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# Isolation, PCR based Diagnosis and Therapeutic Management of Bovine Dermatophilosis

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**ABSTRACT**

Dermatophilosis is an acute or chronic exudative dermatitis caused by the bacterium *Dermatophilus congolensis*. *Dermatophilus congolensis* is a pleomorphic, Gram-positive actinomycete that infects animals and humans. Frequently, there is a misdiagnosis of this infection because of the high similarity of this organism with other members of the family *Actinomycetaceae*. The present study was carried out to isolate and identify the causative agent from a cattle herd clinically suspected for dermatophilosis associated with lower leg dermatitis in the Puducherry region (Southern India), their predisposing factors and treatment. The microscopic examination of skin impression smears from the infected animals did not reveal the characteristic appearance of *Dermatophilus congolensis*. Microscopic examination of the isolated bacteria revealed Gram-positive bacteria with filamentous structures and was further confirmed by polymerase chain reaction (PCR) targeting the 16S rRNA of *Dermatophilus congolensis*. This study supports the PCR technique as a preferable technique to the conventional microscopy and culture techniques for the confirmation of *D. congolensis* infection during epidemiological surveys. The contact of skin with sewage channels in grazing areas and the wetting of skin by rain may be the predisposing factors for transmission by direct contact with clinically infected animals. Based on the antibiogram, the isolates were sensitive to antibiotics such as Gentamicin, Penicillin, Amikacin, Enrofloxacin and Chloramphenicol. The animals with dermatophilosis were treated with fortified procaine penicillin and streptomycin intramuscularly along with supportive drugs and topical dressing with 1% povidone-iodine for successive five days, which resulted in a fast and complete recovery.

**HIGHLIGHTS**

- Confirmatory diagnosis of bovine dermatophilosis was done by PCR targeting the 16S rRNA of *Dermatophilus congolensis*.
- Dermatophilosis affected animals effectively treated with antibiotics along with supportive treatment.

**Keywords:** Bovine, Dermatophilosis, 16S rRNA, PCR, Antibiogram, Therapeutic Management

*Dermatophilus congolensis* is a pleomorphic, aerobic, filamentous Gram-positive actinomycete bacterium that morphologically bridges the gap between bacteria and fungi. Its size (about 1-3µm in diameter) and staining reaction (Gram-positive) distinguish it as a bacterium. Frequently, there is a misdiagnosis of this infection because of the high similarity of this organism with other members of the family *Actinomycetaceae* under the microscopy. However, it resembles the moulds morphologically by forming a branched mycelium during the early stage of growth (Pier *et al*., 1967). This disease affects a wide variety of animals but most commonly associated with goats, sheep, cows, horse and occasionally

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humans (Admasu *et al*., 2011). The first case was reported in Congo in 1915 and later it has been reported in the other parts of the world as a chronic endemic disease and more rarely, as an acute and epidemic infection. The disease has a worldwide distribution, prevailing in tropical areas and associated with humid environments and other factors. It is an obligate parasite of the epidermis and has a distinctive life cycle that includes a motile zoospore stage that is activated under moist conditions (Lunn *et al*., 2016). A serous discharge that dries to mat the hair into tufts resembling paintbrushes or to produce crusts and thick scabs that cause exudative dermatitis are few of its defining characteristics. It is common in tropical regions where vectors are present, but its frequency is primarily seasonal, occurring primarily during the rainy season (Adedeji *et al*., 2017).

The purpose of this study is to describe the clinical signs, isolation and identification of the causative agent from the bovine dermatophilosis suspected lower leg dermatitis in the Puducherry region (Southern India), their predisposing factors and the clinical management. The presumptive diagnosis was based on their morphology, cultural and biochemical characteristics which was further confirmed genetically by PCR. The differentiation and confirmation of the field isolates of *D. congolensis* from the other members of the family *Actinomycetaceae* that can mask the diagnosis of the infection should be done by molecular test like PCR.

