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Identification of Different DermatophytesIsolated From Cattle, Cats and Horses Suffered From Skin Lesions

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ABSTRACT

Dermatophytes are considered one of the important zoonotic superficial fungal skin diseases in different animal species in the world especially among immunocompromised, stressed and senile individuals. The disease affects the animal productivity, leather industry (cattle), animal performance (Horses) and zoonotic importance (cats).our study focused on three main species cattle, horses and cats with skin lesions suspected to be ring worm to determine the different causes. Diagnosis was carried out using direct examination of skin scraping, isolation of the fungus using FungAssay and microscopic determination of fungal spores and microconidia. Different species of dermatophytes were isolated from calves including, *Microsporumcanis* and *Microsporum gypsum*, while in horses *Trichophytonmentagrophytes* and *Microsporum gypsum* were isolated and in cats *Trichophytonmentagrophytes* and *Microsporumcanis* were isolated. Mixed infection was reported in some cases. We could conclude that the isolated fungi are *Microsporumcanis,Trichophytonmentagrophytes* and *Microsporum gypsum*.

Key words: Dermatophytes, Microsporum, Trichophyton, Cattle, Horse, cats.

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1-INTRODUCTION

Dermatophytes are one of the important fungal diseases affecting wide range of animal species including cattle, buffaloes, sheep, goats, dogs and cats. It has a severe zoonotic impact as it induce several affections in human beings. The disease is highly contagious and present all over the world.

Dermatophytosis in animals and human are caused by three main genera of fungi in the class Euascomycetes including Microsporum, Trichophyton and Epidermophyton with each including several species. Dermatophytes are grouped according to their habitat as being either anthropophilic (human associated), zoophilic (animal associated), or geophilic (soil dwelling) as mentioned by Achterman and White 2012.

Most outbreaks of Dermatophytosis in cattle occurred in fall and winter months with inside housing due to over crowdness and contact with infected objects as mangers and walls. Cattle under one year old are more susceptible to the infection especially under stressful conditions like transportation and during weaning. Clinically affected cattle suffered from circumscribed area of alopecia, scaling and crusts with thickness of 8-10 mm in some cases with 10-50 mm in diameter. Lesions exfoliate, exposing dry, airless and floury looking skin and mostly seen on head (around eyes) and in some cases may be seen on chest, rump and dewlap. (Morretti*et al.,* 2013)

Dermatophytosis in cattle is mainly caused by *Trichophytonverrucosum* also *trichophytonmentagrophytes* can cause the disease in some cases and this may be attributed to the presence of rodents. Microsporumcanis isolated from diseased cases in contact with dogs (Scott 2007, Morretti*et al.,* 2013 and ElAshmawy et al. 2015)

Ring worm in cattle is very contagious to farmers, veterinarians, veterinary technicians and workers inside the farms. Employees with occupational Dermatophytosis are entitled for two-week-sick leaves which create difficulties for farm management as temporary workers have to be sourced (Chermette*et al.* 2008).

Dermatophytosis cause reduced weight up to 10-13 kg/butchered animals in beef cattle and lower milk yield in dairy cattle. Poor hide quality for the leather industry as it make the hides unsuitable for tanning so they are discarded or fetch much lower sums. The British leather confederation estimated losses due to poor quality hides at 35 million annually with 5% due to Dermatophytosis (Chermette*et al.* 2008 and Morretti*et al.,* 2013).

In horses, Dermatophytosis predominates during the winter and can rapidly develop into an epidemic.

The infection is more common in livery stables and ridding schools especially in young ages. Lesions mostly appeared on upper chest, flanks, shoulders and areas exposed to trauma and contacts with saddle and bridles. *Trichophytonequinum*, *Microsporumcanis* and *Trichophytonmentagrophytes* are the most common fungi infecting horses and also *M. gypsum* was isolated from some cases (Moretti et al., 1998).

