PEER REVIEWED

Isolation of *Trichophyton* spores from lesions typically classified as resolving in cattle

Renée D. Dewell,[1] DVM, MS; **Darren J. Berger**,[2] DVM, DACVD; **Troy A. Brick**,[3] DVM, MS; **Shae M. Atterberg**,[4] BS; **Charles E. McIntosh**,[4] BS; **Linda D. Zeller**,[5] BS; **Lauren A. McKeen**,[6] BS; **Ronald W. Griffith**,[5] DVM, MS, PhD, DACVM

[1] Center for Food Security and Public Health, Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011

[2] Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA 50011

[3] Department of Veterinary and Diagnostic Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011

[4] College of Veterinary Medicine, Iowa State University, Ames, IA 50011

[5] Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011

[6] Department of Statistics, College of Liberal Arts and Sciences, Iowa State University, Ames, IA 50011

Corresponding author: Dr. Renée D. Dewell, rdewell@iastate.edu

Abstract

Dermatophytosis is an important zoonotic superficial mycosis of cattle, primarily caused by the dermatophyte *Trichophyton verrucosum. T. verrucosum* presents a significant public health risk in the United States. The presence of dermatophyte lesions does not necessarily preclude issuance of certificates of veterinary inspection, and are permitted in some livestock shows and exhibition cattle if the inspecting veterinarian observes hair regrowth within the lesion. The objective of this study was to evaluate dermatophyte lesions, both without hair (alopecic or active) and with hair regrowth (resolving), for presence of *T. verrucosum* spores in calves. Our hypothesis was that the regrowth of hair would not correlate with the absence of infective material. Of the 34 calves sampled, 23 of 34 of the haired/resolving lesions had spores present, while 14/34 of the alopecic/active lesions had spores present. The odds of a haired/resolving sample having spores present was found to be 4 times that of the odds of an alopecic/active sample having spores present (p-value = 0.03516, 95% CI: 1.07, 22.09). Resolving lesions, as evidenced by regrowth of hair, did not correlate with mycologic cure in this study as spores were still recovered in many lesions designated as haired/resolving.

Key words: *Trichophyton verrucosum,* ringworm, cattle, dermatophytosis

Résumé

Chez les bovins, la dermatophytose est une mycose superficielle zoonotique importante causée principalement par le dermatophyte *Trichophyton verrucosum.* Ce dermatophyte pose un risque de santé publique considérable aux États-Unis. La présence de lésions de dermatophyte n'exclue pas nécessairement l'octroi de certificats d'inspection vétérinaire et ces lésions sont tolérées dans des expositions de bétail si le vétérinaire d'inspection observe la repousse de poil au site des lésions. L'objectif de cette étude était d'évaluer les lésions de dermatophyte autant sans repousse de poil (alopécique ou active) qu'avec repousse de poil (en résolution) pour la présence de spores de *T. verrucosum* chez des veaux. Notre hypothèse était que la repousse de poil ne serait pas corrélée avec l'absence de spores infectieux. Sur les 34 veaux échantillonnés, 23/34 des lésions avec repousse de poil/en résolution avaient des spores présentes alors que 14/34 des lésions alopéciques/actives en avaient. Les chances qu'un échantillon avec repousse de poil/en résolution avaient des spores étaient quatre fois plus élevées que celles d'un échantillon alopécique/actif (valeur de p = 0.03516, I.C. 95% : 1.07, 22.09). Les lésions en résolution comme l'indique la repousse de poil n'étaient pas corrélées avec la guérison mycologique dans cette étude car des spores étaient encore présentes dans plusieurs lésions avec repousse de poil/en résolution.

Introduction

Dermatophytosis, commonly referred to as ringworm, is a fungal infection of keratinized tissue such as hair, skin, nails, claws or hooves.[14] Zoophilic species of dermatophytes pose a public health concern because they can be transmitted from animals to humans. Cattle are an important reservoir for the zoophilic dermatophyte *Trichophyton verrucosum,* which also causes primary disease in the species.[4,5,8,12,13] Cattle housed indoors and calves are at higher risk of infection.[5] In cattle, initial lesions associated with dermatophytosis are thickened, dry, scaley to crusted plaques or patches with brittle hairs. Mature lesions are characterized by thick crusts

with scales that eventually fall off, leaving areas of alopecia. A combined cell-mediated and humoral immune response causes the lesions to self resolve and prevents reinfection.[19] Transmission of *T. verrucosum* to humans occurs by direct contact with cattle or indirectly by contact with infectious spores in the environment.[1]

