

Journal of Medicine and Medical Sciences Vol. 5(8) pp. 157-161, August 2014 DOI: http://dx.doi.org/10.14303/jmms.2014.089 Available online http://www.interesjournals.org/JMMS Copyright © 2014 International Research Journals

Case Report

Mycological Studies of *Trichophyton mentagrophyes* var. *mentagrophyes* causing Ringworm in Horses in Al Ahsa Province, Kingdom of Saudi Arabia

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ABSTRACT

The study reports the first confirmed cases of equine ringworm in the Kingdom of Saudi Arabia (KSA) due to lack of reports on dermatophytoses. Extensive lesions of clinical ringworm in two horses were examined in the King Faisal University Veterinary Teaching Hospital equine clinic. Clinical examination showed scales, thick crusts and pruritus on the flanks and back. At the time of presentation, isolated lesions coalesced to form extensive areas with grey, non-exudative and shining patches. Mycological studies were done on skin and hair scrapings using direct, cultural and biochemical methods. It was documented that the causative dermatophyte was *Trichophyton mentagrophytes* var. *mentagrophytes* by various confirmatory tests. This study demonstrates ways for laboratory diagnosis, differential diagnosis and confirmatory tests for horse ringworm dermatophytes. Implications of findings of the study of the zoonotic dermatophyte on Veterinary Health and Public Health are discussed.

Keywords: Ringworm, Horses, Trichophyton mentagrophytes, Extensive infection, Saudi Arabia.

INTRODUCTION

The fungal domain is built up of more than 1.5 million species with a small percentage of pathogens among them. Dermatophytes are a group of related fungi that utilize keratin and adapted to infect superficial layers of the skin of animals and man causing ringworm or tinea. Ringworm is a major Public and Animal Health problem in various regions of the World resulting in great economic loss (Calderone, 1989). The currently known species of dermatophytes, approximately 40 species divided into genera: Epidermopyton, Microsporum, three and Trichophyton, described comyprehensive are in and reviews. The identification textbooks of dermatophytes is based on methods that focus on morphological, physiologic, ecologic, and genetic characteristics (Rebell and Taplin, 1979; Campbell et al., 1996; Kane et al., 1997).

In horses, Microsporum and Trichophyton species have been reported to be the causative agents of dermatophytosis (Quinn and Markey, 2003). *Trichophyton equinum* is the most commonly involved dermatophyte and has been reported in many countries. Other Trichophyton spp. that have been isolated include *Trichophyton mentagrophytes* (Quinn and Markey, 2003). In Sudan, *Trichophyton verrucosum* has been isolated from equine ringworm (Fadlelmula and Idris, 1983). *T. mentagrophytes* is divided into the varieties: *T. mentagrophytes var. interdigitale*, *T. mentagrophytes var. mentagrophytes var. mentagrophytes var. goetzii*, *T. mentagrophytes var. granulosum* and *T. nentagrophytes var, erinacei* to accommodate anthropophilic and zoophilic strains (Rippon, 1988).

Diagnosis of ringworm is often based on the history and clinical presentation, microscopic and cultural features by examination of the obverse and reverse sides of the fungal colonies. In addition, use of selective media, morphological studies, biochemical tests, and hair penetration test are employed (Weitzman and Summerbell, 1995; Shimozawa et al., 1997). Although ringworm in animals is frequently diagnosed in the Kingdom of Saudi Arabia (KSA), reports and investigation on the disease are meagre. For instance, Bagy and Abdel-Mallek (1991) managed to isolate Trichophyton species and Microsporum species associated with apparently-normal horses' hair from Riyadh, KSA. In man, T. mentagrophytes and Microsporum canis were reported to be the most common dermatophytes responsible for tinea infections in a study to determine the characteristics of superficial fungal infections (Abanmi et al., 2008). Another investigation of tinea capitis confirmed the prevalence of zoophilic dermatophytes as T. mentagrophytes and M.canis from the patients (Venugopal and Venugopal, 1993). Thorough search of literature revealed no reports of animal ringworm in KSA in spite of its Veterinary and Public Health importance. Hence, the present study documents the first proven case of horse ringworm and its etiology in KSA.