Ring worm caused by *T. equinum* and *M. canis* are typically dry forms characterized by small tufts of spiky hair which soon fall out leaving alopecic area covered by flaky grayish scales with underlying dry integument. Lesions usually are few in numbers and small size 25 mm in diameter and none itching.

Feline Dermatophytosis is very important zoonotic fungal disease, occurred worldwide. It's caused mainly by *Microsporumcanis* in more than 90% of cases, other species like *M. gypsum*, *T. mentgrophytes*, *T. quinckeanum* and *T. verrucosum* were also isolated from some cases (Frymus*et al.* 2013). It's very common among young ages, immune suppressed animals or animals with malnutrition especially in case of high temperature and humidity (Moriello and DeBoer 2012). Typical presentation of Dermatophytosis in cats is regular and circular alopecia with hair breakage. Clinical lesions ranged from very small size to generalized lesions in some cases due to coalescence of small lesions and occasionally may has diameter of 5-6 cm in diameter. In young ages, lesions are usually appeared on the head localized to the nose bridge then extend to external sides of ear pinnae and auricular margins then to legs and may appear on body and tail in some cases. Pruritis is variable, ranged from mild to moderate in some cases (Frymus*et al.* 2013 and Chermette*et al.* 2008).

Laboratory diagnosis of Dermatophytosis is carried out by different methods including, direct microscopical examination, culturing on sabaroude dextrose agar or other fungal media and by molecular identification using PCR.

Direct microscopical examination is a simple and rapid method carried out on hairs and scales using KOH 20%. It's a rapid method but has poor sensitivity (56%) and may give false positive results especially if saprophytic fungal spores are present in the sample (Sparkes*et al.* 1993).

Fungal culture is carried out on sabaroude dextrose agar or other specific culture media. Samples including hairs and scales should be collected from the margin of the lesions after gentile swabbing with ethyl alcohol 70% to reduce the risk of contamination. It is the gold standard for detection of dermatophytes and this method can determine the species (Moriello and DeBoer 2012).

The objective of our study is to identify the different types of dermatophytes isolated from clinically affected calves, horses and cats with skin lesions suggestive to be ring worm infection.

2- MATERIALS AND METHODS

2-1-Animals

The study was carried out on 5 cats, 2 horses and 20 calves. Calves were belonged to a fattening farm has 200 calves recently received, lesions appeared within 2 weeks of receiving. Cats were admitted to a private clinic while horses are kept in private stables and calves were recently received in a beef farm. All animals were examined clinically and they were suffered from skin lesions suspected to be ring worm. Cats were under 6 months old and lesions are present on the head and face, 2 cats have severe lesions and distributed all over the body. Horses were 6 and 9 months of age and lesions are on the ears and face. Calves were 9-12 months old and lesions are mainly on head and neck, few lesions were seen on the trunk and back.

2-2-Sampling:

Skin scraping was collected from the periphery of the lesions after cleaning with ethyl alcohol 70% using sterile disposable scalpel blade. The collected samples including hair tufts and scales were kept in sterile falcon tubes and sent for fungal examination at laboratory of Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

2-3-Mycological Examination:

The collected specimens were examined for the presence of dermatophytes using different methods including:

2-3-1-Direct Microscopical Examination:

All samples were examined for detection of fungal elements. Some scales and hair tufts were treated with KOH 10% for 10 minutes then examined under microscope for fungal spores and hyphae under 100X and 400X magnifications (Larone, 2011).

2-3-2-Mycological Culture:

FungAssay® Dermatophyte Test Medium is a culture medium that provides the veterinarian with a simple, rapid and practical method for confirming

diagnosis of dermatophytes infections. The test is based on a colour change within the medium from amber to red (Taplin*et al.*, 1969), caused by the growth of pathogenic fungi, such as Microsporum and Trichophyton species. Collected samples were cultured and the tubes were incubated at 25 C for 4 weeks. Tubes were examined daily for fungal growth. The isolates were identified macroscopically according to the colony growth. (Larone, 2011).

