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Molecular characterization of dermatophytes isolated from horses of Nigerian Defence Academy Equitation Stable, Kaduna

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ABSTRACT

Aim: The study was aimed to isolate and characterize equine dermatophytes from the Nigerian Defence Academy Equitation Wing, Kaduna.

Method and Materials: This study was conducted at the Equitation Wing, NDA using 40 infected horses comprising both sexes aged 5 to 15 years and weighing 400 – 600kg. Using standard procedures, body temperatures were taken, skin of each horse was clinically examined and dermatophytes were isolated and characterized from skin scrappings of the head, body and leg.

Results: The average body temperatures recorded ranged from 37.1 - 38.1°C and lesions observed on the skin were hair loss, red sores and excessive sweating. Two different *Aspergillus oryzae* from the head region, *A. flavus* from the body region and *Cladosporium cladosporioides* from the leg region were isolated. The two *A. oryzae* isolated from the head region had base pair lengths of 559 bp and 459 bp, suggestive of mutation. *Aspergillus flavus and A. oryzae* are anthropophilic and more or less conventional form of dermatophytes believed to be opportunistic and a human pathogen.

Conclusion: It was concluded that *A. oryzae* was the most commonly occurring dermatophytes followed by *A. flavus* and *C. cladosporioides.* The isolation of these fungal species revealed that *A. oryzae*, *A. flavus* and *C. cladosporioides* could cause dermatophytosis in horse.

Keywords: *Aspergillus*, *Cladosporium,* Dermatophytosis, Fungi, Horses.

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Introduction

Animals are subjected to infection by several organisms including fungi named dermatophytes which cause dermatophytoses (Cafarchia *et al.,* 2013). Dermatophytosis is a major public and veterinary health problem reported from different parts of the world and causes great economic loss (Cafarchia *et al.,* 2012). Dermatophytes are the main causes of dermatological problems in domestic animals (Nweze, 2011). Ural *et al.* (2009) documented that dermatophytes which belong to the class Ascomycetes, usually colonizes and invade stratum corneum, hair shaft, or hoof, where they invade. The study by Havlickova *et al.* (2008) revealed that there are approximately 40 different species of dermatophytes characterized by their capability to digest keratin and are grouped into three genera: *Trichophyton*, *Microsporum*, and *Epidermophyton*. A wide variety of dermatophytes have been isolated from animals, but only a few zoophilic (*M.canis*, *T. mentagrophytes*, *T. equinum*, and *T. verrucosum*), geophilic (*M. gypseum*) and anthropophilic dermatophytes (*M. equinum* and *T. equinum*) have been reported to frequently cause dermatophytosis in horses (Kwon-Chun *et al.,* 1992; Chermette *et al.,* 2008). The contagiousness among animal populations, high cost of treatment, difficulty of control measures, and the public health consequences of animal dermatophytosis explain their great medical importance (Kwon-Chung *et al.,* 1992).

Animals housed in close proximity to each other for long periods and the presence of infected debris in buildings account for both the higher incidence and the greater infection rate in winter (Macura, 1993). Geographical locations, age of the

animal, and environmental and cultural factors greatly affect the distribution of dermatophytosis (Macura, 1993). Chermette *et al.* (2008) stated that the high resistance of the dermatophyte *Arthroconidia* in the environment, colonization of host species, and the confinement of animals in breeding areas are factors that influence the endemicity of dermatophytosis (Weese and Yu, 2013). Lesions arising from dermatophytosis have many adverse effects besides the discomfort and unsightly nuisance (Cafarchia *et al.,* 2013).