## MATERIALS AND METHODS

### Source of isolates

A total of nine cows, which include eight cross-bred Jersey and one Holstein Friesian cross-bred, in a herd of twelve cattle aged between one to eight years, were presented at the Veterinary Hospital, Department of Animal Husbandry and Animal Welfare, Puducherry. The animals had been presented with the skin lesions confined to the lower leg dermatitis since the last 6 months and gradually progressed to a severe degree of pruritis. The cattle showed clinical manifestations as raised, matted tufts of hair distributed over the lower leg and ventral surfaces of the body. Initially, lesions first appeared in the lower hind limbs and then the forelimbs, inner parts of the thighs and in one animal, lesion were seen near the nostrils and eyes. The skin scrapings, the scab materials and the impression smears from the base of crusty lesions were collected aseptically for microbiological investigation.

### Isolation and identification

The skin scrapings and the scab materials were inoculated into the Luria broth for incubation. The incubated inoculums were cultured onto the Mueller Hinton (MH) agar and 5% blood agar for the isolation of organism. The isolated bacteria were identified phenotypically based on their microscopic morphology, cultural and biochemical characteristics.

### Antimicrobial sensitivity test

Antimicrobial sensitivity test was done as per the Bauer's standard disc diffusion method (Hudzicki, 2009). The test was carried out using 8 antimicrobial agents such as Amikacin, Amoxycillin-clavulonic acid, Chloramphenicol, Co-trimaxazole, Enrofloxacin, Gentamicin, Penicillin and Tetracycline. The inoculated plates were inverted and incubated at 37°C and each plate was examined after incubation for 48 hrs. The diameters of the zones of complete inhibition, including the diameter of the disc, were measured to the nearest whole millimeter with a ruler in a non-reflecting background. The interpretation of zone of inhibition was read as per Clinical Laboratory Standard Institute (CLSI) guideline.

### Polymerase Chain Reaction

### DNA extraction

Suspected bacterial colonies from MH agar / 5% blood agar were inoculated in Luria broth and incubated for 24 h at 37°C. Bacteria were pelleted by centrifugation for 10 min at 5000 rpm and the pellets were used for the genomic DNA extraction using the QIAamp DNA Mini extraction kit (Qiagen Inc. Valencia, CA USA) according to the manufacturer's protocol.

### PCR assay

The assay was carried out using the primers by targeting the 16S rRNA gene *D. congolensis* as described by Han *et al*. (2007). DNA extracted from the suspected

*D. congolensis* was used for the amplification, which amplifies the 475 bp fragment of the 16S rRNA gene of the organism. The PCR reaction mix contained 100ng template DNA, 5µl 10X PCR buffer, 2 mM $MgCl_2$, 2µl of 20 mM dNTPs, 10 µM of forward and reverse primers, 2U of Taq DNA Polymerase (New England Bio Labs) and the volume was made up to 50 µl with Nuclease Free water (NFW). The thermocycling conditions were as follows: 5 min at 94ºC (initial denaturation), 35 cycles of 1 min at 94ºC (denaturation), 45 sec at 56ºC (annealing temperature), 45 sec at 72ºC (extension), followed by final extension (72ºC for 10 min) and hold at 4ºC. The amplified products were confirmed by resolving at 1.5% agarose gels electrophoresis and visualized under UV transilluminator (Syngene, U.K).

### Therapeutic management of the animals

The animals were treated with Inj. Procaine penicillin @ 70,000 IU/kg BW and Inj. Streptomycin (Streptomycin sulphate) @ 70mg/kg BW (Pensbiotic MD™) intramuscularly for five consecutive days (Ilemobade *et al*.,1979; Quinn *et al*., 2011) along with Inj. Ivermectin (200mcg/kg BW) subcutaneously once. As a supportive therapy, Inj. Vitamin $AD_3E$ (Vetade™) 5ml/cattle was injected intramuscularly. Topically potassium permanganate washing, sodium bicarbonate washing, kapicure plus (Herbal spray) spraying and topical dressing with 1% Povidone-iodine were done. The owner was advised to shift the animal to a new grazing area and keep the surrounding area clean and dry.

