



An Over View of Equine Dermatophytosis

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Authors' contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Dermatophytosis is a fungal infection of the skin caused by dermatophytes fungi which have ability to invade the epidermis and keratinized structure derived from it such as hair or nails. *T. equinum* is the main cause of dermatophytosis in equine, all over the world. Clinical signs include mild to severe alopecia associated with erythema. Horses less than 2 years old are more susceptible to infection. The disease can be diagnosed by clinical inspection and conventional methods like direct examination, fungal culture, skin biopsy and molecular diagnosis methods. This review will forecast more light of the different aspects of this disease.

Keywords: Dermatophytosis; equine; clinical feature; diagnosis treatment.

1. INTRODUCTION

Dermatophytosis (ringworm or tinea), is a superficial skin infection caused by closely related keratinophilic fungi [1]. They have the ability to degrade keratin and invade the skin and

its appendages [2]. Ringworm is a major Public and Animal Health problem in various regions of the world which result in great economic loss [3].

Disease in equine has different forms ranging from mild or subclinical disease to severe lesions

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look like pemphigus foliaceus [4]. *T. equinum* is the main cause of dermatophytosis in horses, all over the world [5,6] and it includes 2 varieties which identified as *T. equinum* var. *equinum* and *T. equinum* var. *autotrophicum*. Severe outbreak of ringworm among 69 adult domestic donkeys in Sudan was described due to *T. mentagrophytes* [7]. *Microsporum racemosum* has been recently isolated from naturally infected donkeys [8].

1.1 Impact of the Disease

Ringworm lesions have many effects on horses; a horse with ringworm may be uncomfortable; especially before appearance of lesion. It can prevent the horses from working riding and racing which are the main purposes of horses, so the cost value of horses with ringworm decreased [9,10].

2. EPIDEMIOLOGY

Production of arthrospores is a result of segmentation and fragmentation of the hyphae, so these spores which adhere strongly to keratin are highly resistant, surviving in a dry environment for 12 months or longer [11].

3. RISK FACTORS

- 1- Young animal
- 2- Condition of exposed skin
- 3- nutrition of the animal
- 4- Immunosuppression (including immunosuppressive treatment)
- 5- Poor grooming practice
- 6- Farm size
- 7- Moist condition[12]

4. TRANSMISSION

Dermatophytosis can be transmitted by direct contact with infected animals. Moreover, indirect transmission through fomites like grooming kits and harness as well as the contaminated environment may lead to high prevalence of the disease [13,14].

4.1 Clinical Signs

In horses, most dermatophyte lesions are found in areas of contact with saddle and girth. They usually begin as small patches of raised hairs, and progress to hair loss, with variable amounts of scaling, erythema, crusting and exudation, kerion can be found in some cases [15,16] (Figs. 1-6). In donkey the lesion mainly on head and neck [17] while severe incrustation, scaling, on

the flanks backs, face, ears legs was reported by Elham et al. [18] (Figs. 7-9).

5. ETIOLOGY

Microsporum equinum and *Trichophyton equinum* have been isolated from horses [19,20] *Arthroderma vanbreuseghemii* has been reported Chollet et al. [21]. *Trichophyton verrucosum* [22,23,24] was able to isolate *T. mentagrophytes*. Mixed infection in a horse with *Trichophyton verrucosum* and *T. mentagrophytes* recorded by Umunna et al. [25] Mahmoud [23] added *M. persicolor*. *M. canis* was reported Tartor et al. [26]. *T. violaceum* recorded Abu –Samra and Ibrahim [27]. *M. fulvum*, *T. soudanense* have been isolated [16]. *T. tonsurans* has been reported Balogun et al. [28], while *M. gpseum* isolated [29,30].



