

Phenotyping as a diagnostic tool for *Dermatophilus congolensis* infection in bovines

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Abstract: *Dermatophilus congolensis* is a gram-positive filamentous facultative anaerobic bacterium that infects wide range of animals and humans. Cattle farmers of a village from YSR district of Andhra Pradesh, India, consulted local veterinarian with the history of scabs in few animals, which was referred to college. We investigated the incidence. The scabs and crust formed on the skin of affected dairy cattle was preliminarily indicating *Dermatophilosis* infection. The organisms were isolated and identified by *Haalstra's* method; phenotypic characterization - demonstration of filamentous and motile zoospores from the lesions; and biochemical characterization to arrive at a confirmatory diagnosis of *Dermatophilus congolensis*. Based on antibiotic sensitivity, the antibiotic Amikacin was used successfully to treat the infection along with supportive therapy and better management practices.

KeyWords: *Dermatophilosis, Dermatophilus congolensis, Filamentous Bacterium, Motile-Zoospores.*

I. Introduction

Dermatophilus congolensis, a member of Actinomycetes group, infecting wide variety of animals and humans [1]. It causes severe skin infections of dairy animals that result in economic losses to the dairy farmers [2]. In cattle, these gram positive capnophilic bacterial infections are associated with predisposing factors like high humidity, rain fall, ectoparasites (ticks), poor drainage systems, water logging areas, abraded wounds, stress, lameness etc. [3, 4]. In cattle and buffaloes *D. congolensis* mainly infects the shoulder and thigh regions to the hoof of limbs with characteristic lesions seen as scaling and oozing of scab along with underlying tissue at the infected site. In the present case a herd of dairy cattle were investigated for *D. congolensis* infection as they showed the characteristic lesions. The *D. congolensis* was isolated and identified from the affected skin scab materials.

II. Materials And Methods

2.1 Geographical data

The affected cattle were from Thippaluru village of Yerraguntlamandal of YSR District, Andhra Pradesh, India (geographical coordinates -14°36'40.9"N 78°34'38.4"E)

2.2 Samples

Thirteen animals in a herd of dairy cattle were affected with severe skin infection. Skin scabs from severely affected lesions including the underlying tissues, direct blood smears and impression smears from the affected lesions were collected.

2.3 Direct Microscopic examination

Giemsa and Gram's staining methods were used to examine direct blood smears and impression smears.

2.4 Isolation of *Dermatophilus congolensis* from the infected scabs

The isolation of the *Dermatophilus* species from the infected lesions was carried by the *Haalstra's* method [5], with slight modification. Briefly, the infected scab material with underlying tissues were processed in sterile Petri dishes with BHI broth and made them to small pieces with sterile scissors and forceps. This processed material was transferred to screw cap vials and incubated for 3 hours at room temperature. This processed scab material was exposed to 5% CO₂ tension in a CO₂ incubator for 30 minutes. The motile zoospores at the top layer of the processed sample were transferred on to 10% sheep blood agar using a sterile inoculation loop and incubated at 37°C under 5% CO₂ tension in a CO₂ incubator for 72 hours. The results were recorded after 72 hrs of incubation. Further, few drops of the processed sample from the top most layers were examined by simple Methylene blue stain without heat fixation for the presence of motile zoospores.

2.5 Biochemical characterization

The colonies from blood agar plates were inoculated into Tryptone broth, Urease slant, Gelatin slant, BHI agar, Glucose broth and incubated in a CO₂ incubator at 37°C for 48 hrs.

2.6 Antibiotic resistance pattern and chemotherapy

The antibiotic disc diffusion method was done to test sensitivity of *Dermatophilus congolensis* isolate as per the Kirby and Buear method. The Muller-Hinton agar plates were incubated at 37°C for 48hrs under 5% CO₂ tension and the zone of inhibition of bacterial growth by the antibiotic discs was noted in comparison with the standard charts.

III. Results

3.1 Direct microscopic examination

The direct blood smears were examined by Giemsa stain, revealed no specific Hemoprotozoan infection. The impression smears stained by Gram's stain revealed large gram-positive cocci along with contaminants [3]. The scab material processed in saline was examined after stained with fungal stain Lactophenol Cotton blue, revealed *Mucor* species, and well defined actively motile characteristic zoospores. The specimens examined for other ectoparasitic infections were negative for ticks and mites.

