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ORIGINAL ARTICLE

Mixed rearing correlates with the existence of *Trichophyton verrucosum* pathogens in humans

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ABSTRACT

Background/Objective: *Trichophyton verrucosum* is a serious zoophilic dermatophyte causing dermatophytic infections and skin lesions in humans and animals. Raising small ruminants beside cattle is a common practice in rural areas of Egypt. Therefore, the current study emphasizes the risk of *T. verrucosum* spreading through backyard farming, recognizing the interconnectedness of ruminants and humans.

Methods: A total of 478 cattle, 215 sheep, 186 goats, and 250 human contacts were investigated for the presence of skin lesions, and then the lesions were sampled. All samples were examined by fungal cultures using Sabouraud dextrose agar.

Results: The highest rate of *T. verrucosum* isolates was recorded in cattle that grew up along with sheep and goats (14.5%), whereas the lowest rate was observed in cattle reared as a single species (6.6%), with a significant difference of $p = 0.04$, odds ratio = 2.42 at a 95% confidence interval: 1.03–5.65. In addition, there was a borderline significant difference of $p = 0.05$ between the presence of *T. verrucosum* pathogens in humans in contact with combined species of cattle, sheep, and goats (13.8%) and those in humans in contact with cattle only (3.3%); the odds ratio was 4.66 at a 95% confidence interval: 1.00–22.53. Moreover, the highest rate of *T. verrucosum* isolates in cattle was recorded in young cattle (calves). Tinea barbae was found in 11 human cases, while tinea corporis was determined in seven cases.

Conclusion: Rearing different species of small ruminants with cattle supports the spread of *T. verrucosum* pathogens. Tinea barbae caused by *T. verrucosum* was predominant in the examined humans.

Introduction

Dermatophytosis is a prevalent zoonotic disease spread worldwide, especially in developing countries.[1,2] *Trichophyton verrucosum* is a dermatophyte affecting the skin tissues, including keratin of ruminants.[3,4] Although *T. verrucosum* is adapted to cattle, there is a potential transmission to neighboring sheep and goats that are reared in the same yards.[3,5] *T. verrucosum* infection is considered an occupational hazard for humans in rural areas, and currently, *T. verrucosum* is the most common etiological factor for tinea barbae.[6] This disease is extremely inflammatory and involves the exposed areas of the human body.[7] Other fungal infectious diseases

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caused by *T. verrucosum* have been reported in Egypt; three human cases of tinea corporis caused by *T. verrucosum* were recorded in Ismailia and Port Said Provinces.[8] In addition, *T. verrucosum* was detected in samples collected from the toe and finger nails of students in preparatory schools in Sohag Governorate, Egypt.[9] In Central Italy, Agnetti et al[10] isolated *T. verrucosum* from 14 herdsmen with skin lesions who were in close contact with cattle. In Sfax-Tunisia, *T. verrucosum* was isolated from 178 patients, and the pathogen is in recrudescence in this region.[11] Control strategies should be applied to protect humans against *T. verrucosum* infection who are in contact with ruminants, especially low socioeconomic populations.[8,10,11] Limited data concerning the zoonotic pattern of this pathogen are available, especially in Egypt.

Therefore, the current study aimed to assess the risk of human exposure to *T. verrucosum* pathogens in backyard farming, where mixed species of ruminants are reared, as a novel approach to control zoonotic diseases.

Materials and methods

Study design

This study was conducted during the period from November 2011 to December 2012 in Gharbia Governorate in the Nile Delta region of Egypt. The study population comprised backyard cattle, sheep, goats, and human contacts. The examined villages and backyards were selected based on convenience. All animals were investigated for the presence of skin injuries. The lesions were sampled and prepared for fungal culture. The date of sampling, location, and the species, age, and sex of the animals were recorded. Humans in close contact with these animals were suspected to contract the pathogen. The suspected humans were investigated for the presence of skin lesions, and then the lesions were sampled by a physician. The date of sampling, location, contact species, and the age and sex of the humans were recorded. All samples were transported to Elsalam laboratory, Tanta, Egypt, and to the Faculty of Veterinary Medicine—Kafrelsheikh University laboratory for further identification of *T. verrucosum*. Human sampling procedures were approved by a committee from the Ministry of Health and Population—Gharbia Directorate, for institutional care and concern, and the sampling of ruminants was approved by a committee from the Faculty of Veterinary Medicine—Kafrelsheikh University for institutional animal care and use.