Fig. 1. Lesion 10-20 mm in diameter in head [20]



Fig. 2. Lesion 5-20 mm in diameter in thigh [20]



Fig. 3. Dermatophytosis caused by *T. verrucosum* on the limb of a horse [16]



Fig. 4. Irregular patchy skin lesions are seen on the skin surface of the animal [56]

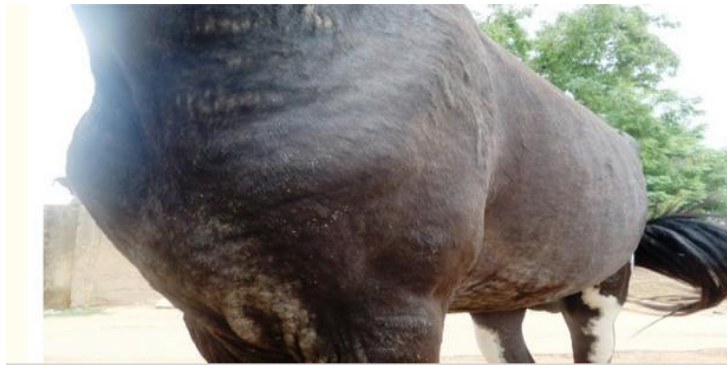


Fig. 5. Areas of hyperkeratosis and alopecia [16]



Fig. 6. Dermatophytosis: scaling of the coronary band caused by *Microsporum gypseum* infection in horse [48]



Fig. 7. Localized skin lesions dermatophytes on the face, neck and fore limbs [18]

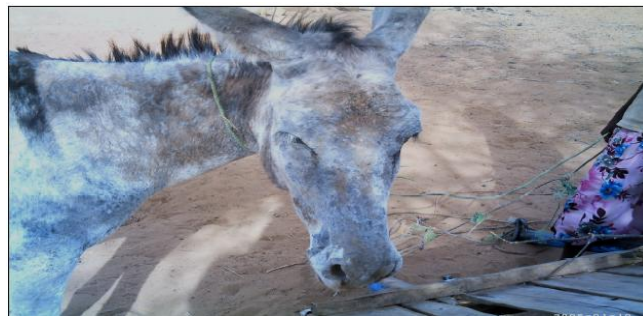


Fig. 8. Ringworm lesions on the head and neck of a donkey



Fig. 9. Generalized skin lesions of dermatophytes in a donkey [18]

5.1 Donkeys

T. mentagrophytes has been isolated [8,18]. While *T. verrucosum* reported Wisal et al. [31]. Recently *Microsporum racemosum* has been isolated from naturally infected donkeys [17].

6. DIAGNOSIS

Diagnosis is based on the history, physical examination, and microscopic examination of scrapings and hairs from the lesions, sometimes in conjunction with fungal culture [13,14].

6.1 Collection of Samples

The surface of the affected area was first rubbed with a cotton swab impregnated with 70% ethyl alcohol to remove surface adhering organisms. Skin scales will be collected by scraping of the margin of the lesion using a sterile scalpel blade into sterile petri dish covered with thin film of paraffin oil. Hairs should be collected by removing dull broken hairs from the margin of the lesion using sterile tweezers as described [32].

6.2 Direct Examination

Hairs and scales can be mounted in potassium hydroxide (KOH) of varying concentrations [33-35]. Infected hairs appear pale, wide and filamentous compared with normal hairs when microscopically examined at x4 or x10 magnification. Arthrospores can be visible on high magnification (x40). Positive result of KOH direct test can lead to positive cultures, which are considered as the gold standard (Fig.10).

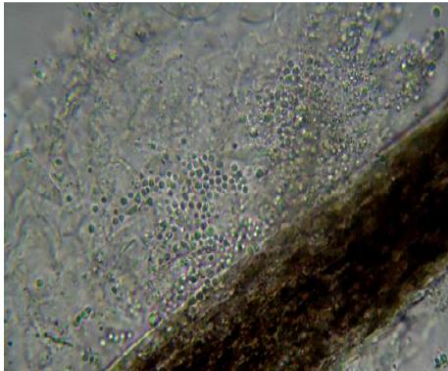


Fig. 10. KOH preparation showing horse hair surrounded with chain of large ectothrix spores [44]

6.3 Fungal Culture

Fungal culture is considered the 'gold standard' for diagnosis [36]. Sabouraud's dextrose agar (SDA) containing cycloheximide, penicillin and streptomycin were used in most diagnostic laboratories [37]. Plates should be incubated at 22-25°C and test weekly for 5 weeks. Dermatophytes test media (DTM) is recommended as the best media for isolation of dermatophytes because the presence of the red color indicated positive result, this can help in early identification of highly suspected cultures [38] (Fig.11). The isolates should be examined macroscopically and microscopically after staining with lactophenol cotton blue using wet mount technique [39] (Figs.12-22).

