

Table of Contents

	Page
List of figures	iv
List of tables	vii
List of abbreviations	viii
1 Introduction	1
2 Literature review	3
2.1 Genus <i>Theileria</i>	3
2.2 <i>Theileria</i> in bovine	3
2.2.1 <i>T.annulata</i>	3
2.3 Life cycle of <i>Theileria</i>	4
2.4 Clinical signs	5
2.5 Pathogenesis and immunity to <i>Theileria</i> infection.....	5
2.6 Host parasite interaction.....	6
2.6.1 Invasion process	7
2.6.2 Induction and maintenance of transformation and proliferation	7
2.7 Apoptosis.....	9
2.7.1 Pathways leading to apoptosis.....	10
2.7.2 Inhibition of apoptosis.....	11
2.8 Stage differentiation	11
2.9 HSPs	12
2.9.1 HSPs and parasitic infection	13
2.10 HSP90.....	13
2.10.1 Structure of HSP90.....	14
2.10.2 HSP90 and parasitic infection	15
2.11 Aim of this study	15
3 Materials and Methods	16
3.1 Materials.....	16
3.1.1 Cells and Cell lines.....	16
3.1.2 Chemicals, reagents and Kits	16
3.1.3 Buffers, solutions and media	18
3.1.4 Primers	20
3.1.5 Antibodies	21
3.1.6 Bacteria, vectors and enzymes	22
3.1.7 Lab supplies.....	23
3.1.8 Equipment	23
3.1.9 Software	24

3.2	Methods	25
3.2.1	Cell culture	25
3.2.2	Sub-culturing and cell counting	25
3.2.3	Molecular biological methods	26
3.2.4	Expression and purification of recombinant <i>T. annulata</i> HSP90	32
3.2.5	Protein analysis	34
3.2.6	Generation of rabbit anti- <i>T. annulata</i> HSP90 antiserum	36
3.2.7	Isolation of <i>T.annulata</i> schizonts	38
3.2.8	Cytospin slides	38
3.2.9	Immunocytochemistry	39
3.2.10	HSP90 inhibitors	41
3.2.11	Statistical analysis and data management	45
3.3	Plan of work	46
4	Results	47
4.1	Functional analysis of HSP90 in <i>T.annulata</i> Ankara 288-infected cells	47
4.1.1	Assessment of apoptosis	47
4.1.2	Quantitative assessment of the proliferation of <i>T. annulata</i> Ankara 288-infected cells	56
4.1.3	Quantitative assessment of the stage progression in <i>T. annulata</i> Ankara 288-infected cells treated with GA	59
4.2	The regulation of HSP90 in <i>T. annulata</i> Ankara 288-infected cells	62
4.2.1	The regulation of bovine HSP90 in GA-treated cells	62
4.2.2	The regulation of <i>T. annulata</i> HSP90 in GA-treated cells	65
4.2.3	Comparison between the expression of bovine HSP90 and <i>T.annulata</i> HSP90 during the parasite differentiation	67
4.3	Detection of the bovine HSP90 protein	68
4.4	Identification and characterization of <i>T. annulata</i> HSP90	71
4.4.1	Detection of <i>T. annulata</i> HSP90 and cDNA cloning	71
4.4.2	Sequencing and sequence analysis	72
4.4.3	Phylogenetic analysis	75
4.5	Expression and purification of recombinant <i>T. annulata</i> HSP90	76
4.5.1	Bioinformatic analysis of TaHSP90-Chr1 and TaHSP90-Chr4	76
4.5.2	Expression and purification of TaHSP90-Chr1 and TaHSP90-Chr4 recombinant protein	76
4.6	Detection of <i>T. annulata</i> HSP90 protein	79
4.6.1	Antigenic determinants of TaHSP90-Chr1 and TahSP90-Chr4	80
4.6.2	Detection of <i>T. annulata</i> HSP90 in cell lysates prepared from <i>T. annulata</i> Ankara 288-infected cells	81
4.7	Assessment of the effect of Novobiocin on <i>T. annulata</i> Ankara 288-infected cells	83

5	Discussion	85
5.1	The role of HSP90 in apoptosis and cell proliferation in <i>T. annulata</i> -infected cells	85
5.2	HSP90 and stage progression of the parasite	88
5.3	The regulation of HSP90 in <i>T. annulata</i> Ankara 288-infected cells	90
5.4	Identification and characterization of <i>T. annulata</i> HSP90	92
5.5	Detection of <i>T. annulata</i> HSP90 and bovine HSP90	93
5.6	Novobiocin	94
5.7	Conclusion	95
6	Summary	96
7	Zusammenfassung	99
8	Reference	102
9	Appendix	115
9.1	Flow cytometric analysis of apoptosis in <i>T. annulata</i> -infected cells incubated with GA 0.05 μ M at 41°C	115
9.2	Quantitative assessment of the stage progression in <i>T. annulata</i> Ankara 288-infected cells treated with GA using TamS1 and TamR1	116
9.3	Quantitative real-time polymerase chain reaction (qRT-PCR)	116
9.4	The alignment of bovine HSP90 with HSP90 β of human	118
9.5	FastDigest® restriction enzymes of purified plasmids from <i>E. coli</i> DH5 α competent cell	119
9.6	Alignment of the amino acid sequence of TaHSP90-Chr1 and TaHSP90-Chr4	119
9.6.1	Comparison of the deduced amino acid sequences of the clones	119
9.6.2	Comparison of the deduced amino acid sequences of TaHSP90-Chr1 with TaHSP90-Chr4	127
9.7	Bioinformatic analysis of TaHSP90-Chr1 and TaHSP90-Chr4	129
10	List of publications	130
11	Acknowledgements	131