



## **RESEARCH ARTICLE**

### Dermatophytosis among Ruminants in Egypt: The Infection Rate, Identification and Comparison between Microscopic, Cultural and Molecular Methods

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### Abstract

Dermatophytosis is a skin disease of farm animals caused by different species of the Microsporum and Trichophyton genera. Although the disease is self-limiting, ringworm has a major public and veterinary health problem with worldwide distribution including Egypt. The objectives of this study were to estimate the infection rate of dermatophytosis in ruminants, identify the incriminated dermatophyte species and differentiate among them using conventional and molecular methods. Moreover, assessment of the role of environmental risk factors in the occurrence of the disease. Cow (n=197), sheep (n=103) and goats (n=70) of different breeds, sexes, and ages in Sharkia and Dakahalia Governorates were clinically examined during the period from June 2018 to July 2019. Out of the examined animals, 111 (30%) have skin lesions consistent with dermatophytosis. The infection rate of the disease was 30, 37.9 and 18.6% in cow, sheep and goat, respectively. The rate of infection was higher in the Holstein breed (36.6%) compared to native breed (23%). The higher rate of infection was found amongst lambs less than 6 months while in cow and goats the infection rate was higher in animals more than 6 months old. Male animals were generally more infected than females in all studied animals, and the infection was more common in winter season. Trichophyton verrucosum was the most commonly identified dermatophyte species (25.6%). Polymerase chain reaction (PCR) of internal transcribed spacer (ITS) along with restriction fragment length polymorphism method (RFLP) succeeded to differentiate between both isolated *Trichophyton* and *Microsporum species*. In conclusion, PCR along with culture results acted as gold standard methods for diagnosis of dermatophytosis. Restriction Fragment Length Polymorphism method was considered as a rapid approach with high specificity and sensitivity to identify and differentiate dermatophytes in ruminants.

Keywords: Dermatophytosis, Egypt, RFLP, Risk factors, Ruminants.

## Introduction

Dermatophytes are considered as one of the most important skin diseases affecting all domesticated animals and human in developing and developed countries. Dermatophytosis belongs to class ascomycetes, Phylum: Ascomycota, family arthrodermataceae and genus Arthroderma and divided into 3 genera: *Epidermophyton*, Trichophyton and Microsporum. Different dermatophytes have zoonotic importance in

which the most species of dermatophytosis public health concern are belonging to *Trichophyton* and *Microsporum* species and its distribution has varied in different animals according to geographical location, age, sex of affected animals [1-5].

Dermatophytosis has a huge economic loss in the form of reduction of weight gain and downgrading of hide and skin that affects marketing show, premature culling and treatment costs with dramatically increase the infection in the last few years all over the world [6, 7]. In addition to easy transmission from animals to human [8, 9]. The British leather confederation evaluated losses due to downgrading of the leather of about 35 million dollars annually, of which 5% due to dermatophytosis [10, 11].

Most of the lesions observed in the affected animals were circumscribed grayish-white area of alopecia, crusty, raised lesions. These lesions commonly appear on the head, neck, chest area, dewlap and limbs [12]. The severity of lesions varies from mild to severe attributed to many reasons as the reaction of the host to metabolic products of fungi, virulence and type of affected strain of dermatophytes along with environmental risk factors [13].

Trichophyton verrucosum, Trichophyton mentagraphytes, Microsporum canis, and Microsporum gypseum are considered the most incriminated dermatophytes species, causing Tinea capitis and Tinea corporis in different parts of the world. Ringworm infection is more prevalent in regions with high warmth and humidity conditions than the cold and dry ones. The direct contact with affected animals and contaminated environment are the most common means to spread the infection in presence of other predisposing factors including age, sex and environmental conditions, other diseases, parasitic infestation, and hygienic measures [14-16].

