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https://dx.doi.org/10.4314/nvj.v44i3.8 **ORIGINAL ARTICLE**

Evidence Of Trichophyton Tonsurans-Like Dermatophyte Isolated From Skin Lesions In A 2½-Year-Old Nigerian Indigenous Male Dog: A Case Report

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SUMMARY

This paper reports indications pointing to a rare case of the isolation of *Trichophyton tonsurans*like Dermatophyte, which is anthropophilic, from skin lesions in a dog. *Trichophyton tonsurans*. Skin scrapings and hair pullouts were collected and processed for mycology. The susceptibility of the isolates to commonly used antifungal agents was tested. Direct microscopic examination of samples digested in 20 % KOH revealed hyaline septate filaments in skin scales and chains of endothrix spores in hair. Colonies on sabourauds dextrose agar were white, flat and velvety with a reddish-yellow pigmentation on the reverse side. Microscopic examination of the isolates stained with lactophenol cotton blue showed numerous inflated microconidia with abundant chlamydoconidia, typical of *T. tonsurans.* The isolate was sensitive to caspofungin and posaconazole but resistant to fluconazole, nystatin, amphotericin B and griseofulvin. *Trichophyton tonsurans* is not a common pathogen of animals. This report provides evidence of the isolation of *T. tonsurans*-like Dermatophyte from skin lesions of a dogs in Nigeria.

Keywords: Canine dermatophytosis, Trichophyton tonsurans- like dermatophyte, endothrix spores, chlamydoconidia.

INTRODUCTION

Dermatophytosis also known as ringworm is one of the most common and wide spread infectious diseases worldwide (Barros *et al.*, 2007). The disease is usually caused by Dermatophytes, a group of closely related fungi that have the capacity to invade keratinized tissue such as skin, hair and nails of humans and other animals. Dermatophytes comprise three genera namely, *Trichophyton*, *Microsporum* and *Epidermophyton* (Irimie *et al.*, 2011, Dalis *et al.*, 2018). Infection is generally cutaneous and restricted to the non-living cornified layers because of the inability of the fungi to penetrate the deeper tissues or organs of immunocompetent hosts (King *et al*., 1975). The dermatophytes are classified based on their natural habitat as anthropophilic, zoophilic and geophilic. Anthropophilic dermatophytes are associated with humans and transmitted among humans but rarely to animals. Zoophilic species are found mainly on animals but are transmitted to humans whereas geophilic species are usually associated with keratinacious or decaying materials in the soil but can cause infection in both humans and animals (Dalis *et al*., 2018). Dermatophytosis in dogs is characterized by round, raised area of hair loss, mild or intense pruritus and scaly lesions with erythematous borders (Brillhante *et al.*, 2006). In some cases, dogs may develop round nodular lesions that may ooze out a substance called kerion (Cornegliani *et al*., 2009). *Microsporum canis*, a typical zoophilic species has been cited as the most common cause of dermatophytosis in dogs and cats (Moriello *et al*., 2017) and less frequently due to *Trichophyton mentagrophytes* and *M. gypseum* (Nweze, 2011). However, *T. tonsurans* is typically an anthropophilic dermatophyte mostly associated with human infection and rarely isolated from animals (Brillhante *et al.*, 2006). This paper describes and documents the isolation of a *T. tonsurans*- like dermatophyte from skin lesions of a dog in Zaria, Nigeria.

MATERIALS AND METHODS

A 21/2-year-old Nigerian indigenous dog weighing 8kg kept as a surgical experimental animal in the Kennel of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, was observed with circumscribed nodular lesions with alopecia characterized with pruritus and erythema on the dorsum and around the lateral aspects of both thighs (Plate I). Skin scrapings and hair pullouts were obtained as described by Brillhante *et al.* (2006). Briefly, the site with lesions was cleaned with cotton wool soaked in 70 % alcohol to remove surface adhering organisms. Skin scrapings were collected from the edge of the lesions using sterile scalpel blade while infected hairs were pulled out using sterile forceps. The samples were placed in clean envelops and labeled. Samples were taken to the Microbiology laboratory of the Department of Veterinary Microbiology, Ahmadu Bello University, Zaria for laboratory analysis.

