

Morphological detection of dermatophytes isolated from cattle in Wasit province

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Abstract

This study aimed to diagnose the skin lesions in cattle which were suspected to be ring worm in order to determine the species causing these lesions. A total of 40 samples of skin desquamation and hair lesions were collected from cattle in a farm located in the Wasit province during the period October 2021 to March 2022. Diagnosis used to be carried out the usage of direct microscopy of potassium hydroxide, isolation of the fungus the use of Sabouraud dextrose agar supplemented with chloramphenicol and cycloheximide, as well as by way of detecting those fungi through staining with Lactophenol cotton blue. The diagnosis results revealed that infection of cattle with ringworm represented 20/40 (50%) of skin lesions and the majority of the common *dermatophytes spp* was identified as *Trichophyton mentagrophytes* 15/20 (75%) and *Microsporum canis* 5/20 (25%) depending on the fungal growth. In conclusion, the most common skin lesions of cattle in Wasit province were identified as ring worm.

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Introduction

The dermatophytes can be considered as one of the most common fungi that affect different animal species such as cattle, buffaloes, sheep, goats, puppies, and cats (1). The condition is regarded as an extremely contagious and prevalent worldwide. According to, dermatophytes are classified as either anthropophilic (related with humans), zoophilic (associated with animals), or Geophilic (associated with earth) (1,2). Dermatophytosis is known to belong to class Ascomycetes, Phylum: Ascomycota, family arthrodermataceae and genus Arthroderma and divided into 3 genera: Epidermophyton, Trichophyton and Microsporum. Different dermatophytes are of zoonotic importance in which the most species of dermatophytosis public health concern are belonging to Trichophyton and Microsporum species and its distribution is reported in different animals according to geographical location, age, sex of affected animals (1,3).

Most of the determined lesions in the affected animals had been circumscribed grayish-white place of alopecia, crusty, raised lesions. These lesions commonly are noticed on different area of the body such as head, neck, chest area, dewlap and limbs (4). The severity of lesions differs from mild to severe, which may be attributed to many reasons like reaction of the host to metabolic products of fungi, virulence and type of affected species of dermatophytes and environmental risk factors (5). Ringworm in cattle is considered to be very contagious to farmers, veterinarians, veterinary technicians and workers inside the farms. Employees who are affected with occupational dermatophytosis are entitled for 14 days-sick leaves leading to difficulties creation for farm management as temporary workers have to be sourced (6). One of the most important techniques for analyzing fungi infections is potassium chloride (KOH) mount, which is used as a primary screening tool. Skin scrapings and hair samples need to be cultured to

discover the fungal isolate the usage of conventional Sabouraud Dextrose Agar (SDA), Dermatophytes Test Medium (DTM), and other fungus culture media depending on the kind of isolate expected (7,8). If not treated, these pathogenic microorganisms may have a deadly effect on hosts and have a significant impact on animal fitness. There are many identified proteolytic enzymes generated from microorganisms (9).

Report the animal diseases that were picked up at the University of Mosul's Veterinary Teaching Hospital between 2017 and 2019., in this analysis, clinical data of 1,280 instances examined and recognized at the inside medicinal drug part and/or medical pathology laboratory had been used, the most acquired instances have been from locations inside the southern part of Mosul, regularly, respiratory infections, blood parasites, and gastrointestinal disorders were the most often reported cases. Skin conditions were also frequently acquired (10). Epidemiological research as measurements of ailment prevalence and threat issue analysis, transmission in cattle herds is of extraordinary significance to be studied, many danger elements had been accountable for prevalence of ailment in animals consist of cattle breed, age. Additionally host unbiased elements are viewed extra vital and encompass herd size, administration practices and cattle buying (11). In many nations around the world, dermatophytes are recognized as one of the leading reasons why domestic animals experience dermatological issues. Additionally, they have been linked to dangerous infections, especially in people with impaired immune systems (such as AIDS, organ transplantation, diabetes mellitus, etc.) (12). Due to its propensity to spread zoonotic diseases and in the context of veterinary care for pets in particular, dermatophytosis is regarded in many regions across the world as a major disease. Because despite their age, gender, or breed, a variety of animal and rooster species are susceptible to infections, though it tends to occur more commonly in young, ill, and older patients. The geographical changes, meteorological variables, and herbal host variances all affect the occurrence of dermatophytosis (13,14).

The main method of diagnosing dermatomycoses is the direct microscopic inspection of scientific samples for the identification of septate hyphae. Direct microscopic inspection is quick and less expensive, but it no longer provides genus or species identification and, in 5% to 15% of cases, the results are mistakenly disastrous (15,16). Cultures are examined macroscopically and microscopically to identify species. Although more accurate, culture-based identification often takes two to three weeks. Sometimes it is necessary to utilize subculture or unusual media to promote condition or the production of pigment, which also delays the prognosis by a few weeks (17). Due to phenotypic pleomorphism and interspecies morphological variability, phenotypic identification can also be challenging (18). In the Netherlands, between 1991 and 2002, only 56% of 1,171 dermatophyte isolates were successfully identified by 50

laboratories in a best management exercise (18). The dermatophyte isolates from the same species differ in terms of genotype and phenotype. These versions' medical software still has to be found. These revisions no longer reflect changes in epidemiological or symptomatological data (19). *Microsporum canis* is the type species in Sabouraud dextrose agar at 25°C, the components of the colony are planar, with a velvety or cottony surface, a white or yellowish hue, and a brown or golden yellow reverse. Macroconidia are characterized by the presence of stiff partitions, which may also be asperulate, echinulate, or verrucose. They might be cylindrofusi form, obovate (egg-shaped), or fusiform. They can range in size from 6 to 106 with the aid of 6 to 25 mm and can have thin, moderately thick to thick partitions as well as 1 to 15 septa. Microconidia are typically arranged singly next to the hyphae or in racemes, and they are sessile or stalked and clavate (20). *Trichophyton mentagrophytes var.* is the kind species. In Sabouraud dextrose agar at 25°C, have flat, white to cream-colored, powdery to granular colony factors and reverse yellowish brown to reddish-brown coloration. When present, macroconidia are borne singly or in clusters, have smooth, regularly narrow partitions, one to twelve septa, and can additionally be elongate and pencil-shaped, clavate, fusiform, or cylindrical. They vary in dimension from eight to 86 via 4 to fourteen millimeters. Microconidia, which are normally large than macroconidia, can additionally be globose, pyriform, or clavate, as properly as sessile or stalked, and are borne singly alongside the hyphal components or in clusters that resemble grapes (20). The objective of our study was to identify the types of dermatophytes isolated from clinically affected cattle with skin lesions suggestive to be ringworm infection.

Materials and methods

Ethical approve

This study ethically approves under the terms license by the following code UM.VET.2021.063.

Animals and clinical examination

The study carried out a total of 40 samples of skin desquamation and hair lesions, which were collected from cattle, aged 2 to 5 years from one farm, in the Wasit province during the period October 2021 to March 2022. The main symptoms were alopecia sites, thickened scaling epidermis in various body regions, primarily on the back, thorax, and neck, with a clear propensity for hair loss (Figure 1). For collection of the samples, areas were perfectly disinfected with 70% ethyl alcohol. Skin scrapings and hairs were collected aseptically using sterilized scalpel from the edges of active lesions placed on a clean fresh carbon paper to avoid being contaminated and samples were labelled with serial numbers. Location, sex, and age of lesions on the body were recorded and stored in 4°C in the cold box to keep

samples during transportation to microbiology laboratory in Veterinary Hospital in Wasit to culture the sample on sabouraud dextrose agar.



Figure 1: The areas of the body most frequently afflicted by ringworm in the facial, back, and area around the nose.

Mycological examination

The collected specimens were examined for the presence of dermatophytes using variable methods including, direct microscopically examination, all samples have been examined to discover the fungal elements. Scales and hair tufts were handled with KOH 10% for 10 minutes and examined under microscope (100x and 400x magnifications) to observe fungal spores and hyphae (7). Collected samples were cultured on a common fungal culture medium Sabouraud Dextrose Agar (SDA), containing chloramphenicol 0.05 g/ml and cycloheximide 0.5 g/ml. Samples were incubated at 25°C and examined for growth for 4 weeks after and the plates which showed no growth were deemed negative (21). Macroscopic Examination of the culture involved the colony character, growth rate, surface and reverse color, and colony consistency. Lacto phenol cotton blue was originally used for microscopic evaluation of positive fungal colonies (22).