Diagnosis of dermatophytosis occurred mainly by direct microscopic examination of scales including hairs along with cultural methods. These conventional methods are time-consuming, high-priced and need skilled staffs [17]. In the early 1980s, several molecular approaches, as restriction fragment length polymorphism (RFLP), restriction enzyme analysis of mitochondrial DNA, internal transcribed spacer PCR (ITS-PCR), random amplified polymorphic DNA and DNA sequencing were practiced as new and accurate diagnostic tools of dermatophytosis [18]. ITSs regions especially ITS1-5.8s-ITS2 along with RFLP has been considered as a rapid and accurate approach to identify the

dermatophytes. Moreover. resolving relationships between close taxonomic dermatophytes and produce specific band on agarose gel electrophoresis to determine dermatophytes species within 5 h. [19-23]. This study was performed to identify and differentiate the isolated dermatophytes from naturally infected cow, sheep and goat at species level using both conventional methods and restriction fragment length polymorphism (RFLP). Moreover, assessment of the role of environmental risk factors in the occurrence of the disease was carried out.

### **Materials and Methods**

The protocol of this study was accepted by Zagazig University Institutional Animal Care and Use Committee (ZU-IACUC/ 2/F/ 103/ 2019)

## Animals and clinical examination

Clinical examination of cow, sheep and goat (n=370) of different breeds, sexes and ages brought to Veterinary Clinics in Dakahlia and Sharkia Governorates during the period from June 2018 to July 2019 was performed in line with Constable [24]. The site, shape, size, distribution and time of the appearance of skin lesions were reported.

# Sampling

Fecal samples (3 g) were collected from all examined animals (197 cow, 103 sheep and 70 goat) and then macroscopically inspected for the presence of parasitic eggs and larvae. Samples were then processed for microscopical examination by a modified McMaster technique [25].

The affected lesions from 111 animals (59 cow, 39 sheep and 13 goats) with skin lesions thought to be ringworm was cleaned with a cotton swab soaked with 70% alcohol. Skin scraping was done by using a sterile scalpel blade from the margin of the lesion until oozing of the blood and hairs also included in the samples [26]. Collected scales were divided into two portions, the first one was subjected to direct microscopic examination, while, the second one was submitted in a sterile Petri dish to the laboratory of Mycology, Animal Health Research Institute, Dokki, Giza, Egypt.

# Direct Microscopical examination of collected samples

A portion of collected scales was put on a clean glass slide with a drop of 10% KOH, covered with a cover slide, heated gently and let for 1 h and then examined for fungal elements (hyphae, ectothrix and endothrix spores) using low and high power of microscopical examination [27].

### Isolation and identification of dermatophytes

The collected specimens from different animals 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 30oC for 4 weeks and examined every three days for evidence of growth.

The isolated dermatophytes were identified by macroscopical examination which comprised of growth rate, surface and reverse colour and consistency of grown colony [28, 29]. While microscopical morphology of the isolates was done by using Lactophenol cotton blue (LPCB) wet mount preparation to demonstrate the presence of hyphae. macroconidia, chlamydospores and other fungal structure [30].

### **DNA** Extraction

DNA from the suspected 21 isolates was extracted using Qiagen Extraction Kit (Hilden Germany) as described in manufacturer's manual.

## Amplification of ITS region

PCR amplification of ITS region of twenty one samples was done by using universal fungal primers ITS 1 (5' – TCC GTA GGT GAA CCT GCG G – 3') and ITS4 (5' – TCC TCC GCT TAT TGA TAT GC – 3') according to White *et al.* [31]. The total reaction mixture of 25  $\mu$ l PCR master mix (Merck genei), 280 pmol of each primer (ITS-1 and ITS-4), 1  $\mu$ l of template DNA and the final volume reached to 50  $\mu$ l with nuclease free water. Amplification was adjusted to initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec, extension at 72°C for 5 min. PCR products were analyzed in a 1.5% agarose gel electrophoresis stained with ethidium bromide for the presence of suspected bands of the expected length.

