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The Use of Polymerase Chain Reaction in the Diagnosis of Dermatophilosis from Cattle, Sheep and Goats in Nigeria

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Abstract: Dermatophilosis an economically important disease of livestock, affecting a wide range of domestic and wild animals including man is caused by an actinomycete *Dermatophilus congolensis*. The disease was previously diagnosed by stained smears of the organism from skin scabs of infected animals and isolation of the organism from the scabs. Polymerase chain reaction was used for the confirmatory diagnosis of dermatophilosis from isolates of *Dermatophilus congolensis* gotten from cattle, sheep and goats in the Northern parts of Nigeria. The pair of primers used previously for the diagnosis of the disease in sheep elsewhere was successfully used for the diagnosis of the disease in cattle and goats as well in Nigeria.

Key words: Diagnosis, *Dermatophilus congolensis*, PCR, cattle, sheep, goats

INTRODUCTION

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 (Zaria, 1993).

The economic importance of the disease is estimated in losses of millions of US dollars, due to loss of productivity in terms of work by infected draft oxen, decreased meat and milk production, a failure of reproduction in cows with severe vulva infection and stud bulls with severe leg lesions making them unable to mount (Oppong, 1976), death due to starvation of calves of dams with infected udders (Lloyd, 1976), loss of hides and skins, cost of chemotherapy and chemoprophylaxis, the cost in time and effort in the control of the disease as well as losses due to the culling and death of infected animals (Zaria, 1993).

The disease was first reported in the Belgian Congo (Van Sacegham, 1915), since then it has been reported worldwide (Zaria, 1993). The disease apart from having a worldwide distribution also has a wide host range. These hosts range from domestic to wild and aquatic animals (Zaria, 1993). The domestic animals include cattle, sheep, goats and horses among others. The agent is also of zoonotic importance (Burd *et al*., 2007). The disease was first reported in Nigeria by Henderson (1927).

Previously, direct smear of skin scabs from dermatophilosis infected materials, staining with Gram stain or Giemsa stain and describing the morphology of the organism sufficed in making a diagnosis (Abu-Samra, 1978). This could be followed by the isolation and identification of the organism on bacteriological media and the use of biochemical reactions (Hudson, 1937; Haalstra, 1965; Gordon, 1976; Kelly and Bida, 1970). Also the use of serological methods in the detection of *Dermatophilus congolensis* antibodies is well documented (Abu-Samra, 1978).

The difficulty in the isolation and conventional biochemical characterization of the organism from skin scabs of infected animals has led to the search for a simpler, rapid and specific method for the diagnosis/ identification of the disease and its agent. The use of the polymerase chain reaction seems suitable for this. Wen-Xing *et al*. (2007) use it in the diagnosis of the disease in sheep in China.

The objective of this study is to use PCR for the identification of isolates in Nigeria with pure isolates of the organism. Secondly to use the same technique for the identification of the disease with isolates from cattle and goats.

MATERIALS AND METHODS

Samples: Nine isolates of *Dermatophilus congolensis* from skin scabs of clinically infected cattle, sheep and

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goats in Jos Plateau State central Nigeria were used for DNA extraction. Three isolates from each of the animal species were used.

DNA extraction: Genomic DNA was extracted using the High Pure PCR Template preparation Kit (Roche). About 200 µL of washed cells suspended in sterile distilled water was used for the extraction. The sample was centrifuged at 3500 g for 5 min. The pellet was re-suspended in 200 µL of Phosphate Buffered Saline (PBS) to the pellet add 5 µL lysozyme (10 mg mL^{-1} in 10 mM Tris-Hcl, PH 8.0 and incubated for 15 min at 37°C. After this 200 µL of binding buffer was added to the sample followed by 40 µL of proteinase k, this was immediately properly mixed and then incubated for 10 min at 70°C. About 100 µL of isopropanol was added and properly mixed. This was then poured into a filter tube with a collection Colum and centrifuged at 8000 g for 1 min. The column was discarded and a new one attached. About 500 µL of inhibition removal buffer was added to the filter tube and centrifuged at 800 g for 1 min. The column was discarded and a new one attached. About 500 µL of wash buffer was added to the filter tube and centrifuged at 8000 g for 1 min. The washing step was repeated and the column containing the eluent was discarded. A new 1.5 mL eppendorf tube was attached and 200 uL of pre warmed elution buffer was added to the tube containing the DNA and centrifuged at 8000 g for 1 min. The eluted DNA was collected in the new 1.5 mL eppendorf tube.

