Dermatophytes
(Superficial Mycoses)

Dermatophytoses or Dermatomycoses relates to the fungal infection caused by three related genera of *Fungi imperfecti namely Trichophyton, Microsporum & Epidermophyton.*

Deuteromycetes (*Fungi imperfecti*)
Genus Trichophyton

Trichophyton rubrum
Trichophyton mentagrophytes
Trichophyton schoenleinii
Trichophyton verrucosum
Trichophyton violaceum
Trichophyton simii

Genus Microsporum

Microsporum canis
Microsporum distortum
Microsporum gypseum
Microsporum audouinii
Microsporum equinum

Genus Epidermophyton

Epidermophyton floccosum

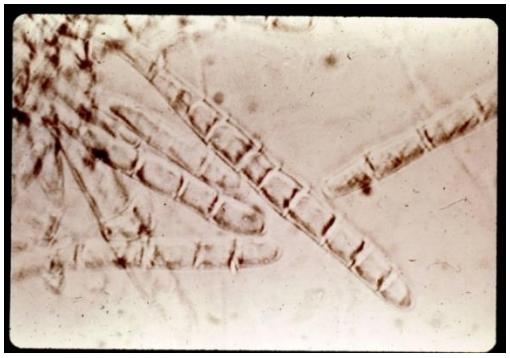
Genus Trichophyton

Trichophyton rubrum
Most cultures have numerous microconidia and moderate numbers of smooth, thin walled multiseptate, slender cylindrical macroconidia.
Colonies are flat to slightly raised, white to cream, with a pinkish-red reverse.

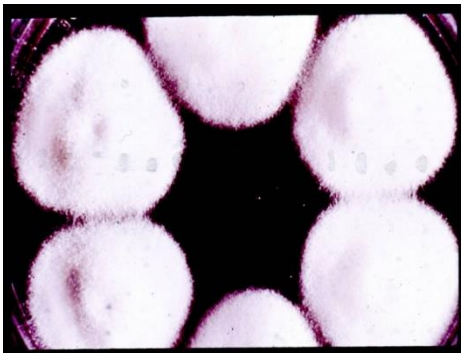
Trichophyton verrucosum
Colonies are slow growing, small, disk-shaped, white to cream coloured, with a velvety surface, a raised centre, and flat periphery with some submerged growth. Reverse pigment may vary from non-pigmented to yellow.
Key features include culture characteristics and requirements for thiamine and inositol, large ectothrix invasion of hair.

Trichophyton mentagrophytes
Numerous spherical to subspherical microconidia are formed, spiral hyphae and smooth, thin-walled, multicelled pencil shaped macroconidia are also present.
Colonies are generally flat, white to cream in colour, with a powdery to granular surface.
Some cultures show central folding or develop raised central tufts or pleomorphic downy areas.
Reverse pigmentation is usually a yellow-brown to reddish-brown colour.

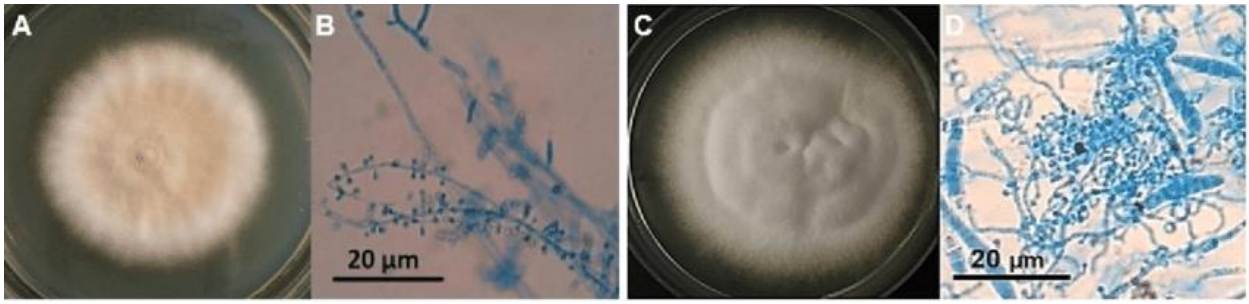
Trichophyton verrucosum



Macroconidia :Large, smooth, thin wall, septate, pencil-shaped



Colonies white to cream coloured, with a velvety surface



Trichophyton genus: *T. rubrum*: A) colony in Sabouraud dextrose agar and B) Microscopy with lactophenol blue solution: tear-shaped hyphae and microconidia. *T. mentagrophytes*: C) colony in Sabouraud dextrose agar and D) Microscopy with lactophenol blue solution: Spiral and thrush hyphae; macroconidia and microconidia found in grape-like clusters (López, et al., 2021).