# **Restriction Fragment length polymorphism** (RFLP) analysis

RFLP procedures were performed according to Jackson et al. [32] as follow; Mixture had 2 µl of 10X FastDigest Green enzyme buffer, 1 µl of FastDigest enzyme (Mva I) and 10µl of PCR product, the volume was adjusted up to 30 µl with nuclease-free water. The reaction mixture was incubated at 37°C for 1 h. Twelve microliters of each RFLP products were loaded into 2% agarose gel with 0.5 µg/mL ethidium bromide. The bands on agarose gel were visualized using gel documentation system and photographed.

### Statistical analysis

The collected data was statically analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA 2011). Quantitative data were represented in form of mean  $\pm$  SD and qualitative data were detected as absolute frequencies (number) and relative frequencies (percentage). For Non- normally distributed variables, Mann Whitney U test was preferred. Mc-Nemar Test was used to compare between two dependent categorical variables. All tests were two sided. *P*-value < 0.05 was considered statistically significant (S) while *P*-value < 0.001 was detected as highly statistically significant (HS), and *P*-value  $\geq$  0.05 was considered statistically insignificant (NS).

## Results

Out of 370 examined cow, sheep and goats, 111 animals (30%) had skin lesions thought to be ringworm and had subjected to examination as illustrated in (Table 1). The rate of ringworm infection varied from species to another, in cow, it was 30% (59/197), while, higher infection rate of 37.9% (39/103) was recorded in sheep, however, goat was the least infected (18.6%, 13/70). The rate of infection was higher in the foreign breed (Holstein breed; 36.6%) compared to native one (23%). Ringworm lesions in cow are usually but not always circular, vary in diameter from (3-5 cm), with hair loss, crusts and scales formation, sometimes lesions may coalesce to

form larger area of alopecia. In cow, lesions mostly found on the head, necks, around-ear, eyes, lips and dewlap and less frequently in flank region, sometimes the entire bodies were affected (Figure 1A). Lesions in sheep and goat also were restricted to head, lips, ear, and neck, limbs and flank regions (Figure 1B). As mentioned in (Table 2), the higher infection rate was found amongst lambs less than 6 months (71.8%) while cow and goats had high prevalence in the age group more than 6 months to 12 month (67.8 and 53.9%, respectively). Male animals were more infected (62.7, 53.9 and 69.2%) than females (37.3, 46.2 and 30.8%) in cow, sheep, and goat, respectively. Ringworm infection was detected all over the year but more common in winter season (35.6, 48.7 and 53.9%) in cow, sheep, and goat, respectively.

 Table 1: Prevalence and comparison of isolated dermatophytes among examined animals using conventional methods in Sharkia and Dakhalia Governorates (2018-2019).

| Animals species |              | Total<br>numbers | Infected animals |      | Microscopic |      |        | culture |  |
|-----------------|--------------|------------------|------------------|------|-------------|------|--------|---------|--|
|                 | -            |                  | Number           | %    | Number      | %    | Number | %       |  |
| Cow             | Native       | 96               | 22               | 23   | 19/22       | 86.3 | 17/22  | 77.3    |  |
|                 | Holstein     | 101              | 37               | 36.6 | 32/37       | 86.4 | 30/37  | 81.1    |  |
|                 | All cow      | 197              | 59               | 30   | 51/59       | 86.4 | 47/59  | 79.7    |  |
|                 | Sheep        | 103              | 39               | 37.9 | 33/39       | 84.6 | 29/39  | 74.4    |  |
|                 | Goat         | 70               | 13               | 18.6 | 11/13       | 84.6 | 10/13  | 76.9    |  |
| Т               | otal animals | 370              | 111              | 30   | 95/111      | 85.6 | 86/111 | 77.5    |  |

Mcnemar test was used to establish the comparison in which there was no significant difference between microscopic and cultural methods in diagnosis of dermatophyte infection in cow, sheep and goats (p>0.05).

