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Original Article

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Prevalence and risk factors associated with canine dermatophytoses among dogs in Kwara and Osun States, Nigeria

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Abstract:

Background: Dermatophytosis (ringworm) is a zoonotic fungal skin infection caused predominantly by *Microsporum canis, Microsporum gypseum* and *Trichophyton* spp. It is highly transmissible and, while normally self-limiting, could be problematic due to its potential to cause disease in certain human populations. The occurrence and associated risk factors of dermatophytoses in dogs presented at three veterinary clinics in Osogbo, and Ilorin, Nigeria between July and November 2019 were investigated in this study.

Methodology: This was a descriptive cross-sectional study of 325 dogs with lesions suggestive of dermatophytosis, selected by simple random sampling from veterinary clinics of two hospitals, purposively selected for the study due to high patronage of the veterinary hospitals by dog owners. Using conventional mycological sampling techniques, plucked hairs and skin scrapings were obtained the dogs. The samples were emulsified in 10% potassium hydroxide, examined microscopically for fungal elements and cultured using standard mycological procedures. Information on dog demographic characteristics and risk factors for dermatophytosis were collected using structured questionnaire. The association between risk factors and demographic variables with the occurrence of dermatophytoses was determined using Chi-square test (with Odds ratio and 95% confidence interval) and p value < 0.05 was considered statistically significant.

Results: Positive cultures for dermatophytes were obtained from samples of 48 (14.8%) dogs with *M. canis* 37.5% (18/48), *M. gypseum* 27.0% (13/48) and *T. mentagrophytes* 8.3% (4/48). Other fungi identified were *Aspergillus flavus* 12.5% (6/48) and *Malassezia canis* 12.5% (6/48). The age distribution of positive dogs were < 1 year (50.0%, n=24), 1-3 years (29.2%, n=14) and > 3 years (20.8%, n=10), while the risk factors associated with dermatophytosis included sex of dogs (p=0.0428), history of dermatophytosis (p<0.0001), clinical presentation (p<0.0001) and lesion type, especially kerion and pustular lesions (p=0.0297).

Conclusion: These findings established the occurrence of dermatophytosis in dogs kept for companionship (i.e., pets), security and breeding purposes in one northern and southern States of Nigeria. Our findings underscore the need for routine mycological investigations in dogs to facilitate early detection of cases and prompt institution of treatment interventions, thereby preventing zoonotic transmission of dermatophytes to their owners, handlers and veterinarians.

Keywords: Dermatophytosis; ringworm; dogs; risk factors

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Prévalence et facteurs de risque associés aux dermatophytoses canines chez les chiens dans les États de Kwara et d'Osun, au Nigeria

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Résumé:

Contexte: La dermatophytose (teigne) est une infection cutanée fongique zoonotique causée principalement par *Microsporum canis, Microsporum gypseum* et *Trichophyton* spp. Il est hautement transmissible et, bien qu'il soit normalement autolimitatif, il pourrait être problématique en raison de son potentiel à provoquer des maladies dans certaines populations humaines. La survenue et les facteurs de risque associés des dermatophytoses chez les chiens présentés dans trois cliniques vétérinaires à Osogbo et Ilorin, au Nigeria entre juillet et novembre 2019 ont été étudiés dans cette étude.

Méthodologie: Il s'agissait d'une étude transversale descriptive de 325 chiens présentant des lésions évocatrices de dermatophytose, sélectionnés par simple échantillonnage aléatoire dans les cliniques vétérinaires de deux hôpitaux, sélectionnés à dessein pour l'étude en raison de la forte fréquentation des hôpitaux vétérinaires par les propriétaires de chiens. En utilisant des techniques d'échantillonnage mycologique conventionnelles, des poils épilés et des raclures de peau ont été obtenus chez les chiens. Les échantillons ont été émulsifiés dans de l'hydroxyde de potassium à 10%, examinés au microscope pour les éléments fongiques et cultivés en utilisant des procédures mycologiques standard. Des informations sur les caractéristiques démographiques des chiens et les facteurs de risque de dermatophytose ont été recueillies à l'aide d'un questionnaire structuré. L'association entre les facteurs de risque et les variables démographiques avec la survenue de dermatophytoses a été déterminée à l'aide du test du chi carré (avec rapport de cotes et intervalle de confiance à 95 %) et la valeur de p < 0,05 a été considérée comme statistiquement significative.

