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Epidemiology, Health Effects and Treatment of Cutaneous Mycoses of Goat and Sheep from Some Eastern States of Nigeria

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Abstract A total of 4860 animals were screened 2570(52.88%) were goats and 2290(47.12%) were sheep. The fungi associated with the infections were identified. Of the 2570 and 2290 of goats and sheep, 80(3.11%) and 144(6.29%) had fungal lesions respectively. Fifty soil samples from the environment were collected for fungal analysis and 31 nomads were checked for fungal lesions. Antifungal biogram and animal pathogenicity studies were also done. Prevalence of fungal infections was higher on the animals from farms than those at the markets. Infection was more prevalent in animals between 13-24 months of age. The glaborous skin was mostly affected (37.5%) in the goats, while in the sheep, the face was affected most (62.5%). Fungi recovered from the animals included *Trichophyton verrucosum* (19.64%), *Trichophyton mentagropytes*,(20.54%), *Microsporum gypsum* (5.80%), *Sporothrix schenckii* (20.98%), *Candida albicans* (7.59%), *Fusarium solanii* (5.36%), *Geotricum candidum* (3.13%) *and Aspergillus species* (16.96%). Almost the same types of fungi were isolated from the nomads and the soil. These parameters when compared statistically using ANOVA was not significant, $P > 0.05$. Pathogenecity studies of the isolates on laboratory mice revealed that *T.mentagrophytes* and *T.verrucosum* were highly virulent. The antifungal biogram test showed the fungal isolates to be more sensitive to Fluconazole than Ketoconazole, Miconazole and Grisofulvin. Fungal skin infections are communicable diseases and poor sanitary conditions promotes there spread but if proper sanitary measures are taken, the infections may be eradicated.

Keywords: *cutaneous mycoses, dermatophytes, goats, sheep, nomads, soil, antifungal drugs*

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1. Introduction

Goat and Sheep are domestic animals which are of great importance in the growth of Nigeria's economy. These animals do not only serve as source of food, but also as sources of income, hides and skin, manure for agriculture and for social or recreational purposes. These animals are exposed to fungal infections that affect the keratinized layers of their skin and appendages. These infections are mostly caused by dermatophytes.

Dermatophytes are filamentous fungi which have the ability to invade the epidermis and keratinized structures such as hair, nails and cutaneous surfaces [1]. They comprise of three genera: *Trichophyton*, *Epidermophyton* and *Microsporum*. Dermatophytes cause infections of the skin like club fungus, athletes foot, tinea infection of some keratinized surfaces and ringworm of the hair and nails. The organisms colonize the keratinized tissues and inflammation is caused by host response to metabolic by-products [2,3]. The infection is usually restricted to the living cornified layer of the epidermis because of their inability to penetrate viable tissue of an immune competent host. Fungal invasion elicits host response ranging from mild to severe lesions. Acid proteinases, elastase, keratinases in the fungus reportedly act as virulence factors.

When the dermatophyte infection is said to be zoophilic if it is of animal origin on is said to be zoophilic dermatophytoses [3]. Animal handlers and nomads are at higher risk of zoophilic dermatophytoses because they are in regular contact with the animals [4]. Similarly, because the animals usually make contact with soil, they are at risk of geophilic dermatophytoses which are fungal infection caused by keratinophilic fungi typically developing in the soil [5]. Zoophilic and geophilic dermatophytoses are enhanced by unhygienic practices of the nomads both on the animal and the environment. Regular contact with wet and dirty surfaces, low temperature, humid environment, crowded living and poor sanitary condition of the animals in the pens are some of the factors that influence the development and spread of fungal skin infection [6,7]. The infection is usually asymptomatic this makes it easier for the rapid spread of the infection [8,9]. The nomads can infect or be infected by the animals especially during

milking which is usually done manually. The asymptomatic carriage of the fungal infection by domesticated animals pose the greatest problem for fungal eradication and prevention [9].

Fungal organisms have ergosterol as an active component of their plasma membrane. The ergosterol synthesis is inhibited by azoles antifungals by inhibiting the cytochrome P-450 –dependent enzyme lanosterol demethylase [10], but sometimes this enzyme is overproduced or the drug target is altered so that the drug cannot bind to the target thereby causing resistance [11]. This makes the treatment of dermatophyte infection difficult, hence the aim of the work, to isolate the fungi causing the cutaneuos fungal disease, to find the best drug of choice for it's treatment and suggest a better way to control the spread or even eradicate the infection.