| Table 2: Frequency distribution of dermatophytes' infected animals in Sharkia and Dakhalia Governorates |  |
|---|--|
| (2018-2019) regarding to risk factors   |  |

| Risk factors                | Parameters   | (   | Cow  |     | Sheep |     | Goat |  |
|-----------------------------|--------------|-----|------|-----|-------|-----|------|--|
|                             |              | No. | %    | No. | %     | No. | %    |  |
| Age                         | < 6 months   | 19  | 32.2 | 28  | 71.8  | 6   | 46.2 |  |
|                             | > 6-12months | 40  | 67.8 | 11  | 28.2  | 7   | 53.9 |  |
| Season                      | Winter       | 21  | 35.6 | 19  | 48.7  | 7   | 53.9 |  |
|                             | Summer       | 8   | 13.6 | 3   | 7.7   | 0   | .00  |  |
|                             | Autumn       | 17  | 28.8 | 9   | 23.1  | 4   | 30.8 |  |
|                             | Spring       | 13  | 22.0 | 8   | 20.5  | 2   | 15.4 |  |
| Sex                         | Male         | 37  | 62.7 | 21  | 53.9  | 9   | 69.2 |  |
|                             | Female       | 22  | 37.3 | 18  | 46.2  | 4   | 30.8 |  |
| Overcrowding                | With         | 41  | 69.5 | 31  | 79.5  | 5   | 38.5 |  |
| C                           | Without      | 18  | 30.5 | 8   | 20.5  | 8   | 61.5 |  |
| Bad hygienic measure        | Yes          | 39  | 66.1 | 33  | 84.6  | 9   | 69.2 |  |
|                             | No           | 20  | 33.9 | 6   | 15.4  | 4   | 30.8 |  |
| <b>Bad ventilation</b>      | Yes          | 35  | 59.3 | 29  | 74.4  | 11  | 84.6 |  |
|                             | No           | 24  | 40.7 | 10  | 25.6  | 2   | 15.4 |  |
| <b>Concurrent infection</b> | Yes          | 21  | 35.6 | 19  | 48.7  | 7   | 53.8 |  |
|                             | No           | 38  | 64.4 | 20  | 51.3  | 6   | 46.2 |  |
| Parasitic infestation       | Yes          | 23  | 39   | 15  | 38.5  | 4   | 30.8 |  |
|                             | No           | 36  | 61   | 24  | 61.5  | 9   | 69.2 |  |
| Insect control              | Yes          | 17  | 28.8 | 19  | 48.7  | 4   | 30.8 |  |
|                             | No           | 42  | 71.2 | 20  | 51.3  | 9   | 69.2 |  |



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

High moisture conditions, bad ventilation, transportation, bad hygienic measures and lack of insect control increased rate of infection among ruminants. The presence of concurrent infection (diarrhea, constipation, pneumonia and cough) or other parasitic infestation were not significantly increased the rate of infection (p > 0.05). A non-significant difference between microscopic and cultural methods for diagnosis of dermatophytosis P> 0.05 was reported. The direct microscopic examination is an easy method to determine the infection with fungi without demarcating between pathogenic and saprophytic one , while, the culture methods had high accuracy level to determine causative dematophyte but it was considered as an expensive and timeconsuming method as shown in Table 1.

Validity of microscopic and culture methods to diagnosis ringworm in comparison to PCR of examined 21 samples donated that microscopic examination had sensitivity 81.3%, specificity 60%, positive predicted value 86.7%, negative predicted value50% and accuracy 76.2% compared to PCR result while culture method had sensitivity 93.8%, specificity 100%, positive predicted value 100%, negative predicted value 83.3% and accuracy 95.2% compared to PCR result (data not shown). The combination of both PCR and culture results acted as a gold standard for accurate diagnosis of dermatophytosis.

