



Prevalence and some risk factors with therapeutic trial of sheep dermatophytosis in Egypt

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Abstract

Dermatophytosis is a fungal disease that affects the superficial skin layers and hair of farm animals all over the world including Egypt. Despite being a self-limiting disease, it has serious effects on public health and devastating economic losses due to its serious skin damage, a long course of treatment, and loss of weight. This study determines the most prevalent species of dermatophyte in sheep and identifies the incriminated species by both microscopic and culture methods with an assessment of animal and environmental risk factors. Moreover, it evaluates the effectiveness of three antifungal compounds (tioconazole cream and clotrimazole spray, and fluconazole capsule), on twenty-four naturally infected sheep. One hundred and three sheep from Sharkia and Dakahalia governorates were examined with clinically suggestive lesions from 2018 to 2019. 47.6% of the cases were positive for the dermatophyte infection either by clinical signs, microscopic or culture, or both. The highest registered infection rate is in males, at the age of < 6 months, and in the winter season. Three antifungal medications are used for the first time in the treatment of ovine dermatophytosis. They are proved to have been effective in subsiding skin lesions with hair growth to return to its normal clinical state with a 100% curative rate. The treatment with preferable and easily applicable topical cures, especially tioconazole cream, is highly effective in the short run. This cream treatment is easily applicable and provides a good alternative to the traditional antifungal medication for sheep. Consequently, such treatment can reduce the possibility of spreading the infection by other animals, and may allow the adaption of efficient control measures.

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Introduction

Ovine fungal infection is commonly known as ringworm or dermatophytosis. It affects the outermost layer of skin and is caused by several species of dermatophyte. According to the conidia morphology and accessory organs, the dermatophytes are classified into three genera; *Epidermophyton*, *Trichophyton*, and *Microsporum* (1). *Trichophyton* and *Microsporum* spp. have a zoonotic

significance and they have effects on public health. Spores can be transmitted by direct contact with the infected animal or during wool shearing. The spores normally are attached to wool shafts (2). The geographical location, exposure to stress factors, environmental conditions, and age play an important role in the spread of dermatophytes (3). Economically, the increasing concern of dermatophytosis is not triggered by its worldwide public health problems in terms of affecting millions of individuals annually, but also being one of the

dermatologic problems in the veterinary field affecting domestic and wild animals (4). In sheep, dermatophytes have negative effects on milk and meat production. The skin lesion scars remain present on leather following the taw and tannin (3). The clinical signs of ringworm appear 1-4 weeks after the contact with fungal spores. In sheep, common clinical signs appear as a circular area of the alopecia and crust formation over the eyes, ears, head, and neck, and a clear fluid or bloody oozing under the scab (5). Conventional methods such as the direct microscopic examination of dermatophytes are simple and can be rapidly carried on the collected skin scrapping samples. Mycological identification using specific culture media is one of the basic standard methods used to detect dermatophytes and identify the different species (6).

Since ringworm is a zoonotic disease, routine screening of all sheep in the pasture and quick treatment to reduce the transmission of the infection to other animals and people have an occupational hazards and close contact one is necessary (3). Treatment includes various topical and systemic antifungal drugs. Systemic treatments include, ivermectin, ethylenediamine dihydride, oral griseofulvin ...etc. Topical applications such as thiabendazole in glycerin, tincture of iodine 10%, 1% tioconazole, 10% enilconazole, propolis ointment, Whitfield's ointment, *Aloe vera* gel ...etc. can also be used (7-9). Therefore, it is necessary to apply new drugs to replace the old and conventional ones or to use herbal ones to control the infection rapidly (10). This study identifies the effective and useful human antifungal compounds such as tioconazole 1% cream, clotrimazole 1% topical aerosols, and fluconazole capsule to be used in the treatment of dermatophytosis in sheep.

Moreover, assessment of the prevalence rate and some environmental risk factors of sheep ringworm. It is worth mentioning here that there are very few research studies on sheep dermatophytosis in Egypt.

Materials and methods

Ethical approve

The protocol was issued by Zagazig University Institutional Animal Care and Use Committee "ZU-IACUC" at approval N. ZU-IACUC/2/F/103/2019.