Genus Microsporum

Microsporum canis

Zoophillic Dermatophyte

Host: Primarily dog,cat & man.Horses less frequently.

Colonies are downy white with yellow pigment on the reverse side by the 2nd week of growth.

Macroconidia are spindle/canoe shaped with thick walls rough exteriors and usually 8 or more cells

***Microsporum* gypseum**

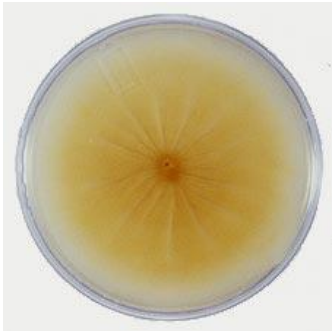
Geophillic dermatophyte

Colonies are usually flat, granular, with a deep cream to orange-tan surface and a yellow brown reverse pigment.

Macroconidia are abundant,thin walls,contain more than 4 cells.

Large spored ectothrix hair invasion.

Front



Backside

Macroconidia: Thick wall, spindle shape, multicellular

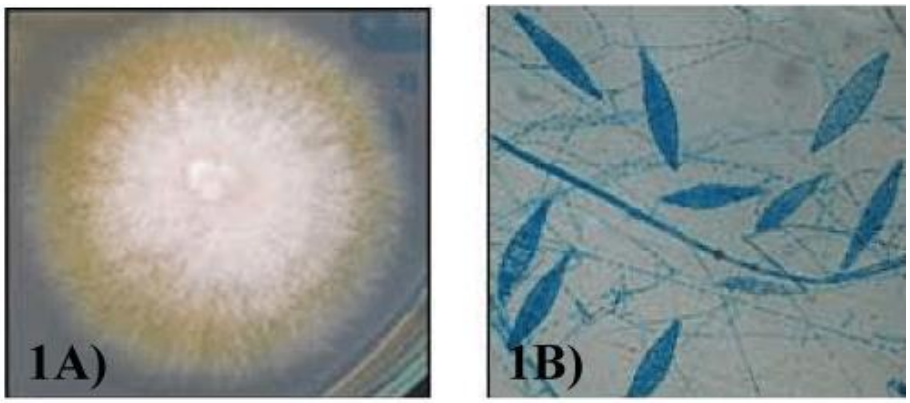


Figure 1: *Microsporum* canis culture, macroscopic colony (A) and microscopic observation in lactophenol (B) (Veterinary Mycology Laboratory, MICVET, Federal University of Pelotas, Rio Grande do Sul, Brazil).

Pathogenesis

Dermatophytes are generally restricted to the cornified, non-living keratin layer of the skin (sufficient availability of iron) and its appendages. They are capable of breaking down the and utilizing as their sole source of nutrients.

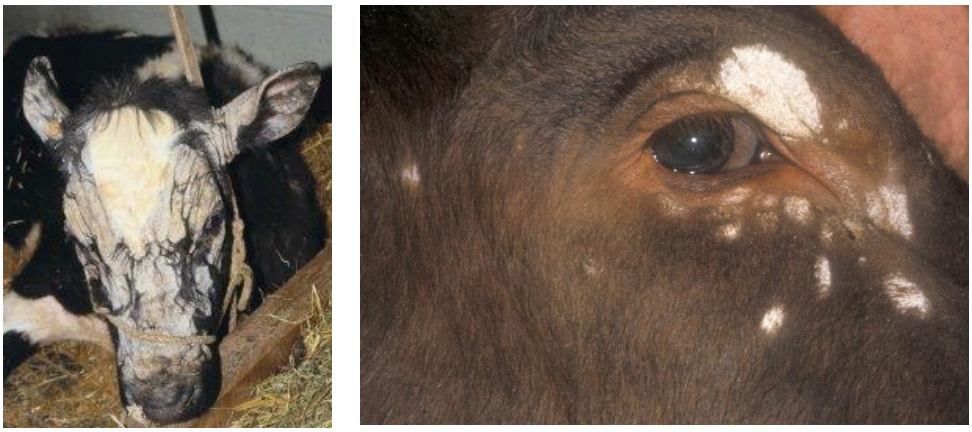
The arthrospores germinate in the s corm\neum and gives rise to long branching septate hyphae which spreads as mycelium through the horny layer and extend radially into adjoining areas of skin.
The localisation of the arthrospores within the shaft of the hair and along the outer surface of the hair gives rise to the conditions i.e., Endothrix and Ectothrix respectively depending upon the species involved.