MATERIALS AND METHODS

Case Study

This report comprises two cases, the first was a 5-yearold male Thorough bred horse referred with skin dermatitis to King Faisal University Veterinary Teaching Hospital. Lesions started as alopecia, severe incrustation, scaling, and pruritus on the left flank and back, which had been in contact with a saddle for 1 year as described by the attendant. Treatment with topical antibiotics by the owner was not effective.

The second case of a 7-year-old male horse with skin lesions.

Microbiological Investigation

Affected areas were cleaned and disinfected with 70% ethyl alcohol. Then, skin scrapings and hair plucks were taken from the active margins of the lesions using sterile disposable scalpel blade. The samples were transported to the laboratory using clean, dry sterile petri dish. In the laboratory, wet mounts were prepared from specimens with 10% potassium hydroxide for direct microscopic examination of typical fungal particles. Cultures were done onto Sabouraud dextrose agar (SDA) (Oxoid) supplemented with cyclohexamide (0.4 mg/L), chloramphenicol (50 mg /L), incubated aerobically at 27 and 37°C and were observed daily for growth of dermatophytes for up to four weeks. For more studies on cultural features, potato dextrose agar (PDA) (Oxoid), cornmeal agar (Oxoid) and 5% horse blood agar was used. The strain was tested for growth on horse hair and on horse stratum corneum at 26 ℃. Natural horse hair was cut into short pieces, washed, rinsed, and autoclaved. Stratum corneum was scraped from healthy and thoroughly cleaned horse skin and autoclaved. This material was placed in sterile distilled water without supplements in petri dishes and inoculated with a suspension of conidia obtained from Sabouraud cultures.

Biochemical study (urease hydrolysis) was done by inoculation parts of colonies from SDA on Christensen urea agar (Difco). As well, culturing on SDA supplemented with 5% salt (Issa and Zangana, 2009) were done to further confirm the identity of the isolated dermatophyte species (Campbell et al., 1996).

RESULTS

In the first case, clinical examination showed scales, thick crusts and pruritus on the flanks and back. At the time of presentation, isolated lesions coalesced to form extensive areas with grey, non-exudative and shining patches (Figure 1).

In the second case lesions were confined to the buttocks part of the body together with the hind limbs (Figure 2). There were scattered small alopecic crusty spots and roughened raised hairy patches that were dark grey in colour.

Direct microscopic examination of hair and skin scrapings showed fungal elements in the form of spores in epithelial cells and exothrix hair sporulation scattered in the tissue (Figure 3). On SDA, at $27 \,^{\circ}$ C growth at a moderate rate was observed for up to three weeks. At 7 days on incubation, white colonies initially then creamy nodular granular on obverse and pale yellowish on reverse were observed. At $37 \,^{\circ}$ C there was no growth but on SDA supplemented with 5% salt, the two isolates yielded growth. On PDA or cornmeal agar, white creamy colonies with granular surface on obverse and pale grey on reverse were seen (Figure 4). On blood agar, as from days 5 a clear zone of β -haemolysis was observed around the colonies (Figure 5).

Wet mounts stained with lactophenol cotton blue showed numerous globose microaleuriospores, sparse mycelia and chlamydospores in old cultures.

A weak positive urease reaction developed on Christensen urea agar within 9 days. Cultures on horse hair showed dense mycelia within 15 days, as did cultures on horse stratum corneum.

DISCUSSION

Conspicuous clinical signs of the ringworm cases of the present study are alopecia, scales and crust formation. Dermatophytes secrete proteolytic and lipolytic enzymes that favour digestion of skin tissue and hair resulting in hair loss, scaly and crusty lesions (Ural et al., 2009) as observed in the present cases. Equine ringworm is a highly contagious infection which is transmitted by direct contact or indirect route through contaminated fomites. Horses of the present study were from different farms in the same area; the possibility exists that source of infection was the spores of dermatophytes that persisted in the farm environment.



Figure 1. Case No.1 horse infected with ringworm showing skin lesions that coalesced to form extensive areas with grey, non-exudative and scaly crusty patches on the back and buttocks.



Figure 2. Case No. 2 horse infected with ringworm showing scattered small alopecic crusty spots and roughened raised hairy patches that were dark grey in colour.