As depicted in Table 3, dermatophytes belong to genus *Trichophyton* and *Microspurum* were equally distributed among the examined animals (50%, each). T. verrucosum was the most predominantly isolated species, where, it was isolated from 25.6% of the total examined samples. From calves the isolated dermatophytes were T. verrucosum (31.9%), Т. mentagrophytes Т. (27.7%),tonsurans (10.6%),М. ferrugineum (12.8%) and M. canis (17%), while, in sheep the isolated fungi were T. verrucosum (17.3%), T. tonsurans (10.3%), M. ferrugineum (24.1%). M. canis (27.6%) and M. gypseum (20.7%) and in affected goats were T. verrucosum (20%), M. ferrugineum (50%), M. canis (50%) and M. gypseum (10%).

 Table 3: The isolated dermatopytes species in cow, sheep and goats from Sharkia and Dakhalia Governorates

 (2018-2019) in Egypt

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| Dermatophytes               | C  | ow   | S  | heep | G  | Goat Total |    | otal |
|-----------------------------|----|------|----|------|----|------------|----|------|
|                             | No | %    | No | %    | No | %          | No | %    |
| Trichophyton verrucosum     | 15 | 31.9 | 5  | 17.3 | 2  | 20         | 22 | 25.6 |
| Trichophyton mentagrophytes | 13 | 27.7 | -  | -    | -  | -          | 13 | 15.1 |
| Trichophyton tonsurans      | 5  | 10.6 | 3  | 10.3 | -  | -          | 8  | 9.3  |
| Microsporum ferrugineum     | 6  | 12.8 | 7  | 24.1 | 2  | 20         | 15 | 17.4 |
| Microsporum canis           | 8  | 17   | 8  | 27.6 | 5  | 50         | 21 | 24.4 |
| Microsporum gypseum         | -  | -    | 6  | 20.7 | 1  | 10         | 7  | 8.2  |
| Total                       | 47 |      | 29 |      | 10 |            | 86 |      |

Dermatophytes belong to genus *Trichophyton* and *Microsporum* were equally distributed among the examined animals (50%, each).

### Effect of probiotic on fecal E. coli count

The effect of probiotic on fecal *E. coli* count for the experimental period is shown in Table 3. The results revealed that calves fecal *E. coli* count 023+ the  $2^{nd}$  week of the experiment, the control and the probiotic group's fecal *E. coli* count were 7.48±0.12 and 7.02±0.22 CFU/g, respectively. While after the

 $3^{rd}$  week of the experiment, the fecal *E. coli* count in the control and probiotic groups were 7.32±0.15 and 6.85±0.40 CFU/g, respectively. Finally, at the 4<sup>th</sup> week of the experiment, the fecal *E. coli* count in the control group and probiotic group were 7.44±0.12 and 6.74±0.15 CFU/g, respectively.

Table 4: Effect of probiotic on mean *Escherichia coli* count (CFU/g ± SE) in feces of neonatal buffalo calves

| Day | non-supplemented gr591oup<br>(control)( n=30) | Probiotic group<br>(n = 35) | Sig (2-tailed) |  |
|-----|---|-----------------------------|----------------|--|
| 7   | 7.40±0.45 <sup>a</sup>                        | 7.12 ±0.22 <sup>b</sup>     | 0.213          |  |
| 14  | 7.48±0.12 <sup>a</sup>                        | 7.02±0.22 <sup>b</sup>      | 0.213          |  |
| 21  | 7.32±0.15 <sup>a</sup>                        | 6.85±0.40 <sup>b</sup>      | 0.213          |  |
| 28  | 7.44±0.12 <sup>a</sup>                        | 6.74±0.15 <sup>b</sup>      | 0.213          |  |

Out of 21 representative (12 Trichophyton and 9 Microsporum isolates), 76.2% were positive by the use of PCR in which 9 samples (42.9%) were confirmed as *Trichophyton spp.* and 7 samples (33.3%) were *Microsporum* spp.

