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MYCOLOGICAL STUDIES OF DERMATOPHYTOSIS ASSOCIATED WITH ALOPECIC ANIMAL HIDES AND SKIN

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ABSTRACT

Ringworm caused by dermatophytes is a contagious fungal infection that affects the skin of animals, the quality of hides and skin in slaughtered animals, and eventual damage to the leather produced. The dermatophytosis associated with slaughtered animal skin suspected with alopecia was studied. Fifty raw goats and sheep skins were collected from both slaughtered sheep and goats at Kano Central Abattoir using aseptic methods. The samples were analysed using standard mycological techniques for isolation and identification; the fungal load was enumerated using hematocytometry. The overall prevalence of dermatophytosis out of 50 analysed samples was found to be 4 (8%), whereas alopecia due to non-dermatophytes accounted for 46 (92%), and the sheep skin samples were found to harbour more fungal load 3 (75%) compared to those of goat skin 1 (25%). The overall fungal load ranges between 3×10^5 to 4.3×10^5 spores/mL. The species of isolated dermatophytes include Trichophyton verrucosum, Microsporum nanum, Trichophyton mentagrophytes, and Trichophyton rubrum. Dermatophytes are among the primary culprits in the causation of animal alopecia. The fungal load was found to be more pronounced in sheep's skin than in goats' skin. The prevalence of dermatophytosis in goats and sheep skins was relatively low in the study area, and the alopecia observed on the skins may be caused by other underlying veterinary medical conditions, such as trauma and nutrient deficiency.

KEYWORDS

Dermatophytosis, hides, skin, alopecia, hematocytometry, leather

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INTRODUCTION

Alopecia is a medical condition that can be congenital or acquired usually occurs in domestic animals, presenting a partial or complete lack of hairs or wools in areas where they usually are present (Tobin, 2009). In living animals, acquired alopecia develops if the disease destroys the hair follicle or hair shaft and interferes with the growth of hair or wool or causes the animal discomfort (e.g., pain, pruritis), leading to self-trauma and loss of hair (Chermette *et al.*, 2008). Diseases that can directly cause destruction or damage to the hair shaft or follicle, such as bacterial skin diseases and dermatophytosis, often destroy the typical structure of slaughtered animal hides. This eventually leads to poor quality of the processed leather whenever such deformed hides and skin are tanned for the production of leather (Bond, 2010).

The diseases caused by dermatophytes are generally called ringworm (from the circular nature of the lesion) or tinea (from the Latin word for the clothes moth, whose feeding habits result in circular holes in woollen cloth). It is the most frequently encountered dermatologic problem in veterinary practice, affecting many domestic and wild animals (Ashwathanarayana and Naika, 2016). The fungus does not invade living skin, but its presence in the dead layers provokes an active inflammation of the underlying living layers. This provides a favourable environment for the invasion of living tissues by secondary bacterial infections. The disease's progress involves wool matting, followed by loss of wool, scaling, and crusting. Early lesions may not be readily seen, but the thickened nodules can be felt. There is usually little evidence of itching in living animals. However, infected sheep and goats will rub along fences and rough surfaces, contaminating the structures with fungal spores (Scott *et al*., 2001).

Dermatophytosis, often called ringworm, caused by dermatophytes, is a contagious fungal infection that affects the skin of animals. This is because most animal breeders rarely seek veterinary assistance when shedding hairs as long as the animals are not losing weight (Rybnikar *et al*., 1991). Nevertheless, the disorder can result in substantial economic loss for the farmers, as most tanners reject deformed animal hides for processing (Bond, 2010). The study was designed to identify the mycological cause of alopecia in animal hides, specifically sheep and goats, to identify the causative agents. The findings can be used to great advantage by tanners and farmers

who engage in animal husbandry by providing baseline information to sensitise farmers on the prompt management of animal skin disorders through veterinary treatment of infected animals.

