the schoolteachers, who are in direct contact with the schoolchildren, to inform them of the ways the disease is transmitted and how to "avoid" contracting the disease, especially by refraining from sharing fomites.

In terms of treatment, griseofulvin, even with the advent of the azoles, is still the drug of choice.

5.1.2 Recommendations.

In the short term: to encourage the treatment of infected children and the barring of pupils from school as an indirect way of making "reluctant" parents seek medical treatment for their infected children.

In the long term: education, involving children, teachers, medical staff and parents, on the preventive measures against tinea capitis.

In conclusion, it is hoped that this study will stimulate interest in human dermatophytoses and mycoses in general, with a view to establishing a more accurate understanding of the epidemiology of mycoses in Zambia and the distribution of the superficial pathogenic mycota.

5.2 SUMMARY.

400 schoolchildren from 14 schools, in the Lusaka area (Zambia) (11 urban, and 3 peri-urban) were examined for tinea capitis.

150 showed symptoms of tinea capitis infection and 67 of these were confirmed by direct microscopy.

Of the 67 positive hair specimens, 52 were endothrix, 7 ectothrix and 8 with mycelium in skin scrapings.

Only positive specimens were cultured on Sabouraud's medium supplemented with chloramphenicol and actidione.

Cultures were subcultured on Sabouraud's medium without antibiotics for macroscopic characteristics and on diluted Sabouraud (Takashio) medium for microscopic identification.

2 species were isolated from the 67 microscopically positive specimens, T.violaceum (35), and M.langeroni (12). 20 of these specimens were negative on culture.

The prevalence of tinea capitis in schoolchildren is about 16.8%, with a peak infection at 9-11 years.

Clinically, most cases were noninflammatory whether due to T.violaceum or to M.langeroni. The inflammatory cases were caused by T.violaceum.

The number of infected boys than girls is not significantly ($P \geq 0.05$) different.

The higher number of infected children from squatter and low-cost areas was statistically significant ($P \leq 0.01$) compared to children from high-cost areas.

All thirteen T.violaceum were inhibited by 4-8µg/ml while four M.langeorni were inhibited by 0.5-1.0µg/ml of griseofulvin.

6. REFERENCES

6. REFERENCES

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7. APPENDIX

7. APPENDIX.

7.1 Direct Microscopy.

Chlorallactophenol

Phenolic acid	1g
Lactic acid	1g
Chloral hydrate	2g

Hairs can be placed in a drop of chlorallactophenol to clear the keratin, on a glass slide for direct examination.

Potassium hydroxide (KOH) 10%.

KOH	10g
Distilled water	100ml

This is specially used for direct examination of the skin scrapings and nail fragments. To stain fungal elements e.g. dermatophytes and Malassezia furfur, a drop of KOH can be mixed with one drop ink (Parker, Quinck permanent blue-blank) on a slide.

7.2 Culture.

Lactophenol.

Phenol crystals	1ml
Lactic acid	1ml
Glycerol	2ml
Distilled water	1ml

This is commonly used for microsporid examination of cultures.

7.3 Isolation.

Sabouraud's Medium + Actidione-Chloramphenicol.

Pure dextrose	2g
Neopeptone	1g
Agar-agar	2g
Distilled water	100ml

-Actidione is dissolved in acetone to give a final concentration of 0.5mg/ml. This is meant to inhibit mould fungal contaminants.

-Chloramphenicol is dissolved in the medium to give a concentration of 0.5mg/ml. This is meant to prevent the growth of bacterial contaminants

Sterilise for about 20 minutes at 115°C and leave the tubes sloped so as to obtain about a 1cm layer at the bottom.

Differentiation Medium (=Dermatophyte Test Medium-D.T.M.)

In this medium an indicator is incorporated to make the colonies of the dermatophytes more distinctive than other fungi, and to have easily recognisable characteristics. Such media are called "differential" media. However, a word of caution is that:- one is better off using the classical methods, that is, Sabouraud's media with and without antibiotics.

The composition of the medium was as follows:-

Mycological peptone	10.0g
Glucose	10.0g
Agar	20.0g

These ingredients were dissolved in 1 000ml of distilled water by steaming for one hour. This was then adjusted to

pH 5.5 using 0.8 Molar HCL. The following basic medium was cooled to 50°C and the following additional ingredients added were:

Chloramphenicol	2.5mg(in 5ml acetone)
Phenol red solution	40.0ml
Hydrochloric acid (0.8 Molar)	6.0ml

This was autoclaved at 115°C for 10 minutes and cooled to 50°C. Other additional ingredients incorporated into the medium were 4mg/ml of both streptomycin sulphate and penicillin G*. These in addition to chloramphenicol, were to prevent growth of bacterial contaminants.