Conclusion: Ces résultats ont établi la présence de dermatophytose chez les chiens élevés pour la compagnie (c'est-à-dire les animaux de compagnie), la sécurité et la reproduction dans l'un des États du nord et du sud du Nigéria. Nos résultats soulignent la nécessité d'enquêtes mycologiques de routine chez les chiens pour faciliter la détection précoce des cas et la mise en place rapide d'interventions thérapeutiques, empêchant ainsi la transmission zoonotique des dermatophytes à leurs propriétaires, maîtres-chiens et vétérinaires.

Mots clés: Dermatophytose; teigne; chiens; facteurs de risque

Introduction:

Dermatophytosis is a superficial fungal infection of the stratum corneum of the epidermis and keratinized tissues including the skin, hair and nails of humans and animals. It is considered as the most commonly encountered fungal infection in veterinary practice affecting a wide range of domestic and wild animals (1,2,3), and is recognized as a major skin disease of zoonotic importance (4,5). Dermatophytosis is caused by a unique group of fungi known as the dermatophytes which can be categorized into one of three genera; Trichophyton, Microsporum and Epidermophyton. While over 20 species of dermatophytes have been found in domestic animals, the most common in dogs are Microsporum canis, M. gypseum and Trichophyton spp (6)

Transmission of dermatophytes occurs rapidly via direct contact with infected animals, fomite-associated contact with infectious spores and sometimes air-borne (7). The close interaction between humans and animals have allowed for easy transmission of dermatophytes to humans through direct contact with cutaneous lesions of infected animals, with *M. canis, Tinea capitis* and *T. corporis*

being the most commonly isolated from humans (8).

Generally, dermatophytes are classified into three ecological groups based on their major natural reservoirs. Those found in the soil, animals and humans are categorized as geophilic, zoophilic and anthropophilic, respectively (9). The zoophilic dermatophytes may precipitate zoonoses in humans with these human infections resulting most often from direct contact with an infected animal or through indirect contact with contaminated environments (10), such as fungus-bearing hair and scales from infected animals. According to Akpolat et al., (11), the prevalence of superficial mycoses caused by zoophilic dermatophytes is significant worldwide, especially in the tropical countries with warm and humid climate, crowded living and poor sanitary conditions (12).

Dermatophytosis often occurs when the host immune system is unable to generate sufficient cell-mediated immune response capable of eliminating the fungi from the host system (13). While the infection is more prominent in younger animals, especially those below one year of age, older animals are less susceptible as incidences of acquired immune response have previously been reported in

them (14). Apart from age, other factors including breed of animal, population density, management system, season of the year, climatic and socio-economic conditions, and natural reservoirs have been reported to play significant roles in determining the prevalence and causative agents of dermatophytosis in different animal species worldwide (15,16).

In recent years, there has been an upsurge in keeping of dogs as pets or for security purposes in Nigeria, with majority of these animals cohabiting and feeding with their owners and household members (17,18,19). In this way, these animals could serve as potential sources of zoonotic diseases including dermatophytoses to their owners and handlers. Despite the existence of several published works on human dermatophytoses in Nigeria, reports of the disease in animals, especially dogs, are limited. Nweze (20) and Chah et al., (21) have previously reported 49. 5% and 12.5% prevalence rates of dermatophytosis, respectively in dogs in Nigeria. Moreover, Adebiyi and Oluwayelu (21) reported the fact that dermatophytes of dogs probably play a significant role in the epidemiology of human dermatophytosis. Our study was therefore conducted to identify the causative fungi and risk factors associated with occurrence of dermatophytosis in dogs presenting to the veterinary clinics in Osogbo and Ilorin, the capital cities of Osun and Kwara States of Nigeria, respectively.

Materials and method:

Study setting and design:

This was a descriptive cross-sectional study carried out in three veterinary clinics; two veterinary clinics located in Osogbo, Osun State, southern Nigeria and the Small Animal Clinic of the Veterinary Teaching Hospital, University of Ilorin, Kwara State, northern Nigeria.

Sample size and dog participants selection:

A purposive sampling technique was used to select a Veterinary Teaching Hospital from Kwara State, and a State Veterinary hospital in Osun State, due to high patronage of dog owners in those hospitals. A total of 325 dogs (125 from Osun and 200 from Kwara) with lesions suggestive of dermatophytosis were selected for the study by simple random sampling technique from each of the selected hospitals between July and November 2019.