This research aimed to investigate dermatophytosis in the hides and skin of domestic animals suffering from hair loss. The objectives were to isolate, characterise, and microscopically identify the causative agents of dermatophytosis in these animals, assess the prevalence of dermatophytosis in their skin, and quantify the dermatophyte population in the skin using hematocytometry.

MATERIALS AND METHODS

Collection of samples

A total number of 385 animal skin samples (n) was screened by Fisher's formula {$n= Z^2 \times p (1-p)/d^2$} using a confidence level (Z) of 95% (1.96), standard deviation (p) of 0.5, and margin error (d) of 0.05 for the unknown population as adopted by Sin-Ho (2013). Out of the total 385 slaughtered animal skins screened, 53 were found to show evidence of hair loss. So, a sample size of 50 was used for the study. Therefore, fifty lesion swabs and hair samples were collected from the fur surface of animal hides and skin with multifocal alopecic patches (Plate 1) presentation from Kano Central Abattoir by purposive sampling (Salman, 2009).

Kano Central Abattoir was chosen as the study area because of the many animals slaughtered daily, including animals raised and sold at Kano and those brought from neighbouring states. The samples were collected from slaughtered sheep and goat skin by scrapping the fur surfaces at the alopecia advancing edge using a blunt scalpel. The hair sample was collected using a pair of tweezers to pluck the hair and transported immediately to the Multi-user Laboratory, Bayero University, Kano, for processing.

Plate 1: Slaughtered animal skin with evidence of alopecia

Laboratory analysis and Identification of the Isolated Dermatophytes

Direct microscopic (M3100, Gogenlab, USA) examination (at magnification ×40) of the scrapping placed on a microscope slide was carried out by processing the sample with two drops of 20% potassium hydroxide (KOH) (Spectrum®, P1315, California, USA) in order to dissolve the cellular matrix of the hair sample. The sample was then treated with Lactophenol in cotton blue (Sigma-Aldrich®, 61335, USA) and covered with a coverslip following the methods of Mackie and McCartney (2023) to recover the type of mycelia, hyphal arrangement and the nature of spores.

Mycelial portion of each isolated fungi was inoculated onto Sabouraud's Dextrose agar (SDA) (Difco™ Cat No. 287710, UK), which was prepared using manufacturer's specifications incorporated with cycloheximide (Sirmaxo Chemicals PVT, India) at 0.5 mg/mL and chloramphenicol (Oxoid™, CT0013B, UK) at 16µg/mL to inhibit growth of unwanted fungal species and bacterial contaminants. The fungal colonies were observed and recorded after incubation at 37°C for two weeks. The isolated dermatophyte species were identified by microscopic morphology and *in-vitro* colonial morphology, where features such as texture, colour, shape and reverse pigmentations were observed as adopted by Dalis *et al*. (2019). The isolates

were confirmed by comparing their cultural morphology and microscopic characteristics with standard reference mycological Atlas (Robert & Pihet, 2008).

Enumeration of Fungal Load

The colonies developed were counted using hemacytometry by microscopic enumeration with a cell-counting hematocytometer (Neubauer chamber; Merck, S.A., Madrid, Spain). Here, the suspension of the isolated fungi was prepared, and then the hemocytometer was placed under a microscope under 10× objective to facilitate grid localisation. The spores were allowed to settle for 2 minutes in the chamber before counting by focusing on a large square consisting of 16 small squares at each corner. The number of spores on each large square was counted using a hand tally counter. The spores within each small square were counted and positioned on the right-hand or bottom boundary line while skipping the ones on the left border and upper boundary lines. The number of spores counted was used to determine the fungal spore count using the equation: Spores per mL of suspension = average spore count per large square × 10^4, as adopted by Umar *et al.* (2016).