PCR: Polymerase chain reaction was carried out using the conditions of 12, from a 16S rRNA of *Dermatophilus congolensis* sequence obtained from the gene Bank. The forward primer 5'-ACATGCAAGTCGAACGATGA-3' and the reverse primer 5'-ACGCTCGCACCCTACGTATT-3' were synthesized by Inqaba biotech South Africa. A total of 25 µL reaction mixture comprising of Buffer 2.5 µL, Mg2+1.0 µL each primer 0.3 µL dNTPs 0.8 µL Taq DNA polymerase 0.4 µL DNA1.0 µL nuclease free water 18.7 µL was used for the amplification (Gene Amp PCR system 9700, Singapore). Template DNA was initially denatured at 95°C for 1 min followed by denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 1 min for a total of 32 cycles and a final extension at 72°C for 7 min. Ten microliters of PCR product was electrophoresed in an agarose gel (1.5%) containing 10 µL of 10 mg mL^{-1} ethidium bromide at 80 volts for 45 min. One hundred base pair marker (Roche, Mannheim, Germany) was used as a molecular size marker. DNA amplifications were examined and photographed using Bio imaging system (Syngene VWR international Japan).

RESULTS AND DISCUSSION

Dermatophilus congolensis isolates from cattle, sheep and goats were amplified by the primer with a band at approximately 500 bp. The following bacteria were included as non specific DNA templates *Staphylococcus aureaus*, *Escherichia coli* and *Salmonella* sp. Water control was also used as negative control. The result of the amplification is shown in Fig. 1.

The results obtained from the amplification of a 500 bp segment of the 16s ribosomal RNA of *Dermatophilus congolensis* gene from sheep isolate of *Dermatophilus congolensis* in this study agrees with the research of Wen-Xing *et al*. (2007), who used primers designed from the 16S ribosomal RNA of *Dermatophilus congolensis* gene, to detect the organism from skin scabs of dermatophilosis infected sheep in china. The result obtained went further to detect the organism in cattle and goats as well. This is an indication of the specificity of the primers, as other organisms that were not *Dermatophilus congolensis* were not amplified. This goes to confirm that PCR can be used as a diagnostic technique in the identification of *Dermatophilus congolensis* isolates. This is also an indication that isolates of *Dermatophilus congolensis* in-respective of the animal species they are derived are closely related. The 16S ribosomal RNA gene has found use in the diagnosis of many bacterial organisms because of the highly conserved nature of this gene in most bacterial organisms.

Some biotech companies have even developed kits for the rapid identification of bacteria, based on this gene.

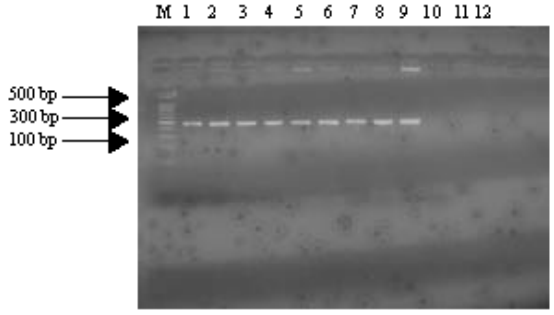

Fig. 1: PCR amplificons of a fragment of 16S rRNA from *D. congolensis* isolates from cattle, sheep and goats. Lane M 100 bp ladder, lanes 1-3 cattle isolates (C, C2 and C6). Lanes 4-6. Sheep isolates (S1, S2 and S3) and lane 7-9 Goat isolates (G1, G2 and G3) and lane 10-12 non specific DNA templates (*Staphylococcus aureaus*, is in lane 10, *E. coli* lane 11 and *Salmonella* sp. lane 12

The pair of primers designed (Wen-Xing *et al*., 2007) seems more specific in detecting isolates of *Dermatophilus congolensis*, compare to that of (Buenviaje *et al*., 2000), who used PCR to detect crocodile and other isolates of *Dermatophilus congolensis* from primers designed from the 16S rRNA of bacteria generally by Marchesi *et al*. (1998) and got amplification which were not specific to only *Dermatophilus congolensis*.

CONCLUSION

It is said that the forward primer 5'-ACATGCAAGTCGAACGATGA-3' and reverse primer 5'-ACGCTCGCACCCTACGTATT-3' designed from the 16S ribosomal RNA of *Dermatophilus congolensis* gene obtained from the gene bank used in this PCR is a good diagnostic technique for *Dermatophilus congolensis* isolates, as it has been able to detect *Dermatophilus congolensis* from cattle, sheep and goats. It is also discriminatory between *Dermatophilus congolensis* isolates and other bacteria (*Staphylococcus aureus*, *E. coli* and *Salmonella* sp.) tested in this study.

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