2. Materials and Methods

2.1. Sample Location

Ethical clearance was got from the Ministry of Veterinary Services Enugu State and Anambra State. Goats and Sheep from New Artisan animal market Enugu, Animal market Amansea Awka, Ugwuoba cattle market Enugu-Onitsha Expressway, Ibeagwa-Nike animal farm Enugu and Akwuke Fulani animal farm Enugu were screened for skin fungal infection. The environmental temperatures ranged from 21-26°C and humidity 60-90%.

2.2. Sample Size and Collection

Evaluation of some clinical signs and symptoms like itching, scaling, ulceration and redness of skin were done on the subjects. Questionnaires were used to obtain information on age, sex, history of any previous skin infection, date of the first signs and symptom and the medication used if any. Out of a population of 4,860 goat and sheep sampled, scrapings were taken from 224 animals which had skin lesions suggestive of fungal etiology, 144 were sheep and 80 were goats. The area was cleaned with 70% alcohol. Samples were placed in paper pockets. Thirty-one scrapings were got from Nomads with fungal skin lesions. Fifty soil samples from the animal environment were also collected into sterile container for analysis.

2.3. Processing of Samples

A portion of each specimen was examined in 20% KOH mount and the remaining portions were inoculated onto slopes of Sabouraud dextrose agar + Chloramphenicol (0.5mg/dl) (S+C), Sabouraud dextrose agar + Chloramphenicol (0.5mg/dl) + Cyclohexamide (0.5mg/dl) (S+C+A) and unto Casein basal medium + thiamin according to known standard procedures [12]. Isolates were identified on the basis of a detailed study of their gross and microscopic morphology and by comparison with standard descriptions [13]. Hair penetration test [14] was used for identification of *T. Mentagrophytes*. Germ tube test according to [15] was used for identification of *Candida albicans*. Slide cultures according to [16] were also done for identification of vegetative forms of the isolates. One way analysis of variance (ANOVA) was used to determine the degree of significance of the different isolates in relation to the sample studied.

Pathogenecity test was done on 30 laboratory animals by making a solution of the fungal isolates and rubbing it on the skin of the albino wister mice to check if the fungi were able to elicit same type of fungal infection as seen in the test animals. The fungal lesion on the mice was scrapped and cultured to know if the causative fungi would be identified as same.

Antifungal biogram was done using four antifungal drugs which were serially diluted. Two tablets of the 200mg Fluconazole, Miconazole and Ketoconazole each were dissolved in 1ml of sterile water to make a concentration of 400mg/ml. Two tablets of the 500mg Grisofulvin tablets were dissolved in 2.5mls of sterile water to make a 400mg/ml concentration. The stock was further diluted down to (200,100 and 50) mg/ml and a sensitivity testing of the isolated fungi was done in duplicate using the bore hole method by(15) after 24 hrs incubation, the zones of inhibition diameter were read and the mean value for the two readings was noted.

3. Results

Of the 4,860 animals screened for skin lesions 224 (4.60%) had cutaneous fungal lesions. The infection was more prevalent on animals from Ibeagwa-nike cattle farm Enugu (8.0%) and lowest on animals from New Artisan cattle market Enugu(3.73%). as shown in (Table 1).

Table 1. Total population of all animals from different animal markets and farms including animals with lesions

Area/Location	Animal type	Total population	Population with lesion
New Artisan cattle	Sheep	500	34
market Enugu	Goat	1000	22
	Total	**1500**	**56(3.73%)**
Anambra Animals	Sheep	450	21
market Amansea Awka	Goat	370	15
	Total	820	36(4.39%)
Ugwuoba cattle	Sheep	420	25
market Enugu/AnambraExpressway	Goat	680	21
	Total	1,100	46(4.18%)
Akwuke Fulani animal	Sheep	520	32
farm Enugu	Goat	520	22
	Total	1,040	54(5.19%)
Ibeagwa Nike	Sheep	400	32
animal farm Enugu	**Total**	**400**	**32(8.0%)**
		4,860	**224(4.60%)**

The fungi isolated from the animals included *Trichophyton verrucosum* 19.64% (n = 44), *Trichophyton mentagropytes* 20.54%,(n = 46), *Microsporum gypsum* 5.80% (n = 13), *Sporothrix schenckii* 20.98% (n = 47), *Candida albicans* 7.59% (n = 17), *Fusarium solanii* 5.36% (n = 12), *Geotricum candidum* 3.13% (n = 7) *and Aspergillus species* 16.96% (n = 38). Same type of fungi listed above and *Trichophyton megninii* were isolated from soil samples but in higher percentage while the nomads samples had fewer numbers of fungal organisms which included *Trichophyton soudenensea* as shown in (Table 2). Animals within the age range of 13-24 months were mostly affected (54.43%). The female animals were mostly affected 51.15% than the males 48.85%. One way analysis of variance was used to determine the level of significance of the different fungal isolate with respect to the different sample studied. Statistically, their mean deference was not significant. P > 0.05.