Microscopic examination of the fungal growth was carried out for identification of different species of dermatophytes using lactophenol cotton blue stain for determination of fungal hyphae, spores and microconidia (Larone, 2011).

3- RESULTS

The study was carried out on 20 calves, 5 cats and 2 horses suffered from skin lesions suspected to be ring worm infection. Skin lesions were examined clinically and proved to be positive by direct microscopical examination of skin scraping. Fungal culture was also applied using FungAssay® and different types of dermatophytes were identified through macroscopical and microscopical examinations of the fungal growth.

Clinical examination of the skin lesions of affected animals revealed that, the examined calves had circumscribed area of alopecia filled with heavy asbestos like scales. Lesions are distributed all over the body but they are more severe at head and neck (fig. 1a). In horses, the examined animals had circumscribed area of alopecia had raised borders and filled with white scales. Lesions are more prominent at head and ears (fig. 1b). In cats, lesions in the examined animals had wide variations in the severity ranged from one or two lesions (circumscribed area of hair loss with white scales) to complete alopecia in some cases. Lesions are commonly seen on head and ears (fig. 1c) or coalesce to form large area of alopecia all over the trunk (fig. 1d).

Microscopical examination of the clinical specimens, clinical specimens (scales and hair tufts) were treated with KOH 10% for 10 minutes then examined microscopically using 100X and 400X magnification for presence of fugal hyphae and spores (endothrix and exothrix) (fig. 3a)

Macroscopic Examination of the culture (Colony character), fungal culture was applied using FungAssay® from the clinical specimens. Different types of dermatophytes were identified according to fungal growth. Colonies of T. Mentagrophytes appeared as moderately rapid, powdery to granular, white to cream coloured on the surface. Colonies of*Microsporumcanis* were downy, rapid wooly with cream to yellow colour. Colonies of*Microsporum gypsum* appeared rapidly and become powdery to granular with cream or pale cinnamon colour on the surface..*Trichophytonmentagrophytes* and *Microsporum gypsum* were identified from horse samples. *Trichophytonmentagrophytes* and *Microsporumcanis* were identified from skin samples of cats (fig. 2 a,b,c) while saprophytic fungal growths were excluded from our study (fig. 2d).

Microscopic examination of the culture, microscopic examination of the fungal growth was carried out to identify the infecting type of dermatophytes. Identification was applied according the characteristic shape of fungal hyphae, macroconidia and microconidia of different types of dermatophytes. Microscopic examination revealed thick-walled macroconidia of *Microsporum spp*. (fig. 3 b,d,e,f), while *Trichophyton spp.* isolated in our study failed to form macroconidia and showed only fungal hyphea and microconidia. The microconidia of T. mentagrophytesare numerous, unicellular, round to pyriform and found in grape like clusters (fig. 3c).

4- Discussion:

Dermatophytes are an important cause of skin superficial fungal infections in animals and man. Diagnosis of dermatophytes depends primarily on microscopical examination of skin scraping using KOH 10%. It's thesimplest and cheapest method as mentioned by Panasiti*et al.* 2006. Identification of dermatophytes is based mainly on cultural and morphological characteristics but it's complicated and laborious due to morphological similarity, variability and polymorphism according to Toshio, 2008.

Figure 1. Clinical examination of the skin lesions of affected animals revealed that, the examined calves had circumscribed area of alopecia filled with heavy asbestos like scales. Lesions are distributed all over the body but they are more severe at head and neck fig (1a). In horses, the examined animals had circumscribed area of alopecia had raised borders and filled with white scales. Lesions are more prominent at head and ears fig (1b). In cats, lesions in the examined animals had wide variations in the severity ranged from one or two lesions (circumscribed area of hair loss with white scales) to complete alopecia in some cases. Lesions are commonly seen on head and ears fig (1c) or coalesce to form large area of alopecia all over the trunk as in fig (1d).

