Dermatophytosis in an adult cattle due to Trichophyton verrucosum

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Abstract
Dermatophytosis is the most commonly occurring highly infectious and contagious cutaneous mycosis of public health and economic significance. This paper describes the etiologic significance of *Trichophyton verrucosum* in bovine dermatitis. *Trichophyton verrucosum*, a zoophilic dermatophyte, was identified in the cutaneous lesions of a crossbred cow by employing standard mycological techniques. The diagnosis was confirmed by demonstration of fungal agents in the skin lesions by direct microscopy in potassium hydroxide and ink solution, and also by isolation of *Trichophyton verrucosum* on Sabouraud dextrose agar supplemented with chloramphenicol, actidione and thiamine. The detailed microscopic morphology of the isolates of dermatophytic fungus in Narayan stain revealed in potassium hydroxide and ink solution, and also by isolation of *Trichophyton verrucosum* crossbred cow by employing standard mycological techniques. The diagnosis was confirmed by demonstration of fungal agents in the skin lesions by direct microscopy in potassium hydroxide and ink solution, and also by isolation of *Trichophyton verrucosum* on Sabouraud dextrose agar supplemented with chloramphenicol, actidione and thiamine. The detailed microscopic morphology of the isolates of dermatophytic fungus in Narayan stain revealed in potassium hydroxide and ink solution, and also by isolation of *Trichophyton verrucosum* on Sabouraud dextrose agar supplemented with chloramphenicol, actidione and thiamine. The animal was treated with topical application of 2% solution of tincture iodine. Direct microscopy in KOH-INK solution is recommended for the rapid presumptive diagnosis of ringworm in rural areas, where laboratory facility of isolation of dermatophytes is not available. As animal dermatophytes are highly communicable to humans, care should be taken when dealing with diseased animals to avoid the infection. It is emphasized that Narayan stain should be widely used in microbiology and public health laboratories to study the morphology of dermatophytes and other fungi, which are implicated in the various clinical disorders of humans and animals.

Introduction
The skin is the largest organ, representing 12 to 14% of the body. It is affected by several diseases commonly manifested by alopecia, pruritis, dermatitis, nodular, crusty, scaly or scabby lesions depending on the underlying causes [1]. Skin diseases cause heavy economic loss to the livestock industry due to direct effects on the quality of hide, skin, wool, and fur of animals [1,2]. Dermatitis is a disease of multiple etiologies, which include virus, bacteria, fungi, algae, and parasites [1,3]. Among the fungi, dermatophytes are recognized as an important cause of dermatitis in humans as well as in animals [3-5]. The disease caused by dermatophytes is known as dermatophytosis (ringworm, tinea), and is more common in tropical and subtropical countries [6]. The dermatophytes are Gram positive, strict aerobic, non-motile, filamentous fungi, which belong to three genera, namely *Epidermophyton*, *Microsporum*, and *Trichophyton*, and attack the keratinized tissues of the skin, hair, nail, etc [3,7]. Based on the primary reservoirs, dermatophytes are classified zoophilic (animals) and geophilic (soil) and anthropophilic (humans) [3]. Transmission of infection can occur through direct contact with infected patients or indirect contact with contaminated fomites [8]. Disease can occur in sporadic as well as in epidemic forms [3,9,10]. Some of the dermatophytes are isolated from the soil [11] and hence, infection can also be acquired from such saprobic reservoirs [3]. The disease is encountered in humans and also in many species of animals, such as cat, dog, cattle, camel, goat, buffalo, sheep, horse, pig, deer, monkey, bear, rabbit, etc [3,7,12-17]. The paucity of published available information on animal ringworm in Meerut region of Uttar Pradesh, India prompted the author to elucidate the etiologic role of dermatophytes in cutaneous lesions of an adult bovine.

Materials and methods
The skin from affected site was thoroughly sponged with 70% ethyl alcohol to remove the surface contaminants. After drying, the skin scrapings along with hairs collected aseptically with the help of sterilized scalpel from the margins of the active lesions of 4-year-old female cow bred cattle on clean carbon paper constituted the material for the investigation. The age, sex, and site of lesions on body were recorded in a Performa. The cow belonged to an animal owner who was residing in Ukhlima village of Meerut district, India. A portion of the clinical material on clean glass slide was digested with 2 drops of mixture containing 4.0 ml of potassium hydroxide (20%), 4.0 ml of Parker blue black ink and 2 ml of glycerol (10%), [3], and examined under light microscope for ectoparasite, fungi, algae and actinomycetes. The specimens were cultured on nutrient agar, blood agar, DTM agar and Sabouraud dextrose agar with chloramphenicol (0.1 mg/ml) and actidione (0.5 mg/ml). The inoculated media were incubated at 25°C and 37°C, and observed daily for microbial growth. The suspected colony was subcultured on Sabouraud medium for further identification. The detailed microscopic morphology of isolates was made in Narayan stain, which contained 0.5 ml of 3% solution of methylene blue, 4.0 ml of glycerine and 6.0 ml of dimethyl sulfoxide [18]. Based on the macroscopic as well as microscopic characteristics, the isolates were identified as *Trichophyton verrucosum* [3,19]. The animal owner was advised to apply 2% solution of tincture iodine daily for 2 to 3 weeks on the lesions after the removal of crusts with disposable spatula, and also to properly destroy the crusted materials and wooden spatula by burning in order to prevent the spread of infection.

