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**HOST-PARASITE INTERACTIONS DURING
ABOMASAL PARASITISM
AND
POTENTIAL ROLES FOR ES PRODUCTS**

A thesis presented in partial fulfilment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY

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WIEBKE BÜRING

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Table of Contents

Abstract	i
Acknowledgements	ii
List of Figures	iii
List of Tables	xi
List of Abbreviations	xiii
Introduction	xxiv
Chapter 1: Literature Review	1
1.1 Parasitic Abomasal Nematodes of Sheep	1
1.1.1 Life Cycle of Abomasal Nematodes	2
1.2 Structure and Function of the Abomasum	3
1.2.1 Pyloric Glands	4
1.2.1.1 The G Cell and Gastrin Secretion	4
1.2.2 Fundic Glands	6
1.2.2.1 Zymogenic Cells and Pepsinogen Secretion	6
1.2.2.2 The ECL Cell and Histamine Secretion	7
1.2.2.3 The Parietal Cell and Acid Secretion	7
1.2.2.3.1 H ⁺ /K ⁺ ATPase, Cl ⁻ Channels and K ⁺ Channels	8
1.2.2.3.2 Mechanism of Acid Production	9
1.2.2.3.3 Regulation of Acid Secretion	10
1.2.2.3.3.1 Histamine H ₂ Receptors	11
1.2.2.3.3.2 Gastrin CCK _b and Acetylcholine M ₃ Receptors	12
1.2.2.3.3.3 Calcium-Sensing Receptor	13
1.2.2.3.3.4 Gap Junctional Channels	13
1.2.2.3.3.5 Inhibition of Acid Secretion	14
1.2.3 Gastric Mucosal Barrier	15
1.2.3.1 Mucus and Bicarbonate	15
1.2.3.2 Properties of the Gastric Epithelium	16

Table of Contents

1.2.3.2.1	Cell-Cell Adhesion	16
1.2.3.2.1.1	Tight Junctions	17
1.2.3.2.2	Cell-Matrix Adhesion	20
1.2.4	Differentiation, Proliferation and Maintenance of Gastric Epithelial Cells	21
1.2.4.1	Cell Death	21
1.2.4.2	Proliferation	22
1.2.4.3	Trophic Agents	23
1.2.4.3.1	Gastrin	23
1.2.4.3.2	Regenerating Protein (Reg)	24
1.2.4.3.3	EGF-Family Peptides	24
1.2.4.3.4	Musashi-1	25
1.3	The Parasitised Abomasum	26
1.3.1	Pathophysiology	26
1.3.2	Histopathology	29
1.3.3	Parasite Excretory/Secretory Products	31
1.3.3.1	Proteases	32
1.3.3.2	Protease Inhibitors	33
1.3.3.3	Antioxidant Enzymes	34
1.3.3.4	Other Enzymes	34
1.3.3.5	Calreticulin	35
1.3.3.6	Non-Protein Compounds	36
1.3.3.7	<i>In vitro</i> Functions - Further Possibilities for <i>in vivo</i> Actions	37
1.3.3.7.1	Effects on Secretion	37
1.3.3.7.2	Immunomodulatory Effects	37
1.3.3.7.3	Effects on Cell Proliferation	38
1.3.3.7.4	Cell Vacuolation	39
1.4	Conclusions	40
	Chapter 2: Effects of Parasitism on the Abomasal Mucosa	41
2.1	Introduction	41

2.2 Early Changes within the Abomasal Mucosa after Infection with Labelled <i>H. contortus</i> L ₃	44
2.2.1 Materials and Methods	44
2.2.1.1 Experimental Overview	44
2.2.1.2 Parasites	44
2.2.1.3 Labelling of <i>H. contortus</i> L ₃	44
2.2.1.4 Infection of Sheep with Labelled <i>H. contortus</i>	45
2.2.1.5 Preparation of Abomasal Tissue for Microscopy	45
2.2.1.6 Microscopy	46
2.2.1.7 Recovery of Labelled <i>H. contortus</i> from the Abomasum	46
2.2.2 Results	48
2.2.2.1 Fluorescent Labelling of <i>H. contortus</i> L ₃	48
2.2.2.2 Identification of Glands Parasitised by <i>H. contortus</i> Larvae	48
2.2.2.3 Counts of Labelled <i>H. contortus</i> in Abomasal Contents and Tissues .	49
2.3 Early Changes within the Abomasal Mucosa after Infection with Adult <i>T. circumcincta</i>	50
2.3.1 Material and Methods	50
2.3.1.1 Experimental Overview	50
2.3.1.2 Infection with <i>T. circumcincta</i>	50
2.3.1.3 Histochemistry	50
2.3.1.3.1 Lectin Staining	51
2.3.1.3.2 Proton Pump and TGF- α	52
2.3.1.4 Statistical Analysis	53
2.3.2 Results	53
2.3.2.1 Lectin Staining	53
2.3.2.2 Proton Pump and TGF- α Labelled Parietal Cells	53
2.3.2.2.1 Abomasal Tissue from Uninfected Control Sheep	54
2.3.2.2.2 Abomasal Tissue 12h after Adult Transplant	55
2.3.2.2.3 Abomasal Tissue 72h after Adult Transplant	55
2.3.2.3 Abomasal pH and Mucosal Thickness	56
2.4 Discussion	57