Animals and clinical samples

From June 2018 to July 2019, a total of 103 sheep were admitted to a veterinary clinic in Dakahlia governorate and the Veterinary Hospital at the Veterinary College; Zagazig University, Egypt. The animals were clinically examined for evidence of dermatophytosis infection according to Constable *et al.* (5). The data on potential risk factors was registered for each sheep, including the breed, age, sex, season, and other related environmental factors that might have contributed to the distribution of ringworm in sheep.

Skin lesions suspected to have ringworm infections were collected. The skin lesions were cleaned with a cotton swab soaked with 70% alcohol (11). By using a sterile scalpel, skin scraping was collected from the margin of the lesion until oozing of the blood. Hairs were also included in the samples (12,13). The collected skin scraping was divided equally into two groups; the first group was subjected to direct microscopic examination, while the second group was submitted in a sterile Petri dish to the laboratory of Mycology, Animal Health Research Institute, Dokki, Giza, Egypt. To identify the presence of parasitic eggs and larvae, 3 grams of fecal samples were collected from all the examined sheep and then microscopically examined by a modified McMaster technique (14). Twenty-four sheep were diagnosed and identified with ringworm infection. They were allocated for dermatophytosis treatment trial with human antifungal drugs.

Examination of the collected samples

The collected samples were examined microscopically. They were put on a clean glass slide with a drop of 10% KOH, covered with a cover slide, heated gently, left for one hour, and then examined for fungal elements (hyphae, ectothrix, and endothrix spores) using low and high power of microscopically examination (12). The collected specimens were cultured on Sabouraud's dextrose agar (SDA) (Sigma) plus chloramphenicol (antibiotics) 50 mg/L (Sigma) and cycloheximide (antifungal) 0.5 g/L (Sigma). The inoculated media were incubated at 30°C for 4 weeks and examined every three days for evidence of growth. The isolates of dermatophytes were identified macroscopically by examining the growth rate, surface and reverse color, and consistency of the grown colony (12). The microscopic identification was done by using Lactophenol cotton blue (LPCB) wet mount preparation to demonstrate the presence of hyphae, macroconidia, chlamydoconidia, and other fungal structures (15).

Antifungal drugs treatment

Twenty-four sheep were subjected to the treatment with antifungal agents after dividing into four groups equally. There were 6 sheep in each group. Group 1 (G1) was treated with tioconazole 1% cream (Trozal 1% cream, Egyptian group for pharmaceutical industries) as a local cream. Group 2 (G2) was treated with clotrimazole 1% topical aerosols (Candistan, ADCO) as a local spray, and group 3 (G3) was treated with capsules (Fungican150mg capsule, Amoun) as 5 mg/ Kg/Bw. There were two doses given in 10-day intervals while topical drugs were applied daily for 10 successive days. Finally, Group 4 (G4) was kept as a positive control without treatment. All sheep groups were kept under observation for ten weeks with monitoring once weekly. Each of the sheep with dermatophytes was assessed by using a modified clinical scoring index from zero to three (0= not affected, 1=mild, 2=moderate, and 3=intense) previously

designed by Mugnaini *et al.* (16). Skin lesions included alopecia, scaling, crust, and the entire affected area. The total score for each animal was the sum of the scores assigned to each clinical lesion (Table 1). The evaluation of the successful treatment was carried out by the reduction of

means and standard deviations of score lesions for the treated groups. In addition, the mycological cure was considered when two consecutive fungal cultures in between one-week intervals were negative.

Table 1: Clinical index scores for evaluation of skin lesions associated with dermatophytosis in sheep

Scores Index	Score 1 (Mild)	Score 2 (Moderate)	Score 3 (Sever)
Alopecia	Mild with marked hair growth	Moderate with partial hair loss	Sever or complete hair loss
Crust	Mild and fine crust	Moderate or marked	Extensive
Scaling	Mild and rarely visible	Moderate and marked	Severe, large, and extensive
Affection area	Localized (One lesion within one area of the skin)	Multiple (Multiple lesions within one area of the skin)	Generalized (Multiple lesions in more than one area of the skin)

Statistical analysis

The data collected were statistically analyzed using the General Linear Model Functions for Windows SPSS 16.0 (SPSS Inc., Chicago, IL) based on a 4×10 factorial design by Snedecor and Cochran (17). The McNemar test was used to compare two dependent categorical variables. The data was expressed numerically and as percentages. The analysis process involved an accurate binomial test in two categories and a chi-square test in three categories to test the differences in proportions of each of the studied factors. Differences among means within the same factor were tested using the post-hoc Newman-Keuls test. $P \leq 0.05$ was considered statistically significant (S), $P \leq 0.001$ was considered statistically more significant (HS), and $P \geq 0.05$ was considered statistically insignificant (NS).

