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Case Report

Mixed infection of *Trichophyton* species in a Nigerian part Arab horse with dermatophytosis

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A five-year-old Nigerian part Arab horse kept in the University of Nigeria, Nsukka demonstration farm was observed to have non-exudative, scaly circumscribed lesions on its body. Two dermatophytic fungi, *Trichophyton mentagrophytes* and *Trichophyton verrucosum*, were isolated from its hair plucking and skin scrapings. This report describes the trend in the diagnosis and differentiation of the two species of dermatophytes in a con-current infection of horse.

Key words: Dermatophytes, horses, *Trichophyton*, mixed infection.

INTRODUCTION

Dermatophytosis is one of the most common and economically important causes of several dermatoses that have been observed in equine species (Fadok, 1995; Ural et al., 2008). In equines, it is caused by the infection of hair roots and follicles by filamentous fungal organisms belonging to the group called dermatophytes (Shimozawa et al., 1997). These organisms are primary cutaneous pathogens and comprise the largest group of fungi that cause skin disease particularly of the stratum corneum (Quinn and Markey, 2003; Ural et al., 2008). They produce keratinase enzyme which have keratinolytic effect enabling them to utilise keratin as substrate (Grappel and Blank, 1972). This enables them to colonize and invade the cornified epidermis and keratinized adnexal structures such as hair and nail that are derived from it (Weeks et al., 2003; Bernado et al., 2005; Issa and Zangana, 2009), producing classical circumscribed, alopecic, crusty and scaly skin lesions generally called ringworm (Fadok, 1995; Quinn and Markey, 2003).

Dermatophytic agents are classified into three ecological groups as anthropophilic (mostly associated with humans), zoophilic (associated with animals) and geophilic (found in soil) (Weitzman and Summerbell, 1995). These ecological adaptations have enabled them to have a wide range of host (Quinn and Markey, 2003), and their zoonotic and public health importance have been well recognized (Shams-Ghahfarokhi et al., 2009).

In horses, *Microsporum* and *Trichophyton* species have been reported to be the causative agents of dermatophytosis (Quinn and Markey, 2003; Ural et al., 2008). *Trichophyton equinum* is the most commonly involved agent and has been reported in many countries (Hasegawa and Usui, 1975; Al-Ani et al., 2002). Other *Trichophyton* spp. that have been isolated include *Trichophyton mentagrophytes* (Shimozawa et al., 1997; Quinn and Markey, 2003) and *Trichophyton verrucosum* (Shimozawa et al., 1997; Khosravi and Mahmoudi, 2003). These fungal species however were isolated from single infections in horses.

Diagnosis of dermatophytosis is often based on the clinical presentation and history, microscopic and cultural (which involves the observation of the obverse and reverse sides of the fungal colonies) morphological studies, biochemical tests, use of selective media and hair penetration test (Weitzman and Summerbell, 1995; Quinn and Markey, 2003; Weeks et al., 2003).

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CASE REPORT

A five-year-old horse in the University of Nigeria, Nsukka demonstration farm was found to have non-exudative, circumscribed, alopecic lesions on its body. Anamnesis revealed that prior to the introduction of the horse into the farm, a mare with generalized circumscribed alopecic lesions was present and both were kept together. Few weeks thereafter, the infected mare died. Several weeks later, patches of raised hair were observed on the head, lateral abdominal region and hind limbs of the horse in the present case (Figure 1A). Consequently, the hairs detached leaving alopecic, grey, and shining areas with diameters of approximately 1 to 3 cm. The lesions progressed to the hind limb, inguinal and ventral abdominal regions of the body. The lesions on the head became encrusted with severe desquamation of the epidermal tissue debris. Ticks were also found attached to the axillary, perineal and navicular regions of the horse.

On presentation to the University of Nigeria Veterinary Teaching Hospital (UNVTH), physical and clinical examination showed that the animal was in good general body condition. De-ticking was done by hand-picking and tick bite wounds were dressed after disinfection by topical application of gentian violet. Systemic chemotherapy given included penicillin/streptomycin combination and multivitamin. However, the circumscribed lesions were observed to have increased to about 4 to 5 cm (Figure 1B) with those on the face and hind limbs coalesced to form large alopecic areas (Figure 1C and D). The horse was observed to be emaciated. A mycotic cause was suspected on the basis of the characteristic annular, alopecic, scaly and non-exudative appearance of the lesions.