Ringworm in cattle

Cattle housed indoor are more susceptible to the ringworm.Cattle of all ages are susceptible but young calves are more susceptible to the ringworm.
The common causative agent found is Trichophyton verrucosum, but T.mentagrophytes and T.rubrum are occasionally associated with the ringworm

Incubation period *is 3-4 weeks.*
Clinical picture includes the affected area appears to be circular, hairs in the affected area falls out, raised scaly lesions appears.The lesions are generally restricted to the hump or dorsal aspect of the body or on head (eyes, muzzle, ears),neck, flanks and limbs. The lesions do recover after 3-4 months naturally.

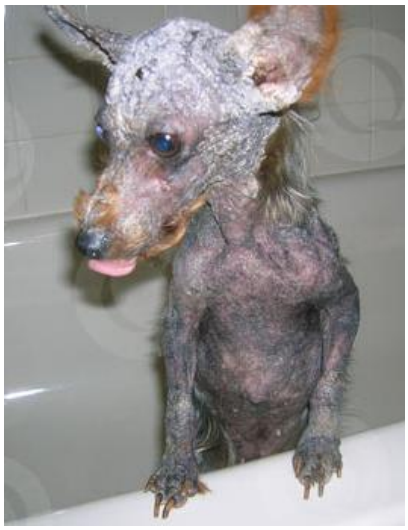
Cattles recovered from the infection are highly resistant to reinfection for

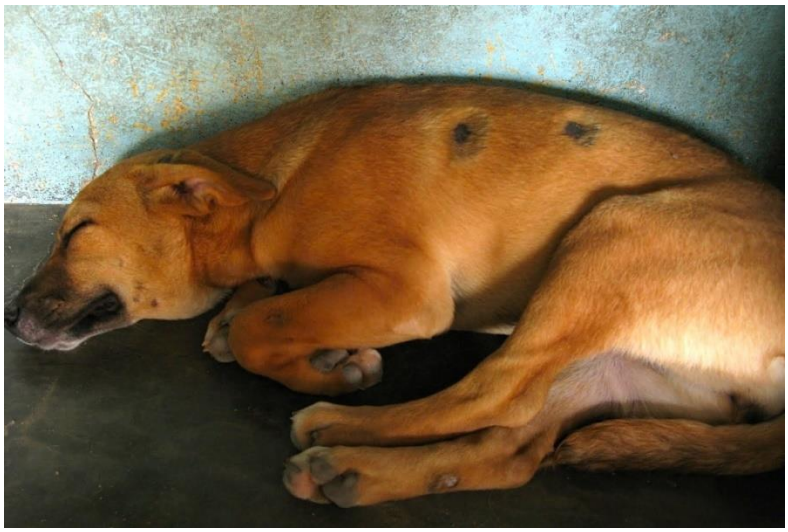


Ringworm in dogs

The common causative agent is *Microsporum canis* and is endemic in most part of the world in dog population.

Lesions in dog appears as thick circular scaly patches upto 3-4 cms in any part of the body. In some cases the lesions are generalized while in some cases the exudative form i.e., 'Wet eczema' is reported. The affected circular area is hairless and vesicles or pustules do appear on the periphery of the lesions.





Treatment

Griseofulvin - An antifungal substance produced by **Penicillium griseofulvin** -oral administration of fungistatic have been reported to be very beneficial in the treatment of Ringworm in case of dogs & cats.
Daily dosage recommended is 20-40mg/kg body weight for 10-15 days.The antifungal is absorbed in to the bloodstream.
Griseofulvin, once the drug of choice for treatment of dermatophytosis,is now used less due to the availability of more effective and less toxic drugs.
Terbinafine and itraconazole are now commonly used in treatment of infections due to Trichophyton spp. and other dermatophytes.

Diagnosis of Dermatophytoses

1.Skin Scrap/Hair Examination

Hydrolysation and partial digestion of keratin by 10-30% Potassium hydroxide can be hastened by gently heating the slide under low flame.
Arrangement of arthrospores in infected hair: Endothrix & Ectothrix.
Staining of fungi In wet mounts with Lactophenol Blue Stain solution

Phenol crystals	20g
Lactic acid	20ml
Glycerol	40ml
Distilled water	20ml
Cotton blue or methyl blue	0.075g

Dissolve the phenol crystals in the liquids by gentle warming and then add the dye.