Laboratory Analysis

Both the skin scrapings and hair pullouts were each divided into two parts. One part was used for direct examination while the other portion was used for cultural isolation of the etiologic agent. Direct examination of samples was performed based on the method described by Robert and Pihet (2008). Each sample was placed in a drop of 20 % potassium hydroxide

(KOH) – (Guangdong Guanghua chemical factory Co. Ltd Shantou, Guangdong, China) on a glass slide and covered with a cover slip. The preparation was gently heated over the flame from a Bunsen burner to facilitate digestion and the preparation was observed using X40 objective of a light microscope (Olympus XSZ-107 BN). The presence of hyaline septate hyphae in skin scale or presence of spores within the hair shaft (endothrix) or outside the hair shaft (exothrix) was indicative of the presence of dermatophytes. The isolation of the etiologic agent in culture was done following standard procedures (Robert and Pihet, 2008). The specimens were inoculated onto Sabourauds dextrose agar (Oxoid^{TM}) supplemented with chloramphenicol (Chlorocarp®) – (Fabrique par Yangzhou, China) at 16 µg/ml and cycloheximide (Oxoid^{TM}) at 0.5 mg/ml. The plates were sealed with a masking tape, labeled and incubated at room Temperature for 14 days. Colony characteristics such as growth rate, topography, texture and pigmentation (surface and reverse) sides were noted. Microscopic examination of the fungal isolate was carried out as described by Dalis *et al.* (2014). A portion of mycelium was removed from pure culture of dermatophyte and emulsified in a drop of lactophenol cotton blue mounting fluid on a clean glass slide. A cover slip was applied and pressed gently to remove air bubbles. The preparation was examined with the X40 objective of the light microscope. The shape, size and arrangement of macro and microconidia were noted.

Antifungal Sensitivity Test

Antifungal sensitivity of the isolate to caspofungin, posaconazole, fluconazole, nystatin, amphotericin B and griseofulvin was carried out using the disk diffusion method as described by Singh *et al.* (2007). Briefly, the suspension of the inoculums was prepared by harvesting hyphal segments and conidia from a 10-dayold pure culture of the dermatophyte grown on potatoe dextrose agar (PDA) and suspended in 5 ml of sterile saline in a test tube and vortexed for 20 seconds. The preparation was kept on the bench at room temperature for 15 minutes to allow heavy hyphal fragments and conidia to settle down. Homogenous suspension of the supernatant was collected in a new sterile test tube and the turbidity was standardized to match that of 0.5 McFarland's standard. A fresh sterile cotton-tipped swab was dipped into the suspension. Excess liquid was removed from the swab by pressing it against the side of the tube. Using the swab, the entire surface of a fresh Sabourauds dextrose agar plate was covered with the inoculum by streaking back and forth from edge to edge. The plate was allowed to dry for 10 – 15 minutes before the disks were applied. Each disk was pressed down firmly to ensure complete surface contact with the media. Diameter of the zones of fungal inhibition was measured to the nearest millimeter using a ruler after 7 days incubation at room temperature.

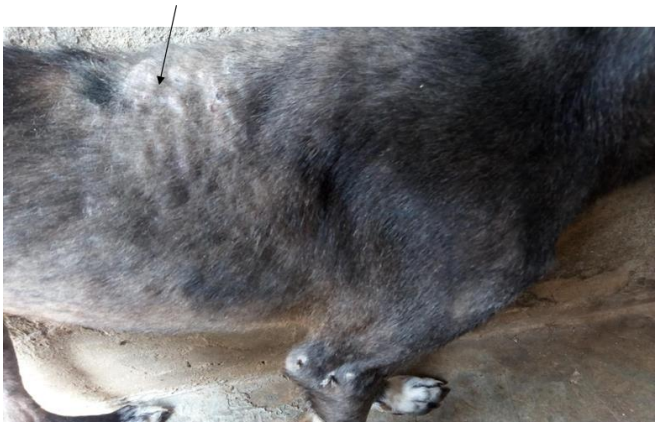

Plate I: Dorsum of a 21/2-year-old Nigerian indigenous dog showing lesions of dermatophytosis. Note the discrete, circumscribed aloepecic regions (arrow).

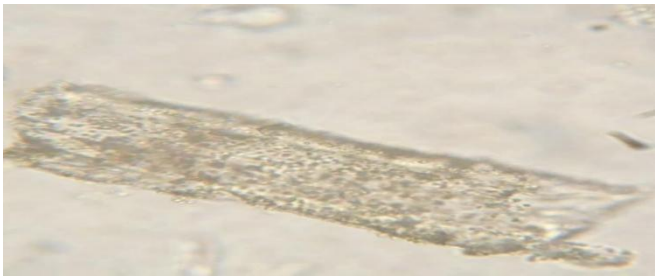

Plate II: Photomicrograph of a dermatophyte infected hair digested with 20 % KOH viewed at X40. Note the numerous spores within the hair shaft.

Plate IIIa: Surface side of a 14-day-old culture of *Trichophyton tonsurans*-like dermatophyte on SDA. Note the wooly whitish surface and a waxy appearance with serrated edges and raised centre.