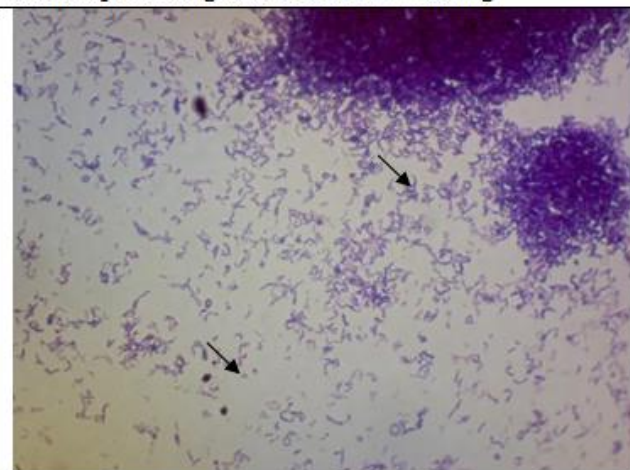
3.2 Phenotypic characterization

On confirmation of the motile zoospores by direct microscopic examination of the skin scabs, attempts were made for isolation of the *Dermatophilus* spp. On examination of few drops from the surface of the medium reveals characteristic actively motile zoospores. Lactophenol Cotton Blue stain was used for the demonstration of the motile spores and any Yeast cells as contaminants. No yeast cell infections were detected. On blood agar plates cultured with motile spores no growth was observed at 25°C that rule out the fungal contaminants and at 37°C a clear greyish white colonies were observed on blood agar within 24 hrs of incubation. These greyish colonies were slowly turned into greyish yellow colour on prolonged incubation. After 72 hrs of incubation, a haemolytic pattern was clearly observed around the colonies of *Dermatophilus congolensis* (Fig.1). The production of haemolysin, a characteristic feature of this bacterium produces β-hemolysis on 10% of Sheep blood agar. On Gram's staining of these colonies a clear gram-positive coccoid cells which are arranged in filamentous form were observed under 100X [3]. The arrangement of bacteria in filamentous form is the characteristic feature of these organisms and the fragmentation of these filamentous forms produces the coccoid cells (Fig.2).

Figure 1. β – Haemolysis on 10% Sheep blood agar developed after 72 hrs. of incubation.



Figure 2. Filamentous and Coccoid morphology of *Dermatophilus congolensis* in Gram's staining



3.3 Biochemical characterization

The morphologically confirmed *Dermatophilus* organisms were biochemically characterized. The results of the biochemical tests were presented in table no.1.

Name of the biochemical test	Result
Indole	Negative
Urease	Positive
Catalase	Positive
Glucose	Positive
Gelatin liquefaction	Positive
Growth in nutrient broth	Growth with Clear turbidity
Growth at 25 ^o C	No growth
Growth at 37 ^o C with 5% CO ₂ on BHI agar	Initially Greyish later turned to Greyish yellow colonies
Haemolytic pattern on 10% sheep blood agar	β-Haemolysis started after 72 hrs
Motility	Motile coccid zoospores

Table No.1 Biochemical Characterization of *Dermatophilus congolensis*

3.4 Antibiogram

A total of Seven antibiotic discs were used namely, Amikacin (30mcg/disc), Oxytetracyclin (30mcg/disc), Penicillin(10units/disc), Cephoxitin (30mcg/disc), Ciprofloxacin (5mcg/disc), Enrofloxacin (10mcg/disc) and Trimethoprim (30mcg/disc). Out of the seven antibiotic discs tested, the susceptibility of the *Dermatophilus congolensis* bacterium was observed only to the Amikacin drug while the remaining six antibiotic drugs shows clear resistance pattern towards the bacterium. Based on this, animals were treated with Amikacin (Nitin, Himalaya Meditek) at the rate of 4 mg/kg.body wt. of the animal per day for 5 days continuously. In addition, Vitamin –A (Vetindia) @6ml/day/animal for 6 days, AVIL (Himalaya Meditek) 10 ml/ animal/ day for 6 days and superficially Zinc Oxide powder mixed with Neem Oil was used as a supportive therapy. The animals were maintained for prolonged periods in grazing and feed changed from groundnut meal to fresh green fodder and concentrate feed. The therapy resulted in a good response in animals. At the same time it was observed that a change in the feeding pattern and long time grazing in dry environment further helped the animals in quick recovery.

IV. Discussion

The *Dermatophilus congolensis*, a facultative anaerobe that grows well under 5% CO₂ tension is a gram-positive bacterium under the *Actinomycete* group. This bacterium commonly exists in two morphological forms, a Filamentous form and a coccoid form [5]. The later is a motile zoospore with flagella. This bacterium has wide range of hosts and infects cattle, sheep, goat, horses, camel and humans [1]. The infection due to the *Dermatophilus* has a zoonotic potential that may be transferred to susceptible humans [7, 8, 9]. High humidity, stall feeding with poor exercise, poor management practices, water logging areas, high tick and mite infections and immunocompromised condition of animals are predisposing factors for these organisms. Along with breed susceptibility, variation within the strains of the *Dermatophilus congolensis* isolates also exists [10]. The Grams staining, Giemsa staining, Lactophenol Cotton Blue and Motility tests used in the present study were confirmatively diagnosed the disease was due to a *Dermatophilus* bacterium. The results of the morphological characterization of this bacterium in both filamentous and motile coccoid forms was the best early diagnostic tool in confirming the disease which was also correlates with the reports of the many authors [1, 6, 11]. Staining of smears with Lactophenol Cotton Blue, a fungal stain, ruled out fungal infections. Results of the biochemical tests and culture on blood agar further confirmed the etiological agent as *D. congolensis*. A further confirmation by molecular diagnostics like PCR test will be helpful.

Treatment of the animals with the antibiotic, Amikacin with supportive therapy showed a very good response in recovery of the infected Cattle. However in earlier reports used different kind of antibiotic therapies like Long Acting Tetracycline [12, 13], Procain Pencillins, Streptomycin [3, 14, 15] etc. were used, which implies the variations in susceptibility to antibiotics among different isolates of *Dermatophilus congolensis*. This difference in susceptibility to antibiotics may be attributed to the strain variations among the *Dermatophilus* isolates. In addition to the chemotherapy, improving the management practices will be suggestive for the effective control of the infected animals as well as preventing the spread of disease to uninfected animals [14].

V. Conclusion

Dermatophilus infection in bovines can be diagnosed preliminarily by demonstration of filamentous and motile coccoid zoospores by direct microscopic examination. Further the infection can be confirmed by biochemical tests and culture on blood agar. In the present incidence, antibiotic amikacin was effective in treating the infection.

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