Ethical approval:

Ethical approval for the study was obtained from the Ministry of Agriculture and Natural Resources, Osun State and the Animal Care and Use Research Ethics Committee, Faculty of Veterinary Medicine, University of Ibadan (UI-ACUREC/19094).

Data and sample collection:

A structured questionnaire was administered to obtain information on the dogs' demographic characteristics and possible risk factors for dermatophytosis from the animal owners and veterinary clinicians. Following detailed examination of the lesions under good illumination and cleaning of the affected area with 70% ethanol, skin scrapings of dogs were collected with sterile scalpel blade from the active edge of the lesions. Hair samples were collected by removing dull broken hairs from the margin of the lesion using sterile tweezers.

The samples were placed in small paper envelopes in separate polythene bags, and transported as dry packages (6) to the Teaching and Research Laboratory of the Department of Medical Microbiology, Ladoke Akintola University of Technology, Osogbo, where they were processed for direct microscopy and culture.

Direct microscopy, fungal isolation and identification:

Each sample was divided into two portions; one portion was used for direct microscopic examination and the other was used for culture. Direct microscopic examination was performed by placing plucked hair strands or skin scrapings on a clean glass slide and 30 μ L of 15% potassium hydroxide (KOH) added. The slide was covered with a coverslip, heated gently for a few seconds and allowed to cool at room temperature for 15 minutes. Thereafter, the wet preparation was carefully examined under both low (10×) and high (40×) power light microscope objectives to observe the fungal elements and diagnostic morphology.

Irrespective of the results of direct microscopy, the samples were cultured on Saboraud's Dextrose Agar (SDA) containing 0.5 mg/mL chloramphenicol and 500 mg/L cycloheximide. The plates incubated at room temperature (25-30°C), and examined daily for fungal growth for up to four weeks (11). Suspected fungal colonies were sub-cultured on SDA slants to obtain pure cultures, which were then sub-cultured on Potato Dextrose Agar (PDA) (Oxoid, UK) to facilitate distinctive spore formation. The PDA plates were incubated at room temperature for up to 4 weeks. Identification of the isolated fungi was based on colonial morphology as well as microscopic features observed after staining with lactophenol cotton blue using standard mycological identification procedures (22).

Statistical analysis:

Statistical analysis of data was perfor-

med using the Statistical Package for Social Sciences (SPSS) version 25.0 with comparison of proportions and Chi-square test (with Odds ratio and 95% confidence interval) to determine association of the risk factors with the prevalence of dermatophytosis in the dogs.

Results:

A total of 325 dogs were examined; 125 from Osogbo and 200 from Ilorin. Of these, 35 (10.8%) were positive for fungal elements by direct microscopy while 48 (14.8%) yielded cultures positive for dermatophytes. Out of the 48 positive cultures, 37.5% (n=18) were identified as *M. canis*, 27.1% (n=13) as *M. gypseum* and 8.3% (n=4) as *T. mentagrophytes*. Other fungi identified include *Aspergillus flavus* 12.5% (n=6), and *Malassezia canis* 12.5% (n=6) (Table 1). It is noteworthy that 18 (37.5%) of the 48 culture-positive

samples were negative by direct microscopic examination.

Furthermore, out of the dog samples from Osogbo (n=125) and Ilorin (n=200) screened for dermatophytes, isolation rates of 16.0% (20/125) and 14.0% (28/200) respectively were obtained (OR=0.8547, 95% CI=0.4583-1.594, p=0.7386). Of the 48 dermatophyte-positive samples, 8 (16.7%) were from dogs kept for companionship (i. e. as pets), while 29 (60.4%) and 11 (22.9%) were kept for security and breeding purposes, respectively (x^2 =0.0159, p=0.992) (Table 2).

Table 3 depict association of risk factors with prevalence of dermatophytosis in the dogs. It shows that sex (p=0.0483), clinical presentation of dermatophytosis (p<0.0001), lesion type, especially kerion and pustular lesions (p=0.0297), and previous history of dermatophytosis in the dogs (p<0.0001) were significantly associated with prevalence of dermatophytoses.

