Diagnosis of dermatophilosis in dairy cattle in Kerala, India

P. V. Tresamol* and M. R. Saseendranath1

College of Veterinary and Animal Sciences,
Mannuthy, Thrissur-680 651, India.
Received: 01-03-2014 Accepted: 06-08-2014 DOI: 10.18805/ijar.5587

ABSTRACT
Skin scabs and scrapings from 82 dermatitis cases in dairy cattle were subjected to detailed bacteriological, mycological, parasitological and molecular studies. Microscopical examination of Giemsa or Gram’s stained smears of scab material from the lesions revealed characteristic gram positive septate branching filaments with typical tram track appearance suggestive of Dermatophilus congolensis in 72 samples (91.5%). Culture of scab materials in sheep blood agar under anaerobic condition yielded typical beta haemolytic colonies of D. congolensis in 75 samples, which were further confirmed by colony morphology, staining characters and biochemical reactions. Molecular confirmation of the isolates was carried out using polymerase chain reaction with primers based on 16S rRNA which yielded specific band of 500bp. The pathogenicity of the isolates was also proved by experimental inoculation into rabbits.

Key words: Culture, D. congolensis, Dairy cattle, Pathogenicity, PCR, Rabbits, Staining.

INTRODUCTION
Dermatophilosis is a skin infection of domestic, aquatic and wild animals caused by D. congolensis. This disease has been reported by the Food and Agricultural Organization (FAO) to be one of the four major bacterial diseases which affect cattle and other animals in the tropical and subtropical regions (Hashemi Tabar et al., 2004). Even though the disease is not fatal, it causes severe economic losses to cattle farmers through inferior quality of hide, reduced milk production, weight loss, cost of treatment and culling of severely affected animals. The present study reports preliminary identification of D. congolensis as the etiological agent of widespread lower leg dermatitis among cattle in Kerala and its molecular confirmation using Polymerase Chain Reaction (PCR).

MATERIALS AND METHODS
A total of 82 cattle with clinical signs of dermatitis such as pustules, matting of hairs, scab formation and cracks or fissures on the skin of legs, udder and hindquarters were included in the study. Sterile Skin swabs, skin scabs and scrapings and impression smears from lesions were collected for laboratory examination.

Direct microscopical examination: Small pieces of skin were taken from the underside of the scabs and softened in few drops of distilled water on a clean microscopic slide; a smear was made with this and stained with Giemsa and Gram’s stains. The impression smears taken from the lesions were also stained with Giemsa’s stain and Gram’s stain and examined under oil immersion objective of microscope. (Quinn et al., 1994). The skin scrapings were also subjected to direct microscopical examination using 10 per cent potassium hydroxide to detect presence of fungal elements or mites.

Cultural isolation and identification: Skin swabs were subjected to isolation of D. congolensis on sheep blood agar as per Haalstra’s technique (Haaalstra, 1965). The isolates were stained by Gram’s method and the preliminary tests were done based on it. The morphological, cultural, biochemical and sugar fermentation tests of the isolates were determined as per the methods described by Barrow and Faltham (1993).

Polymerase chain reaction: Polymerase chain reaction was carried out using the primers based on 16S rRNA of D. congolensis as per Shaibu et al. (2010), with minor modifications. Six of the isolates obtained were inoculated into brain heart infusion broth and incubated overnight. About 200 µl of washed cells suspended in sterile distilled water was used for the DNA extraction. Genomic DNA was extracted using the High Pure PCR Template preparation Kit (Roche, Germany) as per the manufacturer’s directions. The sequences of the primers used were based on 16S rRNA of D. congolensis i.e forward primer 5’-ACATGCAAGTC GAACGA TGA-3’ and reverse primer 5’-ACGCTCGCACC CTCAGTATT -3’.

*Corresponding author’s e-mail: pvtresamol@yahoo.co.in. 1Kerala Veterinary and Animal Sciences University, Wayanad, Kerala.
The reaction was performed in a total reaction mixture of 25µl which consisted of 2.5 µl PCR reaction buffer (10X), 2.0 µl MgCl₂, 1 µl each of forward primer and reverse primer, 0.5 µl dNTP mix, 0.5 µl Taq polymerase and 15.5µl Triple distilled water. To each PCR tube 23 µl of master mix and two µl of template DNA were added. The tubes were spun briefly and placed in the Veriti 96 well Thermocycler (Applied Biosystems, USA).

