Prevalence and distribution of dermatophytes among domestic horses in Kwara state, Nigeria

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Abstract
The study investigated the prevalence and distribution of dermatophytes among domestic horses in Kwara state, Nigeria. A total of 91 samples were collected which comprised of skin scrapings and hair from both infected and asymptomatic animals. The highest dermatophyte isolation rate per total samples collected from each of the 7 different Local Government Area (LGAs) was 25% for Ilorin-East. Statistically significant differences (p < 0.05) were observed in the number of dermatophyte isolates obtained from the seven different LGAs. Dermatophytic lesions were observed on four anatomical sites of the body of horses that were sampled. These sites were the limbs, tail, head and abdominal region with dermatophyte isolation rate per total samples collected being 18.7%, 16%, 15% and 10%, respectively. Out of 85 male horses sampled, 12 were positive, and out of the six female horses sampled, two were positive. However, there was no statistically significant difference (p > 0.05) between the total dermatophytes isolated from male (14.1%) or female (33.3%) horses from the seven LGAs in Kwara state.

Dermatophytes isolated include Trichophyton tonsurans, Trichophyton verrucosum, Trichophyton soudanense, M. gypseum, Microsporum persicolor, Microsporum equinum and Microsporum fulvum with Trichophyton tonsurans and Trichophyton soudanense being anthropophilic.

Keywords: Dermatophytes, Distribution, Horses, Prevalence, Kwara state, Nigeria

Received: 20-10-2016 Accepted: 13-02-2017

Introduction
Dermatophytes are a group of keratinophilic fungi that cause dermatophytoses which are highly contagious fungal infections of the skin that affect horses and other animals of all ages and breeds. Dermatophytes produce proteolytic enzymes, keratinases, which are able to hydrolyze keratin the main protein constituent of hair, nails and skin. The infections can be mild to severe, depending on the host immune response (Akcaglar et al., 2011). Dermatophytosis is a mycotic disease known as ringworm or tinea caused by dermatophytes which comprises a group of closely related fungi in the genera Microsporum, Trichophyton and Epidermophyton (Emmons, 1955; Weitzman & Summerbell, 1995). Dermatophytic agents are classified into three ecological groups as anthropophilic (mostly associated with humans), zoophilic (associated with animals) and geophilic (found in soil) (Weitzman & Summerbell, 1995). These ecological adaptations have enabled them to have a wide range of host (Quinn & Markey, 2003), and their zoonotic and public health importance have been well recognized (Shams-Ghahfarokhi et al., 2009). Zoophilic dermatophytes such as M. canis, T. mentagrophytes and T. verrucosum are significant causal agents in human ringworms in many areas of the world (Nweze, 2011). The incidence of dermatophytosis varies according to climate and natural reservoirs. However, the pattern of the species of dermatophytes involved in dermatophytosis may be
different in similar geographic conditions both in humans and animals. This has been related, among many factors, to the decline in the incidence of animal ringworm in some areas or the degree of closeness of animals to human (Pier et al., 1994). In horses, Microsporum and Trichophyton species have been reported to be the causative agents of dermatophytosis (Quinn & Markey, 2003; Ural et al., 2008). Trichophyton equinum is the most commonly involved agent and has been reported in many countries (Hasegawa & Usui, 1975). Other Trichophyton spp. that have been isolated include Trichophyton mentagrophytes (Shimozawa et al., 1997; Quinn & Markey, 2003) and Trichophyton verrucosum (Shimozawa et al., 1997; Khosravi & Mahmoudi, 2003). These fungal species, however, were isolated from single infections in horses.

Dermatophytosis is not a reportable or notifiable disease in Nigeria and in the tropical areas because the disease is usually self-limiting i.e. usually produces benign skin lesions (Adekeye et al., 1989; Macura, 1993) and as a result, actual prevalence Figures for dermatophytosis are unknown in many endemic areas.

Materials and Methods

Study area
The study area is Kwara state which covers an area of 34,407.5 square kilometers and lies at latitude 8° North and longitude 5° East (Fadeyi, 2009). It has a population of 2,365,353 by 2006 census Figures (NPC, 2006) and accounts for 1.6% of the country’s population. Kwara state has 16 Local Government Area (LGAs), which are grouped into three Senatorial Districts, namely Northern, Southern and Central Senatorial Districts. Agriculture is the major occupation of the people in the state (Fadeyi, 2009).

Sampling and sample size
Based on availability of horses and period of sampling, purposive sampling method was used. Ninety-one skin scrapings and hair samples were taken from both infected and asymptomatic cases of dermatophytosis in horses between March and June, 2013 from different farms, homes and stable from seven LGAs in Kwara state namely: Patigi, Oyun, Baruten, Offa, Ilorin East, Ilorin West and Irepodun.