Amplification of ITS regions of 21 samples resulted in products for the different species such as *T. verrucosum* and *T. mentagrophytes* that were closely similar (690 and 683 bp, respectively) by amplification by ITS primers, while, each one had characteristic pattern by RFLP analysis as *T. verrucosum* was divided into 4 restriction bands (406, 125, 89 and 50 while the RFLP analysis bp) of Т. mentagrophytes gave 4 bands with different bp than T. verrucosum. Also, the similarity was observed between the profiles of the M. canis, T. tonsurans, M. gypseum band (740 bp), while *M. ferrugineum* produce band (590 bp) as detected in (Figure 2A). Digestion of these amplicons based on Mval revealed unique different restriction patterns for each species for easy identification as indicated in (Figure 2B).

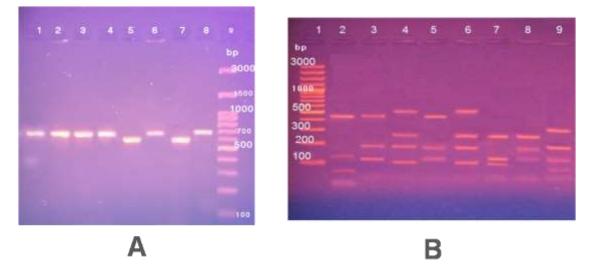


Figure 2: (A) PCR products of ITS1-ITS2 regions from representative clinical isolates of dermatophyte species: Lane 1: *T. verrocosum*, lane 2, 4: *M. ferrugineum*, lane 3.5: *M. canis*, lane 6: *T. tonsurans*, lane 7: *T. mentagrophytes*, lane 8: *M. gypseum* and lane 9: 100 bp DNA ladder; (B) *MvaI* digested products from representative clinical isolates of dermatophyte species: lane1: 100bp DNA ladder , lane 2: *T. verrocosum* , lane 3, 5: *M. ferrugineum*, lane 4,6: *M. canis*, lane 7: *T. tonsurans*, lane 8: *T. mentagrophytess* and lane 9: *M. gypseum*.

#### Discussion

In the current study, infected cow were manifested with typical symptoms of dermatophytosis, which were consistent with other findings [6, 24, 33-35]. In cow, the rate of infection was higher in foreign breed than in native breed and this was consistent with Marai *et al.* [36].

The infection rate of ringworm varied in different animal species, it was; 30, 37.9 and 18.6%, respectively in cow, sheep and goats. This finding was in harmony with Al-Ani et al. [37] who reported that 30.6% of calves were infected with ringworm. Meanwhile, Habb et al. [38] and Abdel-Rady and Kotb [39] recorded that the rate of infection in calves were 7.7 and 17.03%, respectively. In an Indian study the prevalence of infection of various dermatophyte species in goats and sheep was 6.1% and 6.4%, respectively [40]. Also, a low rate of infection (12.55%) in goat was recorded by Rahbari [41]. In another study Mitra et al. [42] recorded only 0.12% infection rate in a population of 1655 goats in India. This variation may be attributed to geographical location, hygienic measures, breed of animals, virulence and species of isolated fungi. In the current study, an incidence of 37.9% of dermatophytosis recorded in sheep was almost similar to 35% documented by Rahbari [41] and much higher than 1.5%-5% reported by Parmanov *et al.* [43] and 0.19% stated by Mitra *et al.* [42]

Higher prevalence of infection was found amongst calves over the age of 6 months compared to age more than 6 months except in infected lambs the opposite was recorded in concern to age. This result was in line with Acha and Szyfres [44] who stated that dermatophytosis is more common in animals under stress as immunocompromised or with a history of transportation under 1 year of age, with poor nutrition and animals kept at high-These results density populations [10]. disagree with Biswas et al. [40] who recorded lambs and kids less than 6 months of age were found to be less susceptible to infection than the older animals.