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Results

On clinical examination, 4-year-old cow showed small, discrete, circumscribed, raised, grayish-white crusty, alopecic lesions, one on the neck, and other on abdomen area. The animal was suffering from dermatitis for the last 60 days. The temperature, respiration, and pulse were in normal range. The animal had good appetite besides normal rumination, defecation, and urination. There was no evidence of ectoparasites on the body of cow. The direct examination of clinical specimens in KOH-INK under light microscope revealed hyaline, thin, slender, branched hyphae, and arthrospores morphologically to dermatophytes. Hair showed ectothrix invasion as arthrospores appeared as chains on the surface of hair shaft. Direct microscopy failed to indicate the presence of ectoparasites, actinomyces, yeasts or algae in cutaneous lesions. Dermatophyge grew in DTM at 25°C by changing its colour from yellow to pink. On Sabouraud dextrose agar with chloramphenicol and actidione, small, compact, heaped, white to gray coloured colonies were observed after 2 weeks of incubation at 37°C. There was no growth of other microbes on the remaining nutrient media. The subculture from the suspected colonies was made on Sabouraud medium for detailed identification. The pure growth of dermatophyte in Narayan stain under light microscope, showed antler like branched hyphae, rat tailed shaped macroconidia, and tear shaped microconidia, and were confirmed as T. verrucosum [3]. The cow showed good clinical response with topical application of tincture iodine after 2 weeks of therapy. The cattle owner did not report any side effects of topical application of tincture iodine. However, no mycological follow up of the animal could not be attempted to assess the efficacy of tincture iodine after the treatment.

Discussion

Among the livestock, cattle play an important role in livelihood of poor farmers, who do not possess enough land for cultivation of various crops. Cattle suffer from a variety of specific and non-specific skin diseases. Among these, dermatophytosis is the most frequently encountered fungal disease of cattle. The disease in cattle is primarily caused by T. verrucosum but occasionally other dermatophytes such as T. mentagrophytes, Microsporum canis, and M. gypseum are also isolated from dermatological disorders of bovine [1,3,6,10,12,20]. Dermatophytosis is reported from over 145 countries of the world including India [3,9,10,21,22]. The global prevalence of dermatophytosis in humans is estimated about 20% [23]. The incidence of disease varies from country to country depending on climatic, social, and economic factors. Dermatophytosis is an important occupational disease of animal handlers, abattoir workers, livestock raisers, veterinarians, dairy farmers, veterinary assistants, and kennel attendants [3,5,17]. In the present investigation, the clinical diagnosis was confirmed by direct microscopy of dermatophyte in cutaneous lesions and by cultural isolation of T. verrucosum on mycological media. This observation is in conformity with the findings of other investigators [5,8,10]. Dermatophytosis is mainly encountered in calves [8,10,12]. However, the current study indicated that adult animal can also be affected with T. verrucosum. This finding goes parallel with the observation of Pal and co-workers [24] who recorded T. verrucosum infection in an adult female cow. This investigation suggests that T. verrucosum is endemic in the study area, and the role of this zoophilic dermatophyte in the etiology of ringworm should be further elucidated in other species of animals as well as in humans.

In the present case, topical use of 2% tincture iodine solution showed good clinical response in cattle having only two lesions on the skin. If the infection is not generalized and lesions are limited in numbers, the treatment of ringworm in animals with topical application with tincture iodine solution is recommended, particularly for the poor resource nations, which can hardly afford expensive antifungal drugs, such as miconazole, clotrimazole, fluconazole, itraconazole, luliconazole and terbinafine. Immunoprophylaxis of cattle against T. verrucosum with live vaccine is considered effective to control the infection [3,25]. It is interesting to mention that immunization of calves with T. verrucosum vaccine showed very encouraging results as it could protect 90% of vaccinated calves against T. verrucosum infection [26].

The isolation of T. verrucosum from cattle is a pertinent observation, as this dermatophyte is of great public health significance. The animal owner narrated that one of his 5-year-old male child who had cutaneous lesions on the head, probably contracted the infection from the diseased cow. Due to ethical restrictions, the scrapings from the head lesions of the child were not obtained for mycological confirmation. It is pertinent to mention that many cases of direct transmission of T. verrucosum from infected animals to humans are documented in the literature [3,5,8,10,20,27-30]. As all animal dermatophytes are highly communicable to human beings, necessary precautions must be exercised to prevent the transmission of infection. The occupationally exposed persons such as veterinarians and other staff are advised to use the disposable gloves, and thoroughly wash their hands with alcohol based hand sanitizer or antiseptic solution like dettol or savlon when examining a sick animal or collecting the skin scrapings for the diagnosis [3,5]. This seems to be the first report of T. verrucosum infection in a cow from this region of India, i.e. Uttar Pradesh.

Conclusion

Dermatophytosis is a global cutaneous mycosis of humans and a variety of animals including birds. The findings of present investigation established the etiologic significance of T. verrucosum in the cutaneous lesions of an adult female cattle, which was suffering from chronic infection for the last 2 months. It is recommended that ringworm should be considered in the differential diagnosis of dermatitis of cattle. The correct rapid diagnosis and prompt treatment is highly imperative to prevent the spread of infection. The emphasis is given to undertake further studies on the role of dermatophytes including T. verrucosum in various dermatological disorders of animals as well as humans. Further work on the development of cheap, safe and potent chemotherapeutic agents for the management of dermatophytosis should be conducted.

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