Sample collection
Skin scrapings and swabs as well as plucked hair were collected from the margins of the lesions after cleaning and disinfecting with 70% alcohol as described by Elewski (1995). Hairs were plucked by pulling them with thumb forceps (Quinn et al., 1994). On the other hand, hair and scale specimens were collected from apparently healthy animals using Mackenzie’s hairbrush technique (Mackenzie, 1963). All collected animal samples were accompanied by data involving location, sex, and anatomical sites of collection of samples from the animals, in addition to date of sample collection.

Collected samples were placed in sterile envelopes in separate polythene bags, and transported as dry packet (Guillot et al., 2001) to the microbiology laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Direct microscopic examination of samples
Small samples of each scraping were placed on a microscope slide and 1 to 2 drops of 10% potassium hydroxide added. A cover slip was applied and the slide gently heated over a flame as described by Hainer (2003). Each treated slide was carefully examined under low objective (×10) and high (×40) power objectives to observe for presence of diagnostic fungal forms.

Laboratory culture of dermatophytes
Sabouraud dextrose agar (SDA) containing chloramphenicol (40mg/L), cycloheximide (500mg/L) and nicotinic acid (100µg/ml) which is a selective media was used for primary isolation. Cycloheximide prevents growth of majority of molds and yeasts, chloramphenicol is an antibacterial agent and nicotinic acid promotes the growth of Trichophyton equinum (Raymond & Piphet, 2008). The SDA slants were inoculated with the sample and incubated at room temperature for one to four weeks.

Identification of isolates
Suspected growths were sub-cultured on PDA (Oxoid, UK) to facilitate distinctive spore formation for identification and pigment production. The subcultures were incubated at room temperature for one to four weeks (Raymond & Piphet, 2008). Identification was based on colony and microscopic characteristics after staining with lactophenol cotton blue and using the Fungal Color Atlas (Baron et. al., 2003).

Data presentation and statistical analysis
The results obtained were described using percentages and presented in charts, tables and plates. Fisher’s exact test was used to measure the association between positively tested samples and sex of the horses. Levels of P<0.05 were considered significant.
Table 1: Percentage distribution of dermatophytes isolated from samples obtained from horses in seven Local Government Areas (LGA) of Kwara state

<table>
<thead>
<tr>
<th>LGAs</th>
<th>Number of samples</th>
<th>Positive samples</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ilorin East</td>
<td>12</td>
<td>3</td>
<td>25.0</td>
</tr>
<tr>
<td>Offa</td>
<td>10</td>
<td>2</td>
<td>20.0</td>
</tr>
<tr>
<td>Irepodun</td>
<td>6</td>
<td>1</td>
<td>16.7</td>
</tr>
<tr>
<td>Oyun</td>
<td>6</td>
<td>1</td>
<td>16.7</td>
</tr>
<tr>
<td>Ilorin West</td>
<td>30</td>
<td>4</td>
<td>13.3</td>
</tr>
<tr>
<td>Pategi</td>
<td>16</td>
<td>2</td>
<td>12.5</td>
</tr>
<tr>
<td>Baruten</td>
<td>11</td>
<td>1</td>
<td>9.1</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>14</td>
<td>15.4</td>
</tr>
</tbody>
</table>

Table 2: Distribution of dermatophytes based on anatomical site of lesions

<table>
<thead>
<tr>
<th>Anatomical site of lesion</th>
<th>Number of samples collected</th>
<th>Dermatophyte positive samples</th>
<th>% positive for dermatophytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limbs</td>
<td>16</td>
<td>3</td>
<td>18.7</td>
</tr>
<tr>
<td>Tail</td>
<td>25</td>
<td>4</td>
<td>16.0</td>
</tr>
<tr>
<td>Head</td>
<td>40</td>
<td>6</td>
<td>15.0</td>
</tr>
<tr>
<td>Abdomen</td>
<td>10</td>
<td>1</td>
<td>10.0</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>14</td>
<td>15.4</td>
</tr>
</tbody>
</table>

Results
The highest dermatophyte isolation rate per total samples collected from each of the seven different LGA was 25%, for Ilorin-East followed by Offa with 20%, 16.7% for each of Irepodun and Oyun. Ilorin-West had the largest number of samples collected (30) but with 13.3% dermatophyte isolation rate. Pategi and Baruten had dermatophyte isolation rates of 12.5% and 9.1%, respectively. Significant differences were observed in the number of dermatophytes isolates that were obtained from the seven different LGAs (Table 1). Dermatophytic lesions were observed on four anatomical sites of the body of horses that were sampled. These sites were the limbs, tail, head and abdominal region with dermatophyte isolation rate per total samples collected being 18.7%, 16%, 15% and 10%, respectively (Table 2). However, there was no appreciable association between the number of dermatophytes obtained and the anatomical sites from where samples were collected.