Results

The clinical examination of 103 sheep illustrated that 49 / 103 sheep with a percentage of 47.6% showed circular areas crusts and scales that varied in diameter from (3-5 cm), with hair loss, and sometimes lesions may have coalesced to form larger area of alopecia. Lesions were mostly restricted to head, lips, ear, and neck sites (Figure 1).

The direct microscopic examination of the collected skin scrapping and hair samples revealed the presence of arthrospores inside or outside the hair shaft in 33 / 49 sheep with a percentage of 67.3% without demarcation between pathogenic and saprophytic ones, whereas the mycological culture revealed that 59.2% (29/49) were positive for dermatophytes infection (Table 2). The identification of mycological culture revealed five zoophilic dermatophytes species of the genus *Trichophyton* and *Microspore* as 17.3% *T. verrucous* (5/29), 10.3% *T. tonsurans* (3/29), 24.1% *M. ferrugineum* (7/29), 27.6% *M. Canis* (8/29), and 20.7% *M. gypsum* (6/29) (Table 2). Statistical analysis showed a non-significant difference between the microscopic and mycological cultural methods in the diagnosis of dermatophytosis in sheep $P \geq 0.05$ by the McNemar test.



Figure 1: The most common affected sites in affected sheep with ringworm (face, around the ear, eyes, and lips).

As mentioned in table 3, the effect of animals' age on the prevalence of dermatophytes shows that the higher prevalence rate is found in lambs less than 6 months 64.08%. Dermatophytosis disease affects male sheep 56.31% more than female sheep 43.69% but it is not statically significant. Dermatophytes in sheep were detected all over the year and the high risk of the disease was recorded in the winter and autumn seasons at 54.43 and 21.36%, respectively. The high moisture conditions, transportation, poor hygienic measures, and lack of insect control significantly increased the rate of infection among sheep. Other risk factors as the presence of concurrent infection (diarrhea, constipation, pneumonia, and cough) and parasitic infestation did not significantly increase the rate of dermatophytes infection ($P \geq 0.05$).

Furthermore, twenty-four infected sheep with ringworm were subjected to a field trial treatment with different antifungal drugs. The diseased sheep showed a typical ringworm lesion on the head and neck. Before starting the treatment trial, the collected skin scrapping and hair samples were examined by direct microscopic and mycological culture methods and were positive. The animals were evenly divided into four groups, each containing six animals. As depicted in Figure 2, the clinical scores of the 4 groups of

sheep did not significantly differ during 0 and 1-week post-treatment (WPT), while the treated groups showed a more significant improvement in clinical scores than the control (untreated) group (G4) from week 2 until 10-week of post-treatment ($P \leq 0.05$). A significant change in the clinical scores ($P \leq 0.05$) was detected in the tioconazole treatment of group (G1) compared to the other treated groups (G3 and G4)

on 4 and 5 WPT. Moreover, a significant improvement in the treated groups [tioconazole treatment group (G1) and fluconazole treatment group (G3)] was noticed when compared with the clotrimazole treated group (G2) during 6, 7 and 8 WPT. No serious or recurrent side effects were observed during the entire period of the treatment trial.

Table 2: Percentage of dermatophytosis among examined animals using conventional methods

Breed	Source		Infected		Microscopic (%)		Culture (%)		Dermatophyte species No. (%)
	Zagazig	Dakahlia	No.	%	+ve	-ve	+ve	-ve	
Osseimi	24	79	49	47.6	33	16	29	20	<i>Trichophyton verrucosum</i> 5 (17.3)
									<i>Trichophyton tonsurans</i> 3 (10.3)
									<i>Microsporium ferrugineum</i> 7 (24.1)
Osseimi	24	79	49	47.6	33	16	29	20	<i>Microsporium canis</i> 8 (27.6)
									<i>Microsporium gypseum</i> 6 (20.7)
Total	103		49 (47.6%)		67.3	32	59.2	40.8	29

