7 SUMMARY

Host species identification of bloodmeals from tsetse (Diptera: Glossinidae) by polymerase chain reaction and restriction fragment length polymorphism analysis (PCR-RFLP).

Tsetse flies are obligatory haematophagous arthropods, feeding only on vertebrate blood. They are responsible for the transmission of Human Sleeping Sickness (HSS) and African Animal Trypanosomosis (AAT) in large areas of sub-Saharan Africa. Information on the feeding behaviour of tsetse is essential in understanding the relationship between hosts and vectors, and their respective roles in a disease transmission cycle. The source of a tsetse bloodmeal might provide important information relating to the epidemiology of trypanosomosis and natural feeding habits of different species of Glossina. The aim of this work was to develop a DNA based assay for the identification of bloodmeals from tsetse flies. A DNA bank from potential vertebrate hosts of tsetse flies was established comprising of 33 wild and domestic vertebrate species. DNA was extracted from biological specimens, such as blood, hair and skin and submitted to PCR using universal *Cytochrome b* primers (*cytb* 1 and *cytb* 2). The primers were complementary to the conserved region of the cytochrome b gene of vertebrates leading to a consistent but variable 359 bp-PCR products in all bovid species tested. The selection of appropriate restriction endonucleases sites was based on the comparison of mtDNA sequence data of bovids drawn from the search tool of the National Centre for Biotechnology Information (USA). Sites for all restriction enzymes that cut the amplified 359 bp sequence were identified by means of the free available programme *NEB cutter V1.0* designed by New England Biolab Incorporation. PCR-RFLP analysis was used to differentiate different species of the family Bovidae by using different restriction endonucleases; *Taq*I, *Alu*I and *Hind*II. The obtained species-specific restriction profiles were suitable for the identification of 10 bovid species tested in this study. Interpretation of the

restriction profiles was performed visually by comparison with reference samples and help of 50 bp molecular size marker, without the need for computer analysis.

The results also demonstrate that it is possible to use universal *cytb* primers to amplify DNA present in bloodmeals from haematophagous tsetse (Diptera: Glossinidae). The detection rate of host DNA in tsetse bloodmeals by PCR-RFLP was 100%, 80%, 60% and 40% at 24, 48, 72 and 96 h post-feeding, respectively. In addition, the technique was successfully used to amplify DNA from blood smeared onto filter paper and subjected to antiseptic solutions without deterioration in the host DNA.

After digestion of PCR amplicons with restriction endonucleases, some non-specific DNA fragments were obtained in some species due to co-amplification of nuclear *cytb* pseudogenes. In addition, incomplete digestion was observed in some species.

The results of this study reveal that the *cytb* PCR-RFLP analysis is a promising tool for the identification of tsetse bloodmeals.