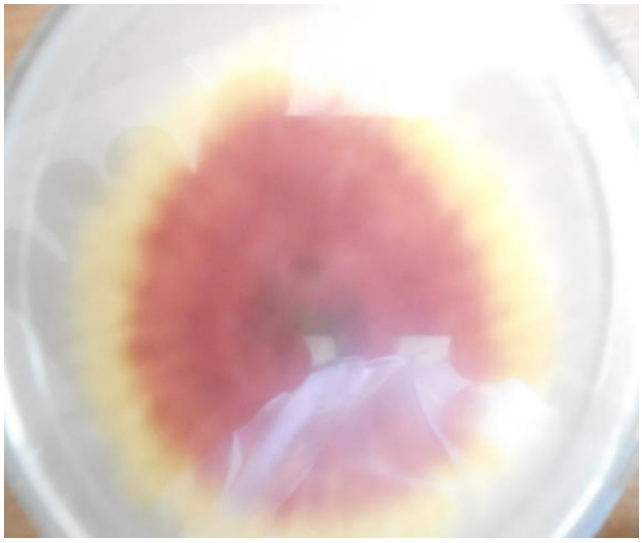

Plate IIIb: The reverse side of a 14-day-old culture of Trichophyton tonsurans-like dermatophyte showing a tan-red and yellowish colour.

Direct microscopic examination of sample revealed hyaline septate hyphae in skin scales whereas chains of endothrix spores were observed in hair suggestive of dermatophyte infection (Plate II). Colonies on Sabourauds dextrose agar were white, velvety and flat with serated edges and raised centres (Plate IIIa). The reverse side was tan-red to yellow in colour (Plate IIIb). Microscopic examination of the isolate stained with lactophenol cotton blue revealed numerous irregularly enlarged and ballon-shaped microconidia (Plate IV) and abundant chlamydoconidia, typical of *T. tonsurans* (Plate V). The isolate was sensitive to caspofungin and posaconazole; intermediately sensitive to voriconazole, ketoconazole and itraconazole, but resistant to fluconazole, nystatin, amphotericin B and griseofulvin (Table 1, Plate VI).

DISCUSSION

Trichophyton tonsurans-like Dermatophyte was isolated from typical lesions of suspected dermatophytosis from a dog in Zaria, Nigeria. The isolate was susceptible to caspofungin and Posaconazole but resistant to fluconazole, nystatin, amphotericin B and griseofulvin. The diagnosis of canine dermatophytosis in this report was based on clinical presentation, direct microscopic examination of the specimen, gross examination of colonies of etiologic agent in culture and microscopic examination of fruiting bodies produced by the isolate. The manifestation of canine dermatophytosis as discrete, circumscribed alopecia in the report agrees with the findings of Brillhante *et al.* (2006), Nweze (2011) and that of Moriello *et al.* (2017). However clinical dermatophytosis in animals may vary widely depending on several factors including age, immunological status of the host, the anatomic site involved and the virulence of the infecting strain (Moriello *et al.*, 2017).

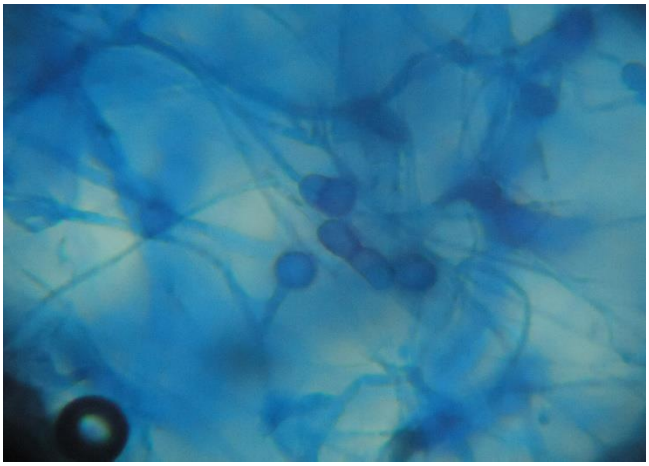

Plate IV: Microscopic appearance of Trichohyton tonsurans-like dermatophyte stained with lactophenol cotton blue and viewed at X40. Note the typical globose microconidia, swollen, elongate, occasionally on match stick-like conidiophores stained with lactophenol cotton blue.