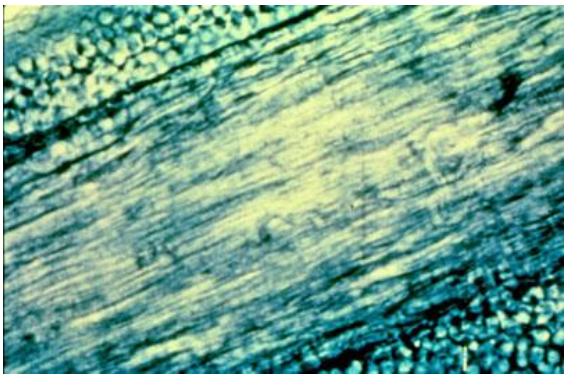
Staining Method

1. Take the scrapping and along with one-two drops of 95% alcohol place with needles or straight wires on the slide. When it is satisfactorily spread, let most of the alcohol evaporate and then add a drop of stain.
2. Apply cover slip, avoiding bubbles and exert gentle pressure if the fungus fragments do not lie flat.

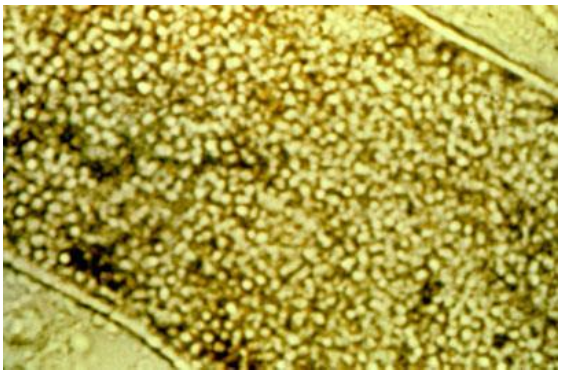
3.Remove any excess stain round the cover slip with the edge of a piece of blotting paper. Let the stain penetrate. For permanent preparations, seal the edges with nail varnish or cellulose lacquer.

Ectothrix and Endothrix

Arrangement of arthrospores in infected hair: Endothrix & Ectothrix.



Ectothrix



Endothrix

Exothrix (ectothrix), where a dermatophyte infection remains confined to the hair surface (**Arrangement of arthrospores on the surface of infected hair**).

Endothrix refers to dermatophyte infections of the hair that invade the hair shaft and internalize into the hair cell (**Arrangement of arthrospores inside the shaft of infected hair**).

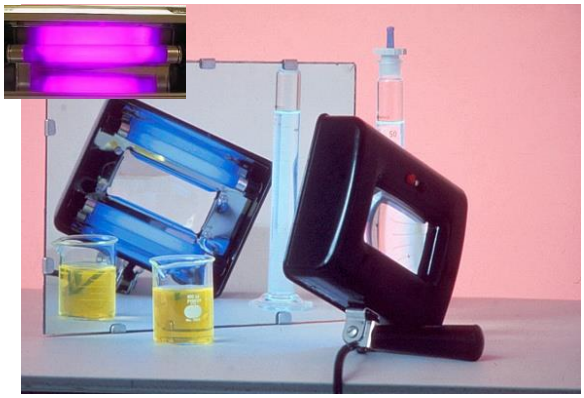
Using an ultraviolet Wood's lamp, endothrix infections will not fluoresce whereas some exothrix infections may fluoresce bright green or yellow-green.

2. Wood's Lamp

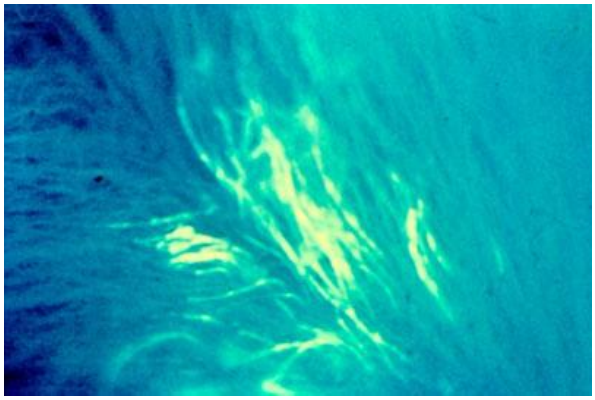
When viewed under the filtered UV rays of Wood's Lamp the hairs infected with M. canis and certain other species of fungi fluoresce with yellow-green coloration while the species of Trichophyton mentioned below either do not show any kind of fluorescence or very poor fluorescence seen with some species.