RESULTS AND DISCUSSIONS

Table 1: Prevalence of dermatophytes associated with slaughtered animal skin

Fungal species isolated	Frequency	Prevalence (%)
Microsporum nanum	1	2
Trichophyton verrucosum	1	2
Trichophyton rubrum	1	2
Trichophyton mentagrophytes	1	2
Non-dermatophytes and absence of growth	46	92%
Total prevalence of dermatophytes	4	8%
TOTAL	50	100%

Table 1 shows the prevalence of dermatophytosis associated with slaughtered animal skin. An overall prevalence of 8% was recorded, with each of the four fungal isolates recording a prevalence

of 2%. The prevalence of 8% concurs with other studies, such as those of Dalis *et al*. (2019), who reported an 11% prevalence of dermatophytosis in Plateau state, Nigeria, and Nweze (2011), who reported a 12% prevalence in Enugu, Anambra, Ebonyi, Abia and Delta States, Nigeria. However, slightly lower rates ranging from 4.5% to 8% have been reported in Perugia, Italy (Moretti *et al*., 1998), while a much higher prevalence of 30.6% in Jordan was recorded by Al-Ani *et al*. (2002).

The low prevalence in Perugia, Italy, may result from proper shelter and management of domestic animals, which is optimal compared to that of Kano state, Nigeria (Righi *et al.*, 2019). Whereas high prevalence in Jordan may be due to weather differences between the two study areas, the aridity index and humidity in Jordan is higher than that of Kano state (De Pauw *et al.*, 2015; Calantoni *et al.*, 2015), thereby resulting in high rainfall allowing dermatophytes to grow optimally at Jordan region, since the fungi require warm, moist and humid environment to grow (Sudan *et al*., 2013), rather than dry temperate region like Nigeria. However, the high prevalence recorded in Jordan may be attributed to the fact that the prevalence of dermatophytosis was analysed in living animals; as such, the fungi may likely colonise living hosts rather than hides and skin of slaughtered animals since dermatophytes are pathogenic fungi rather than saprophytes.

Table 2: Fungal count of dermatophytes associated with slaughtered animal skin

Samples	**Fungi Isolated**	**Fungal count ($\times 10^5$ spore/mL)**	**Remark based on the infective limit ($\geq 3 \times 10^5$ spores/ml)**
A4	*Trichophyton rubrum*	3.5	Slight infectious
A7	*Trichophyton verrucosum*	4.3	Moderate infectious
A19	*Trichophyton mentagrophytes*	3.0	Non infectious
A32	*Microsporum nanum*	3.8	Moderate infectious

Table 2 depicts the fungal count of dermatophytes associated with slaughtered animal skin. The fungal load ranges from 3.0 $\times 10^5$ to 4.3 $\times 10^5$ spores/mL, with *Trichophyton mentagrophytes* recording the lowest count, which is within the safe limit, and *Trichophyton verrucosum* recording the highest count, which exceeds the standard infective limit.

The highest fungal count beyond the infective limit ($\geq 3 \times 10^5$ spores/ml) was recorded by sample A7, where *Trichophyton verrucosum* was isolated. This conforms with the findings of Dalis *et al*. (2019), who reported dominance ($P<0.05$) of *Trichophyton verrucosum* over other zoophilic dermatophytes that are parasitising animal hairs or wool capable of causing alopecia. It is also in agreement with the report of Shams-Ghahfarokhi *et al*. (2009), Swai and Sanka (2012), and Agnetti *et al*. (2014) that *T. verrucosum* is the most common cause of ringworm in farm animals, with *T. verrucosum* recorded the prevalence of 88.3% 71.7% and 98.9% respectively.

The least fungal count of 3.0×10^5 spores/ml was recorded in sample A19, where *Trichophyton mentagrophytes* were isolated. This is in agreement with Dalis *et al*. (2019), who reported that *Trichophyton verrucosum* and *T. mentagrophytes* are often isolated and identified from skin lesions of cattle and other domestic animals with *Trichophyton verrucosum* occurring more frequently (54.2%) than *T. mentagrophytes* (45.8%).