Figure 3. Photomicrograph of a wet mount of skin and hair scrapings from a horse infected with ringworm. There are fungal spores in cells and near hair shaft. (10% KOH,x 200).



Figure 4. Photo of *T. mentagrophytes var. mentagrophytes* colony on potato dextrose agar plate. It shows white creamy colonies with granular surface on obverse and pale grey on reverse.



Figure 5. Colonies of *T. mentagrophytes var. mentagrophytes* on blood agar plate showing a clear zone of β -haemolysis around the colonies.

Clinical signs of ringworm are often diagnostic unless other factors come into play. Hence, laboratory diagnosis by direct microscopic examination of pathological material together with isolation and identification of the causative dermatophyte, is inevitable. Microscopic picture and cultural features of isolates in the present study were suggestive of Trichophyton spp. Other workers reported that many variations of colony morphology are seen among the *T. mentagrophytes* complex (Rippon, 1988). Colonies of isolates in the present study were creamy nodular granular on obverse and pale yellowish on reverse on SDA at 27°C that failed to grow when incubated at 37°C. Growth on salt supplemented SDA has been used to differentiate *T.mentagrophytes* from other Trichophyton spp. (Weitzmann and Summerbell, 1995). Such behavior has been observed on isolates of the present study by ability to grow on SDA supplemented with 5% salt.

Microscopically, salient picture was production of abundant globose microaleuriospores and scarce hyphae. *T. mentagrophytes* zoophilic isolates produce a flat rapidly growing granular cream, yellowish, colony (Rippon, 1988). *T. mentagrophytes var. mentagrophytes* also exhibit a granular colony appearance (Kim et al., 1999),which is demonstrated under the microscope as conspicuous sandy-brown clumps of conidia, which give the colony surface a coarsely granular appearance. Based on microscopic morphology and cultural features. isolates of the present study were identified as T. mentagrophytes var. mentagrophytes constituting the first report of this dermatophyte from horses in the KSA. More studies on prevalence and etiology of ringworm and tinea in man and animals in KSA are needed to plan strategic approaches for control. Further mycological studies and confirmatory tests on isolates indicated their ability to utilize keratin as growth on horse hair and horse stratum corneum was positive. The power of T. mentagrophytes to grow on keratin substrates such as wool, feather, nails, human and animals hairs has been documented (Rippon, 1988). On PDA or cornmeal agar, the isolates produced no pigments which is in agreement with the finding of Philpot (1977) that lack of pigment on cormeal agar and PDA separates T. mentagrophytes from T. rubrum. Urease test was positive indicating the isolates to be T. mentagrophytes as a confirmatory test adopted by other investigators (Issa and Zangana IK, 2009).

Dermatophytes are known to grow best in warm and humid environments and are therefore more common in tropical and subtropical regions but the geographic distribution varies according to the species (Fadlelmula et al., 1994). Zoophilic *T. mentagrophytes* is associated with a wide range of rodents, such as rabbits and relatives, hedgehogs and other small mammals. Although these smaller mammals are probably the primary reservoir, zoophilic *T. mentagrophytes* may also cause infection in horses and other large mammals (Weitzman and Summerbell, 1995). In light of findings of the present study a molecular epidemiological survey of the dermatophytes in KSA is needed to best serve the interest of Veterinary and Public Health.

In conclusion, a detailed mycological study on ringworm of horses using conventional techniques revealed the causative dermatophyte to be *T. mentagrophytes var. mentagrophytes.* It is recommended to carry out investigations on human and animal ringworm in different regions of the KSA due to lack of data on the subject.

ACKNOWLEDGMENTS

The author is thankful to king Faisal University Scientific Research Deanship. The author also thanks Prof. **A. Fadlelmula** for his contribution, and extends thanks to Abdullatif Alsagar for cooperation and help in this study.

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How to cite this article: Shathele M.S. (2014). Mycological Studies of *Trichophyton mentagrophyes* var. *mentagrophyes* causing Ringworm in Horses in Al Ahsa Province, Kingdom of Saudi Arabia. J. Med. Med. Sci. 5(8):157-161