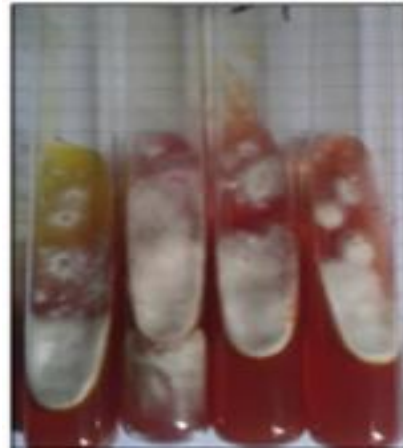


Fig. 11. *M. canis* on dermatophyte test media (DTM) [51]

In addition to technique steps mentioned above, pigment production on corn meal agar, urease activity on urea agar base, growth at 37°C on SDA. It has been recommended that 1–2 drops of a sterile injectable B complex vitamin preparation be added to culture plates when culturing horses, because one strain of *T. equinum* (*T. equinum* var. *equinum*) has a unique niacin requirement [40].



Fig. 12. Surface colony of *T. equinum* var. *equinum* on Sabouraud's dextrose agar [44]



Fig. 13. Downy colony of *M. equinum* [16]



Fig. 14. Pinkish-buff colony of *M. fulvum* [16]



Fig. 15. Colony of *T. verrucosum* on SDA showing button like appearance folded white colour [45]

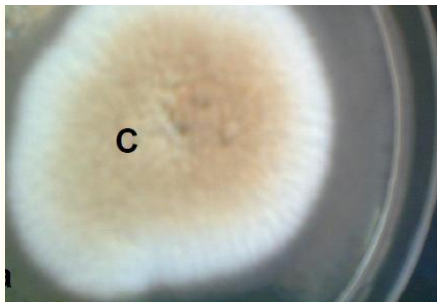


Fig. 16. Colony of *Microsporium gypseum* with flat spreading suede like, deep cream surface, central dome [28]

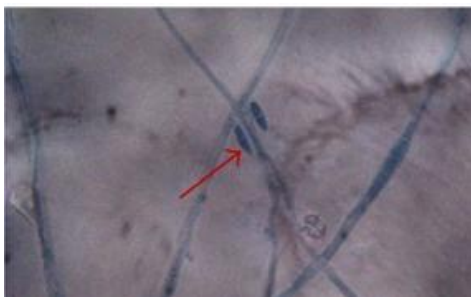


Fig. 17. Two-celled thick walled spindle shaped macroconidia of *M. equinum* [16]

6.4 Skin Biopsy

Skin biopsy from active lesion should be collected after clean with 70% alcohol, the area around the lesion must be injected with local anesthesia drug. 1 mm³ tissue should be cut from the edge of circular raised lesion. Skin samples first fixed in 10% formal saline then 5-6 thick paraffin embedded section should be processed. Before staining, xylene must be used to remove paraffin. Two kinds of stains can be used haematoxylin and eosin for histopathological examination and Grocott methenamine for demonstration of fungal hyphae in tissue sections [40,41]. Diagnosis of dermatophytosis in equine using skin biopsy is less reliable because *Trichophyton* species may cause acantholysis, which look like pemphigus on histopathology [42].

6.5 Molecular Diagnosis

Diagnosis with conventional methods is time-consuming because it might take up to 4 weeks or longer to give the final results [43]. Furthermore, morphological identification may be confusing due to polymorphism of dermatophytes [44]. During the last decade, a wide variety of molecular techniques has become available as possible alternatives for routine identification of fungi in clinical microbiology laboratories [45,46].

T. equinum was identified by application of PCR using non-specific simple repeat sequence (GACA) 4. The result showed the ability of differentiation between of *T. equinum* var. *equinum* and *T. equinum* var. *autotrophicum*, the primer was able to amplify both species forming characteristic PCR profiles for each [47] (Fig.23).

Identification of *T. verrucosum* isolated from infected horses using (GACA) 4 primer amplify two DNA fragments of about 200 and 600 bp that specific to all tested samples [48] (Fig.24).