## RESULTS AND DISCUSSION

In cows, the skin lesions observed were mainly localised on the lower legs, inner aspect of the thigh, and abdominal region (Table 2) (Fig. 1). Appetite, physical examination and vital parameters were within the normal range. The characteristic skin lesions described by other authors were noticed in the present study with the infected site matted in tufts (paintbrush lesions), together with serous exudation and papule formation, leading to the development of raised scab-like crusts (Constable *et al*., 2017; Quinn *et al*., 2011). *D. congolensis* does not usually invade healthy skin. Trauma and persistent wetting predispose to skin invasion (Quinn *et al*., 2011). In this present study, the affected animals were not infested with ticks but had constant contact with the drainage channel near the grazing area and the animals were exposed to rain during the rainy season. These may be the predisposing factors for the incidence of dermatophilosis in this case.

Gram stained smears from dried scabs did not reveal the characteristic appearance of *Dermatophilus congolensis* on microscopic examination. The characteristic appearance

**(A)** **(B)** **(C)**

**Fig. 1: (A)** Scabs around the nostrils **(B)** Lower leg dermatitis in cattle **(C)** Healed lower leg dermatitis (two weeks post treatment)

**Table 1:** Details of primer for amplification of 16S rRNA gene of *D. congolensis*

| Primer | Primer sequence | Amplicon size | Reference |
|---|---|---|---|
| 16S rRNA DC-For | 5'-ACATGCAAGTCGAACGATGA-3' | 475bp | Han *et al.* (2007) |
| 16S rRNA DC-Rev | 5'-ACGCTCGCACCCTACGTATT-3' | | |

**Table 2:** Distribution of lesions in cattle with Dematophytosis

| Animal no | Age | Sex | Breed | Site of infection |
|---|---|---|---|---|
| 1 | 2 years | Female | CBJ | Lower limbs (hind limbs) |
| 2 | 5 years | Female | CBJ | Lower limbs, ventral side, udder |
| 3 | 4 years | Female | CBJ | Lower limbs (fore and hind limbs) |
| 4 | 1.5 years | Female | CBJ | Lower limbs, around nostrils, above right eye |
| 5 | 8 years | Female | CBJ | Lower limbs (hind limbs) |
| 6 | 1 years | Female | CBJ | Lower limbs (hind limbs) |
| 7 | 6 year | Female | CBJ | Lower limbs (fore and hind limbs),thigh region |
| 8 | 7 years | Female | CBJ | Lower limbs (fore and hind limbs) |
| 9 | 3 years | Female | HFCB | Lower limbs (hind limbs) |

of *D. congolensis* was not obvious because of the chronic condition. But further isolation of samples in the MH and Blood agar; the stained smears showed low numbers of Gram-positive bacteria with filamentous structures (Fig. 2).

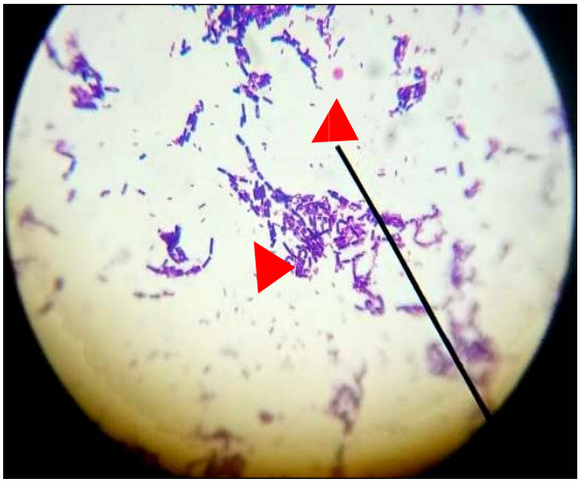

**Fig. 2:** The microscopic examination of Gram stained smears showed Gram-positive bacteria with filamentous structures

Hence, the diagnosis of bovine dermatophilosis was made based on the isolation of *D. congolensis* followed by PCR (OIE manual, 2008). On 5% blood agar; *Dermatophilus congolensis* showed the colonies surrounded by zones of beta hemolysis after 48 hrs of incubation (Fig. 3). The isolates were found to be positive for the biochemical tests such as Catalase, Urease and Sugar Fermentations using Glucose, Fructose and Maltose but give negative for the Indole ring test (Table 3).