Table 2. Frequency of occurrences of fungal isolates on samples

Isolates	**Soil** (50 samples)	**Goats** (80 samples)	**Sheep** (144 samples)	**Nomads** (31 samples)
T. verrucosum	**8** (16%)	**11** (13.8%)	**33** (22.9%)	**3** (9.6%)
Sporothrix schenckii	**2** (4%)	**20** (25.0%)	**27** (18.8%)	**0**
T. mentagrophytes	**11** (22%)	**16** (20%)	**30** (20.8%)	**9** (29.0%)
Microsporum gypsum	**1** (2%)	**0**	**13** (9.0%)	**0**
Fusarium solanii	**6** (12%)	**7** (8.8%)	**5** (3.5%)	**5** (15.7%)
Candida albicans	**7** (14%)	**8** (10.0%)	**9** (6.3%)	**9** (29.0%)
Geotricum candidum	**4** (8%)	**3** (3.8%)	**4** (2.8%)	**0**
Aspergillus species	**7** (14%)	**15** (18.8%)	**23** (15.9%)	**2** (6.1%)
T. megninii	**4** (8%)	**0**	**0**	**1** (3.2%)
T.soudenensea	**0**	**0**	**0**	**2** (6.1%)
Total/percentage number of positive samples	**50 (100%)**	**80(100%)**	**144(100%)**	**31(100%)**

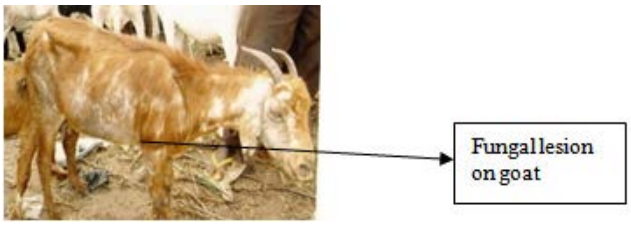

Figure 1. Fungal infection on the glaborous skin of goat

Figure 2. Fungal infection on the face of a sheep

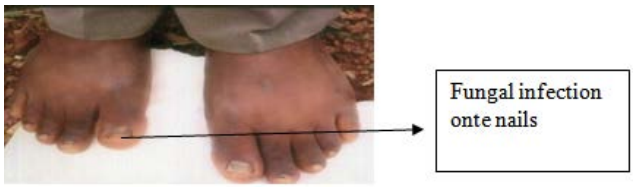

Figure 3. The toe nails of the nomad infected with fungi

The fungal infections were heavy on the glaborous skin of goats and on the face of sheep these are shown in (Figure 1) and (Figure 2). The toe nails of the nomads were mostly infected as shown in (Figure 3).

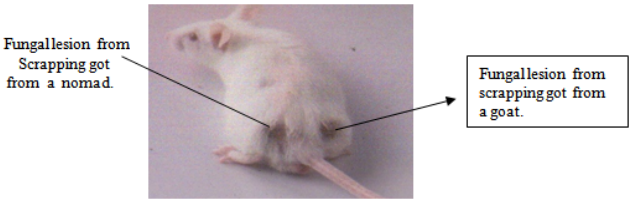

Figure 4. The toe nails of the nomads infected with fungi

Trichophyton mentagrophytes isolated from one of the goats and one of the nomads elicited the same type of fungal lesion on the albino wister mice on the 18th day and 21st after inoculation respectively, this is shown in (Figure 4). Other fungi did elicit lesion on test mice. *Aspergillus fumigatus* was recovered from the lung culture of one of the dead test mice.

The mean value of the zone of inhibition diameter of different antifungals were as shown in Table 3. Fluconazole tablet at the concentration 200mg/tablet gave the highest zone of inhibition diameter with the diameter ranging from 9-21mm for the various fungi. While Ketoconazole and Miconazole at the same concentration gave inhibition diameters ranging from 7-15mm and 5-12mm respectively for the fungal isolate. However, Grisofulvin at the same concentration, did not inhibit the growth of the isolates.