Dermatophytosis was more common in winter months than in hot season and this results may be attributed to high humidity and increase the contact between animals so act as stress which provoke the appearance of disease as recorded by Scott [45] and El-Ashmawy *et al.* [46]. In contrary, Biswas *et al.* [40] in India, found higher rate of infection in the summer and autumn compared to spring and winter due to higher rainfall and humidity along with high temperature which provoked propagation of dermatophytes in animals and man. These results were inconsistent with Papini *et al.* [47] who recorded higher rate of infection in winter season.

Male animals are susceptible to ringworm infection than females in cow, sheep, and goat. This may be attributed to male animals are 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.* [48] who found high rate of infection in males than females in cattle and sheep. However, the results are in contrary to Biswas *et al.* [40] who mentioned that female lambs and kids had higher rate of infection than males.

The predominant isolation of T. verrucosum (31.9%), T. mentagrophytes (27.7%) and M. canis (17%) in this study is in agreement with Abdeen and El-Diasty [49] who mentioned that T. mentagrophytes and M. canis were the most prevalent isolates from skin lesions of infected cows. More and less the same species affected sheep and goat were described in India by Biswas et al. [40] who determined T. verucossum as the most common species isolated from sheep then T. mentagrophytes, M. gypseum, M. canis and T. rubrum. Among goats, M. gypseum was the most common isolated species followed by T. verucossum, T. mentagrophytes, and T. rubrum. In Nigeria, Dermatophytes were recorded in sheep with an incidence of (7.0%) in which T. verrucosum was the most prevalent species (17.5%) followed by M. gypseum (10.0%) and T. mentagrophytes (7.5%). While in goats, the incidence of infection was (8.9%) in which T. verrucosum was the most isolated one followed by T. mentagrophytes and M. canis, otherwise, the incidence in calves was 49.0% in which T. verrucosum had the highest rate 16.4%. Also, M. canis, T. mentagrophytes and M. gypseum were detected [50]. In Libya, Trichophyton spp. (T. rubrum and T. terrestre) isolated from goat and sheep hairs were detected in 12% and 24% of the goat and sheep hair samples, respectively [51].

*T. verrucosum* was the most prevalent species in ruminants then *M. canis*. This result is in agreement with other reports Pepin and Austwick [52], Ranganathan *et al.* [53] and

Cabañes [54]. *T. mentagrophytes* can cause the disease in calves and this may be due to the presence of rodents, but *M. canis* detected in ruminant may be attributed to contact with dogs [11, 45, 54].

Diagnosis of the disease is achieved firstly by microscopical examination of skin scraping but cannot differentiate between pathogenic and saprophytic fungi and need skilled staff as recorded by Panasiti *et al.* [55] and El-Ashmawy and Ali [56]. Cultural and morphological characteristics approach is gold standard identification which is complicated due to similar morphology, variability and polymorphism, time-consuming, and expensive approach [57].

PCR-RFLP methods are a useful assay to identify and differentiate between dermatophyte species within short times even closely related strains for clinical and epidemiological purposes [22, 58]. MvaI revealed unique different restriction patterns of all isolated dermatophytosis species detected in this study for easily identification without further need for other tests as sequence and phylogentic analysis and also, overcome the failure of amplification by ITS primers to identify T. verrucosum and T. mentagrophytes that were closely similar by amplification by ITS primers, also, the similarity was observed between the profiles of the M. canis, T. tonsurans, M. gypseum band (740 bp) were disappeared when used the restiction enzyme of MvaI. This result was similar to El-Damaty et al. [59] who mentioned that the use of RFLP analysis using *MvaI* enables them to differentiate between T. verrucosum and T. mentagrophytes isolated from Arabian horse. Also, Rezaei-Matehkolaei et al. [60] reported the ability to the use of RFLP restriction pattern to differentiate between Trichophyton spp. and Microsporum spp., while, Rezaei-Matehkolaei et al. [60] in contrary to this actual study cannot differentiate between M. canis and M. ferrugineum.