Human infection by *T. verrucosum* occurs worldwide and, in recent years, it has been reported that the rate of infection is increasing.[6,10] In the United States, the prevalence of *T. verrucosum* dermatophytosis in humans is not documented. However, it has been found that individuals with cattle exposure are more likely to be infected by *T. verrucosum.*[16] A study of dermatophytosis in Italian cattle farms found 45.2% of farm employees had dermatophyte lesions and *T. verrucosum* spores were isolated from 100% of the lesions. The same study found dematophytosis in 70.1% of calves under 6 months of age, with *T. verrucosum* accounting for 98.9% of these infections.[2] The United States Department of Agriculture (USDA) reported 882,692 active cattle operations in the United States in 2017.[21] Due to the number of individuals involved in these operations, *T. verrucosum* presents a significant public health risk in the United States. In humans with impaired immune status, infection with *T. verrucosum* can result in extensive and invasive disease.[5] Prevention of *T. verrucosum* dermatophytosis in humans is accomplished by controlling infection in animals, proper sanitation, utilizing protective clothing, and avoiding contact with infected animals.[22]

Dermatophytoses can also adversely affect the skin and hide industry as lesions and scars can persist through processing.[5] The presence of dermatophyte lesions may preclude entrance to shows or exhibitions,[5] but are permitted in some livestock shows and exhibition cattle if the inspecting veterinarian observes hair regrowth within the lesion.[3,17] Currently, there is a lack of data indicating whether lesions with hair regrowth are absent of fungal spores, thus noninfectious. A better understanding of the relationship between gross appearance of dermatophyte lesions in cattle and the presence of *T. verrucosum* spores is an important facet of public health that needs to be investigated. Determining the infectious potential of dermatophyte lesions at various stages of development in cattle may help decrease transmission and prevent *T. verrucosum* dermatophytosis in humans. The objective of this study was to evaluate dermatophyte lesions, both without hair (alopecic or active) and with hair regrowth (resolving), for presence of *T. verrucosum* spores in calves. Our hypothesis was that the regrowth of hair would not correlate with the absence of infective material.

Materials and Methods

The project was approved by the Iowa State University Institutional Animal Care and Use Committee prior to onset of research activities (IACUC-19-244). The study was conducted from August 2019 to September 2019. A dairy in Western Iowa served as the primary sampling venue for this project. A cross-sectional design with a convenience sampling strategy was used for this study. A pen of 88 calves, with approximately 66% dermatophytosis prevalence, was used for sampling. Calves were 3 to 4 months of age and were primarily of Holstein and Holstein-Angus breeding. Individual lock-ups within the pen were used to restrain animals. All calves were examined for clinical signs of dermatophytosis prior to sampling. Specifically, researchers examined calves for evidence of dermatophytosis such as discrete round or oblong lesions with patches of thick adherent crusts or scale.[13] To be enrolled in the study, calves had to have at least 1 lesion with alopecia and at least 1 lesion with evidence of hair regrowth (Figures 1 and 2). Prior to sampling, an image of each enrolled lesion was taken using a digital camera. The images were reviewed by a board certified dermatologist (DB) for preliminary dermatophytosis diagnosis. Only samples from lesions that were approved and designated with a preliminary dermatophytosis diagnosis of "active" or "resolving" by the boarded dermatologist were subjected to further laboratory analysis.

Figure 1. Hairless ("active") dermatophytosis lesion on a study calf.

Figure 2. Haired ("healing") dermatophytosis lesion on a study calf.

Haired lesions were sampled on each calf first, followed by sampling of the hairless lesion. Lesions were sampled using a modified Mackenzie method.[11] Briefly, any visible debris within a 2 inch (5.08 cm) margin around the lesion was removed with a gloved hand. Next, a clean cotton 4 inch by 4 inch (10.16 × 10.16 cm) gauze pad soaked with 70% isopropyl alcohol was used to further clean the lesion and area immediately surrounding the lesion, as well as to reduce potential growth of saprobic fungi. A sterile, individually wrapped plastic pediatric toothbrush was used to vigorously brush the lesion for 10 to 15 seconds. Following sampling, the individual brush was placed in a separate, clean, plastic bag and labeled with calf ID, farm ID, and sample number. This procedure was repeated with a second sterile, individually wrapped pediatric toothbrush so that 2 samples were obtained from each lesion. After changing gloves, the sampling procedure was repeated for the lesion showing no evidence of hair regrowth. Gloves were changed after sampling each lesion. Samples were stored in a covered polystyrene foam cooler for transport to the laboratory.