Sample collection for microbiological examination was done as were described by Weeks et al. (2003). Following disinfection with 70% alcohol, skin scrapings and hair plucks were taken from the advancing border of the lesions using sterile disposable scapel blade. The samples were transported to the laboratory using clean, dry sterile petri dish.

Mycological examination was done by digesting a portion of the sample with 10% potassium hydroxide (KOH) for direct microscopic examination of typical hyphae or arthroconidia (Quinn and Markey, 2003). Isolation of the fungal agents were done by culturing using Sabouraud dextrose agar (SDA) supplemented with cyclohexamide (0.4 mg/L), chloramphenicol (0.05 g/L), and gentamicin (0.16 mg/L) (Nweze, 2011). Cultures were incubated aerobically for 2 weeks at 25 and 37°C, and were observed daily for growth of dermatophytes (Weeks et al., 2003). Identification of dermatophyte species was performed by macroscopic examination of colonial appearance. Culture slide smear stained with lactophenol cotton blue was used for microscopic identification following the methods described by Bernado et al. (2005) and Issa and Zangana (2009). Biochemical study (urease hydrolysis) and culturing on SDA supplemented with 5% salt (Issa and Zangana, 2009) were done to further confirm the identity of the isolated dermatophyte species (Campbell et al., 1996; Weeks et al., 2003; Issa and Zangana, 2009).

Hair plucked from the margin of the head lesion and scales collected from this site did not reveal fungi by direct microscopy (KOH). At 8 days of incubation, two different fungal isolates were observed macroscopically at the spots where hair strands touched the agar (Figure 2A and B). Both isolates had similar obverse morphology - white, buff initially but later became folded with raised centre (Figure 2C). On the reverse, one of the isolates gave colourless/tan pigmentation, whereas the other gave wine red (reddish-brown) pigmentation (Figure 2D and E).

The isolates were purified using SDA supplemented with chloramphenicol (5%) to ensure that further studies were conducted using pure isolates (Figure 2C and D). Of the two isolates, only one (with reddish-brown reverse) yielded growth on SDA supplemented with 5% salt while, only the other (tan coloured) isolate gave characteristic heaped/"button-shaped" growths at 37°C (Figure 3A). Microscopic examination of the stained culture smear of the non-pigmented isolate showed chlamydospores arranged in chain-like appearance, whereas that of the red wine pigmented isolate revealed multi-septated, clavate, smooth and thin-walled macroconidia (Figure 3A and E). The isolates were also observed to be urease positive (turned urea agar from yellow to pink) after 7 and 15 days of incubation, respectively (Figure 3B and C).

DISCUSSION

Equine dermatophytosis is caused by dermatophytic fungi which are keratinophilic and keratinolytic (Connole, 1990; Ural et al., 2009). It has been well documented that apart from keratinase, dermatophytes also produce proteolytic and lipolytic enzymes that have major roles in mycotic invasion and pathogenesis (Weitzman and Summerbell, 1995). These factors enable them to destroy skin and associated structures in a centrifugal pattern giving rise to alopecic, scaly or crusty annular "ringworm" lesions as were observed in the present case (Quinn and Markey, 2003; Ural et al., 2009).

Diagnosis of dermatophytosis is based mainly on history and clinical manifestation, direct microscopic examination, cultural and microscopic studies (Quinn and Markey, 2003).

Characters that have been employed in identifying dermatophytes include colony pigmentation, texture, growth rate, and distinctive morphological features such as microconidia, macroconidia and nodular organs (Weitzmann and Summerbell, 1995). The macroscopic features observed were typical for *Trichophyton* spp.

