



Dermatophytosis Caused by *Trichophyton Mentagrophytes* in an 8-Month-Old Friesian-Bunaji Cross-Breed Calf in Jos, Plateau State, Nigeria

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SUMMARY

Dermatophytosis is a contagious, zoonotic skin disease affecting a variety of domestic and wild animals including man. It is caused by a group of morphologically and physiologically related fungi in the Genera *Trichophyton*, *Microsporum* and *Epidermophyton*. *Trichophyton mentagrophytes* was responsible for dermatophytosis on an 8-month-old Friesian-Bunaji cross bred calf in Jos, Nigeria. The diagnosis was based on clinical signs, direct microscopy and isolation of aetiologic agent on Sabouraud's dextrose agar supplemented with chloramphenicol and cycloheximide. The lesions involving the face, muzzle and jaw were circular, alopecic, thickly crusted, and grayish-white raised above the skin. Direct microscopic examination of skin scrapings and hair pullouts in 10% KOH revealed chains of arthroconidia on the surface of infected hairs. Colonies were flat, white, and granular to fluffy with yellow reverse side. The isolate was urease test positive when inoculated on to Christensen's urea agar. Microscopically, many spherical microconidia arranged in dense grape-like clusters with few multiseptate, thin and smooth walled macroconidia, typical of *Trichophyton mentagrophytes* were observed. Bovine dermatophytosis is economically important both in the livestock and leather industries as well as in public health. The need for prompt diagnosis and treatment of infected animals as preventive and control measures was emphasized

Key words: *Trichophyton mentagrophytes*, dermatophytosis, calf, Jos, Nigeria

INTRODUCTION

Dermatophytosis also known as tinea or ringworm is a disease involving the superficial keratinized layer of the skin, hair and nails of humans and animals (Weitzman and Summerbell, 1995). The disease is caused by a group of physiologically and morphologically related fungi called dermatophytes in the genera *Trichophyton*, *Microsporum* and

Epidermophyton (Chah et al., 2012; Habeb et al., 2016). The dermatophytes are both keratinophilic and keratinolytic, which means they are able to digest keratin in vitro in their saprophytic state as well as *in vivo* in their parasitic state to produce ringworm (Weitzman and Summerbell, 1995)

The disease is transmitted either directly by contact with an infected host or indirectly by

contact with formites contaminated with arthrospores (asexual spores formed in the hyphae of the parasitic stage) or conidia (asexual spores formed in the free living environmental stage) Ringworm in cattle is characterized by circumscribed, scaly, alopecic, thickly crusted, grayish- white lesions raised above the skin, mostly occurring on the head and neck (Haggag *et al.*, 2017). It is highly contagious, and could spread easily from a single infected animal to affect an entire herd population (Pal, 2017). Animals serve as reservoirs of the zoophilic dermatophytes, and constitute a constant source of infection to humans (Hameed *et al.*, 2017). According to Peres *et al.* (2010), dermatophytes affect about 25% of the world population, with an annual USD500 million spent worldwide for diagnosis, treatment and prevention of dermatophytosis. Apart from its zoonotic potential, bovine dermatophytosis causes high economic losses in the livestock and leather industries due to reduced quality of hides, reduced milk yield in lactating animals, poor growth rates in calves and culling of stock for welfare reasons (Bond, 2010; El-Ashmawy *et al.*, 2015). The prevalence and etiologic agents of dermatophytosis in animals may vary from one geographical location to another and appears to be influenced by trade, exchange of animals for the purpose of reproduction, exhibition and sportive activities (Nweze 2011). *Trichophyton verrucosum* is the most common cause of dermatophytosis in cattle (Dalis *et al.*, 2014; Pal, 2017). However, *T. mentagrophytes*, *Microsporum canis*, *M. gypseum* and *M. nanum* are occasionally isolated (Pal and Dave, 2013). In spite, of its significance as a major cause of economic loss, animal ringworm, particularly bovine dermatophytosis is rarely reported (Chah *et al.*, 2012). This paper reports and documents the isolation and identification of *Trichophyton mentagrophytes* from ringworm lesions in an 8-month-old Friesian-Bunaji cross bred calf in Jos, Nigeria.

MATERIALS AND METHODS

Examination of Animal and Collection of Sample

An 8-month-old Friesian-Bunaji cross bred calf was presented with skin lesions suggestive of dermatophytosis. Skin scrapings and hair pullouts were aseptically collected from the edge of the lesions into a clean envelop and transported at room temperature to the Veterinary Microbiology Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for confirmation of diagnosis.

Laboratory Examination

The sample was divided into to two parts. One part was used for direct microscopic examination while the other portion was used for isolation of the etiologic agent in culture.