T. mentagrophytes and *T. verrucosum* isolated from infected donkeys were characterized by molecular markers using (β tubulin gene) A pair of primers, β t2a and β t2b Their sequences were 5'-GGTAACCAAATCGGTGCTGCTTTC-3' and 5'- ACCCTCAGTGTAGTGACCCTTGGC-3' respectively. PCR amplicons by agarose gel electrophoresis revealed amplicon fragments of about 500bp [18] (Fig.25-27).

DNA extraction directly from thirty hair samples was performed using specific primers for



Fig. 18. *M. fulvum* showed one- to three-celled macroconidia with tapered ends (c) (LCB ×400) [16]

dermatophytes group. This PCR resulted in 22 (73.3%) positive samples within 8 hours. While the result of culture examination of horse samples showed that 13 (43.3%) out of 30 were positive [9] (Fig.28).

ITS-based PCR was used for identification of different dermatophytes species isolated from Arabian horses in Egypt [49] (Fig.29).

The dermatophytes isolated from Arabian horses were identified based on PCR of the ribosomal region spanning internal transcribed spacer (ITS1 and ITS2), the 5.8S rDNA and subsequent restriction analysis using *Mva*I and sequence analysis. [50] (Fig.30).

7. TREATMENT

In racing stables, riding schools and stud farms care should be taken to prevent horses from becoming infected. Wherever possible, infected animals should be quarantined. The infected animals should be given a full treatment, particular attention being paid to the lesions but preferably covering the entire surface of the skin. [51].

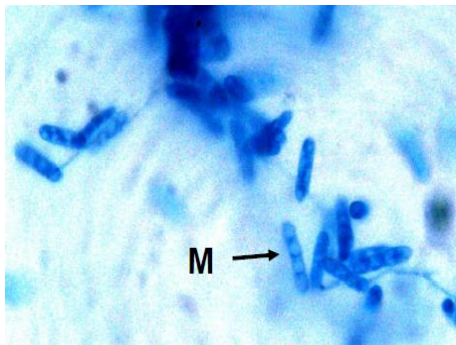


Fig. 19. Lactophenol cotton blue stained smear of *Microsporium gypseum* ×400 showing 4 celled macroconidia (M) with truncated distal ends [28]

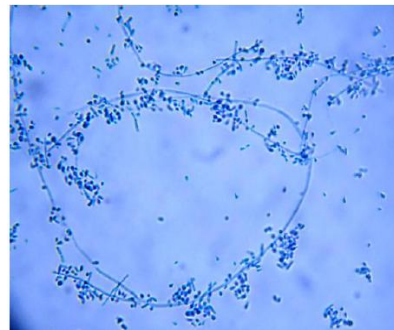


Fig. 20. Microconidia of *T. equinum* stained with LCBP [44]

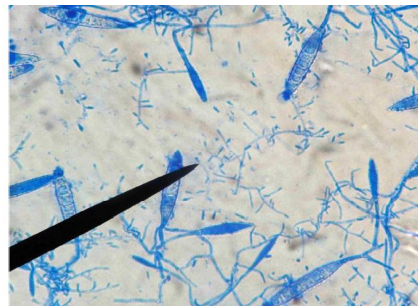


Fig. 21. Microscopic morphology of *Microsporium racemosum* [17]



Fig. 22. Lactophenol cotton blue mount Shows chains of chlamydospores of *T. verrucosum* culture incubated at 37°C [31]

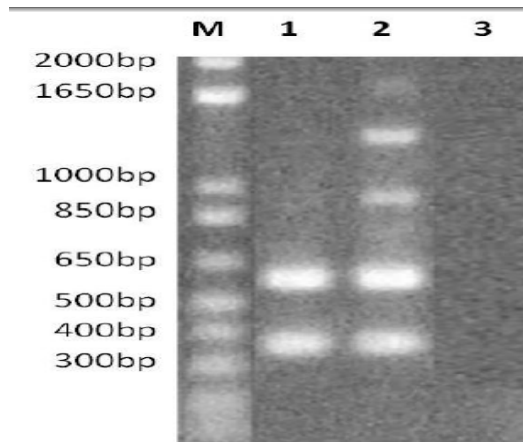


Fig. 23. PCR amplification of genomic DNA samples was carried out with Simple repetitive Oligonucleotide (GACA)₄. Lanes: M, molecular weight marker; 1, *T. equinum* var autotrophicum; 2, *T. equinum* var equinum; 6, and 3, control negative [47]