M.canis	Bright yellow-green fluorescence
M audouinii	Bright yellow-green fluorescence
M.distortum	Bright yellow-green fluorescence
M.equinum	Bright yellow-green fluorescence
M.gypseum	Weak fluorescence
T rubrum	No fluorescence
T verrucosum	No fluorescence
T mentagrophytes	No fluorescence
T.violaceum	No fluorescence
T.Schoenleinii	Very poor fluorescence/None
T.simii	Bright yellow-green fluorescence

As many species do not fluoresce on the exposure to the Wood's UV Lamp, Negative results with Wood's Lamp does not rule out the presence of ringworm infection.



Wood's Lamp



Fluorescing hair (under Wood's lamp) is seen in dogs and cats infected with some dermatophytes

Wood's lamp emits light, from **320nm to 400nm**, with a peak wavelength of 365nm. **[**A Wood's lamp is a source of ultraviolet radiation of wavelengths centered around 3,650 Angstrom units, and ranging from approximately **3,200 A to 4,000 A**]

3. Isolation & Identification

Sample collection: Sample taken for the laboratory diagnosis should include the material from all parts of the lesion. Preliminarily cleanse the affected part/lesion with 70% ethanol .It helps in reducing the bacterial contamination. Scales and crusts should be scrapped with the blunt scalpel and hairs should be plucked from the lesions (never cut).Collect the scales, crust/hairs in a paper envelope and never collect the material in a bottle with closed cap.

Medium Used

Dermatophytes Test Medium supplemented with Cyclohexamide & Chloramphenicol.

Dermatophyte Test Medium (DTM)

Remel Dermatophyte Test Medium (DTM) is a solid medium recommended for use in qualitative procedures for selective isolation of pathogenic fungi (dermatophytes) from cutaneous sources.

The dermatophytes are fungi that possess keratinolytic properties that enable them to invade skin, nails, and hair.

The infections caused by these organisms are commonly referred to as ringworm and are classified by the Latin word **tinea** followed by the area of the body infected.

Dermatophyte Test Medium (DTM) was formulated by Taplin et al. for use in locations where specialized training and microscopic examination is not available.

A pH indicator and three antimicrobial agents are incorporated into the agar to provide a differential and selective medium for isolation of dermatophytes belonging to the genera Microsporum, Trichophyton, or Epidermophyton.

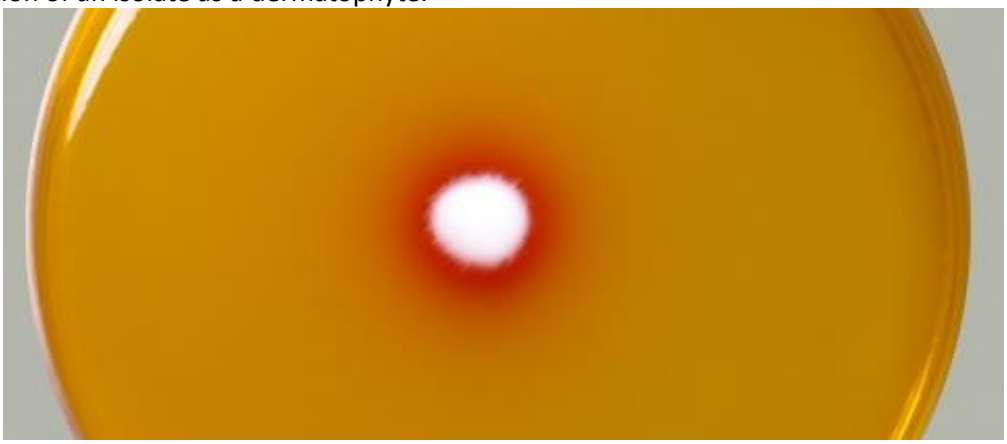
Principle

Soy peptone supplies the nitrogen and carbon compounds necessary for the growth of microorganisms. Dextrose is an energy source.

Phenol red is a pH indicator that detects alkaline metabolites produced by dermatophytes, resulting in a red color development of the medium. Cycloheximide, chloramphenicol, and gentamicin are selective agents that inhibit most saprophytic fungi and many gram-positive and gram-negative bacteria, including some Pseudomonas spp.