Animal sampling and laboratory procedures

A pair of sterile forceps and a scalpel were used to collect skin lesions by scraping the margin of the affected skin. Dull broken hairs were removed from the margin of the lesion using sterile tweezers. Each sample was placed in a separate sterile plastic container and transported to the laboratory for fungal investigation within 24 hours. The samples were examined microscopically under a light microscope at 400× magnification after adding 10% Potassium hydroxide (KOH) and incubation at 37°C for 30 minutes in a humidity chamber to detect the hyphae and arthroconidia. Each sample was cultured on Sabouraud dextrose agar supplemented with chloramphenicol 0.05 g/L and cycloheximide 0.5 g/L (Mycobios Selective Medium; Oxoid Ltd., Basingstoke, Hampshire, United Kingdom). Each medium was supplemented with inositol and thiamine to enhance the growth of *T. verrucosum* isolates, and then incubated at 37°C for 2–4 weeks and examined every 2–3 days for colony formation. On Sabouraud dextrose agar, colonies were slow growing, small, button or disk shaped, white to cream colored, with a suede-like to velvety surface, a raised center, and a flat periphery with some submerged growth. Characteristic chains of chlamydoconidia were identified, and terminal vesicles at the tips of hyphae were observed (chains of pearls).[7,12] The characteristics of *T. verrucosum* that were observed in the KOH mount examination were chains of chlamydoconidia and antler-like hyphae. Culture characteristics and microscopic appearance are the gold standard diagnostic tools for such fungal occurrence.[13]

Human sampling and laboratory procedures

The humans who were in close contact with animals were examined by a specialized physician following their consent. The body of each human was examined for crusting, scaling, follicular inflammation, erythema, or hair loss. A sterile scalpel was used to collect skin scrapings from body lesions. Hair roots were pulled out using sterile tweezers. All samples were transported to the laboratory in sterile containers within 24 hours. *T. verrucosum* was identified as previously discussed.

Ruminant and human samples were considered positive when *T. verrucosum* isolates could be detected by KOH examinations and/or fungal cultures.

Statistical analysis

Univariate categorized analysis was used to calculate odds ratios to assess the risk of cattle and human exposure to *T. verrucosum* via different farming groups of ruminants. The effects of age and sex on the presence of *T. verrucosum* in ruminant groups and humans were also assessed. Statistical analysis was performed using MedCalc-version 12.6.0 statistical software (MedCalc Software bvba, Maria Kerke, Belgium).

Results

The *T. verrucosum* vaccine was not used for the examined animals and humans. *T. verrucosum* was the only dermatophyte isolated from the ruminants and humans in this study.

A total of 478 cattle, 215 sheep, and 186 goats were investigated for the presence of *T. verrucosum* isolates. Combining the results of KOH examinations and fungal cultures, 44 (9.2%), 12 (5.6%), and seven (3.8%) of the examined cattle, sheep, and goats were positive, respectively. Two cattle samples and one sheep sample were KOH positive but negative for the culture, while one cattle sample was positive for the culture and KOH negative.

The highest occurrence rate of *T. verrucosum* in cattle was recorded in cattle that grew up with sheep and goats (14.5%), whereas the lowest rate was recorded in cattle reared separately (6.6%). The cattle that were raised with affected sheep or goats also showed positive mycological results (Table 1).

A comparison of the biodiverse groups of ruminants revealed a significant difference at $p = 0.04$ in the occurrence rates of *T. verrucosum* between cattle raised with sheep and goats and those raised separately (Table 2).

Regarding the age of the positive cattle, the highest occurrence rate was observed in those younger than 12 months (calves) in all biodiverse groups. There was a borderline significant difference at $p = 0.05$ between the positivity of *T. verrucosum* in calves <12

Table 1 Presence of *Trichophyton verrucosum* in ruminants.

Raised species	No. of positive ruminants/total		
	Cattle	Sheep	Goats
Cattle	9/137 (6.6%)	—	—
Cattle with sheep	10/109 (9.2%)	6/112 (5.3%)	—
Cattle with goats	8/115 (6.9%)	—	3/89 (3.4%)
Cattle with sheep and goats	17/117 (14.5%)	6/103 (5.8%)	4/97 (4.1%)

Table 2 Risk of cattle exposure to *Trichophyton verrucosum*.

Raised species	*T. verrucosum* in cattle		
	OR	95% CI	*p*
Cattle only (baseline)[a]			
Cattle with sheep	1.44	0.56–3.67	0.45
Cattle with goats	1.06	0.40–2.85	0.90
Cattle with sheep and goats	2.42	1.03–5.65	0.04

CI = confidence interval; OR = odds ratio.
[a] Univariate categorized analysis was used to calculate the OR.

Table 4 Risk of human exposure to *Trichophyton verrucosum*.