Horses are being used in polo, racing, and riding. Equine dermatophytosisis worldwide in distribution, more prevalent in hot humid climates than in cold dry regions and prevents horses from working and interferes with their use (Macura, 1993). Although equine dermatophytosis decreases the economic value of a horse, it also has considerable zoonotic importance as they serve as reservoirs of the zoophilic dermatophytes (especially those caused by members of the *Microsporum* and *Trichophyton* genera) (Seker *et al.,* 2011; Cafarchia *et al.,* 2013). Cafarchia *et al.* (2012) suggested that the increasing number of reports of infections due to zoophilic dermatophytes in humans is directly linked to the persistence of these fungi in animals. Equine dermatophytosis has received little attention in Nigeria especially in the northern part of the country where a large population of horses are located and used for ceremonial purposes. Despite the high prevalence of dermatophytoses in Nigeria, few studies have been carried out to identify the fungal species causing cutaneous lesions in horses and their prevalence (Nweze *et al.,* 2011).Therefore, there is need to identify and characterize equine dermatophytes so as to be able to adopt effective strategy for the treatment of dermatophytosis. Therefore, the study was aimed to isolate and characterize equine dermatophytes from the Nigerian Defence Academy Equitation Wing, Kaduna.

Materials and Methods

Study area

The study was conducted at the Equitation Wing and Equitation Wing Laboratory of the Nigerian Defence Academy (NDA), Kaduna with Latitudinal coordinates 10º 41′43″ and Longitude 6º 38′58″(Umar *et al.,*2013). The horses used for this study were kept and maintained in the Equitation Department within the Academy. The management systems in the stables were intensive and semi-intensive. Routine veterinary care was provided for the horses at the stables in accordance with internal best practice for animal care (Cabanes *et al*., 1997).

Animal Selection

A total of 40 apparently healthy horses of both sexes age range of 5 to 15 years weighing 400 – 600kg were selected for the study. Prior to the onset of the study, all horses were bathed with anti-tick shampoo. The horses were well-fed and provided water *ad libitum*. Horses within this age range were selected because of their high exposure risk in continues military engagements.

Clinical Examination of Horses

Evaluation of the general physiology of the horses which include body temperature, alertness, appetite and respiration, were all observed. The skin of the infected horses was examined and a record of all affected areas was documented appropriately in a laboratory record book as described by Hamidreza and Salman (2012). The shape, size, position, distribution and the appearance of skin lesions as well as the age of the animals were recorded.

Collection of Skin Scrapping

Skin scrappings were collected from lesions in the head, body and leg. The surface of the affected area was first swabbed with a cotton swab treated with 70% v/v ethyl alcohol to remove any form of surface adhering organisms. Skin scales and scrapings were collected by scrapping off the margin of the lesion using a sterilized scalpel blade into sterile Petri-dish. Hairs were collected by removing dull broken hairs from the margin of the lesion.

Cultural Isolation and Identification

Each sample collected from a specific body region was used for direct microscopic examination with 20% v/v wet mount preparation using Mueller Hinton Agar (MHA). Also, some portions were cultured into (MHA), incubated at 28°C for 1-4 weeks and checked daily for colony formation and identification. The time of appearance and growth, colony morphology, and also color, shape, size and colony of reverse side morphology were observed and recorded. Microscopic examination for positive fungi cultures was carried out using Lactophenol cotton blue wet mount as described by Cabanes *et al*. (1997).

Deoxyribonucleic Acid Extraction

The fungal deoxyribonucleic acid (DNA) was

extracted using 5 g of each of sample according to the method described by Apfalter *et al*. (2001). The extracted DNA in labeled tubes was stored at -20˚C until used for polymerase chain reaction (PCR).

Polymerase Chain Reaction

Polymerase chain reaction (PCR) was performed according to the method described by Maurya *et al*. (2005) with slight modification using specific set of forward (VR5SR-5′-CCATCAGAACTCCGCAGTTA-3′) and reverse (VR5SR-3′-GGATCCGGTGCATTAGTGCT- 5′) primers. Amplification was done in a DNA thermal cycler and included the following steps: 95˚C for 4 minutes (initial denaturation), 35 cycles of 94˚C denaturation for 4 minutes, 55˚C annealing for 1 minute, 72˚C for elongation for 1 minute and final extension period of 10 minutes at 72˚C. The amplified PCR products were visualized on 1.5% agarose gel stained with ethidium bromide.