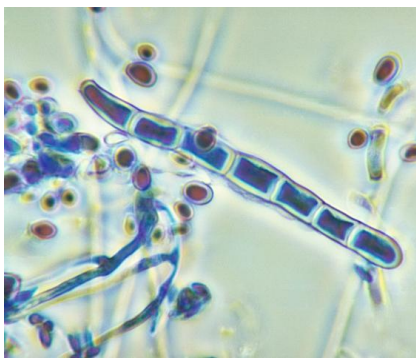
Dermatophyte Test Medium (DTM)
Composition
Dextrose..... 10.0 g
Chloramphenicol .....0.1 g
Soy Peptone..... 10.0 g
Gentamicin .....0.1 g
Cycloheximide..... 0.5 g
Agar .....20.0 g
Phenol Red ..... 0.2 g
Demineralized Water .....1000.0 ml
pH 5.5 ± 0.2 @ 25°C

Procedure
1. Collect specimen following established procedures.
2. Allow DTM to equilibrate to room temperature prior to use. The agar surface should be dry before inoculation.
3. Place the specimen centrally on the surface of the medium and press into the agar to ensure firm contact.
4. Incubate in ambient air at 25-30°C for up to 14 days. Allow the cap on the tube to remain loose so that air may be exchanged during incubation.
5. Examine the medium at regular intervals for a red color development. On DTM, dermatophytes elaborate alkaline metabolites which elevate the pH of the medium and change the phenol red indicator from yellow to red. 5 Note: Microscopic examination (e.g., wet-mount, KOH) is required for presumptive identification of an isolate as a dermatophyte.



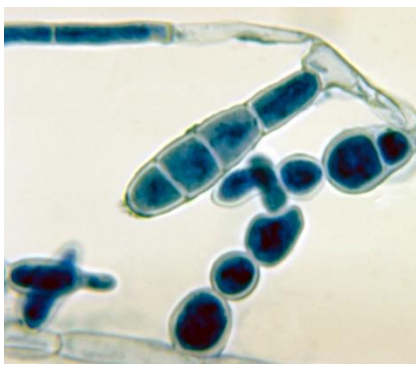
Growth of Trichophyton on Dermatophyte Test Medium Agar with Cyclohexamide and Chloramphenicol

Microscopic after staining with Lactophenol Cotton Blue stain



Trichophyton rubrum

Microsporum canis



Epidermophyton floccosum

Epidermophyton floccosum

Epidermophyton floccosum (*E. floccosum*) is an anthropophilic dermatophyte that causes tinea pedis, tinea unguium, tinea corporis, tinea cruris, and tinea manuum in humans. *E. floccosum* is the only species in the genus *Epidermophyton*, is the third most common cause of tinea pedis worldwide after *Trichophyton rubrum* and *Trichophyton mentagrophytes*.

*Epidermophyton floccosum i*s the only species pathogenic to animals or human beings. This anthropophilic dermatophyte preferentially infects humans and **rarely infects animals**, thus lab animal experiments are found to be unsuccessful. *E. floccosum* is more infective than most dermatophytes.

Morphology and colony characters

Macroscopically, colonies are usually slow growing without any specific growth condition in culture. The colonies appear to form a dark yellow to greenish-brown or khaki-colored with a suede-like surface, raised and folded in the center, with a flat periphery and submerged fringe of growth. The opposite color of the colonies is from a burnt orange appearance to sienna brown.

Microscopically, *E. floccosum* is a filamentous fungus with septate and hyaline hyphae microscopically. Hyphae are characterized by the smooth, thin-walled, clavate, club-shaped macroconidia and the absence of microconidia, which also is a distinguishing feature of this species from the other dermatophytes.

Epidermophyton floccosum

Macroconidia and chlamydoconidia of *E. floccosum*

https://www.adelaide.edu.au/mycology/fungal-descriptions-and-antifungal-susceptibility/dermatophytes/epidermophyton

Pathogenesis of *E. floccosum*

Similar to other fungal dermatophytes, *E. floccosum* contains keratinase giving it the ability to break down keratin within the skin, nails, and hair. Wood's lamp can't be applied for the diagnosis of the *E. floccosum* infection as the fungus does not fluoresce under ultraviolet light.

Reference

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https://www.creative-biolabs.com/drug-discovery/therapeutics/epidermophyton-floccosum.htm

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