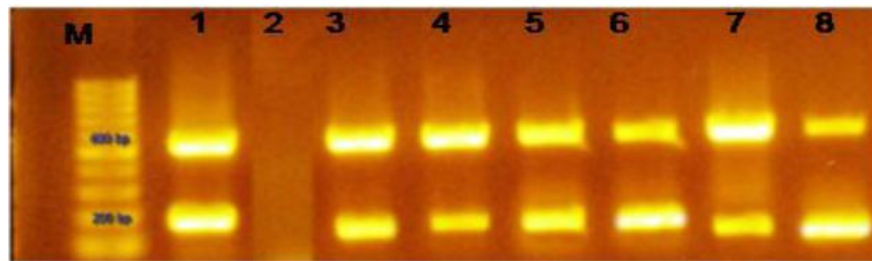


Fig. 24. PCR implication of genomic DNA samples was carried out with simple repetitive Oligonucleotide (GACA)₄ primer lanes M molecular weight marker, C+ control positive, C- control negative and 1,2,3,4,5 and 6 tested samples

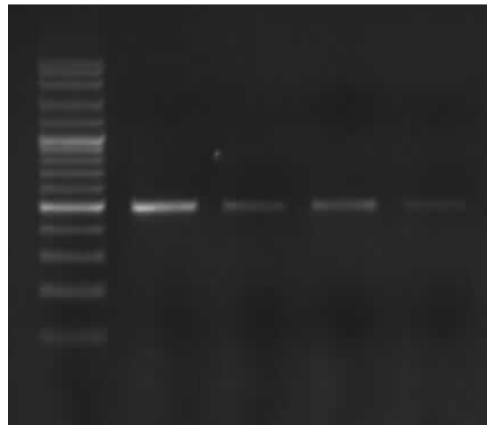


Fig. 25. Gel image of dermatophyte DNA amplified with β tubulin primers with a product size of approximately 500 bp [18]

7.1 Topical Therapy

- 1- 50% captan (2 tablespoons of the powder in 1 gallon of water) [52].
- 2- Lime Sulfurd(1 cup to 1 gallon of water) or bleach (1:10 with water) [52].
- 3- Miconazole or ketoconazole preparation [52,53].

- 4- Enilconazole rinse Wash or spray with diluted emulsion (2000 ppm.) four times at 3-4 day intervals [51].
- 5- Ointment containing benzoic acid 6 g, salicylic acid 3 g, Sulfur 5 g, iodine 4 g and vaseline 100 g [19].
- 6- 2% tincture iodine [30].

days, 1–2 times) should be used in pregnant mares [52].

7.3 Environmental Decontamination

Hypochlorite bleach and enilconazole environmental sprays may be used for environment decontamination [52].

7.2 Systemic Therapy

- 1- Griseofulvin 100 mg/kg daily for 7–10 days [52].
- 2- Instead of Griseofulvin 20% Nal may be given IV (250 ml/500 kg horse every 7

7.4 Vaccination

Inactivated vaccine of *T. equinum* reduced the incidence of new infections and protects 80% of

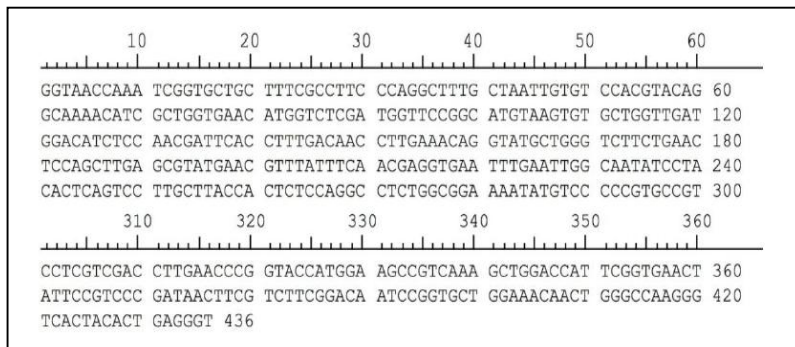


Fig. 26. Partial sequence of *T. mentagrophytes* [18]