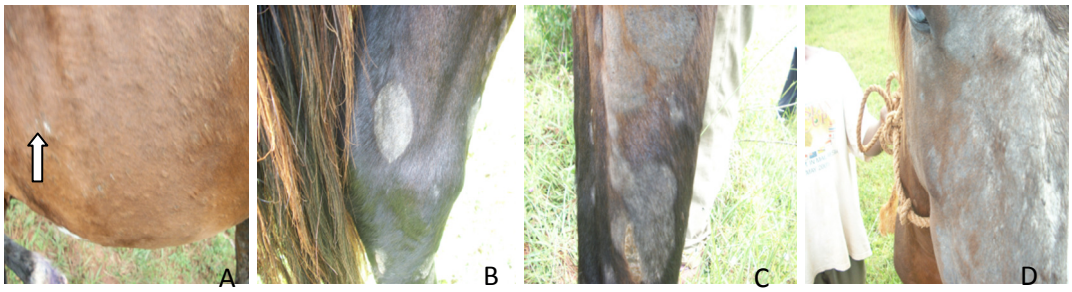

Figure 1. (A) Initial lesion of raised hair patches and focal alopecic patch (arrow). (B) Annular (circumscribed) alopecic, greyish, shinning characteristic "ringworm" lesion on the hind limb. (C) Coalesced lesions on the same limb after 2 weeks. (D) Crusty, coalesced alopecic lesion on the head.

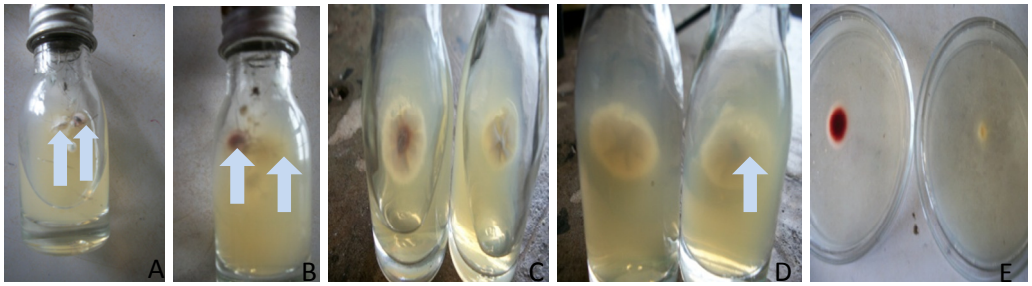

Figure 2. Primary culture, (A) Obverse: Growth of two dermatophyte species (arrows) at spots where hair strands touched the agar after 8 days of incubation at 25°C; (B) Reverse: Wine red and colourless pigmentation of the growths. Pure culture, (C) Obverse: White, powdery, folded, velvety with central folding similar to both isolates after 12 days of incubation at 25°C; (D) Reverse: Colourless (left) and wine red pigmentation (right with arrow) of the isolates; (E) Reverse of the isolates on SDA agar plates.

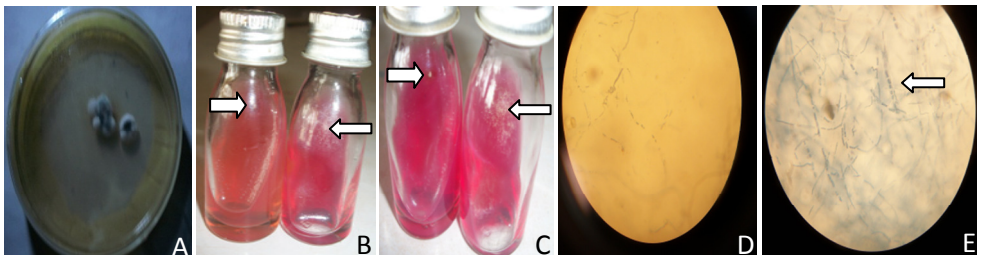

Figure 3. (A) Heaped "button" morphology of *T. verrucosum* cultured at 37°C after 4 days of incubation; (B) Urease positive test of *T. verrucosum* (left) and *T. mentagrophytes* after 7 days of incubation at 25°C, observe that *T. verrucosum* which is glabrous (left) showed slow hydrolysis; (C) Complete hydrolysis after 15 days of incubation (arrows point at fungal growth); (D) *T. verrucosum* chlamydospores in ''antler-like" arrangement; (E) *T. mentagrophytes* multinucleated smooth, thin-walled clavate ''cigar shaped" macroconidia (x400).