Table 1: Dermatophytes and other fungi isolated from skin lesions of dogs in Kwara and Osun States, Nigeria

Organism	Ilorin	Osogbo	Total	Percentage (%)	
M. canis	11	8	19	37.5	
M. gypseum	7	6	13	27.1	
T. mentagrophytes	3	1	4	8.3	
Aspergillus flavus	4	2	6	12.5	
Malassezia canis Total	3 28	3 20	6 48	12.5 100	

Table 2: Distribution of dermatophytes based on purpose for keeping dogs

Purpose	Number of dogs	No of positive dogs (%)	<i>x</i> ²	p value	
Companionship	56	8 (14.3)	0.01598	0.9920	
Security	194	29 (15.0)			
Breeding	75	11 (14.7)			
Total	325	48 (14.8)			

	Table 3: Risk fac	ctors associated wi	th dermatophytosi	is in dogs from Kwara	a and Osun States, Niger	ia	
Variables	Parameters	Frequency	Positive (%)	Negative (%)	OR (95% CI)	<i>X</i> ²	p value
Location	Ilorin	200	28 (14.0)	172 (86.0)	0.8547 (0.4583-1.594)	0.113	0.7401
	Osogbo	125	20 (16.0)	105 (84.0)	(0.4363-1.394)		
Period	July	65	9 (13.8)	56 (86.2)		4.058	0.3893
	August	65	10 (15.4)	55 (84.6)			
	September October	65 65	12 (18.5) 5 (7.7)	53 (81.5) 60 (92.3)			
	November	65	12 (18.5)	53 (81.5)			
	November	03	12 (10.3)				
Breed	Caucasian Rottweiler	48 64	5 (10.4) 7 (10.9)	43 (89.6) 57 (89.1)		10.144	0.3389
	German Shepherd	71	8 (12.7)	55 (87.3)			
	Lhasa Apso	18	1 (5.6)	17 (94.4)			
	Cane Corso	16	4 (25.0)	12 (74.0)			
	Boerboel	2	0	2 (100.0)			
	Samoyed	12	4 (33.3)	8 (66.7)			
	Mongrel	43	9 (20.9)	34 (79.1)			
	Labrador	2	0	2 (100.0)			
	Mastiff	2	0	2 (100.0)			
Sex	Female	168	18 (10.7)	150 (89.3)	0.508	1.616	0.0428*
	Male	157	30 (19.1)	127 (80.9)	(0.2704-0.9543)		
Age	1-9 months 1-3 years	141 135	24 (17.0) 14 (10.4)	117 (82.9) 121 (89.6)		3.881	0.1436
	≥ 3 years	49	10 (20.4)	39 (79.6)			
Purpose	Companionship	56	8 (14.3)	48 (85.7)		0.02014	0.9900
•	Security	194	29 (14.9)	165 (85.1)			
	Breeding	75	11 (14.7)	65 (85.3)			
Location of lesion	Face Back	116 89	20 (17.2)	96 (82.8)		3.063	0.3819
lesion	Side	93	12 (13.5) 10 (10.8)	77 (86.5) 83 (89.3)			
	Leg	27	6 (22.2)	21 (77.8)			
	_09	_,	0 (==:=)	21 (77.10)			
Description of lesion	Alopecia Kerion	160 1	22 (13.8) 1 (100.0)	138 (86.3) 0		12.399	0.0297*
	Pustular	1	1 (100.0)	0			
	Itchy	60	7 (11.7)	53 (88.3)			
	Scaly	53	9 (16.9)	44 (83.0)			
	Others	50	8 (16.0)	42 (84.0)			
Presented for	Yes	48	48 (100.0)	0		325.0	<0.0001*
dermatophytosis	No	249	0	249 (100.0)			
	Others	28	0	28 (100.0)			
Previous history	Yes	89	2 (2.3)	87 (97.7)		19.419	<0.0001*
	No	223	46 (20.6)	177 (79.4)			
	Others	13	0	13 (100.0)			
Previous medication	Acaricide Antibiotics	84 74	9 (10.7) 10 (13.5)	75 (89.3) 64 (86.5)		6.541	0.2570
medication	Ointment	68	10 (13.5) 9 (13.3)	59 (86.8)			
	Honey	2	9 (13.3) 1 (50.0)	1 (50.0)			
	Anti-inflammatory	38	9 (23.7)	29 (76.3)			
	Ivermectin	59	10 (16.9)	49 (83.1)			
Housing type	Inside house	115	10 (8.7)	105 (91.3)		1.830	0.4006
nousing type	inside house	115	10 (8./)	105 (91.3)		1.830	0.4006