Samples were stored at room temperature for 3 days prior to plating them on Mycosel and Trichophyton-3 agar plates.[a] Plates were incubated at room temperature for 4 weeks. Typical colonies of *Trichophyton verrucosum* were identified both visually and microscopically by a veterinary microbiologist who was unaware of the lesion type associated with the sample. Gross morphology included the presence of tomentose white, fluffy colonies which were whitish to yellowish brown on the reverse[12] (Figure 3). Chlamydoconidia chains of *T. verrucosum* were identified microscopically using lactophenol cotton blue stain. Lesions were determined to be positive if they exhibited at least 1 *T. verrucosum* colony.

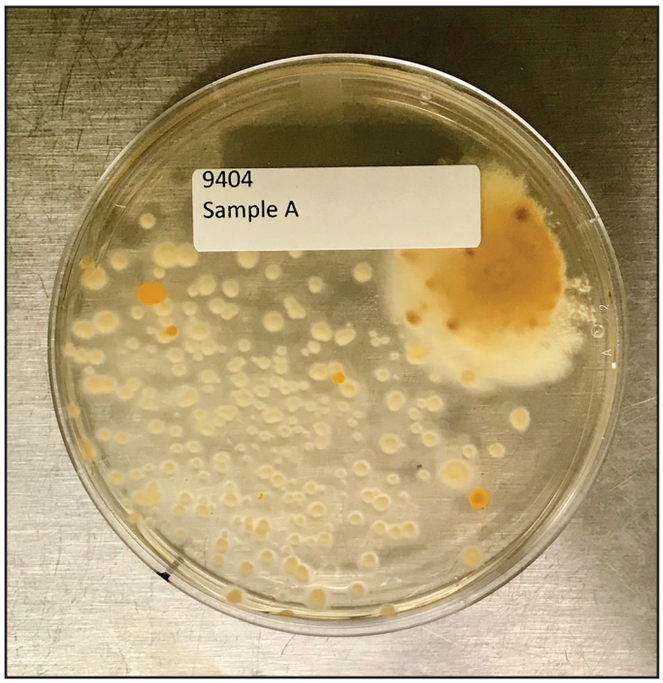

Figure 3. Plated sample exhibiting tomentose, white, fluffy colonies on Mycosel agar plate.

Statistical Analysis

All data were analyzed using R.[b,20] A 2-sided exact version of McNemar's Test was used to assess the association between sample type (haired/resolving or hairless/active) and the presence of *T. verrucosum* spores.[7] Significance was set at $p < 0.05$.

Results

Paired lesions from 34 calves were obtained. Thirty-four paired samples were plated. Table 1 outlines the results. Of the 34 calves sampled, 23 of 34 haired/resolving lesions had spores present, while 14 of 34 hairless/active lesions had spores present. Spores were present on both the haired/resolving and hairless/active lesions in 11 of 34 calves, while 8 of 34 calves did not have spores present on either the haired/resolving or hairless/active lesion. Spores were present on the haired/resolving lesion only in 12 of 34 calves, while 3 of 34 calves had spores present only on the hairless/active lesions. The odds of a haired/resolving sample having spores present are 4 times that of the odds of a hairless/active sample having spores present (p-value = 0.03516, 95% CI: 1.07, 22.09).

Discussion

In this study, the designation of lesions as haired/resolving or hairless/active did not correlate with the presence/absence of infectious material. Thus, "resolving" as evidenced by regrowth of hair did not correlate with mycologic cure in this study, as spores were still recovered in many of the lesions designated as haired/healing. Spontaneous healing has been described as hair regrowth beginning in the center of the lesion. Hyphae and arthrospores can penetrate deeply within the hair shafts and even the follicles,[4] thus even though a lesion may appear to be healing, it may still contain infective fungal elements and may continue to contaminate the environment as well as cause disease in humans or other animals. The hypothesis that haired/resolving lesions may be "inactive" may stem from attempted extrapolation for human medicine where the fungus is likely to be found in the stratum corneum. In contrast, animals with dermatophytosis often demonstrate significant follicular invasion when infected.[4]

Current guidance allows for certificates of veterinary inspection to be written for animals with evidence of haired/resolving lesions. Dermatophytosis may presumably be spread by animals that are permitted to move with haired/resolving lesions. Many environments, such as cattle markets and exhibition sites, are constructed of porous materials such as wood, which can easily harbor spores and are difficult to

Table 1. Descriptive statistics for the presence of *Trichophyton* spores in haired and unhaired bovine skin lesions.

