Isolation and Identification of *Microsporum canis* in Companion Animals from Selected Local Government Areas in Abia State

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Authors' contributions

This work was carried out in collaboration between both authors. Author NPO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OB managed the analyses of the study and equally managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The aim of the study is to isolate and identify *Microsporum canis* from companion animals (dogs and cats) in three local government areas of Abia State. A total of one hundred and fifty skin scrapings from infected dogs (100) and cats (50) were screened. Saboraud destroxe agar was used for the culture and Needle mount technique was adopted. Lactophenol Cotton Blue (LCB) was used for staining. Demographic indices like: age, sex, and breed of the animals were considered. This organism at macroscopy appears as white, light yellow, cottony to powdery colonies. At microscopic view, the spores of *M. canis* appear as large and spindle shaped with thick wall. The dogs has a predominant isolation rate of 36.0%. The female dogs and cats presented the highest frequency of occurrence at 58.2% and 63.6% respectively. Dogs of 9months old and above had more *M. canis* isolation rate at 70.0%, while cats between 5 and 8months of age had the highest isolation rate at 33.3%. Dogs and cats at 1 to 4 months of age had the least *M. canis* isolation rate at 7.5% and...
14.5% respectively. The indigenous breeds of dogs had the highest isolation rate of *M. canis* at 53.8% while the Caucasian breed was the least at 7.7%. Statistical analysis shows that (p=.05) there is significance in isolation rate of *M. canis* in dogs and cats.

Keywords: *Microsporum canis*; skin scrapings; dogs; cats; isolation; Saboraud destroxe agar.

1. INTRODUCTION

*Microsporum canis* is a pathogenic asexual fungus in the phylum Ascomycota that infects the upper, dead layers of skin on domesticated cats, and occasionally dogs, humans and other animals [1]. Dermatophytosis, also called ringworm or tinea is the disease caused by this organism and is of public health and economic importance [2]. It is the most frequently occurring infectious and highly contagious mycosis of man and animal [3]. There are three important genera of dermatophytic fungi: Epidermophyton (infects skin and nail), *Microsporum* (attacks the hair and skin) and *Trichophyton* (invades (hair, skin and nail) [4]. *Microsporum canis* is the most commonly isolated pathogen causing dermatophytosis in dog and cats. The tineas (ringworm) are frequent in domestic and savage animals: the most affected are the small species, such as dogs, cats and rodents [5]. Infection occurs by direct transmission of infective spores to a susceptible host through cuts on the skin. Mere exposure to dermatophyte spores does not guarantee infection. A critical mass of the spores must come in contact with susceptible host. The spores must evade host defense mechanism that include mechanical removal, competition with normal bacterial and fungal flora, exposure to fungistatic properties of epidermal lipids, low humidity of the skin surface and acquired host immunity [6]. Factors that predispose to infection include any pre-existing disease that will cause an increase in surface humidity, cause micro-trauma (cuts) to the skin and compromise host immune surveillance [6]. It has been estimated that dermatophytosis accounts for approximately 2% of all skin infection in which *Microsporum canis* contributes a great deal [7]. However, prevalence of the disease tends to be more common in warm tropical/subtropical climates and or where large number of feral animals [8]. Although cat is regarded as the natural host, and even as a reservoir for *Microsporum canis* [9] dogs are also incriminated though without obvious clinical symptoms. The presence of other diseases may also affect susceptibility to infection: dermatophytosis is three times more prevalent in cats with feline immunodeficiency virus than in uninfected cats [10]. The infections caused by this organism are hardly fatal but mostly debilitative and disfiguring that can give rise to permanent deformation if not treated [11]. There had been increasing complaint by dog and cat owners of skin related infections in the study area and the poor management by animal health care givers, hence the study. The aim of this study is to isolate and identify *Microsporum canis* in skin lesions of dogs and cats amongst other dermatophytes, and to determine the rate of occurrence of *Microsporum canis* in the animal under study.

2. MATERIALS AND METHODS

2.1 Study Area

Three (3) Local Government Areas of Abia state was used for the study. The study area includes, Umuahia North, Umuahia South and Osisioma local Government Area. Abia State in Nigeria is located in a tropical rainforest between latitude 543E, in the south eastern part of the country and longitude 752E. The average annual temperature and rainfall are 26.9°C and 2193 mm respectively [12]. Sample Collection: The sample collection and study protocol used in this study was as described [13] with some modifications. A purposive sampling method was employed. A total of 150 animals were sampled and this comprised of 100 dogs and 50 cats. The sampling cut across the three selected Local Government Areas of the state. Skin scraping was collected from obviously infected dogs and cats with lesions. Samples were collected with relevant socio-demographic data such as age, sex, breed, by observation or from the owners. Only animals that had no history of antifungal or antibacterial therapy in the previous months were included in the study. The representative samples were collected at varying occasions as reported clinical cases arise from various veterinary clinics in the location under study. Ethical clearance was obtained from the zonal director of veterinary division of the ministry of Agriculture in the state. Sample collection lasted for 8 months from April to November, 2019.