	Outside House	52	8 (15.4)	44 (84.6)		
	Kennel	158	20 (12.7)	138 (87.3)		
Frequency of sanitation	Weekly Monthly	41 118	2 (4.9) 22 (18.6)	39 (95.1) 96 (81.4)	4.607	0.09
	Scarcely	166	24 (14.5)	142 (85.5)		
Frequency of grooming	Weekly Monthly	42 200	5 (11.9) 35 (17.5)	37 (88.1) 165 (82.5)	3.194	0.20
	Scarcely	83	8 (9.6)	75 (90.4)		
Total		325	48 (14.8)	277 (85.2)		

^{*}: statistically significant: x^2 : Chi square; OR: Odds ratio; CI: Confidence interval

Fig 1 represents the typical dermatophyte lesion on the skin of the dog infected with *M. canis* showing scales and skin crusts (A), with typical yellowish tinge on Saboraud Dextrose Agar (B and C), and spindle shaped macroconidia on lactophenol cotton blue stain.

Fig 2 shows inflammation of the skin and alopecia in dogs infected with *T. menta-grophytes* (A), with growth and reverse growth on SDA (B and C) and typical macroconidia on lactophenol cotton blue stain (D).



Fig 1: A: Dog skin showing alopecia and scaly lesion, B: Microsporum canis growth on Saboraud Dextrose Agar, C: Reverse growth of Microsporum canis on Saboraud Dextrose Agar, D: Macroconidia of Microsporum canis stained with lactophenol cotton blue

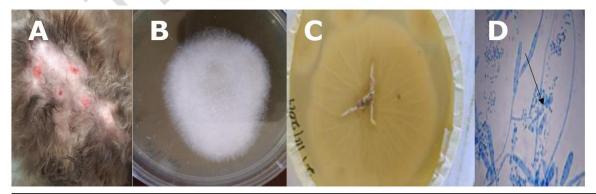


Fig 2: A: Dog skin showing alopecia and inflammation, B: *Trichophyton mentagrophytes* whitish cottony growth on Saboraud Dextrose Agar, C: Reverse growth of *Trichophyton mentagrophytes* on Saboraud Dextrose Agar, D: Microconidia of *Trichophyton mentagrophytes* stained with lactophenol cotton blue

Discussion:

Dermatophytosis has been recognized as a major public health hazard worldwide and has become endemic in some countries, especially in Africa. In Nigeria, it is a major etiology of skin infection in domestic animals and humans, especially children (19,23,24). This study was designed to investigate the occurrence and distribution of dermatophytes in dogs with suspected dermatophytosis and the prevalence of infection in relation to some epidemiological variables in Osogbo, Osun State and Ilorin, Kwara State, Nigeria.

Although the distribution of zoophilic dermatophytosis is worldwide, its incidence varies with the type of animal and geographical location. Murmu et al., (25) reported that the incidence of dermatophytosis in cats was higher (55.5%) than in dogs (37.8%) in Kolkata, India. However, in the present study, we obtained 14.8% prevalence of dermatophytosis in the dogs. This relatively low prevalence rate is comparable to the 8.2%, 18.7%, 12.5% and 8.2% reported by Khosravi and Mahmoudi (26), Seker and Dogan (27), Chah et al., (20) and Sigirci et al., (13) in symptomatic dogs in Iran, Western Turkey, Southeastern Nigeria and Istanbul, Turkey, respectively. According to previous workers (14,15, 24), differences in the prevalence of dermatophytoses can be attributed to age and breed of the animal, clinical and living conditions of the animal, geographical location, season of sampling, socio-economic conditions, and whether the animal is confined or free-roaming, among others.

The isolation of dermatophytes in dogs used essentially for companionship (i. e. as pets), security and breeding purposes in our study is of public health significance as they can serve as source of infection to their human owners, handlers, or veterinarians (28). Previous studies have established a direct correlation between dermatophytoses in dogs and humans. For instance, in a study in Italy, Cafarchia et al., (29) conducted a dermatophytic search in dogs and cats whose owners were either diagnosed with tinea corporis caused by M. canis or those without dermatophytosis. They found M. canis in 53.6% of cats and 36.4% of dogs whose owners had tinea corporis, and in only 14.6% of cats and in none of the dogs whose owners were not infected. They concluded that these pets shou-Id be recognized as a major source of dermatophytosis for humans even when they are asymptomatic.