	Number of calves		Total
Haired lesion WITH spores present	23		34
Haired lesion WITH NO spores present	11		
Hairless lesion WITH spores present	14		34
Hairless lesion WITH NO spores present	20		
Both haired AND hairless lesions WITH spores present	11		34
Both haired AND hairless lesions WITH NO spores present	8		
ONLY haired lesion (of the pair) WITH spores present	12		
ONLY hairless lesion (of the pair) WITH spores present	3		

clean and therefore eradicate potential infectious material. The spores may be dispersed at markets or exhibition areas where they may persist for years[5] and continue to cause disease.

Show cattle may be at increased risk for contracting dermatophytosis from contaminated exhibition sites because concurrent microtrauma to the skin is typically needed to cause disease.[15] Many show cattle are subjected to grooming practices (repeated bathing, brushing, and application of hair and skin products) that may damage the epidermis and allow for introduction of the infectious spores. Infection with *T. verrucosum* is more prevalent in the winter months and is most likely to affect cattle younger than 12 months.[4] Thus, younger cattle at winter shows or maintained in air conditioned environments may be at greater risk of infection.

Dermatophytosis is often self-limiting with apparent resolution in 1 to 4 months.[4] There are no drugs approved by the FDA to treat ringworm in cattle; topical antifungals are regulated by the EPA, thus not applicable to the FDA. If considering the use of a systemic or topical treatment for ringworm in cattle, the Food Animal Residue Avoidance Databank (FARAD) should be contacted for appropriate withdrawal times. Combined topical and systemic treatments for at least 10 weeks is recommended for canine and feline dermatophytosis;[5] however, the cost of antifungal drugs, need for individual treatment, treatment duration and frequency, and lack of approval, make this impractical for most cattle operations. Other treatments include extra label drug usage (ELDU) of oral administration of griseofulvin, topical treatments such as application of tincture of iodine, chlorhexidine, povidone-iodine, dilute bleach, antifungal cream, lime sulfur, or thiabendazole have also been used with varying results. The use of a pomade containing 3% enilconazole has also been shown to be curative for bovine dermatophytosis.[9] Topical treatments sometimes discolor cattle hair, making it an unattractive treatment option for many people who exhibit cattle. Additionally, topical treatments can be expected to reduce contamination and transmission potential by functioning against spores in the stratum corneum as well as the hair surface, but are likely not effective against infection within the hair shaft[4]. Further research is needed to develop viable dermatophytosis treatments for cattle that will not damage or discolor hair, are effective against infection within the hair shaft, and do not pose a food safety risk to humans. Additional research to determine the range of the infective period is also needed.

This study, though small, provides important information for veterinarians regarding potential infectivity of dermatophytosis lesions that appear to be healing. It is possible that the frequency of plates with spores was under-reported in this study, due to potential overgrowth of bacteria on some plates where the presence of spores were not noted. This potential was minimized by cleaning the sampled area with alcohol prior to sampling and using selective growth media. This study is also limited to a single farm, and perhaps the *T. verrucosum* spores recovered in these lesions were not typical of spores found in other infected cattle. This is less likely because, though small, the study only enrolled calves which were naturally infected with dermatophytosis. Additionally, since samples from haired/resolving lesions as well as hairless/active lesions were obtained from the same calf, it is possible that cross-contamination could have occurred, perhaps due to grooming. However, though calves normally perform allogrooming or may groom themselves, it is unlikely that they transferred spores between types of lesions because all samples were obtained from the head and neck area where allogrooming and self-grooming are unlikely.

These study results may provide guidance for veterinarians in writing certificates for veterinary inspection for animals with haired/resolving dermatophytosis lesions. The results indicate that precautions to prevent horizontal transmission of infective material should continue to be prac-

ticed for dermatophytosis even when evidence of healing is present. Though dermatophytosis is recognized as a zoonotic disease that causes treatable skin lesions in humans, the actual rate of transmission from infected animals to humans is not known.[15] Based on the results of this study, humans should continue to take steps to prevent zoonotic infection even when dermatophytosis lesions appear to be resolving. Additional research is needed to determine the actual risk of horizontal transmission from cattle with evidence of healing dermatophytosis lesions as well as further definition of what clinical stage correlates with a lack of infectious material. Further research to evaluate and culture lesions following the use of commonly used dermatophytosis treatments would also be of practical value.

Endnotes

[a] Becton, Dickinson and Company, Difco Laboratory subsidiary of Becton, Dickinson Company, Sparks, MD

[b] Version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria

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