Nucleotide Sequencing and BLAST

The DNA fragments were separated by agarose gel electrophoresis using 1 % w/v agarose in Tris-acetate-EDTA (TAE) buffer. Automated DNA sequencing was performed after which the sequences were compared to other sequences in the GenBank databases using the Basic Local Alignment Search Tool (BLAST) package at http://www.ncbi.nlm.nih.gov/blast/ and Clustal X were used for 16S rDNA sequence alignments. For phylogenetic tree construction, multiple sequences were obtained from GenBank and the alignments were performed using MEGA6.

Results and Discussion

Clinical manifestations

The clinical manifestations of the lesions were characterized by red sores and itch, excessive sweating, hair loss and circular skin rashes (Table 1).

Table 1. Clinical manifestations classified by anatomical site

Region of isolation	Age of horse	Body temp.	Clinical manifestation of lesion
Head, Body and Leg	Randomly	37.2°C	Normal
Head 1	5-8	37.2°C	Red sore and itchy scabby circular skin rash
Head 2	8-10	38.1°C	Red itchy, excessive sweating and hair loss
Body	10-13	38.3°C	Excessive sweating, circular skin rashes and hair loss
Leg	13-15	37.1°C	Red itchy, skin rashes and hair loss

Morphological characteristics and molecular identification of the dermatophytes

The morphological characteristics revealed that the dominant fungi isolates from the head, body and leg regions were distinct (Fig. 1 to 4).

Molecular characterization

Molecular characterization revealed that the fungi isolates from the head region were *Aspergillus oryzae*, *Aspergillus flavus* from the body region and *Cladosporium cladosporioides* from the leg region (Fig 5).

Phylogenetic analysis

The phylogenetic trees of the isolates were shown the ancestry and their relatedness with other fungi organisms. *Aspergillus oryzae* isolated from head region 1 was different from the *Aspergillus oryzae* isolated from head region 2. The base pair length of *A. oryzae* (H1) was 559 bp while that of *A. oryzae* (H2) was 459bp.

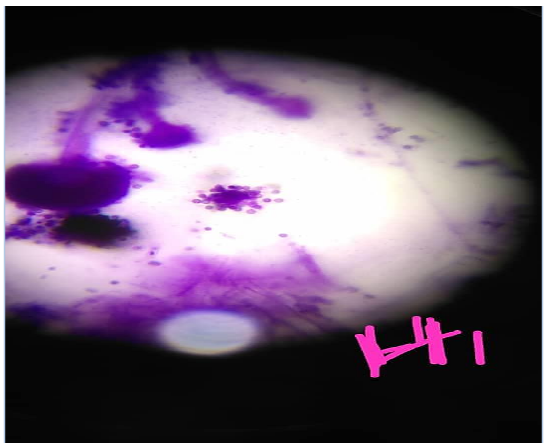

Fig.1 Morphological feature of *Aspergillus oryzae* isolated from the head region 1 (H1) a

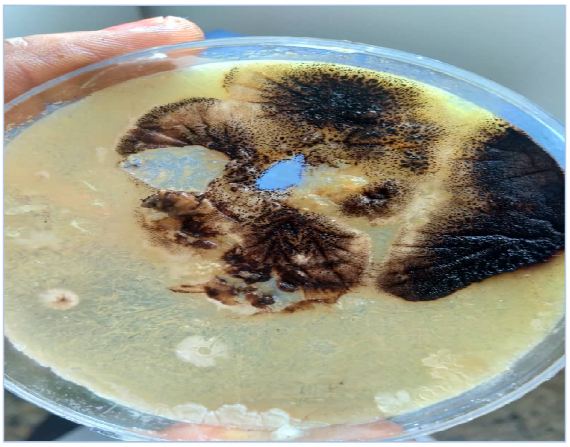

Fig.2 Morphological feature of *Aspergillus flavus* isolated from the body region

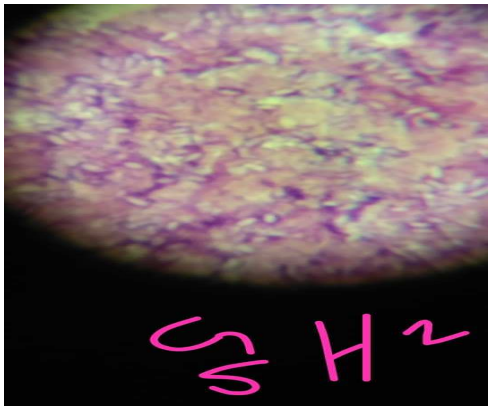

Fig. 3. Morphological feature of *Aspergillus oryzae* isolated from the head region 2 (H2)

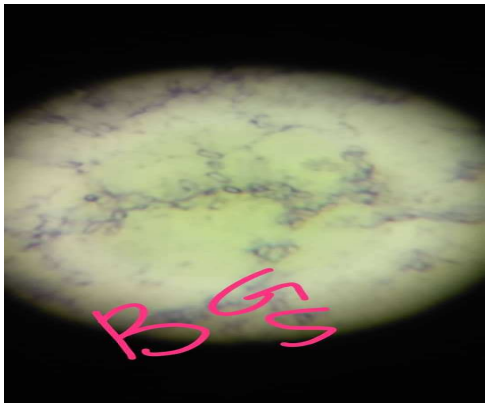

Fig. 4. Morphological feature of *Cladosporium cladosporioides* isolated from the leg region

Sequences of the identified organisms from the various body parts

Head 1: Aspergillus oryzae

GCGCGTTCCTCGGTCCAGGCTGGCCGCATTGCACTCCCGGCTATAARGTGCCCCGGAGGGCACTACA
TTCCGGGAGCCTTTGACCGGCCGCCCAAACCGACGCTGGCCCGCCCCCAGGGAAGTACACCGGCAC
GAATGCCGGCTGAACCCTGGAGGCGAGTCTGGTCGCAAGCGCTTCCCTTTCAACAATTTCACGTGCT
TTTTAACTCTCTTTTCAAAGTGCTTTTCATCTTTCGATCACTCTACTTGTGCGCTATCGGTCTCCGGCCA
GTATTTAGCTTTAGATGAAATTTACCACCCATTTAGAGCTGCATTCCCAAACAACTCGACTCGTCGA
AGGAGCTTCACACGGGCGCGGACACCCCATCCCAGACGGGATTCTCACCCTCTCTGACGGCCCGTTC
CAGGGCACTTAGACAGGGGCCGCACCCGAAGCATCCTCTGCAAATTACAATGCGGACCCCGAAGG
AGCCAGCTTTCAAATTTGAGCTCTTGCCGCTTCACTCGCCGTTACTGAGGCAATCCCGGTTGGTTTCT
TTTCCTCCGCTTATTGATATGCA

Head 2: Aspergillus oryzae

GCGCCCCGGAGGGCACTACATTCCGGAGCTTTGACCGGCCGACCAAGCTGACGCTGGCCGCCCCCA
GGGAAACACCGGACCAATGCCGGCTGAACCCCGGGTGAGTCTGGTCGCAAGCGCTTCCCTTTCAAC
AATTTCACGTGCTTTTAACTCTCTTTTCAAAGTGCTTTTCATCTTTCATCACTCTACTTGTGCGCTATCG
GGCTCCGGCAGATTTACTTTAATGAAATTTACCCCCATTTAGAGTGCTTCCCAAACAACTCGACTCG
AAGAGCTTACACGGCGGACACCCCCCCCAAAGGGATTCTCACCCTCTYTGAGGGCCCGTTCCAGGSGC
TTAAGGGGCCGCACCCAACATCCTCTGCAAATTACAAGGACCCCGAAGAGCCGATTAAATTTAGCT
TTGCCCTTCMCTCCCGACTGAGGCATCCCGGTTGTTTCTTTCTCCGCTATGATATGCAAAA

Body: Aspergillus flavus

GTCGAGCGCGTTCCTCGGCCAGGCTGGCCGCATTGCACCCCGGTATAAGGCCCCGGAGGGCACTAC
ATTCCGGGAGCCTTTGACCGGCCGCCCAAACCGACGCTGGCCCGCCCCCCGGAAGACACCGGCACGA
ATGCCGGCTGAACCCGAGGCGAGTCTGGCGCAAGCGCTTCCCTTTCAACAATTTCACGTGCTTTTAA
CTCTCTTTTCAAAGTGCTTTTCATCTTTCGATCACTCTACTTGTGCGCTATCGGTCTCCGGCCAGTATTT
AGCTTTAGATGAAATTTACCACCCATTTAGAGCTGCATTCCCAAACAACTCGACTCGTCGAAGGAGC
TTCACACGGGCGCGGACACCCCRTCCCAGACGGGATTCTCACCCTCTCTGACGGSCCGTTCCAGGGC
ACTTAGACRGGGGCCGCACCCRAAGCATCCTCTGCAAATTACAATGCGGACCCCGAAGGAGCCAGC
TTTCAAATTTGAGCTCTTGCCGTTCACTCGCCGTTACTGAGGCAATCCCGGTTGGTTTCTTTTCCTCCG
CTTATTGATATGCA

Leg: Cladosporium cladosporioides

CGCGTACTAGTCGAGGCGTACCTCGGCCGCGGCTCGCCGCATTGACCAGGGTAAACTCCCCCCGGA
GGGCGTTACCTTCCGGGGTCCAGGACCGGCCGACCATGCTGGGCTGCAACGCGGGATGAGACCGGA
CCGAACAACGGCGGAACGCCGGAGCAAGCTGGGTGGAATCCCTTCCCTTTTAACAATTTCACGTGCT
TTTTAACTCTCTTTCCAAAGTGCTTTTCATCTTTCGATTACTCTACTTGAGCGCTATCGGATTCTGGTCA
ATATTTAGCTTTAGAAGAAATTTACCTCCCATTTAAATTTGAATTCCCTAACAAATCAACTCGACTAA
GGAACTGCGTTAGAACAGATTTCCGACCGCCTACGGAAATGGCACCCTCTCTCTCTGCCTGGCCCCA
GGAAAGTACGTCGASGGKTGCTCAGAACCATCCTCTTCATTACAATTCGACGCGGAACCCGAGGAT
AATTTAATTGCTGTTTCCTTCCCTCCTATACAAGCCAGTCCCGTGTTTCCTTTCTTCG

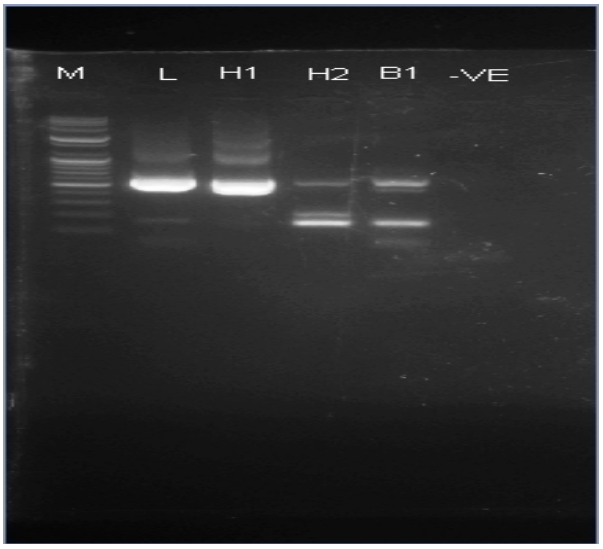

Fig 5: Gel electrophoresis of DNA of the isolates. M= Molecular lather, L= Leg region, H1= Head region 1, H2= Head region 2, B1= Body region 1, -VE= Negative control.

Dermatophytes are one of the commonest skin diseases affecting horses (Pilsworth and Knottenbelt, 2007). The lesions suggestive of dermatophytosis in this study were areas of scaling, crusting, and alopecia with some kerion formation. This resulted from utilization of the skin, hair and hoof of animals as sources of carbon and digestion using proteolytic and lipolytic enzymes by dermatophytes (Hubka *et al*. 2014). Dermatophytes cause the formation of single or multiple ulcerated cutaneous or subcutaneous nodules in the regions of the head and neck, occasionally, lymphangitis and regional lymphadenopathy (Valentine *et al*. 2006). The foci of infection caused by dermatophytes in the studied horses were head, body region and legs. Abdalla *et al*. (2005) reported that skin infections in horses mainly occur on the saddle area, shoulders, chest and withers, and less commonly on the head, neck, buttocks and extremities. However, organisms exposed to different environmental conditions and people at different period could bring about mutation and genetic variation as observed by the mutation of *A. oryzae* in this study. George *et al.* (1997) reported that infections in donkeys are manifested predominantly by lesions not only on the head and neck but also on the forelimbs and buttocks.

In agreement with the findings of this study, George *et al.* (1997) reported circular arrangements in lesions covered by crusts or pustules and characterized by loss of hair and a hyperaemic margin. Following the acute period, the skin becomes scaly and red itchy. Hubka *et al*. (2014) stated that the infected hairs are microscopically described as large-spored ectothrix (ectothrix megaspore) with the size of arthroconidia ranging from 5 to 10 µm in diameter.

Aspergillus oryzae, Aspergillus flavus and *Cladosporium cladosporioides* were the most common dermatophytes detected in this study. This finding is contrary to previous study by Pilsworth and Knottenbelt (2007) who reported *Trichophytonver rucosum* and *Microsporum equinum* as the common agents of equine dermatophytoses. Also, Oke *et al*. (2014) stated that that the aetiological agents of dermatophytosis are *M. gypseum*, *M. canis*, *T. verrucosum*, *T. equinum* and *T. mentagrophytes*. However, similar species of dermatophytes detected in this study have been isolated from horses in other parts of the world (Cabanes *et al*., 1997). The dermatophytes isolated in this study belong to three ecological groups and are geophilic. They might have infected the horses directly from the soil and through spores infested fomites as they were all housed in different stables. Also, asymptomatic carriers in the stables could possibly serve as another source of the infection.

Aspergillus flavus and Aspergillus oryzae are anthropophilic dermatophytes and are more or less a conventional form of dermatophytes believed to be opportunistic and associated with humans. Their presence in horses in this study could be attributed to the close contact that existed between these horses and humans during grooming, riding, and exercising of the horses which predisposed to the infection. These dermatophytes have previously been isolated from prepubescent children in Nigeria by Nweze (2001). This study revealed that human pathogens like *Aspergillus flavus and Aspergillus oryzae* could cause equine dermatophytosis. *Cladosporium cladosporioides* isolated from the leg of the horsesis zoophilic and might have been contracted from rodents that have free rein of the stables.

The difference in the *Aspergillus oryzae* isolated from the head is in agreement with the report of Ozumba and Nlemadim (2005) who stated that genetic modification or changes in sequence of DNA of dermatophytes species could be due to transmission of fungi from one host to another. This will result in the fungi species trying to adapt to the new host, thus leading to a change in the genetic sequence. More so, genetic modification could as well be associated to genetic variation and

progressive evolution arising from the theory of natural selection.

Conclusions

Dermatophytes *Aspergillus* and *Cladosporium* genera were found in isolated samples from horses. *A. oryzae* was the most common dermatophytes followed by *A. flavus* and *C. cladosporioides.* The isolation of these fungal species revealed that *A. oryzae*, *A. flavus* and *C. cladosporioides* could cause dermatophytosis in horse.

Reference

Abdalla W, Suliman E and Gabbar A (2005). A report on *Trichophytonver rucosum* in donkeys in the Sudan. *Sudan Journal of Veterinary Research*. 20: 83-85.

Apfalter PF, Blasi J, Boman CA, Gaydos M, Kundi M and Maass A (2001). Multicenter comparison trial of DNA extraction methods and PCR assays for detection of *Chlamydia pneumoniae* in endarterectomy specimens. *Journal of Clinical Microbiology,* 39: 519-524.

