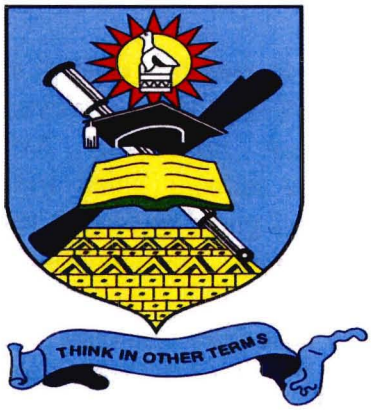

NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY

Faculty of Applied Sciences

Department of Applied Biology and Biochemistry

MOLECULAR CHARACTERISATION OF PARAMPHISTOMES FROM CATTLE FROM MATEBELELAND REGION USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPDS) AND AMPLIFIED RIBOSOMAL DNA RESTRICTION ANALYSIS (ARDRA)

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Abstract

A total of 18 paramphistome isolates collected from Grills and CSC abattoirs were genetically characterised using the Random Amplified Polymorphic DNA (RAPD) technique and Amplified Ribosomal DNA Restriction Analysis (ARDRA). These isolates were morphologically characterized using median sectioning and five putative species were identified. Of the 18 isolates that were being investigated, 16 were positively identified: 3 belonged to *Calicophoron calicophorum*, 2 were *Paramphistomum microbothrium,* one was *Gigantocotyle symmeri* and two other species that were identified as *Calicophoron raja* and *Paramphistomum clavula* both having a total each of 6 paramphistomes. A restriction digest of the amplified ITS-2 region (ARDRA) of all isolates was done using two restriction enzymes *Hae III* and *Sau 3A1* and the fragments obtained did not show any detectable polymorphisms on all isolates. ARDRA was therefore not sufficient to distinguish between the different paramphistome isolates. A total number of 110 bands were generated by RAPD-PCR and 91.82% of these were polymorphic with an average genetic distance of 0.4810+/-0.185 that showed substantial variability among the paramphistome isolates. The RAPD-PCR technique however, gave banding patterns that on analysis were able to cluster (on the dendrogram) the isolates into their respective species groups and even aid in identifying the two isolates that were not positively identified morphologically as *Calicophoron raja*. A fragment of approximately 1300bp was generated from primer OPB 07 on *Paramphistomum microbothrium* isolates which can be used as a selectable marker for this species. The findings of the present study therefore showed that the RAPD- PCR technique can be used for molecular identification of paramphistomes and can be particularly used for developing epidemiological techniques for the study of *P. microbothrium* transmission patterns and prevalence in definitive and intermediate hosts.