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Direct detection of *Dermatophilus congolensis* from skin scabs of dermatophilosis infected animals by polymerase chain reaction

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Abstract

Dermatophilosis is an economically important disease of livestock caused by *Dermatophilus congolensis*, an actinomycete Gram-positive bacterium that produces motile zoospores which invade the skin and cause an acute, sub acute or chronic skin disease resulting in an exudative epidermitis with scab formation. The organism has also been reported to be of zoonotic importance. The economic effects of the disease is seen in all facets of the livestock industry which include milk, meat, hides and skins, reproduction, draught power, besides premature culling and high cost of veterinary treatment. The presence of *Dermatophilus congolensis* was previously diagnosed from clinical signs, stained smears of the organism from skin scabs of infected animals and through isolation of the organism from the scabs on microbiological media. It is also rarely diagnosed through serology and animal inoculation. Beside the direct smear, which requires the presence of the characteristic morphology of the organism for it to be diagnostic, the isolation of the organism on microbiological media is often very difficult and requires an active form of the disease. It also requires at least forty eight to seventy two hours. Polymerase chain reaction, a molecular biology technique, which is simple and specific, was used for the confirmatory detection of *Dermatophilus congolensis* directly from skin scabs of dermatophilosis infected animals (cattle, sheep, goats and horses) in Nigeria. This was based on the pair of primers used for the detection of the organism in sheep elsewhere, using the same technique. It is concluded that this technique is good enough for the successful detection of the organism in dermatophilosis infected animals in Nigeria.

Key words: *Dermatophilus congolensis*, dermatophilosis, cattle, sheep, goats, horse, skin scabs, polymerase chain reaction.

Introduction

Dermatophilus congolensis (VanSacgham) is the causative agent of dermatophilosis, a skin infection, affecting a wide variety of wild, aquatic and domestic animals and also man. The diagnosis of this organism is usually based primarily on clinical signs and the demonstration of characteristic septate, branching filaments which are longitudinally, as well as transversely divided, to form spherical or ovoid cocci, each about 0.5μm in diameter, in multiple rows [1-3]. The cultivation of this organism is quiet tedious and cumbersome as it requires an active form of the clinical disease for ease of isolation, though researchers have reported the isolation of the organism after prolonged storage under laboratory condition [4]. It requires at least 48 to 72 hours for the colonies to come up on blood agar when cultivated. The difficulty in the isolation of the organism has been confirmed by other researchers [2, 3, 5]. Due to the difficulty in the isolation of the organism, diagnosis was usually accepted on the basis of direct stain smear from skin scabs of infected animals. The characteristic morphological appearance of the organism is diagnostic, provided that cocci are found in transverse rows of two or more and readily seen in stained preparations [6]. Though the diagnosis of dermatophilosis is routinely made using conventional methods like clinical signs, direct smear and isolation of the organism, other methods like serology and animal inoculation have been employed, though rarely used routinely [11-20].

The difficulty in the isolation of the organism from skin scabs of infected animals, has led to the search for a simpler, rapid and specific method for the diagnosis of the disease. The use of Polymerase Chain Reaction (PCR) became feasible for the detection of *D. congolensis* following [7] design of a pair of primers from the 16S rRNA gene of *D. congolensis* and its use for the detection of the organism in sheep in China.

This study was therefore undertaken to see if PCR can be used to detect *D. congolensis* from skin scabs of different species of domestic infected animals viz- a- viz direct stain smears from the same skin scabs using the same primers.

Materials and Methods

Direct smear: Fresh skin scabs collected from dermatophilosis infected cattle, sheep, goats and horses were used for direct smear. Three skin scabs from each of the animals were used. About 5 g of each scab was pulverized and put in a bijou bottle and 5 ml of sterile distilled water added. A loopful of the suspension from each scab was used to make smears on clean grease free microscopic slides. The slides were heat-fixed and Gram stained

for the identification of *D. congolensis*. The remaining skin scab suspensions were used for DNA extraction.