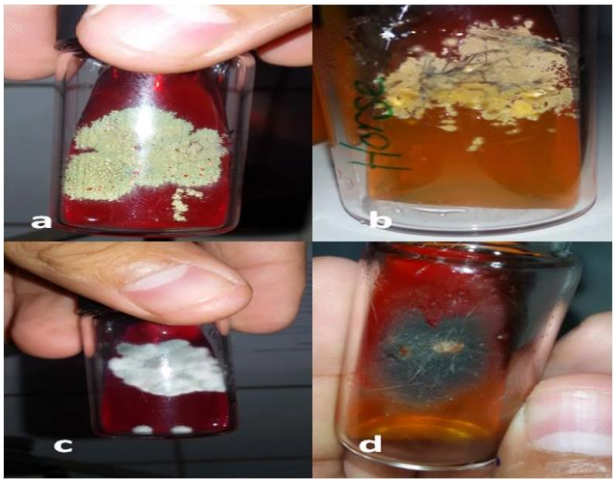

Figure 2. Fungal culture was applied using FungAssay® from the clinical specimens. *Microsporumcanis* and *Microsporum gypsum* were identified according to the morphology of the grown colonies from calves' samples. *Trichophytonmentagrophytes* and *Microsporum gypsum* were identified from horse samples. *Trichophytonmentagrophytes* and *Microsporumcanis* were identified from skin samples of cats fig (2 a,b,c) while saprophytic fungal growths were excluded from our study fig (2d).

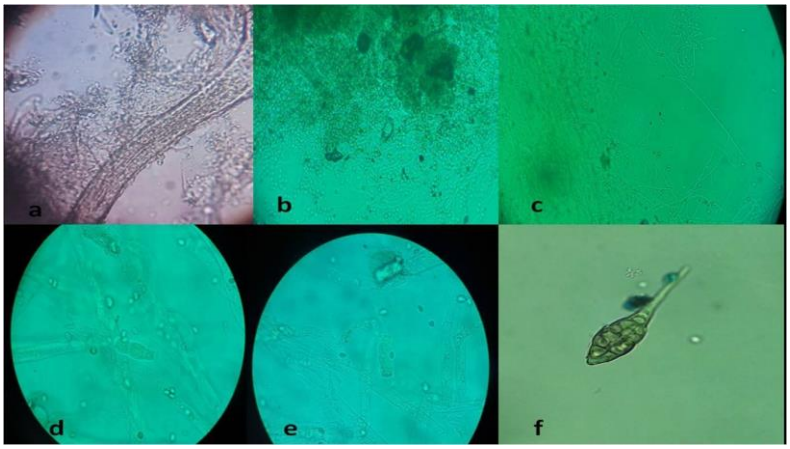

Figure 3. Fugal hyphae and spores (endothrix and exothrix) from clinical samples examined microscopically using 400X magnification 3(a). Microscopic examination revealed thick-walled macroconidia of *Microsporum spp*. as in fig (2 b,d,e,f), while *Trichophyton spp.* isolated in our study failed to form macroconidia and showed only fungal hyphea and spores as in fig (2c)

According to clinical examination of the diseased calves, lesions appeared on 20 calves out of 200 (10%). The examined herd has calves recently received and they were stressed due to

transportation for longer periods and changing of housing and feeding system. Affected calves suffered from circumscribed lesions of alopecia filled with heavy white scales. Lesions are commonly distributed on head and neck and this agreed with Aghmirian and Ghiasian, 2009. Lesions appeared due to several stress factors as winter months which increase the contact between animals and help in transmission of infection also lesions appeared after 2 weeks of transportation as recorded by Radosttis et al., 2007, Scott 2007 and ElAshmawy et al., 2015.

Clinical examination of horses revealed circumscribed area of alopecia with raised borders and filled with white scales on head and ears as described elsewhere in Abdallah (1971);Refai (2013).