Direct examination

The portion for direct examination was placed in a drop of 10% KOH on a clean glass slide and covered with a cover slip. The preparation was gently heated for few seconds over flame from a Bunsen burner to facilitate digestion and examined with x10 and x40 objective of a light microscope (Nikon, ECLIPSE- E100, 824592, China) for fungal elements. The presence of hyaline septate hyphae in skin scales or either ectothrix or endothrix spores in hairs was considered positive for dermatophyte infection (Robert and Pihet, 2008)

Isolation of etiologic agent in culture

The part for culture was inoculated onto plates containing Sabouraud's dextrose agar incorporated with cycloheximide at the rate 0.5 mg/ml and chloramphenicol at 16µg/ml. The plates was sealed with masking tape and incubated at room temperature for 14 days. Colony features such as rate of growth, texture, topography and color were noted.

The use of a selective medium is critical for the successful isolation of dermatophytes. This is because skin and hair are susceptible to contain many bacteria and spores of saprophytic fungi which may prevent the growth of dermatophytes in culture (Chah *et al.*, 2012). The addition of

chloramphenicol to the medium inhibits the growth of bacteria while cycloheximide prevents the growth of saprophytic molds and allows the isolation of the etiologic dermatophyte (Robert and Pihet, 2008)

Microscopic identification of etiologic agent

A portion of mycelium was removed from the plate using a sterile needle and emulsified in a drop of lactophenol cotton blue stain on a clean glass slide. The preparation was covered with a cover slip and examined with the x10 and x40 objectives of a light microscope (Nikon, ECLIPSE- E100, 824592, China). The shape, size and arrangement of macro- and microconidia were noted.

Urease Test

Urease test was carried out as described by Ates *et al.* (2008). Briefly, the dermatophyte isolate was inoculated onto Christensen's urea agar slants in a universal bottle and incubated together with an uninoculated control at room temperature for 7 days. Urease production was determined by a change in the color of the medium from orange to pink.

RESULTS

Examination of the animal revealed circular, grayish-white, alopecic and thickly crusted skin lesions involving the head region especially around the eyes, muzzle and jaw (plate I).

Direct microscopic examination of specimen in 10% KOH revealed branching mycelium and arthrospores in skin scales whereas infected hairs showed branching mycelium in their interiors and their surface covered with a sheath of small spores about 3 to 5 microns in diameter, arranged in chains and packed together in a mass (the so called small spored ectothrix) suggestive of *Trichophyton* infection (Chermette *et al.*, 2008)..

Colonies on Sabouraud's dextrose agar were white, flat and granular with yellow reverse colour (plate II). Microscopically, many globose microconidia arranged in dense grape-like clusters were observed with an elongated, pencil-shaped, multisepted, thin and smooth

walled macroconidia typical of *T. mentagrophytes* (plate III). Christensen's urea agar inoculated with the test isolate changed from orange to pink color indicating urea hydrolysis while the control remained unchanged (plate IV).

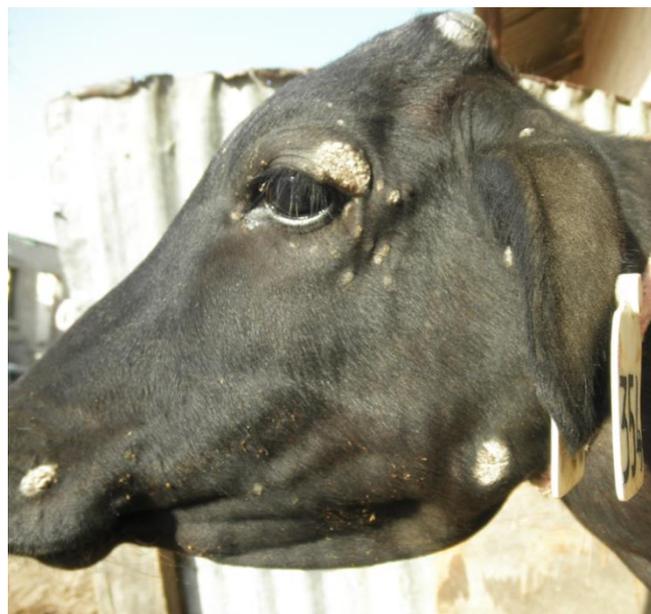


PLATE I: An 8-month-old Friesian Bunaji cross bred calf affected by dermatophytosis. Note the grayish-white, circumscribed, thickly crusted skin lesions around the eye, muzzle and jaw.

DISCUSSION

This study reports dermatophytosis caused by *Trichophyton mentagrophytes* in an 8-month-old Friesian-Bunaji cross bred calf. The diagnosis was based on clinical signs, direct microscopic examination of clinical specimen, isolation and microscopic identification of etiologic agent supplemented with biochemical test.