Sabouraud's Dextrose Medium.

Same formula as above, but without antibiotics

Diluted Sabouraud's (=Takashio's) Medium .

It has the same composition as Sabouraud's medium, but is 10 times less concentrated in dextrose and peptone, and is enriched with 0.1% magnesium sulphate and potassium dihydrogen monophosphate.

Pure dextrose	2.0g
Mycological peptone	1.0g
MgSO ₄ .7H ₂ O	1.0g
KH ₂ PO ₄	1.0g
Agar	20.0g
Distilled water	1 000ml

Note:-

*A stock solution of penicillin-streptomycin of 500mg/ml was as supplied by Microbiological Associates, Maryland 21793 for in vitro diagnostic purposes.

7.2 LIST OF THE PRIMARY SCHOOLS.

URBAN SCHOOLS

1. Chibelo
2. Chamba Valley
3. Chankunkula
4. Chisengalumbwe
5. Emmasdale
6. Muleya
7. Northmead
8. Kamwala
9. Kaunda Square
10. Lusaka Boys
11. Lusakasa

PERI-URBAN SCHOOLS

12. Chilanga
13. Musamba
14. Mt.Makulu

DERMATOPHYTIC EPIDIOMOLOGY AND DIAGNOSTIC RESEARCH 1986-87

CASE NUMBER:

PATIENT CONSENT FORM

We are carrying out a research to find more information on the occurrence and spread of the infection that is found normally on the head of young school children in Zambia. We hope, from the study, to recommend ways of controlling and treating the infection and the use of new medicines for its eradication.

For the purposes of this study we shall require hair samples from the head of infected patients for laboratory identification of the infectious agent. This information will assist us, in the near future, to have effective therapeutic management of the disease in young Zambians.

If you will agree to having your head examined and hair specimens taken for further examination, then we shall be pleased if you will sign below, this indicating your consent to take part in the research.

We will also require the signature of your Parent (or Guardian) and that of your Head Teacher, with another teacher as witness:

Signature of Parent/Guardian

Signature of Head Teacher

Signature of Patient

Signature of WitnessDate: 19

FORM YA NGWILIZANO NDI ODWALA

Tili kufufuza-fufuza kuti tupeze njila m'mene matende abwelela ndiponso ndi m'mene amwazilikila ndiku pezeka nthawidzambili mumitu ya ana ang'ono a sukulu muno mwathu mu Zambia. Matenda amenewa adziwika ndi dzina lakuti CHISESEYA. Tikhulupilila, kuti mukufufuza kwathu, tizatha kupeza njila zovomeletsedwa monga zoyendeselamo ndi kachilitsidwe kamatenda amenewa ndiponso kagwilitsidwe ka mankwala atsopano pakuyesa kugonjetsa nthendayi.

Pa nchito yakufufuza iyi, tizafunako tizidutswa ta tsitsi kuchokela m'mutu wa odwalayo ndi kulipima kuti tidziwe ndikalombo lotani kotani kameneka kamene kaku lengetsa matendawa. Njila zimenezi zizatithandiza ife kutsongolo, ku chinjilidza matenda amenewa ku ana ang'ono mu Zambia.

Ngati inu muvomela kuti mutu wanu ndi tsitsi tidzifufudze, tidza khala okondwa ngati musaina dzina pansipa, ichi chizasonyedza kuti inu muli ogwilizana nafe kufufuza kwathu.

Ndiponso tizafuna makolo, kapena kholo, kapena aliyense amene alikutsunga mwana, kuti nayenso asaine dzina lache, akulu apunzitsi pamodzi ndi muphunzitsi nao afunika kusaina kukhala ngati mboni:

Kuvomeledza ku ckokela ku makolo/osunga

Kuvomeledza ku ckokela kwa m'phuzisi mukulu

Kuvomeledza ku ckokela ku odwala

Kuvomela kuchokela kwa mboni

CASE CARD FORM FOR TINEA CAPITIS

Number:	Microscopic Examination
	Ectothrix:
Name of pupil:	Endothrix:
Age: Sex: M/F:	Microscopic Morphology
Tribe (enthnic group):	Microconidia:
School address:	Macroconidia:
Grade:	Colony Morphology
Home address:	Texture:
Parental occupation:	Topography:
Father:	Rate/tyoe of growth:
Mother:	Marginal Features:
Guardian:	Surface Pigmentation:
Domesticated animals:	Reverse Pigmentation:
cat(s):	Additional Diagnostic
	Techniques:
Dog(s):	Dermatophyte Test*Medium
chicken:	Urease Test:
Infected relative(s):	In vitro Hair
	Perforation Test:
Clinical Presentation:	Vitamin/amino acid Test: