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Prevalence of mycotic agents isolated from skin lesions of trade horses in Obollor-Afor, Enugu State, Nigeria

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Abstract

The study was aimed at identifying mycotic agents that colonize skin lesions in trade horses found in south eastern Nigeria. Skin scrapings were collected from seventy (70) horses with skin lesions in Obollo -Afor market, Enugu state, south eastern Nigeria. Portions of the skin specimen were treated with 10% KOH for microscopic identification of typical hyphae. Sabouraud dextrose agar (SDA) slants, supplemented with cycloheximide were used as a standard substrate for the cultures. Cultures were incubated aerobically for 2 weeks at 37°C and were observed daily for growth of fungi isolates. Identification of fungal species was done based on their cultural and morphological characteristics. From the seventy (70) skin scraping samples studied, fifty-six (56) species of fungi belonging to 6 genera were recovered in different frequencies including *Aspergillus sp (54%), Mucor spp (32%), Rhizopus oryzae (7%), Penicilium marneffi (2%), Microsporum fulvum (2%) a*nd *Tricophyton equinium (4%)*. More of the isolates were from the female horses than male horses. At $p > 0.05$ there was no significant difference in the distribution of fungal isolates between females and male horses. The season of the year had no notable impact on the occurrence or frequency of isolation of the fungi. The isolated dermatophytes may be possible aetiological agents of dermatomycoses in horses, while the Saprobes isolated may be contaminants associated with skin infections in horses. These mycotic agents isolated are not known to primarily, affect humans however there may be a possibility of transmission to human and other susceptible animals that cohabit with these horses.

Keywords: Equine, Mycotic agent, Prevalence, Skin lesion

Introduction

Fungi are groups of organisms that can infect the skin, hair, horns, nails and feathers in man and animals. Most fungal agents do not cause infections in healthy individuals, but become invasive in conditions of decreased resistance (Kushida *et al*., 1972). Worldwide research has described infections caused by saprophytic fungi in domestic and wild animals over the last two decades. Previous investigations have shown that the most commonly isolated fungi from the skin or hair of different animals are *Penicillium, Aspergillus, Alternaria, Mucor, Scoupolariopsis and Chrysosporium* (Aho 1983;

Efuntoye & Fashanu, 2002; Bourdeau *et al.*, 2004; Samson & Varga, 2007).

Dermatophytes are among the most frequent cause of dermatological problems in domestic animal (Ranganthan *et al.*, 1997; Cabañes 2000a; Cabañes 2000b). They are primary cutaneous pathogens that cause disease particularly of the stratum corneum (Quin *et al.*, 2003; Ural *et al.*, 2008). They 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 & Zangana, 2009), producing circumscribed, alopecic, crusty and scaly skin lesions generally called ringworm (Fadok, 1995; Quin *et al.*, 2003). *Microsporum* and *Trichophyton* species have been reported to be the causative agents of dermatophytosis in horses (Ranganathan *et al*., 1997; Weeks *et al*., 2003). *Trichophyton equinum* is the most commonly involved agent and has been reported in many countries (Al-Ani *et al*., 2002). Other *Trichophyton* spp. that have been isolated include *Trichophyton mentagrophytes* (Ranganathan *et al*., 1997; Shokri & Khosravi, 2011) and *Trichophyton verrucosum* (Khosravi, 1996; Shokri & Khosravi, 2011). These fungal species however were isolated from single infections in horses.

Animal ringworm is of great concern because the majorities of dermatophytes isolated from animals are zoonotic and have been recognized as a public health problem in many parts of the world and have reached endemic proportions in Africa (Pitt, 1994; Nweze, 2011; Nweze & Okafor, 2005). For instance, in Nigeria, many surveys have confirmed this finding, especially among children (Nweze, 2011). Human transmission could be possible through direct contact or fungus-bearing hair and scales from infected animals.

In the last few years, the interest in having animals as pets has increased dramatically in Nigeria and many other developing countries with increasing number of such animals co-habiting and feeding with their owners especially in the rural areas (Pitt, 1994). Owing to such close contact between animals, their owners and the rest of the household members, there is a high possibility of transmission of fungal dermatophytic infection to humans, especially from animals that are asymptomatic carriers, hence the need to investigate the mycotic agents that colonizes the skin lesions of trade horses and draw attention to risks involved.

Materials and Methods

Study area/population

A total of Seventy (70) horses with evidence of skin lesion, were sampled in Obollo-Afor market located in Udenu local area of Enugu State south east Nigeria. Tropical forest and savannah predominate the study area, ecologically. The wet season lasts from April to early October while the dry season lasts from end of October to early April. The town is a popular place of business activity, a gate way from the North to the South eastern Nigeria. All animals coming from the North pass through or stop at Obollo-Afor market before getting to their destination. Horses and donkeys are brought on weekly basis from the North to Obollo-Afor market. Only horses with skin lesions were included in the study. The study was conducted in mid- rainy season and early dry season.

Sampling/sample size

Based on the availability of the horses and period of sampling, purposive sampling technique was used. Skin scrapings were taken from Seventy (70) trade horses kept for sale in Obollor-Afor Udenu LGA of Enugu State during the mid-rainy season to the beginning of dry season.

Specimen collection

The affected skin was cleaned with alcohol and the advancing border of the lesion was scraped with the blunt edge of a sterile disposable scalpel. Hairs and scales were plucked with sterile tweezers. All collected animal specimens were accompanied by data involving sex and season of the year. Collected samples were placed in clean, dry and sterile paper envelopes and transported to the Microbiology Laboratory of the Faculty of Veterinary Medicine University of Nigeria Nsukka.

Laboratory examinations

Portions of specimens were treated with 10% KOH for microscopic identification of typical hyphae or arthroconidia at ×/100 x/400 magnifications. SDA (Oxoid, UK) slants, supplemented with cycloheximide (Sigma, Steinhim, Germany), 0.4 mg/L, chloramphenicol (Fluka, UK) 0.05 mg/L and gentamicin (Sigma) 0.16 mg/L were used as a standard substrate for the cultures. Cultures were incubated aerobically for 2 weeks at 37°C and were observed daily for growth of fungi isolates (Weitzman & Summerbell 1995). Identification of fungal species was done on the basis of cultural and morphological characteristics. Macroscopic features like colony colour, texture and margins, Culture slide

smear stained with lactophenol cotton blue was used for microscopic identification following the methods described by Bignell (2010) and Khosravi & Mahmoudi (2003).

Statistical analysis

Statistical analysis was done using descriptive statistics (percentages in table, pie charts and bar charts) and Statistical Package for Social Sciences (SPSS) version 16 for windows (SPSS Inc. Chicago, Illinois). Data generated from sex distribution of fungal isolates were analyzed using the Students'-test which was performed at a 5 % level of probability.

Results

A total of 56 isolates represented by 6 genera were recovered from the seventy skin scraping samples studied. They include the saprophytic fungi; *Aspergillus sp (niger)* and (*flavus)*, *Mucor spp*, *Rhizopus oryzae* and *Penicillium marneffei.* The dermatopytes were *Tricophyton equinium and Microsporum fulvum.* The percentage distribution of the fifty-six (56) fungal isolates recovered from the samples studied shows that *Aspergillus niger* among the others saprophytic fungi was the most frequently isolated while among the dermatophytes, *Tricophyton sp* were the most frequently isolated (table 1). The percentage (94.60%) of occurrence of saprophytic fungi was more than that of the dermatophytes (5.4%) isolated from the samples studied (Figure 1).

A greater percentage of isolated fungi; *Aspergillus sp* (53.2%), *Mucor sp* (31.8%), *Penicillium marneffei* (2.1%) and *Microsporum fulvum (2.1%)* were from the female. Whereas *Trichophyton equinium and Rhizopus oryzae* were isolated mainly from the male horses (Table 2). However, based on the students t-test the mean percentage of occurrence of the fungal isolates for females is 12.49 ± 6.88% and that of males is 9.26± 3.30%, this shows that there was no significant difference (P>0.05) in the distribution of fungal isolates between females and male horses (Figure 2).

Table 1. Percentage distribution of fungal isolates in sampled horses

S/N	Species	Frequency (%)
1	*Aspergillus niger*	27 (48)
2	*Mucor sp*	18 (32)
3	*Rhizopus oryzae*	4 (7)
4	*Aspergillus flavus*	3 (5)
5	*Tricophyton equinium*	2 (4)
6	*Microsporum fulvum*	1 (2)
7	*Penicillium marneffei*	1 (2)
	Total	56

Table 2: Sex distribution of fungal agents isolated from apparently healthy horses

Fungal agents	Female n=58	Male n=12
Aspergillus niger	25 (53.2%)	2(16.7%)
Mucor sp	15 (31.9%)	3 (25.0%)
Rhizopus oryzae	2 (4.3%)	2 (16.2%)
Aspergillus flavus	2 (4.3%)	1 (8.3%)
Trichophyton equinium	0 (0.-0%)	2 (16.7%)
Penicillium marneffei	1 (2.1%)	0 (0.0%)
Microsporum fulvum	1 (2.1%)	0 (0%)
Total	47 (81.0%)	9 (75%)

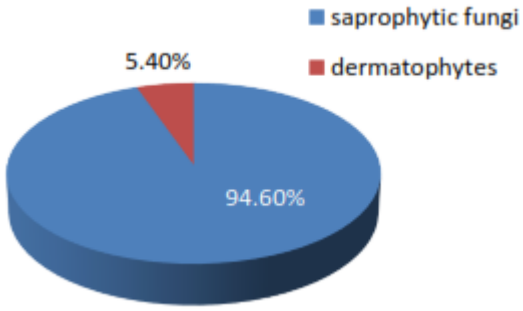

Figure 1. The percentage distribution of fungi isolated from sampled horses

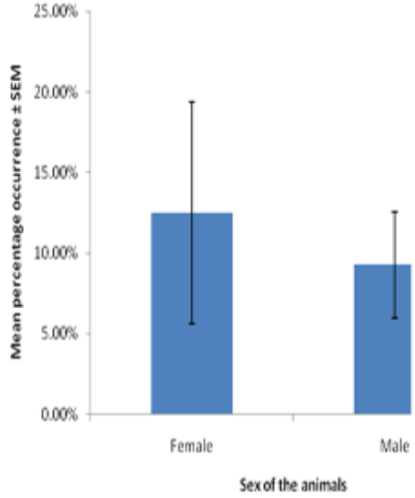

Figure 2. Sex Distribution of fungal isolates showing no significance at p ≤ 0.05

Twenty-six (26) and thirty (30) isolates were recovered in the mid-rainy and dry season respectively. *Trichophyton spp* was most commonly isolated in both mid-rainy and dry season. However, *Rhizopus spp*, *Penicllium spp* and *Microsporum spp* occurred more frequently in the mid-rainy season, while *Aspergillus spp and mucor spp* occurred more frequently in the dry season (Figure 3).

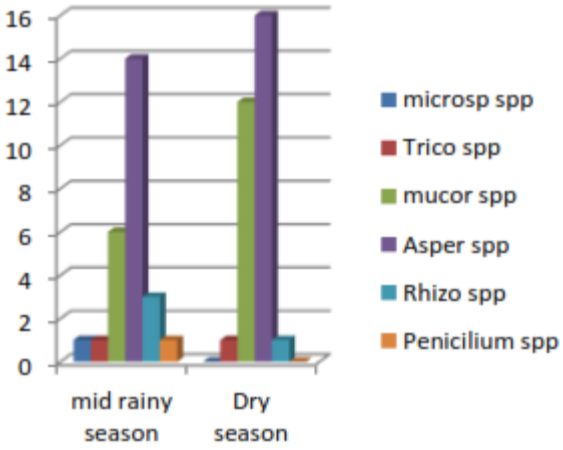

Figure 3: Bar chart showing distribution of fungi isolates from sampled horses in the mid rainy and dry season

Discussion

Fifty-six (56) species of fungi belonging to 6 genera were recovered in different frequencies, which includes dermatophytes; *Microsporum fulvum and Tricophyton equinium* which are the dominant species colonizing animals and have often been classified as natural pathogens affecting both humans and animals (Ross, 1951). The saprobes; *Aspergillus sp, Mucor spp, Rhizopus oryzae* and *Penicillim marneffi* are saprophytic organisms as well as environmental contaminants from soil or plant materials, on the skin of horses, under special circumstances could become invasive on the hair or skin, thus causing a primary or secondary infection. (Aho 1983).

The lower percentage (6%) of occurrence of dermatophytes in horses in the study area, contrasts with the findings of (Pitt, 1994) and (Al-Ani *et al.*, 2002) that had a higher percentage of 44.0% and 18% respectively. The variability in percentage occurrence of dermatopytes in horses in Nigeria and other countries (Aho 1983; Faggi *et al*., 1987; St-Germain & Summerbell 1996; Cabañes 2000a; Rene *et al.,* 2008) clearly shows that geographic location is an important factor affecting the findings.

Though *Microsporum fulvum is* not a common cause of dermatophytoses in horses based on the reports of earlier workers in Nigeria (Pitt, 1994) and other countries (Larone, 2011), however, it has been suggested (Pitt, 1994) that with the passage of time and human population migration there may be a change in the etiology of dermatophytoses in horses. Isolation of saprophytic fungi which includes *Aspergillus spp,Mucor spp,Rhizopus spp* and *Penicillium spp* from skin of large animal as in this present study is a consistent finding reported by previous workers.(Aho 1983; Sparkes *et al*., 1993; Popovic & Lazarevi, 1999; Ural *et al.,* 2008). This is because these fungi are frequently found in soil, air, plants and on other materials, man and animals are always in constant contact with them (Mancianti & Papini, 1996).

Even though more spent mares were brought to the market for sale and chances of isolating from these females higher than for males, the frequency of isolation was evenly distributed between both sexes therefore the was no significant difference in the distribution of fungal isolates between females and male horses at $p \leq 0.05$ which was similar with the report of (Pitt, 1994), however Al-Ani *et al.* (2002) reported that there was a significant difference between males and females, which may be due to the disparity in sample size for both sexes in the present study. The higher frequency of occurrence of dematophytes; *Microsporum spp* and *Trichophyton spp* and some saprobes such as *Rhizopus sp and Penicllium sp* in the rainy season was similar to the report of Kushida *et al.* (1972) who observed greater abundance of certain species of fungus during summer time. This shows that high humidity during the rainy season may support the growth of dermatopytes.

In conclusion, the results show that dermatophytes, *Microsporum fulvum and Trichophyton equinium* are possible aetiological agents of dermatomycoses in Horses. Saprobe fungi, such as *Aspergillus niger, Aspergillus flavus, Mucor spp, Rhizopus oryzae* and *Penicillim marneffi* may be contaminants that are found in skin infections in horses, the fungal agents isolated are not known to affect humans primarily however there is possibility of transmission to human and susceptible animals who cohabit with these horses.

Sex and season of the year had no notable impact on the distribution of the isolated species.

Conflicts of Interest

The authors declare no conflicts of interest.

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