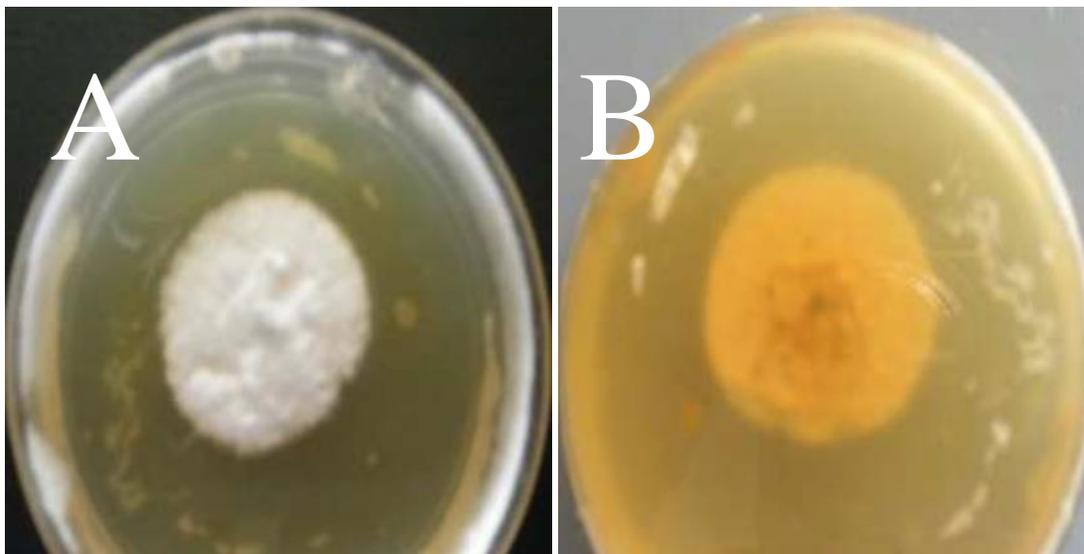


PLATE II: Surface (A) and reverse (B) colony sides of a 14 day- old culture of *T. mentagrophytes*. Note the white, flat and granular surface and the yellow reverse side of the colony

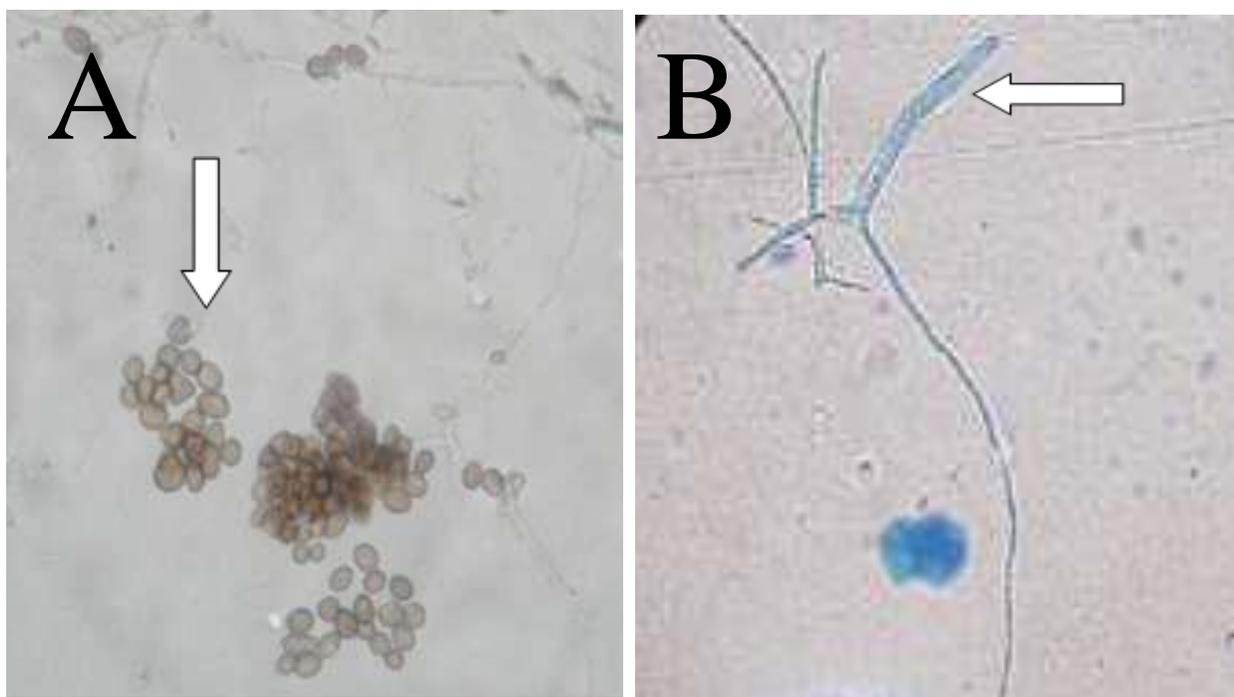


PLATE III: Microscopic morphology of *T. mentagrophytes* stained with lactophenol cotton blue x400. Note: many globose microconidia arranged in typical grape-like clusters (A) and an elongated pencil shaped smooth and thin walled macroconidium (B).