2.4.1	Early Changes within the Abomasal Mucosa after Infection with Labeledled <i>H. contortus</i> L ₃	57
2.4.2	Early Changes within the Abomasal Mucosa after Infection with Adult <i>T. circumcincta</i>	59
2.4.2.1	Identification of Functionally Different Parietal Cells	59
2.4.2.2	The Fate of the Parietal Cell and H ⁺ /K ⁺ -ATPase Effect	61
Chapter 3: Effects of <i>H. contortus</i> ES Products <i>in vitro</i>: Cell		
	Vacuolation	67
3.1	Introduction	67
3.2	Materials and Methods	70
3.2.1	ES Preparations of Adult <i>H. contortus</i>	70
3.2.2	Further Modifications or Treatments of ES Preparations	71
3.2.2.1	Different Storage Conditions	71
3.2.2.2	Dilutions of ES Preparations	71
3.2.2.3	Ammonia	71
3.2.2.4	Fractionation of ES Products	71
3.2.2.5	TLC (Thin-Layer Chromatography)	73
3.2.2.6	Protease and Phosphatase Inhibition	73
3.2.2.7	Proteinase K Digestion	74
3.2.2.8	Heat-Treatment	74
3.2.2.9	Acid-Treatment	74
3.2.3	Protein Concentration of ES Preparations	74
3.2.4	Ammonia Determination in ES	75
3.2.5	Cell Culture	75
3.2.6	Incubation of Cells with ES Preparations	75
3.2.7	Neutral Red Uptake	76
3.2.8	Microscopic Examination of Cells	76
3.2.9	Electrophoresis	76
3.2.9.1	Sodium Dodecylsulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)	76
3.2.9.2	Blue Native-Polyacrylamide Gel Electrophoresis (BN-PAGE)	77

Table of Contents

3.2.9.3	Two-Dimensional (2D) BN/SDS-PAGE	78
3.3	Results	78
3.3.1	Cell Vacuolation Due to ES Products	78
3.3.2	Stability of the Vacuolating Factor	79
3.3.3	Vacuolating Ability of ES Preparations	80
3.3.4	Time Course of Vacuolation	81
3.3.5	Reversibility of Vacuolation	81
3.3.6	Vacuolating Ability of ES at Different Time Points of ES Harvest	82
3.3.7	Ammonia and ES	85
3.3.8	Fractionation of ES Products	86
3.3.9	Lipids and Prostaglandins - A Possible Role in Vacuolation?	88
3.3.9.1	Lipid and Prostaglandin Detection via SDS-PAGE	88
3.3.9.2	Lipid and Prostaglandin Detection via TLC	89
3.3.9.3	Vacuolating Activity of Prostaglandins and Extracted Lipids	89
3.3.10	Protease and Phosphatase Inhibition	90
3.3.11	Proteinase K Digestion	91
3.3.12	Heat-Treatment	92
3.3.13	Acid-Treatment	93
3.4	Discussion	94
3.4.1	Characteristics of the Vacuolation	94
3.4.2	Characteristics of the Vacuolating Factor of <i>H. contortus</i> ES Products	96
3.4.2.1	Ammonia and Vacuolation	96
3.4.2.2	Differences in ES Products from Successive <i>in vitro</i> Incubations	97
3.4.2.3	Fractionation of <i>H. contortus</i> ES Products	99
3.4.2.4	Do Lipids Play a Role in Vacuolation?	100
3.4.2.5	Is the Vacuolating Factor of <i>H. contortus</i> ES Products a Protein?	101
Chapter 4: Effects of <i>H. contortus</i> ES Products <i>in vitro</i>: Cell		
	Detachment	103
4.1	Introduction	103
4.2	Materials and Methods	105
4.2.1	ES Preparations of Adult <i>H. contortus</i>	105