Samples were gotten during ambulatory services (House calls), while others were collected by submitting sample bottles to veterinarians
working in different clinics. The animals showing marked lesion was carefully examined for areas with loss of hair, erythema, scaling or heavy crusts. Animals of this form may or may not show suppuration beneath them. Animals of all ages showing areas of infection were sampled. The samples were collected aseptically by cleaning the area with cotton wool soaked in alcohol and then, using a sterile scalpel blade, areas of skin lesion were gently scrapped especially at the advancing border of the lesion making contact with inner layers of the skin (blood). Collected samples were placed into bijou bottles. The bottle containing normal saline was transported to the laboratory in a cold-chain within 4 to 10 hours aseptically for processing in the laboratory. Culture and identification of the fungal genera was carried out in the department of Veterinary Microbiology Laboratory of Michael Okpara University of Agriculture.

2.2 Laboratory Procedure

Sabouraud dextrose agar medium was used for the isolation of fungi in the skin scrapings. The medium was prepared aseptically following the manufacturer’s description of 65 grams in 1000 mls of distilled water. In addition, gentamicin (2ml) was included in the media to inhibit bacteria growth and make the media selective for fungi organisms. The medium was autoclaved at 15 pounds pressure, temperature of 121°C for 15minutes and gradually dispensed into sterile petri dishes in hood chamber and allowed to set.

Portions of the sample were treated with 5% potassium hydroxide for microscopic identification of the hyphae or the arthroconidia at ×10 and ×40 magnifications. A portion of the skin scraping was placed on a glass slide and with the aid of a thumb forcep, a drop of 5% potassium hydroxide (KOH) was dropped and allowed for 5minutes. It was then covered with a cover slip and viewed under the microscope. Another portion of the sample was seeded into the already prepared media and tightly sealed with a masking tape to avoid contamination and to create a humid environment necessary for growth. The sealed plates were incubated at room temperature for 7-14 days and observed at intervals for growth of the organism. After culturing for 7days the plates were opened and with the aid of a sterile wire loop, a loopful of the culture was picked and placed on a glass slide. A drop of alcohol was added and the sample was teased to remove any excess and then allowed for 5minutes. Afterwards a drop of Lactophenol Cotton Blue (LCB) was added and cover with a cover slip. Isolates were then identified based on atypical colonial and microscopic morphology of the culture at 100-1000 magnification [14]. Microscopic examination of the isolates was done using direct microscopy and Needle mount. Culture of specimens is necessary not only to allow direct microscopic confirmation of the diagnoses but also to allow identification.

2.3 Statistical Analysis

the results obtained from the study was analyzed using student ‘T’ test for two variables and analysis of variance (ANOVA) for three variables. Other results were presented in tables and bar chart.

3. RESULTS

Microsporum canis isolated from dog sample shows fungi spores which are large, spindleshaped and thick walled, with six or more internal cells (LCB ×100) for Fig. 3, while Fig. 4 obtained from cat sample, showed a more larger spores with broader shape (LCB×1000).

The result (Table 1) suggests that there were more positive samples in dogs than in cats, with dogs presenting 36% isolation rate.

From (Table 2), the result, out of the 100 dogs that were sampled, 21 representing (58.2%) was positive for female while 15 representing (41.6%) was positive for male. Out of the 50 cats, 4 male was positive representing 36.3% while 11 females was positive representing 63.6%. This result suggests that female dogs were more susceptible to Microsporum canis than males. Statistical analysis shows (p=.05) there exist a significance in occurrence in dogs than cats.

The result in (Table 3) shows that dogs of 9months of age and above were more affected. A total of 21 dogs representing 70% recorded the highest occurrence of Microsporum canis, hence, statistical analysis shows that (p=.05) there is significance. Dog age 1 to 4months recorded the least of 3 dogs representing 7.5%, Fig 1 shows light yellow, cottony to powdery colonies, while Fig 2 shows white powdery surface with red spot. Fig 3 shows spores that are spindle shaped with thick wall, while Fig 4 shows spores with large colonies.