DNA extraction: The skin scabs suspensions above were vigorously shaken (vortexed) and allowed to stand at room temperature for one hour. Two ml of the suspension was put in Eppendorf tubes and centrifuged at 14,000 x g for 5 min. The supernatant was discarded and the pellet washed two more times with sterile distilled water. The pellet was then used for DNA extraction. DNA extraction was carried out using the High Pure PCR Template preparation Kit (Roche, Mannheim, Germany), according to the manufacturers instructions.

PCR assay: This was carried out according to the method and primers designed by Han [7], with slight modifications. This was done in a total reaction mixture of 25 µl, consisting of buffer 2.5 µl, Mg^{2+} 1.0 µl, primer 0.3 µl each, dNTPs 0.8 µl, Taq 0.4 µl, sample DNA 1.0 µl and nuclease free water 18.7 µl. The DNA was initially denatured at 95°C for 1 min, followed by denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min. This was done for 32 cycles, and a final extension at 72°C for 7 min. Ten µl of the PCR products was electrophoresed in 1.5% agarose gel containing 10 µl of 10 mg/ml ethidium bromide at 80V for 45 min. A one hundred base pair (bp) marker was used as a molecular size ladder. DNA amplifications were examined and photographed using Bio imaging system (SyngeneVWR international Japan).

Results

Direct smear: All the smears from the animal species demonstrated Gram positive, filamentous branching, septate arrangement enclosing 2-4 rows of coccoid cells indicating the characteristic microscopic morphology of *D. congolensis*.

PCR: A 500 bp fragment of the 16S rRNA gene of *Dermatophilus congolensis* was amplified from all the scabs of the animal species but not from *Staphylococcus aureus* and *Escherichia coli* which were included as non-specific DNA templates. The PCR result is presented in Fig. 1.

Discussion

The detection of the causative agent of dermatophilosis is done primarily as mentioned earlier on the demonstration of the causative organism in scabs from the lesions or in exudates beneath the scabs. The characteristic microscopic appearance of the organism was observed in the direct smears from all scabs examined and is in agreement with previous workers [1-3,6]. It is, however, sometimes difficult to observe the characteristic microscopic appearance of the organism on direct smear due to the process of preparing the smears. This coupled with the difficulty in isolation and identification of the organism from skin scabs of infected animals on bacteriological media and other methods [1, 8-10], makes the use of PCR a better alternative. The amplification of a 500 bp fragment of the 16S rRNA gene of *D. congolensis* directly from skin scabs of infected animals is a confirmation of this fact, as other bacteria which were included as non-specific DNA templates were not amplified (Fig. 1). This is in agreement with the work of Han [7]. The 16S rRNA gene is a highly conserved region in most bacteria and is of diagnostic significance. This can be seen in the fact that, though the technique was originally used in sheep, it was successfully used in the amplification of the same segment from skin scabs of dermatophilosis infected cattle, goats and horses as well, suggesting that it is the same organism responsible for the disease in these animal species.

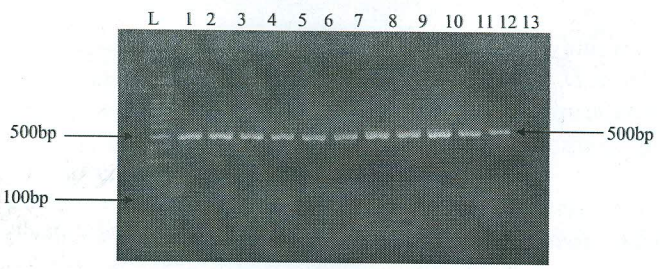

Figure 1. PCR amplifications of a 500 bp fragment of 16S rRNA gene of *Dermatophilus congolensis* from scabs of dermatophilosis infected animals.

Lane L 100bp ladder, Lanes 1 – 3 cattle scabs, 4 – 6 sheep scabs, 7 – 9 goats' scabs and 10 – 11 horses scabs, 12 *Staphylococcus aureus* and 13 *Escherichia coli*.

Conclusions

It is therefore concluded based on the results of this study that, the pair of primers designed by Han [7], from the 16S rRNA gene of *D. congolensis*, is suitable for the specific and direct detection of *D. congolensis* from skin scabs of dermatophilosis infected animals.

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