Table of Contents

4.2.2	Cell Culture	105
4.2.3	Incubations with ES Preparations on Cells	105
4.2.4	Actin Staining Using Alexa Fluor® Phalloidin	106
4.2.5	Determination of Cell Numbers Using the CyQUANT® NF Assay ..	106
4.2.6	Statistical Analysis	107
4.3	Results	107
4.3.1	Staining of the Actin Cytoskeleton	107
4.3.2	Quantification of Detachment	108
4.4	Discussion	109
	Chapter 5: Tight Junction Permeability	116
5.1	Introduction	116
5.2	Materials and Methods	119
5.2.1	ES Preparations of Adult <i>H. contortus</i> and <i>T. circumcincta</i>	119
5.2.2	Preparation of Sheep Gastric Mucosa	119
5.2.3	Cell Culture	119
5.2.4	Incubations with ES Preparations on Cells	120
5.2.5	TEER Measurements	120
5.2.6	Antibody Staining of Tight Junction Proteins (Occludin and ZO-1)	120
5.2.7	Microscopic Examination of Cells and Tissue	121
5.2.8	Statistical Analysis	121
5.3	Results	121
5.3.1	Changes in Tight Junction Permeability Shown by TEER Measurements	121
5.3.2	Changes in Tight Junction Permeability Shown by Tight Junction Protein Staining	122
5.3.2.1	CaCo-2 Cells	122
5.3.2.2	Fixed Abomasal Tissue Folds	123
5.4	Discussion	123
	Chapter 6: General Discussion	129
	References	136
	Appendix I: Parasitology	187

Table of Contents

1.1	Animals	187
1.2	FEC	187
1.3	Infections	188
1.4	Labelling of <i>H. contortus</i>	188
1.4.1	Solutions	188
1.5	ES Preparations of Adult <i>H. contortus</i> and <i>T. circumcincta</i>	188
1.5.1	Solutions	188
1.5.2	Method	189
1.6	Larval Culture Stock	190
	Appendix II: Cell Culture	191
2.1	Cell Culture Media and Solutions	191
2.2	HeLa Cell Passage	193
2.3	AGS Cell Passage	194
2.4	CaCo-2 Cell Passage	194
2.5	Cryopreservation of Cells	195
2.6	Thawing of Cryopreserved Cells	195
	Appendix III: Gel Electrophoresis	196
3.1	SDS-PAGE	196
3.1.1	Solutions	196
3.1.2	Gel Composition	197
3.2	BN-PAGE	198
3.2.1	Solutions	198
3.2.2	Gel Composition	200
3.3	Gel Staining Methods	201
3.3.1	Coomassie Brilliant Blue	201
3.3.1.1	Solutions	201
3.3.1.2	Method	201
3.3.2	Silver Staining	201
3.3.2.1	Solutions	201
3.3.2.2	Method	202
3.3.3	Modified Carbohydrate Silver Staining	202

Table of Contents

3.3.3.1	Solutions	202
3.3.3.2	Method	203
3.3.4	Sudan Black	204
3.3.4.1	Solutions	204
3.3.4.2	Method	204
Appendix IV: Assays		205
4.1	Bradford Assay	205
4.2	Ammonia Assay	205
4.2.1	Solutions	205
4.2.2	Method	206
Appendix V: Histochemistry		207
5.1	Solutions	207
Appendix VI: Chemicals		209

Abstract

Parasite excretory/secretory (ES) products are believed to play a role in the initiation of the host response to the abomasal parasites *Haemonchus contortus* and *Teladorsagia circumcincta*. Both parasites inhibit and cause loss of the acid-producing parietal cells. Three days after transplantation of adult *T. circumcincta* into parasite-naive sheep, a subpopulation of their parietal cells no longer expressed the proton pump β -subunit, but still stained for Transforming Growth Factor- α , suggesting loss of the proton pump preceded cell death. To investigate the ability of parasites to modify the function of mammalian cells *in vitro*, HeLa, AGS and CaCo-2 cells were exposed to ES products. ES products vacuolated all three cells, causing the development of large numbers of small vacuoles, which differed in appearance from those produced by *Helicobacter pylori* bacterial toxin VacA or ammonia. The vacuoles were unlike those which develop in parietal cells in the parasitised abomasum. Neither lipids nor prostaglandins appeared to play a role in vacuolation and the vacuolating factor *in vitro* is likely to be a protein because of its heat and acid lability. Vacuolation occurred within one hour and was partially reversible. ES products were also able to cause cytoskeletal rearrangement and detachment of HeLa cells, similar processes to those caused by bacterial pathogens, which also disrupt tight junctions in mammalian cells. *H. contortus* ES products also disrupted tight junctions of CaCo-2 cell monolayers, a model cell system used for these studies. The increased epithelial permeability was associated with structural rearrangements of the tight junction proteins occludin and ZO-1. This could explain protein loss and back-diffusion of pepsinogen into the blood, a marker of abomasal parasitism. Cell detachment and disruption of cell-cell adhesion in parasitised sheep may inhibit acid production by parietal cells, which cannot function when separated from adjacent cells. Increased permeability of the surface epithelium would allow parasite ES products to penetrate the mucosal barrier, causing further damage. This could also allow inhibition of parietal cells deeper in the abomasal glands and also allowing adult parasites living in the gastric lumen to modulate host immunity to enhance their survival.