Fig. 5 from the bar chart, the Nigerian indigenous breed and the Cross breed were more susceptible to Microsporum canis at 53.8% and 23.0% respectively, while the Caucasian was the least susceptible at 7.6%
Fig. 1. Colonies appearing after 7 days are white, light yellow, tan cottony-to-powdery

Fig. 2. Colonies appearing after 10 days are cottony white powdery surface with red spot is visible

Fig. 3. Microsporum canis (LCB x100), When we see through the pictures, we notice there is a difference in the power of the magnification
Fig. 4. *Microsporum canis* (LCB ×1000)

Table 1. Overall Percentage isolation rate of *Microsporum canis*

<table>
<thead>
<tr>
<th>Animals</th>
<th>Positive samples</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs (100)</td>
<td>36</td>
<td>36.0%</td>
</tr>
<tr>
<td>Cats (50)</td>
<td>11</td>
<td>22.0%</td>
</tr>
</tbody>
</table>

Fig. 5. Percentage occurrence of *Microsporum canis* in different breeds of dogs
Table 2. Percentage occurrence of *Microsporum canis* based on sex

<table>
<thead>
<tr>
<th>Species of animal</th>
<th>Sex</th>
<th>Number positive.</th>
<th>Percentage of occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs (100)</td>
<td>Male</td>
<td>15</td>
<td>41.6%</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>21</td>
<td>58.2%</td>
</tr>
<tr>
<td>Cats (50)</td>
<td>Male</td>
<td>4</td>
<td>36.3%</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>7</td>
<td>63.6%</td>
</tr>
<tr>
<td>Total (150)</td>
<td></td>
<td>47</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 3. Percentage occurrence of *Microsporum canis* Based on age

<table>
<thead>
<tr>
<th>Age range</th>
<th>No. of dogs sampled</th>
<th>No. of dogs positive</th>
<th>(%) occurrence for dogs</th>
<th>No. of cats sampled</th>
<th>No. of cats positive</th>
<th>(%) occurrence for cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4 months</td>
<td>40</td>
<td>3</td>
<td>7.5%</td>
<td>14</td>
<td>2</td>
<td>14.5%</td>
</tr>
<tr>
<td>5-8 months</td>
<td>30</td>
<td>12</td>
<td>40.0%</td>
<td>18</td>
<td>6</td>
<td>33.3%</td>
</tr>
<tr>
<td>≥9 months</td>
<td>30</td>
<td>21</td>
<td>70.0%</td>
<td>18</td>
<td>3</td>
<td>16.6%</td>
</tr>
</tbody>
</table>

4. DISCUSSION

The study revealed high isolation rate of *Microsporum canis* in dogs and cats at 32.4% and 12.5%, and this is in agreement with the findings [4]. Cabanes in his study conducted across seven states in Nigeria reported high prevalence rate of *Microsporum canis* at 37.4% in dogs. Similar result was reported by Seker et al. in India with *Microsporum canis* isolation being the predominant species of dermatophyte in dogs [15,16]. Other studies have also reported high recovery rates from these animals. There are contradicting reports on the prevalence of *Microsporum canis* on dogs, while some authors report low prevalence of between 4-10%, others found higher values [17]. This may be due to the fact that geographical distribution varies with the organism, as they grow best in warm and humid environments and are therefore more common in tropical and subtropical regions [13].

In this study the difference between male and female dogs was statistically significant. This finding is in agreement [18,19]. There was higher isolation rate of *Microsporum canis* in those dogs between the ages of 9 months and above, this is in agreement with [20], who reported that adult dogs were more prone to the infection. However, this is in disagreement with [21,15], who stated that puppies and kittens below 6 months of age showed a significantly higher isolation rate than those greater than 6 months old. This might be due to their poorly developed immune system and deficiency of fungistatic linoleic acid [22].

According to the study, mongrels (Nigerian indigenous dogs) had the highest isolation rate, followed by terrier dogs and this is in disagreement with [23]. Cabarcha reported that terrier breed of dogs were more susceptible because of their long hairs which protects the organism and allows it to establish undetected. The mongrels despite being domesticated are freely allowed to move around the neighborhood and return to their owners at intervals to feed, they had higher chance of exposure to the infection compared to other breeds which are mostly under confinement. Similar view was supported [24].

The appearance of the colonies are white, light yellow, tan, cottony-to-powdery. The diameter of the colony reaching 3 to 9cm following incubation and this is in consistent with [25]. The microscopic view revealed large, spindle shaped, and thick walled spore with six or more internal cells and often has a terminal knob, similar to the findings [26]. This is confirmatory for *Microsporum canis*.

5. CONCLUSION

*Microsporum canis* is an important pathogen because of its zoonotic importance and transmission is through contact with the spores in infected animals. There was high isolation rate of *M. canis* in this study. Nigerian indigenous breed and female animals above 9 months old were more susceptibility and instructions available at www.le probably due to underfeeding in the former and pregnancy stress in the later which affects their immune system.
ETHICAL APPROVAL

Ethical clearance was obtained from the zonal director of veterinary division of the ministry of Agriculture in the state. Sample collection lasted for 8 months from April to November, 2019.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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