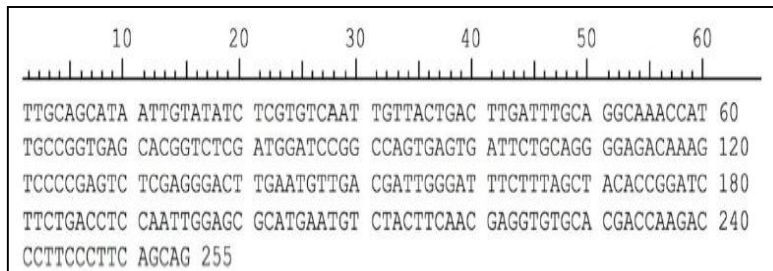


Fig. 27. Partial sequence of *T. verrucosum*[18]

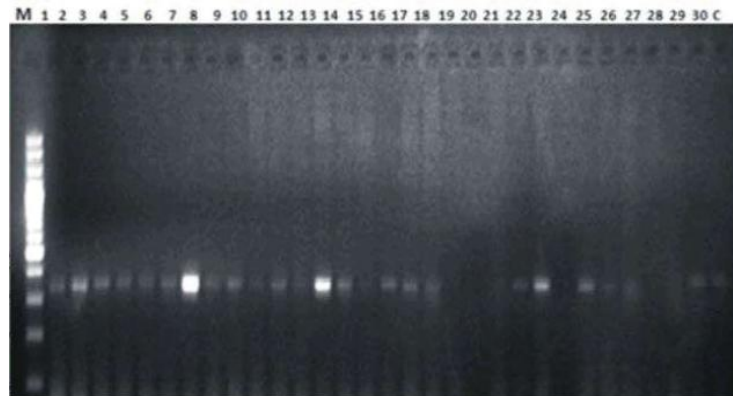


Fig. 28. Agarose gel electrophoresis showing amplification of 366 bp. Fragment of dermatophyte. M: 1 kb marker, lane 1- lane 30: NNA samples. C. Positive control [9]

horses from infection. The vaccine was used for treatment of suspected clinical cases of dermatophytosis 95 % of the cases showed a successful treatment four weeks after the 2nd vaccination [54,55].

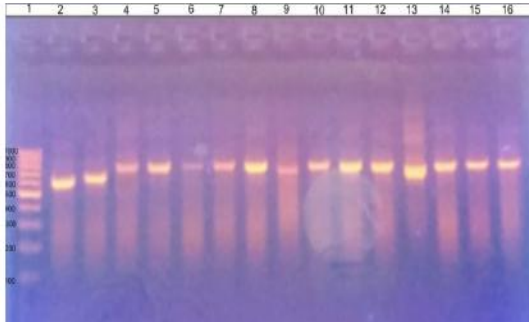


Fig. 29. Intergenic spacer (ITS)-based PCR product analysis for DNA of dermatophyte isolates. Lane: 1 molecular size marker (fragment sizes, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1,000 bp); lanes: 2, 3, 13: *Trichophyton mentagrophytes* at 680 bp; 4, 6, 7: *Microsporum equinum* at 750 bp; 5, 8, 10, 11, 12, 14, 15 and 16: *Microsporum canis* at 720 bp; and lane 9: *Trichophyton verrucosum* at 685 bp [49]

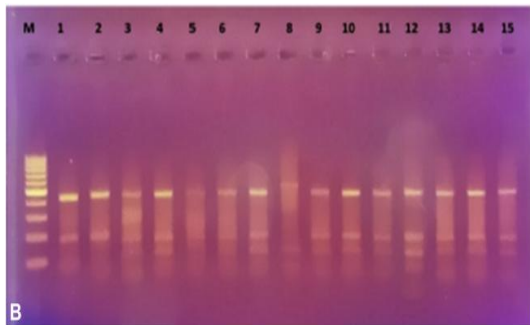


Fig. 30. RFLP patterns of dermatophyte species by Mva1. Lane M, 100-bp marker; lanes 1, 2, *Trichophyton mentagrophytes*; lanes: 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, and 15: *M. canis* and lane 8: *T. verrucosum*. [50]

8. CONCLUSION

Dermatophytoses are the most common fungal infections in equine. Many studies were done considering different aspects of the disease (eg. epidemiology, clinical presentation and diagnosis, treatment, prevention, and control). Due to economic impact and health problem of the disease in equine vaccination and improved hygiene, may be useful for managing ringworm

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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