**Table 3:** Details of Biochemical test result

| Biochemical tests | Result |
|---|---|
| Catalase test | Positive |
| Urease test | Positive |
| Indole ring test | Negative |
| **Sugar fermentation test** | |
| Glucose fermentation | Positive |
| Fructose fermentation | Positive |
| Maltose fermentation | Positive |

The isolates of *D. congolensis* were found to give variable, inconsistent biochemical reactions that are not specific for characterization and identification of the organism. For example, varying results have been reported by different researchers with maltose, sucrose, galactose, fructose and other reagents (Van Sacegham, 1934; Macadam and Haalstra, 1971; Gordon, 1976).The tendency of a mixed infection and the difficulty in isolating the organism from field cases using conventional techniques is the drive for a search for a more effective diagnostic approach.

**Fig. 3:** Suspected colonies of *Dermatophilus congolensis* on 5% blood agar showing the colonies surrounded by zones of beta hemolysis after 48 h of incubation

## PCR amplification

The scabs/isolates were subjected to polymerase chain reaction (PCR) targeting the 16S rRNA, and were found positive for *D. congolensis* amplifying a 475 base pairs (bp) product. The results of PCR amplification of the isolates from infected cattle are shown in Fig. 4. The PCR assay for the 16S rRNA gene of *D. congolensis* was done in accordance with the method described by Han *et al*. (2007); Shaibu *et al*. (2010). Molecular diagnostic methods like polymerase chain reaction can be used for the definitive identification of *D. congolensis* (Samon *et al*., 2010).

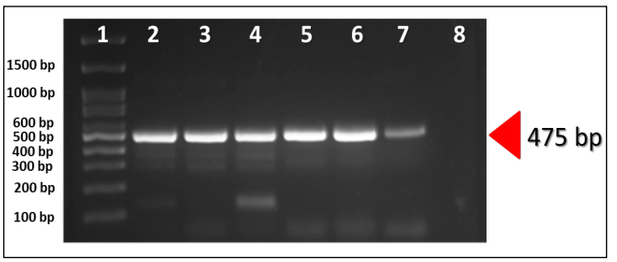


**Fig. 4:** PCR amplification of a fragment of 16S rRNA gene from *Dermatophilus congolensis* isolates. **Lane 1:** 100 bp ladder, **Lane 2-7:** Isolates of *D. congolensis* from cattle and *Lane 8:* Nuclease free water (Negative control)

## Antibiogram

The *D. congolensis* isolates were sensitive to antibiotics such as Gentamicin, Penicillin, Amikacin, Enrofloxacin and Chloramphenicol but were resistant to Amoxycillin-clavulonic acid, Tetracycline and Co-trimaxazole. As per the other researchers; the most effective drugs in treating dermatophilosis are Gentamicin, long-acting oxytetracycline and the combination of penicillin and streptomycin (Hamid M.E & Musa M.S, 2009).

## Therapeutic management

After the treatment with fortified procaine penicillin and streptomycin, the lesions began to heal during the course of the treatment and topical dressing with 1% povidone-iodine for successive five days, resulted in a fast and complete recovery. High doses of penicillin-streptomycin combinations on three consecutive days was used by others for successful therapeutics (Quinn *et al*., 2011). The direct contact of the lesion with povidone-iodine helps in inhibiting and destroying *D. congolensis* (Awad *et al*., 2008). The supportive therapy like the use of Ivermectin helped to prevent the flies from infesting the lesions.

## CONCLUSION

The present study indicates that the PCR assay is the preferable technique in comparison to microscopy and the conventional culture technique for confirmation of *D. congolensis* organisms during epidemiological surveys. The contact of skin with sewage channels in grazing areas and the wetting of skin by rain may be the predisposing factors for transmission by direct contact within clinically infected animals. Based on the antibiogram, the isolates were sensitive to antibiotics such as Gentamicin, Amikacin, Enrofloxacin and Chloramphenicol. The animals with dermatophilosis were treated with fortified procaine penicillin and streptomycin intramuscularly along with supportive drugs and topical dressing with 1% povidone-iodine for successive five days, which resulted in a fast and complete recovery. Managemental practices like keeping the animal and the surrounding area dry and clean are also important factors for the prevention and control of dermatophilosis.

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