# Conclusion

*Trichophyton* and *Microsporum* spp. are predominant among ruminant in Egypt. PCR-

RFLP is considered as rapid approach with high specificity and sensitivity for identification and differentiation of dermatophytes in ruminants.

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### **Conflict of interest**

The authors have no conflict of interest to declare.

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القراع في المجترات في مصر :معدل الإصابة والتصنيف والمقارنة بين الطرق التقليدية والجزيئية

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القراع هو مرض جلدي يصيب حيوانات المزرعة يسببه أنواع مختلفة من أجناس ميكروسبورم و تر ايكوفايتون. على الرغم من أن هذا المرض محدود ذاتيًا ، إلا أن القراع مشكلة صحية عامة كبيرة مع انتشار عالمي واسع يشمل مصر . هدفت هذه الدراسة إلى تقدير معدل الإصابة بمرض القراع في المجترات، وتحديد وتمييز الفطريات الجلدية باستخدام كل من الطرق التقليدية والجزيئية علاوة على ذلك تقييم دور عوامل الخطر البيئية في حدوث المرض . تم فحص 197 من الأبقار و ١٠٣ من الغرق من الأغنام و ٢٠ من المرض . تم فحص 197 من الأبقار و ١٠٣ من الغرق على ذلك تقييم دور عوامل الخطر البيئية في حدوث المرض . تم فحص 197 من الأبقار و ١٠٣ من الأغنام و ٢٠ من الماعز التي لها سلالة وجنس وأعمار مختلفة في محافظتي الشرقية والدقهلية اكلينيكيا وجزيئيًا خلال الفترة الأغنام و ٢٠ من الماعز التي لها سلالة وجنس وأعمار مختلفة في محافظتي الشرقية والدقهلية اكلينيكيا وجزيئيًا خلال الفترة القراع . حيث كان معدل الإصابة بالمرض ٣٠ معدل الإعدار ٣٠ من العرض . ٢٠ من يونيو ٢٠١٨ إلى يوليو ٢٠١٩ من الحيوانات التي خضعت للفحص، ١١١ (٣٣%) لديهم اصابات جلدية مشابهه لمرض القراع .حيث كان معدل الإصابة بالمرض ٣٠ ، ٣٠ ٣٠ و ٢٨٠ ٪ في الأبقار والأغنام والماعز على التوالي .كان معدل الإصابة أعلى معدل الإصابة بالمرض ٣٠ ، ٣٠ ٣٠ و ٢٨٠ ٪ في الأبقار والأغنام والماعز على التوالي .كان معدل الإصابة أعلى معدل القراع في الملالة البلدى. و كان أعلى معدل للإصابة بشكل عام أقل من ٦ أشهر بينما الأبقار والماعز أعلى معدل انتشار في الفئة العمرية الأكثر من ٦ أشهر .الذكور أكثر إصابة بشكل عام من الإناث في جميع الحيوانات التي خضعت للدراسة ، والعدوى أكثر شيوعا في فصل الشتاء ..كان تر ايكوفايتون فيروكوسم من الأناث في جميع الحيوانات التي خضعت الدراسة ، والعدوى أكثر شيوعا في فصل الشتاء ..كان مراكم معدل الألم ما من الأدام في الفرين من ٦ أشوع على المتاء ..كان على معدل للإصابة بشكل عام من الإناث في جميع الحيوانات التي خضعت للدراسة ، والعدوى أكثر شيوعا في فصل الشتاء ..كان تر ايكوفايتون فيروكوسين في مان ألفر ما ٦ أشهر بين المعزو في المعزولين ترايكو في فصل الشتاء ..كان ترايكوفايتون فيروكوسم ما من الألم ما من الألم مان المرض القراع في المعزولين تر ايكوفايتون والميكر وسبورم. الحرم الع مام مان اكثر المسباس الفرل المرض المرض المرون ما الممز والم ما م