Table 3: Risk factors associated with dermatophytosis in sheep

Risk factor		Sheep (No.)	Sheep (%)	χ^2	df	P-Value
Age	< 6 months	66	64.08	8.17	1	0.004**
	> 6 -12 months	37	35.92			
Sex	Male	58	56.31	1.64	1	0.200 ^{NS}
	Female	45	43.69			
Season	Winter	54	52.43	45.54	3	< 0.001**
	spring	19	18.45			
	Summer	8	7.77			
	Autumn	22	21.36			
Moist condition	Yes	92	89.32	63.70	1	< 0.001**
	No	11	10.68			
Overcrowding	With	83	80.58	38.53	1	< 0.001**
	Without	20	19.42			
Bad hygiene	Yes	89	86.41	54.61	1	< 0.001**
	No	14	13.59			
Insect control	Yes	47	45.63	0.79	1	0.375 ^{NS}
	No	56	54.37			
Concurrent infection	Yes	56	54.37	0.79	1	0.375 ^{NS}
	No	47	45.63			
Parasitic manifestation	Yes	36	34.95	9.33	1	0.002**
	No	67	65.05			

X²: Chi-square test, df: degree of freedom, **: highly significant, NS: non-significant.

Group 1 (G1) was treated with tioconazole 1% cream. The animals revealed an improvement of lesion gradually from the 1st week with a full growth of hair during 6-7-week post-treatment (WPT) (Figure 3). G2 was locally treated with clotrimazole spray, and the lesions began to respond to the treatment later within 7-8 WPT. The complete regression to normal state was seen during 9-10 WPT. G3 was treated orally with fluconazole, and the lesions improved gradually within 5-6 WPT with complete recovery achieved within 7-8 WPT as illustrated in Figure 4. G4 was kept as a positive control without treatment, and the lesion improved less with no hair growth until the end of the experimental observation

(10 weeks) with some progress and enlargement of the lesion were observed in some of the examined sheep of this group (G4).

The mycological examination of the treated groups revealed that the mycological cure with minimum environment contamination and less possibility of human infection was noticed within 1 month in G1 and within 5-6 weeks in G3 while the period extended to 8 weeks in G2. In the untreated control G4, on the other hand, some of the lesions were repeatedly positive for mycological examination during the entire period of the study.

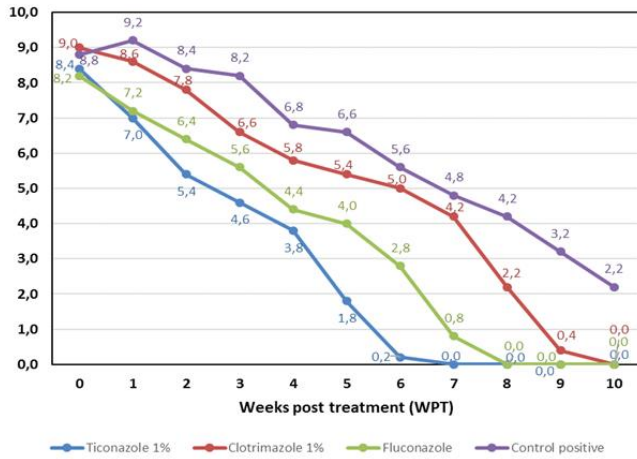


Figure 2: Clinical scoring in naturally infected treated and untreated sheep through 10 weeks of the experiment.



Figure 3: The efficacy of treatment with tioconazole 1% cream in sheep. A. before treatment & B. in 6th week post-treatment.



Figure 4: The efficacy of treatment orally with fluconazole in sheep. A. before treatment and B. in 7th week post-treatment.

Discussion

The infected sheep with dermatophytes usually suffer from circumscribed lesions of alopecia with scales and crust formation. Lesions are commonly distributed on the head, lips, ear, and neck (5,18). In the current study, the prevalence of dermatophytosis infection in the examined sheep was 47.6% which was higher than the recorded percentage registered in an Indian study in which the prevalence of infection of various dermatophyte species in sheep was 0.19% as mentioned by Mitra *et al.* (19) and 6.4% as stated by Biswas *et al.* (20). This variation may have been attributed to the geographical location, hygienic measures, breed of animals, virulence, and species of isolated fungi (21). Furthermore, dermatophytosis in sheep and goats is undoubtedly an underdiagnosed disease and many episodes of infection have not been mentioned in the official literature (22). The difference in prevalence may vary from one site to another due to the type, age, and the number of the examined animals, location, and environmental conditions (23,24).