T. verrucosum colonies have been reported to be glabrous, folded, heaped, velvety, wrinkled and white, with an unpigmented (colourless/tan) reverse (Quinn and Markey, 2003).

Enhanced growth and formation of "button-shaped" and/or heaped colonies on culturing at 37°C, has been widely utilized in differentiating *T. verrucosum* from other *Trichophyton* spp. (Khosravi and Mahmoudi, 2003). *T. mentagrophytes* complex, both zoophilic (var. *Interdigitale*) and anthropophilic (var. *mentagrophytes*) strains have been described. The former strains produce colonies that are granular and off-white colour, while the latter are white and fluffy with wine red colour pigment on the reverse side (Weeks et al., 2003). Again, growth on salt-

supplemented SDA has been used to differentiate *T. mentagrophytes* from other *Trichophyton* spp. (Weitzmann and Summerbell, 1995). These characters were observed in the present case.

The microscopic "antler-like" arrangement of chlamydospores (chlamydoconidia) observed in the present case has been a feature used for identifying *T. verrucosum* (Quinn and Markey, 2003; Shams- Ghahfarokhi et al., 2009). It is also well documented that this *Trichophyton* spp. rarely produces macroconidia and often times do not produce microconidia (Weitzmann and Summerbell, 1995; Quinn and Markey, 2003). *T. mentagrophytes* produce macroconidia which are clavate, multicelled (multi-septated), cylindrical, singly-borne, and smooth-thin walled (Weitzmann and Summerbell, 1995; Quinn and Markey, 2003). Both species have also been reported to produce urease enzyme (as virulence factor) which hydrolyses urea (Issa and Zangana, 2009; Weeks et al., 2003). Weitzmann and Summerbell (1995) reported that urease test for *T. verrucosum* is slow to develop. These features were very similar to our observations, and thus enabled us to identify the isolates as *T. verrucosum* (colourless pigment) and *T. mentagrophytes* (wine red pigment).

Although, there are slight resemblance in cultural (observe) and microscopic features of *Trichophyton* spp. incriminated in equine dermatophytosis (Weeks et al., 2003), we were able to distinguish our isolates from *T. equinum* because its cultures usually have a deep-yellow reverse with dark red centre (Amor et al., 2001; Ural et al., 2009). It also produces microconidia which usually forms nodular bodies (Weitzmann and Summerbell, 1995).

The horse in the present case most likely contracted the infection from the previously-infected horse, which may have served as source of contamination of the formites, trees and the ground where these animals lie and scratch themselves. Trichophytosis is a highly contagious disease which affects horses of all ages and transmission is by direct (especially when animals are grouped together) or indirect contact with a source of infection (Shimozawa et al., 1997; Quinn and Markey, 2003). It is also possible that the spores of the dermatophytes contaminated and persisted in the farm environment (Arslan et al., 2007).

The ticks and their bite wounds observed may have also facilitated infection, by serving as vector and providing portal of entry for the fungal agents. *Trichophyton* infection relies upon the presence of active (live) spores and skin damage (Pascoe, 1979; Hainer, 2003; Weeks et al., 2003). It has been noted that biting insects could be potential vectors for dermatophytes (Weitzmann and Summerbell, 1995). As observed in the present case, initial lesions in equine *Trichophyton* infections appear as erect hairs in circular areas often with a degree of localized inflammation, resulting in thickening of the skin within the infected area (Radostis et al., 1997). The annular and coalesced lesions observed later in this case have been reported to be the characteristic of *Trichophyton* infection in horses, the infected areas expand centrifugally and may lose the circular appearance, becoming diffuse and ill-defined (Radostis et al., 1997).

For each animal species, the dermatophytes involved depend on the host studied and on the geographical, environmental conditions and husbandry (Bernado et al., 2005; Shams-Ghahfarokhi et al., 2009). The two isolates obtained in the present case have been reported to occur worldwide (Quinn and Markey, 2003; Weeks et al., 2003). However, although *Trichophyton* infection is common in horses, this report describes an unusual mixed infection involving two different species.

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