Moreover, Adesiji et al., (30) recently reported a significant association between the playing habits of children and acquisition of dermatophytoses. Their study revealed that

children who played with animals were at a higher risk of dermatophytoses compared with children who did not play with animals. As the contact between humans and pets is becoming more intimate, dermatophytoses have become significant public and animal health concern globally. It is believed that the incidence of mycotic zoonoses such as dermatophytoses is increasing because of such direct interspecies interaction as is the possibility of reinfection due to indirect spread of spores in the environment (29,31,32).

Three major species of dermatophytes were identified in our study with M. canis being the most prevalent, followed by M. gypseum while T. mentagrophytes was the least prevalent. Other fungi detected include Aspergillus flavus and Malassezia canis. It has been reported that M. canis, M. gypseum and T. mentagrophytes are the fungi responsible for more than 95% of all dermatophytoses cases in companion animals (33). Further, analysis of the results of the present study revealed a statistically significant association between the breed of dogs and occurrence of dermatophytosis as German Shepherd breed had the highest prevalence of dermatophytic infections while Lhasa Apso breed had the least prevalence (34). It has been reported that dog breed plays a significant role in the acquisition of dermatophytosis. For example, there have been reports of superficial dermatophytosis and subcutaneous dermatophytic infections in Yorkshire terrier dogs caused mainly by M. canis in Brazil and Italy (29,35,36). Also, Cafarchia et al., (29) reported a higher prevalence of geophilic dermatophytes in pure breed dogs compared to cross-breed dogs.

A few studies also showed that dermatophytosis occurs more often in female animals than in males (37,38). However, our findings revealed that the prevalence of 19.1% for dermatophytosis in the male dogs was significantly higher than 10.7% in the female dogs (OR=0.5080, 95% CI=0.2704-0.9543, p=0.0483), although other workers did not detect any association or correlation between sex and occurrence of dermatophytoses (27, 33). Some studies have indicated that age could be a factor in the incidence of dermatophytoses in domestic animals. Mattei et al., (33) determined that animals younger than one-year-old appeared to be susceptible to dermatophytoses. This is clearly at variance with the results from our study which showed that there was no statistically significant relationship (p=0.144) between the age of the animals and the prevalence of dermatophytoses. Our result corroborates the findings of Seker and Doğan (27) who detected no statistically significant difference between the animal age groups and prevalence rate in a similar study. It has been reported that the higher susceptibility of young animals to dermatophytoses may be related to immunological immaturity, the deficiency of fungistatic sebum or linoleic acid, biochemical exchange on the skin, being of the anagen phase of hairs and physiological situation (7).

Other factors significantly associated with dermatophytosis in our study include previous history of dermatophytosis in the dogs (p<0.0001), clinical presentation of dermatophytosis (p<0.0001) and dermatophytic lesion type, especially kerion and pustular lesions (p=0.0297). However, the housing types (p=0.4006) and frequency of sanitation (p=0.099) were not significantly associated with the prevalence of dermatophytosis. This contrasts previous studies that report housing type and sanitation as two factors critical to the incidence of dermatophytosis. For instance, in identifying the predispositions for the development of dermatophytoses in cats and dogs, Moriello et al., (24) underlined being puppies and kittens, lifestyle, free-roaming animals and warm locations as risk factors. Seker and Dogan (27) also opined that in addition to age, animal management and living conditions were risk factors for the acquisition of dermatophytoses by dogs. Moreover, our findings clearly showed that anatomical sites of lesions from where the dermatophytes were obtained had no significant association with the disease prevalence (p=0.3819), agreeing with previous studies which showed that pets harbor dermatophytic spores in both their hair coats and skin (27,39).

In conclusion, the results of this study revealed that dermatophytoses occur commonly in dogs in the study area, and that M. canis is the most prevalent fungus encountered. These findings, especially in dogs kept for companionship (i.e., as pets), security and breeding purposes, have significant implications for animal-to-human transmission of the disease and underscore the fact that canine dermatophytoses is a potential public health hazard in Nigeria. Routine screening of dogs followed by prompt treatment with appropriate anti-fungal agent in cases where infection is established are advocated to reduce incidence of dermatophytosis in dogs and prevent subsequent spread from these animals to humans.

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Contributions of authors:

AYO conceived, designed, collected the data, and wrote the manuscript; ODO participated in design, coordination and critical review of the manuscript; and AJO collected data, coordinated sample collection from animals and reviewed the manuscript. All authors read and approved the final manuscript.

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Authors declare no conflict of interest

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