The most important risk factor that affects the prevalence of ringworm infection among animals is age. In this study, sheep < 6 months are most likely to have a higher prevalence of infection compared to older sheep with a significant difference ($P < 0.004$). This result is in line with Eissa *et al.* (25) who state that dermatophytosis is more common in young sheep. This may be due to their weak immunity and the alkaline pH of their skin which is a suitable media for mycotic diseases (5). On the contrary, Biswas *et al.* (20) record that the prevalence of ringworm is higher in older animals > 6 months.

The effect of sex on the prevalence of ringworm is illustrated in this study as male sheep are more susceptible (56.3%) without a significant difference in the sex ($P > 0.2$). This may be attributed to male animals being housed in close contact for long periods for the fattening purpose and the contagious aspect of the disease. These results are in agreement with those obtained previously by Abou-Eisha *et al.* (26) who find a higher rate of infection in males than in female sheep. However, the results are contrary to Biswas *et al.* (20) who mention that female lambs and kids had a higher rate of infection than males.

Furthermore, in the present research, a significant difference between the year seasons ($P < 0.001$) in the spread rate of ringworm among sheep is found. Dermatophytosis is more common in the winter months. This may be attributed to high humidity and increased contact among animals. It also acts as stress and provokes the appearance of disease as recorded by previous authors (27-29). Biswas *et al.* (20), on the other hand, mention that India has a higher infection rate in summer and autumn than in spring and winter, due to the high rainfall, humidity, and temperatures, which provoke the propagation of dermatophytes in animals and humans.

The development of dermatophytosis infection in sheep is associated with other risk factors such as the presence of

parasitic manifestation and overcrowding that is commonly related to high humidity and bad ventilation as predisposing factors for ringworm infection (30,31). With more focus on other predisposing factors, concurrent infection and insect control provoke ringworm infection without a significant difference ($P>0.375$).

The diagnosis of the ringworm is achieved firstly by the microscopic examination of the skin scraping samples with a percentage of 67.3% of the examined samples. This result is in line with the other studies that state a range from 60 to 71% (32,33). Although the direct microscopical examination is a simple and easy method for the diagnosis of fungal spores, it cannot differentiate between the saprophytic and pathogenic fungi and it requires a skilled and qualified staff (33).

The cultural and morphological characteristics method make it the most appropriate approach for identification. However, it is complicated due to the similar morphology, variability, and polymorphism, time-consuming, and high cost. Despite all these disadvantages, it is still a highly precise approach (34). Mycological examination of skin scraping samples on specific media provides a macroscopic identification of fungal spp. as have been described earlier in our study (35). The study results indicate a 59.2% difference in the growth of mycotic spp., *M. Canis* 27.6% is a predominant specie orderly followed by *M. ferrugineum* 24.1%, *M. gypsum* 20.7%, *T. verrucous* 17.3% and *T. tonsurans* 10.3%. Other studies in Nigeria, India and Libya (3,18,36) determine that *Trichophyton spp.* are the main species isolated from dermatophytosis infection in sheep. *M. Canis* detected in ruminants may be attributed to the contact with dogs (28,29).

The efficacy of the three compounds (tioconazole, fluconazole, and clotrimazole) is considered a good choice with satisfied antifungal activities in humans (37), but the use in sheep not achieved. So, the trial to use these compounds in treatment of sheep dermatophytosis is needed. Tioconazole 1% cream is an effective topical drug with a complete remission in 5-6 weeks/ post-treatment. This result is in line with Kirmizigül *et al.* (7) who report that the first use of 1% tioconazole in the treatment of bovine dermatophytosis is highly effective due to its feasibility in terms of application and the powerful therapeutic effect. All the cattle treated with tioconazole in this study exhibited new hair growth in the lesion site within 3rd-4th weeks and a complete recovery within 6-7 weeks. This finding is in line with Pal and Dave (38) who proved the effective use of 2% miconazole ointment to treatment of dog dermatophytosis and Tartar *et al.* (31) who detected the high effectiveness of miconazole spray to treat dermatophytes in calves within 2 to 3 weeks. The efficacy of tioconazole 1% cream can be explained by the fact that this compound is a synthetic antifungal preparation containing imidazole as an active element. It is also topical, safe, and effective with a long curative effect (39). The study results recommend the use of

tioconazole 1% cream. The local use of clotrimazole spray is mentioned by Alchorne *et al.* (40) who highlight the effective curative effect of tioconazole 1% cream with no adverse reaction to treat the patients with tinea rather than the clotrimazole spray.

