

CONTENTS

ABBREVIATIONS	IV
1 INTRODUCTION	1
2 OBJECTIVES.....	5
3 LITERATURE REVIEW.....	7
3.1 African Animal Trypanosomosis	7
3.1.1 Definition and aetiology	7
3.1.2 Morphology	8
3.1.3 Host range	10
3.1.4 Transmission and distribution	11
3.1.5 Pathogenesis and clinical signs	11
3.1.6 Epidemiology	13
3.2 Molecular biology of trypanosomes.....	14
3.2.1 Nucleus.....	14
3.2.2 Kinetoplast DNA.....	15
3.3 Diagnosis	16
3.3.1 Clinical diagnosis	17
3.3.2 Parasitological diagnosis	17
3.3.3 DNA-based techniques.....	19
3.4 Control of African Animal Trypanosomosis	23
3.4.1 Vector control.....	23
3.4.2 Trypanotolerant cattle breeds	25
3.4.3 Vaccination.....	25
3.4.4 Chemotherapy and chemoprophylaxis	26
3.5 Drug resistance in trypanosomes	30
3.6 Mechanism and genetics of resistance to trypanocides.....	35
3.6.1 Alteration of drug transport.....	35
3.6.2 Adenosine transport and drug resistance.....	37
3.7 Laboratory induction of drug resistance in trypanosomes	40
3.8 Cloning of trypanosomes	42
3.9 Methods of detection of drug resistance in trypanosomes.....	42
3.9.1 Tests in ruminants	43
3.9.2 Tests in mice.....	44
3.9.3 <i>In vitro</i> assays.....	45

3.9.4	Trypanosomal drug ELISAs.....	46
3.9.5	Longitudinal field studies.....	47
3.9.6	Genetic markers for drug resistance in trypanosomes	48
3.9.7	Potential new tests for detection of resistance to isometamidium	50
4	MATERIAL AND METHODS.....	51
4.1	Origin and history of <i>T. congolense</i> and <i>T. brucei</i> stocks and clones	51
4.2	Origin and history of <i>T. congolense</i> and <i>T. brucei</i> reference clones	53
4.3	Experimental animals	56
4.4	Propagation of <i>T. congolense</i> and <i>T. brucei</i> stocks in mice	56
4.5	Cloning of trypanosome stocks	56
4.6	Induction of isometamidium resistance in <i>T. congolense</i> in experimentally infected mice	58
4.7	Standardised drug sensitivity tests in mice	61
4.8	Molecular characterisation of the genetic identity of the <i>T. brucei</i> and <i>T. congolense</i> study clones and stocks	62
4.9	Characterisation of the TbAT1 gene in <i>T. b. brucei</i>.....	65
4.9.1	Genomic DNA amplification of the TbAT1 gene fragments in <i>T. b. brucei</i>	66
4.9.2	Sequence analysis of TbAT1 gene fragments in isometamidium-sensitive and -resistant <i>T. b. brucei</i>	66
4.9.3	Sfa NI Restriction Fragment Length Polymorphism (RFLP) analysis of TbAT1 gene fragments in <i>T. b. brucei</i>	67
4.10	Characterisation of putative target sequences in <i>T. congolense</i> homologues to <i>T. brucei</i> adenosine transporter gene (TbAT1)	67
4.10.1	Genomic DNA amplification of the TbAT1 in <i>T. congolense</i> isometamidium-sensitive and -resistant clones using the primers Sfa-s and Sfa-as	68
4.10.2	Investigation of TbAT1 homologous gene transcripts in <i>T. congolense</i> using flanking primers.....	68
4.10.3	Genomic DNA analysis of <i>T. congolense</i> for the presence of TbAT1 homologous sequences using degenerate primers.....	69
4.11	Data management and analysis.....	71
5	RESULTS.....	72
5.1	Propagation of <i>T. congolense</i> stocks and clones	72
5.2	Cloning of <i>T. congolense</i>	72
5.3	Isometamidium sensitivity of <i>T. congolense</i> clones	73

5.4	Experimental induction of isometamidium-resistance in <i>T. congolense</i> using immunosuppressed mice.....	80
5.5	Molecular characterisation of genetic identity of the <i>T. brucei</i> and <i>T. congolense</i> study clones	85
5.6	Detection of a fragment of TbAT1 gene in <i>T. b. brucei</i> stocks by PCR.....	88
5.7	Sequence analyses of the TbAT1 gene fragments from the genomic DNA of isometamidium-sensitive and -resistant <i>T. b. brucei</i> stocks.....	90
5.8	Sfa NI RFLP analysis.....	93
5.9	Characterisation of putative target sequences in <i>T. congolense</i> homologues to <i>T. brucei</i> adenosine transporter genes (TbAT1).....	95
5.9.1	PCR analysis of the genomic DNA of <i>T. congolense</i> for the presence of the 677 bp TbAT1 transcripts	95
5.9.2	Investigation of TbAT1 homologous gene transcripts in <i>T. congolense</i> using sets of primers flanking different regions of the gene	96
5.9.3	Genomic DNA analysis of <i>T. congolense</i> for the presence of TbAT1 homologous sequences using degenerate primers.....	98
6	DISCUSSION	100
6.1	Cloning of trypanosome stocks	100
6.2	Isometamidium sensitivities of clones of <i>T. congolense</i> from East and West Africa	101
6.3	Induction of isometamidium resistance in <i>T. congolense</i>	103
6.4	Molecular characterisation of isometamidium-sensitive and –resistant trypanosomes	107
7	CONCLUSIONS.....	114
8	SUMMARY.....	116
9	ZUSAMMENFASSUNG	118
10	REFERENCES	120
11	ANNEXES.....	142
	ACKNOWLEDGEMENTS.....	147
	CURRICULUM VITAE	148
	SELBSTÄNDIGKEITSERKLÄRUNG	149