The PCR programme consisted of initial denaturation at 95°C for one minute followed by 32 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for one minute and then a final extension at 72°C for seven minutes. The PCR products were detected by electrophoresis in a 1.5 per cent agarose gel in TBE buffer (1X). Five microlitre each of the PCR products was mixed with one microlitre of 6x gel loading dye and the samples were loaded into the respective wells carefully. A 100 bp DNA ladder was used for detection of 500 bp product as molecular size marker. Electrophoresis was carried out at 70V for 45 minutes. DNA amplifications were visualised under Ultra violet transilluminator and the results were documented in a gel documentation system (Alpha inotech Corporation, USA).

Pathogenicity Study: The inoculum of six representative isolates of D. congolensis was prepared by dissolving the culture material in two millilitres of distilled water and was then painted on to the scarified area at the flank region, ears or legs of rabbits with a swab (Haalstra, 1965). The animals were examined for development of lesions. The scab materials from the lesions were collected and subjected to direct microscopic examination and cultural isolation.

RESULTS AND DISCUSSION

Microscopic examination of Giemsa or Gram’s stained smears of the scab material from the lesions revealed characteristic Gram positive septate branching filaments with typical ‘tram- track appearance’ suggestive of Dermatophilus congolensis in 72 cases (Plate 1). Skin scrapings from two animals were positive for fungal spores on examination with potassium hydroxide and none was positive for mites. The distinctive morphology of tram track appearance of the organism was demonstrated by most of the workers as the most practical diagnostic method for dermatophilosis (Quinn et al., 1994; Award et al., 2008). This appearance was clearly evident only in scabs from fresh active lesions. But, in other cases the branching filaments had undergone disintegration and clumps of zoospores were seen. This might be due to the collection of specimens during chronic or healing stage of the disease as suggested by Award et al. (2008) or due to rough treatment of the scabs during smear preparation which resulted in disintegration of the filaments into coccoid form as suggested by Quinn et al. (1994).

Culture of the scab materials in sheep blood agar in presence of 10 per cent carbon dioxide yielded typical beta haemolytic colonies of D. congolensis in 75 samples (Plate 2). Microscopical appearance of organisms in Gram stained smears from colonies were also highly variable depending on the age of the culture and strain of the isolate (Plate 3). The organisms appeared in any of the following forms, such as Gram positive branching filaments in different stages of segmentation, filaments broken down into coccoid forms, packets of coccoid forms, germinating spores or combinations of the above forms. Because the organisms are facultatively anaerobic, isolation by the technique described by Haalstra (1965) was found to be useful and it also reduced the contamination by other bacteria. There was great variation in colonial morphology of different isolates and even of same isolate on the same agar plate. Similar observations were also made by Amor et al. (2011).
All the isolates were haemolytic in sheep blood agar and positive in tests for motility, catalase, oxidase, urease, gelatin hydrolysis, starch hydrolysis and digestion of Loefflers coagulated serum. They showed negative reaction in tests for nitrate and indole. All the isolates produced acid from glucose, fructose and sucrose within 24 hours of incubation. But variable results were obtained with maltose and mannitol. All isolates except two were unable to produce acid from lactose. The isolates were unable to produce acid from sorbitol and xylose. None of the isolates produced gas from the sugars. The biochemical characteristics showed by the isolates were in agreement with the findings by many workers (Pal, 1995; Mannan et al., 2009; Nath et al., 2010).

Experimental inoculation of rabbits by application of inoculums on scarified skin produced skin lesions by fourth day. The lesions were characterised by erythema, pustules and scab formations. But spontaneous healing of lesions took place after one week without any treatment. Application of inoculum on healthy intact skin did not produce any lesions. Direct microscopical examination of smears of scabs from rabbits stained with Gram’s stain revealed characteristic appearance of coccoid organisms arranged in parallel rows. The cultural examination of the scabs yielded typical colonies which were identified microscopically as D. congolensis. Development of lesions on rabbits after two to three days following experimental inoculation as a zone of intense erythema around scarification, pustules and scab formation which remained in place for six to seven days and regressed after 20 to 52 days was also reported by several workers (Salkin et al., 1981). The present study confirmed the existence of D. congolensis infection among cattle population of Kerala.

ACKNOWLEDGMENT
Authors wish to thank Dean, College of Veterinary and Animal Sciences for the facilities provided and Animal Husbandry Department of Kerala for providing the financial assistance.
REFERENCES