The use of Fluconazole orally in the treatment of sheep dermatophytosis seemed to be effective after the application but the response to the therapeutic effect starts later than compared to G1 that was treated locally by tioconazole 1% cream. Accordingly, its use is not practical due to the side effect of this drug affecting the cross-brain membrane barrier (41). Despite its therapeutic effect and no adverse reaction, this drug is not preferable to be used due to the possibility of having problems in near future in the treated ruminant. Hence, the topical treatments are thought to speed the resolution of clinical lesions and may help prevent zoonotic contagion (42). Tartar *et al.* (31) reveal that fluconazole has the highest value of minimum inhibitory concentration test (MIC) ranging from 8-64 µg/ml making it a less effective drug to be used in the treatment of calve dermatophyte.

The mycological cures in G1 and G3 were faster than the cures in G2 that was treated locally with clotrimazole spray, yet, all the treated groups achieved no fungal growth after culture in comparison with the untreated control group. The result of this study concerning the untreated control group agrees with the results of Borman and Summerbell (43) who show that the untreated sheep are usually more self-healing but with a long duration for 2-4 weeks than the treated groups. The possibility of increased environmental contamination and zoonotic spread is suspected to be high.

Conclusion

This study clearly shows that sheep dermatophytosis is a public health concern in both Sharkia and Dakahlia Governorates. *Trichophyton* and *Microsporum* spp. are predominant among sheep in Egypt, and they achieve good hygienic measures by avoiding overcrowding and paying attention to the treatment of concurrent diseases. The rapid treatment of infected sheep can aid in the achievement of effective control measures and prevent epizootic in humans and sheep. The local application of tioconazole 1% cream for 10 days allows sheep skin to return to its normal appearance within 6 -7 weeks. It is, therefore, considered a potent alternative, easily applicable, and economic in terms of labor and cost of antifungal medicine for the treatment of sheep dermatophytosis.

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Conflict of interest

The authors have no conflict of interest to declare.

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معدل انتشار وبعض عوامل الخطر مع التجربة العلاجية لمرض قراع الأغنام في مصر

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الخلاصة

يعد مرض القراع واحدا من الأمراض الفطرية المنتشرة في جميع أنحاء العالم بما في ذلك مصر والذي يصيب طبقات جلد الإنسان والحيوان. يؤثر مرض القراع تأثيرا سلبيا على الصحة العامة بجانب الخسائر الاقتصادية الناتجة عن شكل الجلد وفترة العلاج الطويلة بالرغم من كونه مرض ذاتي العلاج. تهدف هذه الدراسة الى تحديد نوع الفطر الأكثر انتشارا والمسبب لمرض القراع في الأغنام بواسطة كلا من اختبار الفحص المجهرى والزرع على الأوساط الزرع الخاصة. كما تهدف الدراسة الى التعرف على بعض العوامل الحيوانية والبيئية المرتبطة بانتشار الفطر الجلدي بين الأغنام وتقييم فاعلية كل من (كريم تيكونازول وسبراى كلوتريمازول وكبسولة فلوكونازول) على أربع وعشرين حالة من الأغنام المصابة كأول تجربة لاستخدامهم في علاج مرض القراع في الأغنام. وتبين الدراسة معدل انتشار مرض القراع ما بين ١٠٣ حالة من الأغنام المصابة بمرض جلدي بمحافظة الشرقية والدقهلية بمصر خلال الفترة الزمنية من ٢٠١٨ ولغاية ٢٠١٩ والتي تم فحصها مجهريا وبزرعها على الأوساط الزرع الخاصة بإيجابية ٤٧,٦٪ حيث تم تسجيل أعلى معدل أصابه في سن أقل من 6 أشهر وخاصة بفصل الشتاء. ونجاح استخدام (كريم تيكونازول وسبراى كلوتريمازول وكبسولة فلوكونازول) في تهدئة الفطر الجلدي مع نمو الشعر بصورة كاملة بمعدل علاجي ١٠٠٪ وخاصة استخدام كريم التيكونازول حيث يساعد في عودة الجلد إلى المظهر الطبيعي في غضون ٦-٧ أسابيع. نستخلص بأن سرعة علاج الحيوانات المصابة باستخدام العلاج المناسب مع تدبير الإجراءات الوقائية يقلل من دور الحيوان في نقل وانتشار العدوى.