Results

The study was carried out on 40 cattle, which were affected with skin lesions suspected to be ringworm infection. Skin lesions were clinically examined and proved to be positive by direct microscopical examination of skin scraping. Fungal culture was also applied using sabouraud dextrose agar and different types of dermatophytes were identified through macroscopical and microscopical examinations of the fungal growth. Following incubation at 25°C for 4 weeks, the results of skin scraping lesion culture were 20/40 (50%). The majority of the common dermatophytes spp was identified as *Trichophyton mentagrophytes* 15/20 (75%) and *Microsporum canis* 5/20 (25%) were identified according to fungal growth, Colonies of *T. mentagrophytes* on sabouraud dextrose agar appeared as moderately rapid, powdery to granular, white to cream coloured on the surface. Colonies of *Microsporum canis* were flat to slightly groove with a fuzzy to cottony feel, the

color ranges from white to yellowish on the front and from deep yellow to orange on the reverse. Under microscopic examination of the culture, colonies of *T. mentagrophytes* gave rise to branched conidiophores. Sessile microconidia were formed on the conidiophores in quite dense, grape-like clusters. While colonies of *Microsporum canis* were characterized by septate hyphae, long macroconidia, stiff, and had strong exterior cell walls. They were spindle-shaped, with an asymmetrical apical knob (Figures 2 and 3).

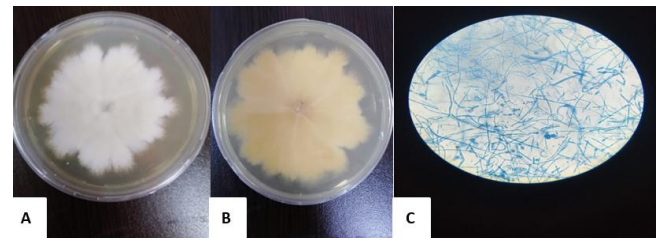


Figure 2: *Trichophyton mentagrophytes* - 14 days growth on Sabouraud dextrose agar at 25°C, A- front colony, B- Revers colony, C- Showing production of sessile microconidia along septate hyphae stained by LPCB.

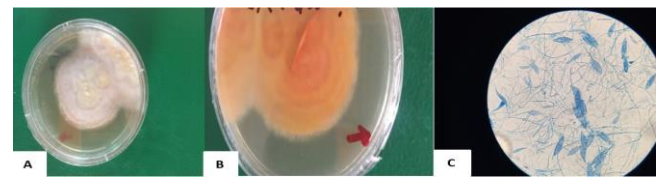


Figure 3: *Microsporum canis*- 10 days growth on Sabouraud dextrose agar at 25°C, A- front colony, B- Revers colony, C- Typical spindle-shaped macroconidia of *Microsporum canis* stained by LPCB.

Discussion

Many years ago, veterinary publications have been described ringworm, which can be considered an important animal illness. Several factors such as restricted herd movement, hygiene practices, and treatment of diseased animals have a little have an impact on on the disease condition globally (23).

In this study, mycological examination of the skin lesion lesions revealed that *Trichophyton mentagrophytes* 15/20 (75%) was endemic in the studied area and *Microsporum canis* 5/20 (25%). This result showed higher than reported in Baghdad, Iraq, Sudad and Mohammed (21), who isolated *Trichophyton mentagrophytes* 5 (14.3%) and *Microsporum canis* 1 (28%) from cows. in Southern Iraq, Al-Duboon and Farhan (24), proved that most frequently employing the species of dermatophytes *Trichophyton mentagrophytes*, *Microsporum gypseum*, and *Trichophyton verrucosum*, were isolated from 182 specimens representing the global incidence of dermatophytosis in 4 ruminants (buffalo, camel,

cow, and sheep). These different results could be attributed to many factors such as a seasonal variation and their effect on microbial composition, economic conditions, and disease incidence which varied from country to country. In the present study, the percentage result of *Trichophyton mentagrophytes* was higher than these obtained in Baghdad, Iraq by Al-Samarrae *et al.* (25), who reported that the proportion was 5 (3.6%). These differences in the proportions of each study may result from the difference in the number of isolates enrolled in each study and the skills of laboratory investigators.

In the current study, the proportion of each of the isolated *Trichophyton mentagrophytes* 15/20 (75%) and *Microsporum canis* 5(25%), which were incompatible with documented in Egypt, Mohammed *et al.* (1), who reported the percentage of *Trichophyton mentagrophytes* was 13(27.7%) and *Microsporum canis* 8(17%). This distribution sample may have also been influenced by the recent and widespread dissemination of these specific strain types, which may also exhibit increased virulence, invasiveness, or other virulence-related traits. *T. mentagrophytes* and *T. verrucosum* were two successfully described species, as mentioned in Plateau State, Nigeria (26). In the present study the percentage of obtained *Microsporum canis* was in agreement with a previous study done in Egypt, Wagdy and Mohamed (6) who isolated this species from cattle skin lesions.