Table 3.The mean value of the zone of inhibition diameter of different antifungals on isolates (recorded in mm)

	Fluconacole 200mg	Ketoconazole 200mg	Miconazole 200mg	Grisfulvin 200mg
T. Mentagrophytes	**4**	**8**	**6**	**0**
T. verrucosum	**6**	**5**	**7**	**0**
T. soudanense	**7**	**7**	**4**	**0**
T. Megninii	**3**	**3**	**2**	**1**
Sporotrix schenckii	**9**	**8**	**5**	**1**
Fusarium solanii	**10**	**11**	**10**	**0**
Candida albican	**15**	**16**	**9**	**1**
Geotricum candidium	**13**	**19**	**9**	**0**
Aspergillus Spp	**6**	**4**	**6**	**0**

4. Discussion

This study has shown public health importance of cutaneous fungal infection of goats and sheep in animal markets and farms. The etiologic agents recovered from this study supported to what was earlier reported by MacKenzie *et al* [17]. The study revealed a high prevalence of a dermatophyte T.*verrucosum* and *T.mentagrophyte* in the animals as compared to other dermatophytes. This partly agrees with earlier work done [18] which isolated only *T. verrucosum.*

This work showed that fungal skin infection was more prevalent with animals in the farms than those in the markets. The reasons for this could be that the animals in farms most often move from one bush part to another in search of pasture, hence exposing themselves to different environmental hazards like cuts on their skin, these skin aberration often becomes an easy entrance for fungal spores [19]. Another reason might be that animals usually have regular health checks by veterinary doctors before they are taken to the market.

Zoophilic dermatophytes *T.verrucosum* and *T.mentagrophytes* were isolated from soil samples collected from the farms and markets. This further confirmed the role soil plays as a reservoir of the fungi and as a medium for the spread of fungal infections to both humans and animals [18], because these fungi were also heavy in the animals.

It has been established that environmental factors like pH, temperature and humidity enhance the existence and spread of fungal infection [20,21]. The farms where the animals were kept were hot and humid thereby supporting the fungal growth, propagation and transmission.

Female animals were more infected than males probably due to the fact that gestation lowered their immunity and level of fungistatic fatty acids. It has been established that insufficient fatty acids predisposes animals to fungal and other opportunistic infections [22].

In Nigeria and other developing countries, milking of animals are done manually with hands by uninformed nomads under unhygienic conditions. These hands could have been the source of fungal contamination or the soil where the animal lay often. High contamination of milk sample by Candida species from soil was recorded in work done in southern Iran [23].

The heavy infection glabrous skins of the goats were heavily infected. This could be as a result of large surface area of the skin which was making contact with infected soil, equipment and animals. This confirms the role of contaminated environmental in spreading skin fungal infections [24].

The younger animals aged 13 to 24 months had higher prevalence of infection than older ones. This may be as a result of their immune status still developing because according to [25] immunity grows with age.

The positive results got from the pathogenicity test of *T.mentagropytes* was an evidence of the infectivity of these pathogens. The isolation of *Aspergillus fumigates* from the culture of internal organs of the dead test rats after some days is in line with an earlier reported work [26,27] which reported that *Aspergillus fumigatus* can cause kidney disfunction and death in test laboratory animal.

The antifungal biogram showed that fluconazole tablets had the highest inhibitory property against the isolates. The zones of inhibition diameter of the conventional drugs tested were generally higher than what was previous reported [28]. This could be as a result of the emergence of new drugs whose efficacy might be stronger than that used by Oyeka and Gugnani [28] at their time of study. Generally, the zone of inhibition diameter decreases as the concentration of the drug decreases, it could be said that the zone of inhibition diameter was directly propotional to the concentration of the drug.

Fungal organisms have ergosterol which an active component of their cell wall [12]. Fluconazole, Ketoconazole and Miconazole which are azoles antifungals, were ergosterol biosynthesis inhibitors, while Grisofulvin was not, hence explaining the reason for its inability to inhibit the growth of any of the dermatophytes. This azole antifungals have been reported to have an adverse effect on the renal tubules after a long term use, it was therefore advisable not to use them for a long time.

In conclusion, mycotic public health hazards could be got from contact with the environment (soil) and environmental conditions (humidity and temperature) played major role in it's propagation. These hazardous conditions could be averted if proper hygienic measures were taken in the animal farms.

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