Table 3: Microscopic and cultural characterisation of dermatophytes associated with slaughtered animal skin

Cultural and morphological characteristics	Suspected Organism
Flat, creamy colonies with powdery to suede-like surface and pinkish brown reverse pigment. Microscopically, it appears with numerous sub-spherical microconidia.	*Trichophyton mentagrophytes*
White, fluffy, and downy colonies with yellow-brown reverse pigmentation. Microscopically, it appears with scanty numbers of slender microconidia.	*Trichophyton rubrum*
White, grey colonies with a velvety appearance and heaped, smaller colonies with white reverse pigmentation. Microscopically, it appears with long, thin, smooth-walled macroconidia and chlamydospore chains.	*Trichophyton verrucosum*
White, fluffy centre with golden yellow border colonies and closely spaced radial grooves, with yellow reverse pigmentation. Microscopically, it appears with few microconidia that form along round hyphae.	*Microsporum nanum*

Table 3 shows the microscopic and cultural characterisation of dermatophytes associated with slaughtered animal skin. The isolated dermatophytes include *Trichophyton mentagrophytes*,

Trichophyton rubrum, *Trichophyton verrucosum* and *Microsporum nanum* by comparing the features of microscopy and culture obtained with standard guidelines of mycological atlas.

This finding agrees with previous studies by Ashwathanarayana and Naika (2016), who documented that geophilic and zoophilic dermatophytes are associated with mycosis in living ruminant animals raised for domestic farming. *Trichophyton verrucosum, Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum nanum, Microsporum canis* and *Epidermophyton floccosum* are the primary culprits in the causation of dermatophytosis among livestock and other domesticated animals.

Table 4: Prevalence of dermatophytosis on slaughtered animal skin in relation to the animal breed

Type of hide/skin	No. of samples collected	Frequency of dermatophytosis (%)	Prevalence (%)
Sheepskin	25	3 (75%)	6%
Goat skin	25	1 (25%)	2%
TOTAL	50	4	8%

The highest prevalence of dermatophytosis was found in slaughtered sheep skin compared to goats. This finding agreed with Dalis *et al*. (2009), who reported a high prevalence of dermatophytosis in the sequence of cattle, sheep, and goats. This may be due to the physiological differences between sheep and goats, where sheep wool is aerially arranged, giving more porosity that allows microbial invasion.

In contrast, goat skin is arranged axially, protecting it from microbial colonisation. The overall prevalence of dermatophytosis was 8%, though the samples analysed were collected from alopecic animal skin. Probably, the alopecia on the slaughtered animal skin must have been caused by other underlying medical conditions, parasitic disease, trauma and nutrient deficiency.

CONCLUSION

The overall prevalence of 8% was obtained from the study area, where dermatophytes of species *Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum nanum* and *Trichophyton verrucosum* were isolated. *Trichophyton verrucosum* recorded the highest fungal loads of 4.3 ×

10^5 spores/mL, which is beyond the average fungal counts, hence signifies its infectious potential. Trichophyton and Microsporum genera species were found to be common fungal species associated with slaughtered animal skin in the study area. The fungal load was found to be more pronounced in slaughtered sheep's skin (75%) compared to those of slaughtered goat skin (25%). However, the prevalence of dermatophytosis in slaughtered animal skin is relatively low in the study area compared to other areas, and the alopecia observed on the animals may be caused by other veterinary medical conditions such as parasites, cannibalism, trauma and nutrient deficiency.

The following recommendations are therefore made:

1. People engaged in animal husbandry should be enlightened by animal scientists and veterinarians on the need to treat any skin disorder on live animals using commercially available antifungal agents such as miconazole, ketoconazole, clotrimazole and itraconazole in order to improve the quality of the animals' skin.
2. Animals suspected of alopecia should be isolated and quarantined from healthy ones, and those with skin-related diseases should be treated appropriately before butchered.
3. Further studies should be conducted to ascertain the level of damage caused by dermatophytes on finished leather to compare the quality, tensile strength and malleability of healthy and alopecic animal hides.

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