Cabanes FJ, Abarca ML and Bragulat MR (1997). Dermatophytes isolated from domestic animals in Barcelona, Spain. *Mycopathologia*, 137(2): 107-113.

Cafarchia CS, Weigl S, Figueredo LA and Otranto DO (2012). Molecular identification and phylogenesis of dermatophytes isolated from rabbit farms and rabbit farm workers. *VeterinaryMicrobiology*,154(3-4): 395-402.

Cafarchia CS, Figueredo A and Otranto D (2013). Fungal diseases of horses. *Veterinary Microbiology*, 167(1-2): 215-234.

Chermette R, Ferreiro L and Guillot J (2008). Dermatophytoses in animals. *Mycopathologia*, 166(5-6): 385-405.

George LK, Kaplan W and Camp L (1997). Equine ringworm with special reference to *Trichophyton equinum*. *American Journal of Veterinary Research*, 18(69): 798-810.

Hamidreza F and Salman AR (2012). A comparison between the routine treatment of equine dermatophytosis and treatment with Galic-Aloe vera gel. *International Research Journal of Applied and Basic Sciences*, 3(11): 2258-2261.

Havlickova B, Czaika VA and Friedrich M (2008). Epidemiological trends in skin mycoses worldwide. *Mycoses*, 51(4): 2-15.

Hubka V, Dobiasova S, Dobias R and Kolarik M (2014). *Microsporum aenigmaticum sp. nov*. from *M. gypseum* complex, isolated as a cause of tineacorporis. *Medical Mycology*, 52: 387-396.

Kwon-Chung K J and Bennett JB (1992). *Medical Mycology*. Lea and Febiger, Philadelphia, Pa, USA.

Macura AB (1993). Dermatophyte infections. *International Journal of Dermatology*, 32 (5): 313-323.

Maurya R, Singh K, Kumar B, Salotra P, Rai M and Sundar S (2005). Evaluation of PCR for diagnosis ofIndian Kala-Azar and assessment ofcure. *Journal of Clinical Microbiology*, 43(7): 3038-3041.

Nweze EI (2001). Etiology of dermatophytoses amongst children in northeastern Nigeria. *Medical Mycology*, 39 (2): 181-184.

Nweze EI (2011). Dermatophytoses in domesticated animals. *Revista do Instituto de Medicinal Tropical de Sao Paulo*, 53 (2): 95-99.

Oke O, Onayemi OO, Olasode OA, Omisore AG and Oninla OA (2014). The prevalence and pattern of superficial fungal infections among school children in ile-ife, south-western Nigeria.*Dermatology Research and Practice*, vol. 2014, Article ID 842917, 7pp.

Ozumba UC and Nlemadim R (2005). Prevalence of dermatophytosis in University of Nigeria Teaching Hospital, Enugu, Nigeria: any change in pattern? *Nigerian Journal of Clinical Practice*, 8(2): 83-85.

Pilsworth RC and Knottenbelt D (2007). Dermatophytosis (ringworm). *Equine Veterinary Education*, 19(3): 151-154.

Seker E and Dogan N (2011). Isolation of dermatophytes from dogs and cats with suspected dermatophytosis in Western Turkey. *Preventive Veterinary Medicine*, 98(1): 46-51.

Umar YA, Maikaje DB, Garba UM and Alhassan MAF (2013). Prevalence of gastro-intestinal parasites in horses used for cadets training in Nigeria. *Journal of Veterinary Advances,* 3(2): 43-48.

Ural KB, Ya GCI and Ocal N (2009). Cellular enzyme values inhunter/jumper and dressage horses with dermatophytosis. *Arquivo Brasileiro de Medicina Veterinariae Zootecnia*, 61(5): 1233-1237.

Valentine BA, Taylor GH, Stone JK and Halse RR (2006). Equine cutaneous fungal granuloma: a study of 44 lesions from 34 horses. *Veterinary Dermatology*, 17: 266-272.

Weese JS and Yu AA (2013). Infectious folliculitis and dermatophytosis. *Veterinary Clinics Equine*, 29(3): 559-575.

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