Raised species	No. of positive humans/total	*T. verrucosum* in humans		
		OR	95% CI	*p*
Cattle only (baseline)[a]	2/60 (3.3%)			
Cattle with sheep	4/63 (6.3%)	1.97	0.35–11.15	0.44
Cattle with goats	3/62 (4.8%)	1.47	0.24–9.15	0.68
Cattle with sheep and goats	9/65 (13.8%)	4.66	1.00–22.53	0.05

CI = confidence interval; OR = odds ratio.
[a] Univariate categorized analysis was used to calculate the OR.

months old and that in cattle >18 months old that grew up with sheep. Furthermore, a clear significant difference at $p = 0.04$ was recorded between the same age groups when cattle were raised with sheep and goats together (Table 3).

In humans, 18 out of 250 (7.2%) samples tested positive for *T. verrucosum*; one case was KOH positive and culture negative.

Humans in contact with a combination of backyard ruminants such as cattle, sheep, and goats had a higher occurrence rate of *T. verrucosum* with a borderline significant difference at $p = 0.05$, compared with humans in contact with cattle that were reared separately (Table 4).

Eleven humans tested positive for tinea barbae, which was limited to the bearded areas of the face and neck with inflammatory deep plaques and noninflammatory patches. Only four cases of tinea barbae were unilateral. Another seven humans tested positive for tinea corporis, which affected the whole body (except the scalp, beard, and feet) as an annular plaque. No cases of tinea capitis or tinea pedis were recorded in the examined humans.

The positive humans were aged between 23 years and 57 years, and the positivity rate was 8% (13/163) in males and 5.7% (5/87) in females. In cattle, *T. verrucosum* was isolated from 9.9% (28/283) of males and 8.2% (16/195) of females. In addition, in sheep, the rate was 6.1% (8/132) in males and 4.8% (4/83) in females, whereas in goats, it was 4.1% (4/97) in females and 3.4% (3/89) in males. No significant difference was observed between the positivity rates of males and females of each species of the ruminants as well as humans.

Discussion

The current novel study was established to assess the zoonotic risk of humans' exposure to *T. verrucosum* during the rearing of mixed species of ruminants in backyard farming.

Table 3 Effect of cattle age on the presence of *Trichophyton verrucosum*.

Age of cattle (mo)	No. of positive cattle/total	*T. verrucosum* in cattle		
		OR	95% CI	*p*
Cattle				
<12 (baseline)[a]	4/27 (14.8%)			
12–18	3/58 (5.2%)	0.31	0.06–1.51	0.15
>18	2/52 (3.8%)	0.23	0.04–1.35	0.10
Cattle with sheep				
<12 (baseline)[a]	7/36 (19.4%)			
12–18	2/38 (5.3%)	0.23	0.04–1.19	0.08
>18	1/35 (2.8%)	0.12	1.00–1.05	0.05
Cattle with goats				
<12 (baseline)[a]	5/31 (16.1%)			
12–18	2/45 (4.4%)	0.24	0.04–1.34	0.10
>18	1/39 (2.6%)	0.14	0.01–1.24	0.08
Cattle with sheep and goats				
<12 (baseline)[a]	9/33 (27.3%)			
12–18	3/31 (9.7%)	0.29	0.07–1.18	0.08
>18	5/53 (9.4%)	0.28	0.08–0.92	0.04

CI = confidence interval; OR = odds ratio.
[a] Univariate categorized analysis was used to calculate the OR.

Most of the ruminants had annular skin lesions that were ignored by their owners or went unnoticed. *T. verrucosum* is considered a serious pathogen due to its ability to survive on the skin or hair of cattle and the wooden parts of hedges for 15–54 months.[14] During active infection, the behavior of the infected animals was changed; animals rubbed their legional areas against posts, gates, feed bunks, walls, and other neighboring animals. The fungal spores deposited on these objects or animals may remain infective for a long time. Consequently, the risk of occurrence increases not only among existing mixed-species animals, but also in animals new to the region and in exposed humans.[15]

In Egypt, it is common that small backyards hold different livestock species that are raised together to optimize the use of resources and maximize economic benefits. These mixed backyards usually contain cattle, sheep, and a smaller number of goats.[16] Rearing sheep and goats with cattle may also be considered a risk factor for the spreading of other diseases such as brucellosis.[17]

Our results showed that the occurrence of *T. verrucosum* pathogens was higher in cattle, followed by in sheep, and finally in goats. Accordingly, cattle could be considered as the main host for the circulation of *T. verrucosum*, playing an important role in the epidemiology of this dermatophytosis. Baxter and Rush-Munro[18] stated that *T. verrucosum* pathogens were isolated from sheep and goats. Whereas the presence of sheep and/or goats with cattle may enhance the transmission of this pathogen, current results indicated that sheep could be considered more important in spreading the pathogen compared to goats.

