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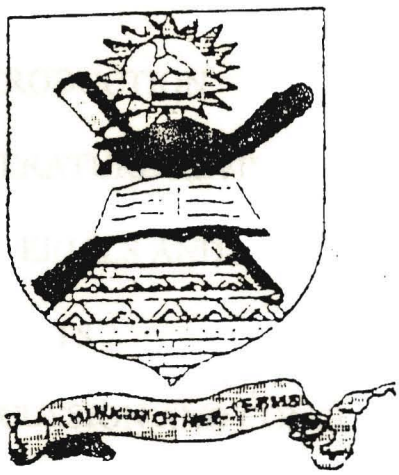

BACHELOR OF APPLIED SCIENCE IN APPLIED BIOLOGY AND BIOCHEMISTRY (HONOURS)

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PROJECT TITLE: TAXONOMIC DETERMINATION OF CATTLE AMPHISTOMES BY R.F.L.P.

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ABSTRACT

Genetic variability between organisms has been assessed using RFLP. The aim of the present study was to extend the technique to taxonomic characterization of paramphistomes. Its application depends on the ability to isolate DNA from many individuals, hence this is the rate-limiting step. Three methods of DNA isolation were used and compared before arriving at the protocol which was used for the study.

The initial method involved extraction from five grams of paramphistomes from one animal. Chloroform : isoamyl alcohol (6:1) was used for protein extraction. On spectrophotometric analysis of the DNA solution, the absorbance at 280nm (protein absorbance) was high. The other problem with this method was that an error had been introduced when phenotypically different paramphistomes, which could also be genetically different, were used. So, in the second method, DNA was extracted from one parasite from each animal, and phenol:chloroform : isoamyl alcohol (25:24:1) was used for protein extraction. On spectrophotometric analysis of the DNA solution there was a large quantity of phenol contamination. This was confirmed by scanning the protein extraction solution spectrophotometrically. This problem was overcome in the third method whereby the residual phenol was extracted using chloroform. DNA was then isolated from three morphologically different parasites from one animal. The extractions were spectrophotometrically assayed and the amount of DNA in each was determined. The solutions were then digested with 10mg/ml of RNAse. The DNA samples were digested with Eco R1 and then another set double- digested with Eco R1 and Bam H1. Agarose gel electrophoresis of the digested samples was then performed. Not all the samples gave results, but those that did had one spot with Eco R1 and those double digested with Eco R1 and Bam H1 had two or three spots.

A second digestion of the samples was performed and agarose gel electrophoresis performed. The spots obtained in the first run were not reproduced. A third run gave spots which were not comparable to the first run. RFLP can therefore be used for the taxonomic variation of paramphistomes but according to this study this has to be supplemented by morphological classification as is detailed by Narsmark (1937).