Two anthropophilic dermatophytes were isolated, namely *T. soudanense* from the head and *T. tonsurans* from the abdominal region (Table 3). Seven different dermatophyte species were identified from the 14 isolates that were obtained in the study (Table 3). These are *T. tonsurans* (1), *T. verrucosum* (5), *T. soudanense* (1), *M. gypseum* (1), *M. persicolor* (2), *M. equinum* (1) and *M. fulvum* (3). Out of 85 male horses sampled 12 were positive, and out of the six female horses sampled, two were positive. However, there was no statistically significant difference (p > 0.05, P=0.2293) between the total dermatophytes isolated from male (14.1%) or female (33.3%) horses from the seven LGA in Kwara state (Table 4).

Discussion
Cultivation of the collected specimens revealed seven isolates made up of four *Microsporum* species and three *Trichophyton* species, with isolation rate of 15.4% (14 out of 91 positive samples) which is closely similar to the result obtained by Chah *et al.* (2012) who examined 46 domestic animals (sheep, dogs and goats) and found 6 (13%) positive for dermatophytes but lower than the report of Nweze (2011), who examined 25 horses out of which 11 samples (44 %) were positive as well as Hassan (2011), who isolated dermatophytes from 36.5% of total horse samples in Cairo, Egypt. Furthermore, El-Yazeed (1990) obtained an isolation rate of 49% for dermatophyte species from horses. The lower isolation rate obtained in this study can be attributed to the fact that samples were collected from both infected and asymptomatic cases coupled with the management practice by most horse groomers in Kwara state with most horses being kept in separate stalls and with different grooming equipment.

The 14 isolates made up of *T. verrucosum* (5, 35.7%), *T. tonsurans* (1, 7.1%), *T. soudanense* (1, 7.1%), *M. gypseum* (1, 7.1%), *M. persicolor* (1, 14.3%), *M. equinum* (1, 7.1%) and *M. fulvum* (3, 21.4%) confirm
the etiological agents of equine dermatophytosis as reported by El-Yazeed (1990); Pilsworth & Knottenbelt (2007) and Nweze (2011). The observation in this study of *Trichophyton verrucosum* being the most prevalent etiological agent of dermatophytosis in equine (5 isolates out of 14) is in contrast with the reports of El-Yazeed (1990); Kane *et al.* (1997) and Nweze (2011) who observed that *T. equinum* and *T. equinum var autotrophicum* were the most commonly isolated dermatophyte species from horses and *Trichophyton verrucosum* in cattle. This can be due to the management practice by most horse owners in Kwara state as they keep many species of animals together including horses and cattle and this play an effective role in cross infection through several routes from cattle to horses as suggested by Mantovani (1978). *Microsporum fulvum* is a cosmopolitan geophilic dermatophyte species and with similar clinical disease is similar to that of *M. gypseum* but less common. However it was the second most causative agent of dermatophytosis in this study and this is a rare occurrence. The infection is suspected to have been contracted by the affected horses rolling in the sand as they do sometimes with this sand already contaminated by anthrospores and infection aided by skin abrasions. *M. persicolor* infection will ensue since it is known to be a zoophilic and geophilic fungus. Based on anatomical location, the limbs showed the highest distribution rate (18.7%) than the other body locations where samples were collected from (tail, head and abdomen). This is contrary to The CFSPH (2005) report which stated that most dermatophyte lesions are found in areas on the back of horses in contact with saddle. The reason for this higher distribution on the limbs may be due to contamination of the hay given to the horses and also the floor of the stalls as confirmed by the isolation of two *M. fulvum* a geophilic dermatophyte, from the limbs. The observation of Ilorin-East having the highest incidence rate of 25% is possibly due to the high concentration of stables in that LGA as this can facilitate the spread of infections. Further studies are therefore required to establish antifungal sensitivity of commonly available drugs to the isolates recovered in our study.

In conclusion, the dermatophytes affecting horses in the seven LGAs of Kwara state were *T. verrucosum*, *T. tonsurans*, *T. soudanense*, *M. equinum*, *M. gypseum*, *M. persicolor* and *M. fulvum*. Ilorin-East LGA had the highest distribution (25%) of suspected cases of dermatophytosis while Baruten LGA had the lowest (9.1%). Female horses had higher rate of infection (33.3%) than male horses (14.1%). Anatomically, the limbs had the highest frequency of dermatophyte infection (18.7%) while the abdomen had the lowest frequency (10%).

**Acknowledgements**

We are grateful to the Technical staff of the Department of Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for providing guidance in the laboratory during the study.
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Sakota Journal of Veterinary Sciences, Volume 15 (Number 2). August, 2017

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