T. verrucosum is the main cause of ringworm in cattle worldwide and creates skin lesions in bovine species and humans.[15] This mycosis may circulate between different species of ruminants, increasing the risk of the disease in exposed animals and/or humans. However, cattle are still considered the main reservoir of *T. verrucosum*.

Furthermore, gathering of cattle with sheep and/or goats during rearing increases the threat of the occurrence of *T. verrucosum* in cattle significantly compared to the threat in cattle that were raised as a single species. In addition, inside mixed ruminant housing, the risk of contagion is increased due to backyard overcrowding and close contact with contaminated objects such as mangers and walls in addition to the diseased cases. In mixed backyards, small ruminants are set free and not tied, so they can move freely between the cattle and to different sections of the backyard due to their relatively small size. Furthermore, small ruminants chewing on feeds may provide the moisture that is needed to nurture the fungus and allow it to live in pens for prolonged periods. Also, the use of topping rams in breeding between different backyards may aid in the spreading of the disease.

The risk of *T. verrucosum* was also increased significantly in humans in contact with mixed species of cattle, sheep, and goats, in comparison with those in contact with cattle reared as a single species. *T. verrucosum* pathogens may be transmitted to healthy humans through contact with diseased animals, producing inflammatory lesions in the exposed areas of the skin.[15] This indicates

an important association between *T. verrucosum* occurrence in the examined cattle and humans which was affected by the presence of mixed species of small ruminants.

Our results indicated that the occurrence rate of *T. verrucosum* was increased in calves in all the examined groups. Aghamirian and Ghiasian[19] recorded a high proportion of dermatophytoses in calves younger than 12 months. The pH value of the skin reduces with age; young animals are at greater risk due to the high pH of their skin in addition to their weak immunity.[20] Calves are more sensitive to any stress and do not adapt well to environmental changes (heat stress and high humidity), prolonged transportation, and/or changes in diet.[21]

This study specified that sex was not a determining factor for the infection in the examined ruminants and humans.

In addition, the present study pointed out the presence of unilateral lesions of tinea barbae on one side of the face and/or neck of humans that might turn toward the flank of the animal during the milking process. This might be a potential zoonotic pathway of *T. verrucosum* pathogens during contact between cattle and humans. Sabota et al[22] found five cases of tinea barbae in human milkers due to *T. verrucosum*; all lesions were unilateral in the part of the face or neck that was rubbed against the cow during the course of milking.

The results of this study pointed out the absence of tinea capitis or tinea pedis in the examined humans. Inversely, a previous study showed that one tinea cruris, six tinea corporis, two tinea pedis, one tinea unguium, and four tinea capitis cases in humans involved in dairy activities were positive for *T. verrucosum*.[23] They isolated different dermatophyte species and strains from the examined patients, indicating that the reported occurrences of tinea capitis and tinea pedis may be due to the presence of mixed species and strains of tinea. In addition, the severity of the strain may affect the occurrence of a disease and its symptoms. *Trichophyton rubrum*, *Trichophyton mentagrophytes* var. *interdigitalis*, *Epidermophyton floccosum*, and *Trichophyton tonsurans* are the etiological pathogens responsible for tinea pedis.[4] Therefore, *T. verrucosum* is not a common causative agent for tinea pedis. In addition, tinea capitis occurred only in children of school age, whereas adults and the elderly were rarely infected.[22] Humans that tested positive were adults and elderly people, who were involved in rearing ruminants. Our results indicated a higher positivity of *T. verrucosum* pathogens in human males than in human females. In addition, in Australia, *T. verrucosum* was isolated from cases of tinea corporis, tinea faciei, and tinea capitis (17 males and 9 females).[15]

The isolation of *T. verrucosum* pathogens from ruminants and humans in the current study does not indicate the occurrence of infection, but it still a potential risk factor or correlation to the infection and disease.

Finally, veterinary services, and public health and environmental authorities must encourage the application of new biosecurity measures to overcome this zoonotic problem. Isolation of animals with active lesions, use of separate grooming equipment and feeding utensils, proper cleaning and disinfection of backyards, and placing of newly purchased animals in quarantine for up to 6 weeks may be considered as suitable biosecurity measures. The animal reservoir must be reduced using *T. verrucosum* vaccine, and applying preventive and hygienic measures.

Conclusion

This study indicated a higher occurrence rate of *T. verrucosum* in cattle and humans in contact with mixed farming species. Rearing mixed ruminant species in the same backyard must be restricted as an important biosecurity measure. Tinea barbae caused by *T. verrucosum* predominated in the examined human cases.

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