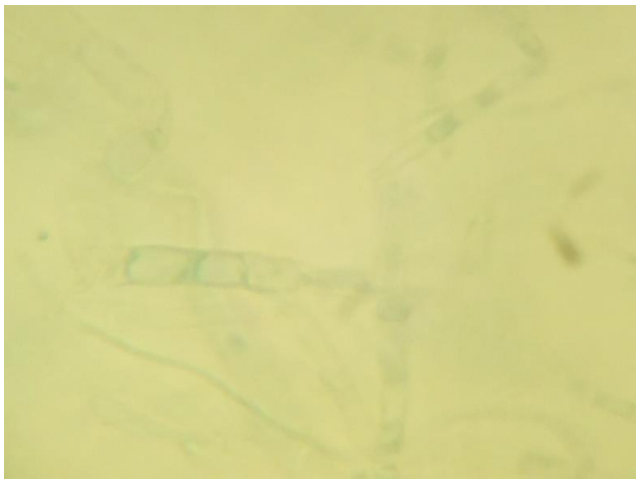

Plate V: Microscopic appearance of Trichophyton tonsurans-like dermatophyte showing typical few macroconidia arising at right angles to hyphae.

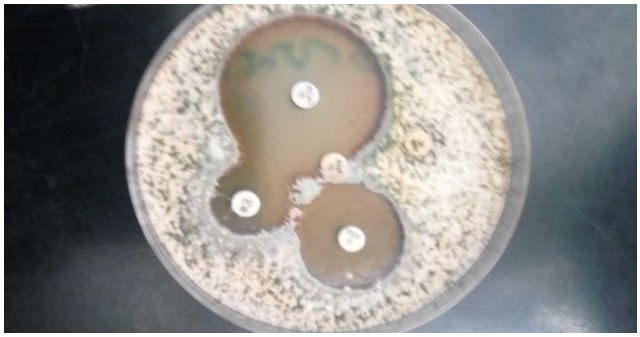

Plate VI: A representative plate showing the antifungal susceptibility testing of routinely used antifungal agents on *Trichophyton tonsurans*-like dermatophyte.

TABLE I: Antifungal sensitivity test of routinely used antifungal agents on *Trichophyton tonsurans*-like dermatophyte isolated from a Nigerian indigenous male dog.

Antifungal agent	Concentration (µg)	Zone of inhibition (mm)	Interpretation
CAS	5	20	S
VOR	1	15	I
FCA	25	0	R
NS	100 units	0	R
POS	5	25	S
KCA	10	16	I
ITC	50	16	I
AMB	20	8	R
AGF	10	0	R

Key: CAS=Caspofungin, VOR=Voriconazole, FCA= Fluconazole, NS= Nystatin, POS= Posaconazle, KCA= Ketoconazole, ITC=Itraconazole, AMB=Amphotericin B, AGF=Griseofulvin. S= Sensitive, I=Intermediate sensitivity, R= Resistance; according to standards by CLSI, 2010.

The presence of hyaline septate hyphae in skin scales and chains of endothrix spores in hair suggestive of *Trichophyton* infection is consistent with other reports (Dalis *et al.,* 2014, Moriello *et al*., 2017). According to Moriello *et al.* (2017), arthroconidia have different dimensions and dispositions on the hairs depending on the species. *Microsporum canis* produces clusters of very small arthroconidia whereas, members of the Genus *Trichophyton* form chains of arthroconidia. Although direct examination is a good method for presumptive diagnosis of dermatophytosis, it is not possible to determine the species by this technique. Therefore, the isolation and identification of the etiologic agent to species level is critical especially for the purpose of therapeutic interventions. The dermatophyte isolated and being presented in this report produced numerous balloon-shaped microconidia and abundant chlamydoconidia typical of *T. tonsurans*. This observation is in agreement with the report of Balogun *et al.* (2017). The shape, size and arrangement of macro and microconidia have been used to identify dermatophyte species (Robert and Pihet, 2008, Dalis *et al.,* 2014, Balogun *et al.,* 2017). However, in the phase of modern diagnosis, molecular techniques need to be employed to conclusively determine the identity of the etiologic agent. Antifungal susceptibility testing in this report suggests that caspofungin and posaconazole are the drugs of choice for the treatment of dermatophytosis caused by *T. tonsurans*-like dermatophytes. Antimicrobial susceptibility testing is important to confirm susceptibility to empirical antimicrobials or to detect resistance in individual fungal isolates. Dermtophytosis is transmitted by direct contact with infected hosts or indirectly with contaminated fomites or the environment (Robert and Pihet, 2008). We do not know the source of infection to the dog in this study, whether it was from the attendant or from the environment, it is difficult to say since neither of these sources was examined. *Trichophyton tonsurans* is a common source of Tinea capitis in humans especially children (Nweze and Okafor, 2005) but very rarely isolated from animals

(Brillhante *et al.*, 2006). As far as we know, this is the first report providing evidence of the isolation of *T. tonsurans*-like Dermatophyte from skin lesions of dogs suspected of Dermatophytosis in Nigeria.

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