Dermatophytosis could be recognized below direct microscopic examination of skin scraping and detection of fungal spores distributed in relation to hair. This technique was rapid, simplest and most inexpensive method as mentioned by Panasiti *et al.* (27). In current find out about result unwell matched with they find out reveals *T. verrucosum* in cattle for the first time reveals that the incidence rate of cattle fungal skin disease is higher in the summer and winter in China's Ningxia Hui Autonomous Region Guo *et al.* (28). The summer months are hot, humid, and full of sunshine, which speeds up the metabolism of skin cells and causes them to produce more sebum, a lot more sweat, and other waste products. As a result, the skin's subcutaneous environment is altered, which decreases the cattle's disease resistance and encourages the growth of fungus. Because it's chilly outside and the herd has gathered, dermatophytosis is more likely to develop and spread during the winter. Meanwhile, the frequency is higher in the iciness than in the summer (29). With a global distribution, including Egypt, dermatophytosis is a fungal disease that affects the hair and superficial skin layers of farm animals. Despite the disease's self-limiting nature, it has a lengthy recovery period and devastating economic losses in the skin's structure and concealment damage (30). Furthermore, dermatophytes identification is based mainly on cultural and morphological characteristics however, it is viewed as a complicated and laborious technique due to morphological similarity, variability and polymorphism (6).

In order to determine the source and capacity of dermatophytosis transmission in both humans and animals, diagnostic and epidemiological research must now go beyond simple species distinction of isolates however additionally differentiation inside speciesese discrepancies in the consequences amongst these special learn about from the contemporary ones basically may end result from the variations of the samples quantity enrolled in every study, hygienic condition, climate impact and intraspecific variant of the isolates (31). The control of dermatophyte recurrence in herds as well as in a provider state is an intriguing issue related to ringworm in cattle, this issue is particularly significant in cowsheds where both vaccinated and non-vaccinated animals are housed, the use of traditional methods for dermatophyte detection and identification in cattle herds has a number of drawbacks because they are time-consuming, labor-intensive, and require a mycological diagnostician's expertise (32). Conidia from the surroundings or from present hosts can unfold dermatophytic infections to new recipients immediately thru contact with contaminated animals or circuitously thru contaminated fomites, all occasions that prefer these contacts serve as predisposing factors. Conidia are extraordinarily resilient and can also persist in the surroundings for years (33). This explains why using cloth that animals share encourages infection, the soil serves as the reservoir in which the fungi grow for geophilic dermatophytes. Therefore, the risk of infection is higher for animals having contacts from the outside world (33). Animals with ringworm typically have a circular, daily alopecia with an erythematous edge and a thin desquamation. Despite being reported in a large percentage of animals in some surveys, pruritus is frequently absent in animals. Lesions can be solitary or many and localized on any part of the animal, although the head and the front of the body seem to be more frequently affected. Lesions commonly unfold in a centrifugal fashion. While many lesions might also additionally merge, a spontaneous recuperation at the core with hair regrowth is generally found by Kano *et al.* (34). Kerion (nodular dermatophytosis) is characterized via a granulomatous dermatitis to a deep pyogranulomatous infection and is a spherical and nodular edematous response to dermatophyte contamination with a patch of erythematous scaling alopecia. It is more often than not brought about in animals with the aid of the dermatophyte species *M. gypseum*, *M. canis*, and *T. mentagrophytes* (35).

Martinez-Rossi *et al.* (36) showed the establishment of infection depends on the dermatophytes' potential to stick to these substrates and adapt to the host environment. This adaptive response to the surroundings and to keratin degradation includes a variety of proteins and enzymes from the fungal kingdom. In the early ranges of the fungal-host interaction, transcription elements, which include PacC and Hfs1, as properly as warmth shock proteins, are essential for detecting and adjusting to the acidic pH of the skin. When

keratin serves as the sole carbon supply for dermatophyte growth, the extracellular pH adjustments from acidic to alkaline. This produces conditions that favor the best action for the majority of known keratinolytic proteases. The establishment and renovation of the infection, which may be persistent, is the result of these activities (36).

Animals of many kinds are now affected by dermatophytosis in addition to small animals. A study on dermatophytosis in farm animals conducted in Beheira, Egypt, revealed that 74% of 150 samples collected from certain animal species were dermatophyte positive. The majority of suggested dermatophytosis cases occurred in sheep 78%, followed by cattle 72%, buffalo 76%, and horses 68% (37). In Irbid, Jordan, cattle farms, the prevalence of dermatophytosis was originally estimated to range from 10 to 100%. Al-Ani *et al.* (38) research also revealed that out of 375 cattle, 115 (30.6%) were found to have a typical macroscopic dermatophytosis lesion.

Theel *et al.* (39) have proposed the development of alternative molecular methods has allowed for the quick and accurate detection of dermatophytes. They include DNA hybridization, PCR fingerprinting, sequencing of the internal transcribed spacer regions, sequencing of the large-subunit rRNA gene or the gene encoding chitin synthase, and limit fragment size polymorphism analysis (ITS1 and ITS2). The need for novel methods for identifying these prevalent fungal diseases is highlighted by the fact that present molecular methodologies are time-consuming and expensive for the identification of all isolates regularly recovered from scientific materials.

The identification of more than a few animal species that are prone to dermatophytosis contamination and that are in shut contact with people is deemed indispensable for each animal and human fitness primarily based on the findings of a learn about performed in Ankara by using Sever *et al.* (40). Additionally, laboratory diagnoses need to be acquired in instances of suspected dermatophytosis, and it would be fantastic to make bigger recognition of this trouble amongst animal proprietors and keepers owing to the practicable for zoonotic humans, which is vital for each the fitness of animals and humans. Additionally, laboratory diagnostics ought to be acquired in instances with suspected dermatophytosis, and it would be very advisable to increase attention of this difficulty amongst animal proprietors and keepers due to the opportunity of zoonotic transmission (40). Since all animal dermatophytes are very contagious to people, appropriate precautions must be taken to stop the spread of infection. When examining a sick animal or taking skin scrapings for the diagnosis, occupationally exposed individuals like veterinarians and other staff are advised to wear disposable gloves and thoroughly wash their hands with alcohol-based hand sanitizer or an antiseptic solution like dettol or savlon (41).

Conclusion

Trichophyton mentagrophytes and *Microsporum canis* were predominant species among infected cattle in Wasit province, skin scraping using KOH 10 % was an appropriate method for early diagnosis of dermatophytes. Identification of dermatophytes used to be primarily based frequently on cultural and morphological characteristics which used to be a simplest and cheapest method.

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Conflict of interest

The authors declare no conflict of interest.

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الكشف الشكلي عن الفطريات الجلدية المعزولة من الأبقار في محافظة واسط

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الخلاصة

هدفت هذه الدراسة إلى تشخيص الأوقات الجلدية في الأبقار المشتبه في كونها مصابة بمرض السعفة فضلاً عن تشخيص أنواع الفطريات الجلدية التي سببت هذا المرض. خطة الدراسة تضمنت جمع ٤٠ عينة من تقشرات الجلد وكذلك الشعر من تلك الماشية (الأبقار) المرباة في إحدى المزارع الواقعة في محافظة واسط العراقية وخلال المدة الزمنية التي تراوحت من تشرين الأول عام ٢٠٢١ إلى آذار عام ٢٠٢٢. شخّصت الفطريات الجلدية بالاعتماد على الفحوصات المظهرية سواء كانت العيانية منها أم المجهرية وفيها تم استخدام هيدروكسيد البوتاسيوم لإجراء الفحص المجهرية المباشر بينما استخدم وسط أكار دكستروز السابرويذ المدعم بالمضاد الحيوي الكلورامفينيكول والسايكلوهكسمائيد لعزل تلك الفطريات فضلاً عن استخدام صبغة اللاكتوفينول الزرقاء لإيضاح الأشكال المجهرية للفطريات التي عزلت بواسطة أكار السابرويذ. أظهرت نتائج الدراسة الحالية ٢٠ عينة إيجابية الاختبار من أصل ٤٠ عينة وإن عدداً من الأبقار المفحوصة كانت مصابة بمرض السعفة حيث تم تشخيص نوعين من الفطريات هي فطريات القوباء الحلقية بنسبة ٧٥% أي شخص في ١٥ من أصل ٢٠ عينة إيجابية الاختبار بينما نوع البويغاء الكلبيّة استحوذ على المرتبة الثانية فتواجد في ٥ من العينات العشرين الإيجابية واحتل نسبة ٢٥% وبهذا فقد استنتجت هذه الدراسة أن هناك إصابات بمرض السعفة في أبقار محافظة واسط.