The clinical signs observed in this report were consistent with the findings of Potkonjak *et al.*

(2013), Dalis *et al.* (2014) and Pal (2017). However, the clinical presentation may vary

depending on the immune status of the host, the virulence of the infecting strain and the anatomical site affected (Simpanya, 2000). Furthermore, it is often not possible to differentiate between clinical dermatophytosis and other non-mycotic dermatoses (Robert and Pihet, 2008), necessitating further studies by direct examination and in vitro culture.



PLATE IV: 7 day- old culture of *T. mentagrophytes* on urease agar. Note the pinkish coloration of the test medium compared to uninoculated control medium

The presence of a sheath of small spores arranged in chains and packed together in a mass on the surface of infected hairs as observed in 10% KOH is in agreement with the report of Chermette *et al.* (2008). This type of hair invasion was referred to by Sabouraud as small-spored ectothrix in contrast to the large-spored ectothrix as seen in hairs infected by *T. verrucosum*. It is important to state that infection of hairs by *Microsporum canis* also produces small-spored ectothrix, however, in this case, the spores are smaller, 2 to 3 microns in diameter and are packed together in clusters but not in chains (Chermette *et al.*, 2008).

The macroscopic characteristics of the colonies which were white, flat and granular with yellow reverse sides agree with those reported by Maikha *et al.* (2018) who described the colonies of *T. mentagrophytes* as white, flat with cottony texture which later became powdery to granular with radial grooves and yellow reverse sides. Nevertheless, colony pigmentation may vary from white to cream or yellow. The reverse sides also can vary from yellow to reddish brown to brown or ochre (Robert and Pihet, 2008). In contrast, colonies *T. verrucosum* are glabrous, raised at the center and flat periphery with some submerged growth (De Hoog *et al.*, 2011).

Since culture characteristics such as surface texture, topography, and pigmentation are often influenced by temperature variation, medium and chemotherapy, they are the least criteria for identification of dermatophytes (Faggi *et al.* 2001). Therefore, a study on the microscopic morphology of the micro and macroconidia which is the most reliable identification character for dermatophytes (Robert and Pihet, 2008) is imperative.

The presence of many globose microconidia arranged in dense grape-like clusters with elongated, pencil-shaped, multiseptate, thin and smooth walled macroconidia observed microscopically in this study concurs with the report of Ates *et al.* (2008), Cafarchia *et al.* (2012) and Guadalupe *et al.* (2020). The differences, especially in the shape, size and arrangement of microconidia are the main characters used to differentiate between species of *Trichophyton*. The production of many microconidia that tend to arrange in loose, grape-like clusters by *T. mentagrophytes* distinguishes this dermatophyte from closely related anthropophilic *T. rubrum* which produces tear-shaped microconidia that are borne laterally and singly from the hyphae in what is referred to as “bird on the fence” arrangement (Larone, 2011). The microconidia of *Trichophyton tonsurans* are also borne singly

and laterally from the hyphae, however, in contrast to those of *T. rubrum*, they are irregular in size with many enlarged and either club-shaped and/ or balloon-shaped (De Hoog et al., 2011; Larone, 2011). *Trichophyton verrucosum* produces clavate to pyriform microconidia borne singly along the hyphae. Macroconidia are only rarely produced, but when present they are sinuous (having many curves) and have a characteristic tail or string bean shape. This feature separates *T. verrucosum* from other *Trichophyton* species (Larone, 2011). It is pertinent to state that some atypical isolates of *T. mentagrophytes* may not be easily distinguishable from *T. rubrum*. In this circumstance, further biochemical and physiological tests are necessary for identification (Robert and Pihet, 2008). The hydrolysis of urea by the dermatophyte isolate in this study is in agreement with the report of Summerbell et al. (1988), Ates et al. (2008) and Robert and Pihet (2008) who tested several isolates of *T. rubrum* and *T. mentagrophytes* for their ability to split urea. They found that all the isolates of *T. mentagrophytes* hydrolyzed urea while *T. rubrum* did not, and concluded that the urease test was a rapid and reliable method for separating these two species.

Bovine dermatophytosis is highly contagious. Once the disease is introduced into a herd, it spreads rapidly among susceptible animals and could be easily transmitted to humans.

It is recommended that, animals especially young cattle should be screened routinely for evidence of ringworm, so that positive cases are isolated and treated promptly to avoid spread of the disease to other animals as well as humans.

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