Clinical examination of affected cats' revealed circumscribed area of hair loss with white scales appeared on head and ears. Lesions coalesce to form large area of alopecia all over the trunk in some cases and this may be due to either the cat is young, sick, debilitated or old animals. On the other hand, concurrent diseases, as immunodeficiency virus, may increase the susceptibility to infection (Manciantiet *al.* 1992). Longhaired animals are at higher risk due to hereditary factors and/or the fact that spores stick to their hairs (‘fungus friendly’ environment) as mentioned byMoriello (2004). Cats that failed to groom themselves are at more risk as grooming recognized to be host defense mechanism as stated by DeBoer and Moriello (1994).

Dermatophytosis can be diagnosed direct microscopic examination of skin scraping and detection of fungal spores distributed in relation to hair, either endothix or ectothrix. It's a rapid method but the sensitivity is low so it confirms the presence of dermatophytes but not exclude the infection with them and it may detect saprophytic fungi as mentioned by Sparkeset *al.* 1993.

Dermatophytes produce colonies that vary in texture. M. canis colonies have wooly fluffy filamentous appearance with cream to yellow colour, M. gypseum shows velvety-powdered filamentous colonies with cream or pale cinnamon colour on the surface, while T. mentagrophytes colonies have granular-powdery appearance white to cream coloured on the surface and these results agreed with Dion (1978), Dvorak and Otcenasek (2013) andRefai (2013).

Pathogenic dermatophytes change the colour of the media of FungAssay® into red colour while Non-dermatophyte*spp.* can't so the FungAssay® media has the ability to distinguish pathogenic from non pathogenic fungi. This is attributed to that it contains a selective antifungal antibiotic, cycloheximide, which suppresses the growth of many saprophytic fungi, and an indicator, phenol red, which changes the colour of the medium from yellow to red above pH 7. All dermatophytes (ringworm fungi) release ammonia when digesting peptone thus turning the medium red, whereas most nonpathogenic fungi tend to maintain a slightly acid environment during the early stages of growth as mentioned by Dion, 1978 and Guillot*et al.* 2001.

Microscopical examination of the isolated colonies showed hyphae and spores of different fungi. Macroconidia of *Microspourmspp.* appeared as spindle-shaped or fusiform with rough walls, while the macroconidia in *Trichophyton spp.,* may be absent or present in a few number this may be due to special medium requirement and these results agreed with Kane and Fischer (1973);Refai (2013).

In calves different species of dermatophytes were isolated including, *M. canis* and *M. gypsum*. They are not the common dermatophytes as the most common species in calves is T. verrucosum and this may indicate that the source of infection may be infected dogs as mentioned by Moretti*et al.,* 2013.

In horses *T.mentagrophytes* and *M. gypsum* were isolated and those species were also isolated from horses as reported by Moretti et al., 1998. In cats *T. mentagrophytes* and *M. canis* were the isolated dermatophytes and these results agreed with the results of Frymus*et al.* 2013.

Ring worm infections in calves and horses usually associated with stress factors such as transportation, bad weather specially winter months and malnutrion while in cat's lesions appeared as localized or generalized and are common in young ages and long hair breeds.

5- CONCLUSION

We could conclude that dermatophytes infection is a common problem in calves, cats and horses. Different species of dermatophytes were isolated from calves including, *Microsporumcanis* and *Microsporum gypsum*, while in horses *Trichophytonmentagrophytes* and *Microsporum gypsum* were isolated and in cats

Trichophytonmentagrophytes and *Microsporumcanis* were isolated.

Ring worm infections in calves and horses usually associated with stress factors such as transportation, bad weather specially winter months and malnutrion while in cat's lesions appeared as localized or generalized and are common in young ages and long hair breeds.

Skin scraping using KOH 10 % is an appropriate method for early diagnosis of dermatophytes but it can't differentiate between pathogenic and saprophytic fungi, while FungAssay® is considered more rapid and applicable method for identification of fungi as it depend on color change of the media and the colony characteristic. It can differentiate between pathogenic and saprophytic fungi.

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