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List of Figures

	Facing page
1.1 Anterior morphology of <i>H. contortus</i> .	2
1.2 Adult <i>H. contortus</i> - female.	2
1.3 Generalised life cycle of Trichostrongylidae.	2
1.4 Sheep abomasum and adult <i>H. contortus</i> 21d p.i.	3
1.5 Layers of the gastric wall (schematic, human stomach) and tunica mucosa (tissue section, sheep fundus).	3
1.6 Regulation of gastrin secretion.	5
1.7 Gastric gland of the fundus (schematic).	6
1.8 Regulation of histamine secretion and scheme of histamine synthesis by the ECL cell.	7
1.9 Scheme of the transformation of the parietal cell tubulovesicular membrane system into the intracellular canaliculus during acid secretion.	8
1.10 The parietal cell at rest and after stimulation (schematic).	8
1.11 Mechanism of acid production by the parietal cell.	9
1.12 Overview of the principal pathways involved in the regulation of acid secretion.	10
1.13 Intracellular pathways involved in acid secretion by parietal cells.	10
1.14 Components of the gap junction (schematic).	13
1.15 Overview of cell adhesion mechanisms.	16

1.16	Schematic model of adherens junctions.	17
1.17	Schematic model of a desmosome.	17
1.18	Freeze-fracture electron micrographs and schematic model of tight junction strands.	17
1.19	Transmembrane proteins of tight junctions.	17
1.20	Model of tight junction pores formed by claudins.	18
1.21	Tight junction model with associated tight junction plaque proteins.	19
1.22	Schematic model of focal adhesions (focal contacts).	20
1.23	Stages of cell-matrix adhesion.	20
1.24	Overview of proliferation, differentiation and maintenance of gastric epithelial cells.	23
1.25	Putative proteolytic cascade for hemoglobin degradation by blood-feeding nematodes.	33
2.1	Characteristic autofluorescence in the gut region of sheathed (A) and exsheathed (B) <i>H. contortus</i> L ₃ .	48
2.2	Hoechst 33258 labelled sheathed (A) and exsheathed (B) <i>H. contortus</i> L ₃ .	48
2.3	Razor blade-cut sections of fundic mucosa collected 24h p.i. (A. and B.) and 30h p.i. (C.) from sheep infected with <i>H. contortus</i> L ₃ .	48
2.4	Luminal surface of a nodular area of fundic mucosa from a sheep 18h after infection with 30,000 labelled and unlabelled (1:1) <i>H. contortus</i> L ₃ .	48
2.5	Lectin stained (DBA) fundic tissue 30d p.i. from a sheep infected with 35,000 <i>T. circumcincta</i> L ₃ .	53

2.6	Lectin stained (SBA) fundic tissue 30d p.i. from a sheep infected with 35,000 <i>T. circumcincta</i> L ₃ .	53
2.7	Lectin stained (PNA) fundic tissue 30d p.i. from a sheep infected with 35,000 <i>T. circumcincta</i> L ₃ .	53
2.8	Lectin stained (DBA) ovine fundic tissue before (control) and 12 and 72h after transplantation of 10,000 adult <i>T. circumcincta</i> .	53
2.9	Lectin stained ovine fundic tissue before (control) or 6h after transplantation of 10,000 adult <i>T. circumcincta</i> .	53
2.10	TGF- α and H ⁺ /K ⁺ -ATPase positive parietal cell staining of successive ovine fundic sections (12h after transplantation of 10,000 adult <i>T. circumcincta</i>).	54
2.11	Numbers of parietal cells - TGF- α positive vs. H ⁺ /K ⁺ -ATPase positive of individual sheep (A) and group mean (B) before (control) and 12 and 72h after transplantation of 10,000 adult <i>T. circumcincta</i> .	54
2.12	Fundic mucosa of uninfected control sheep.	54
2.13	Numbers of TGF- α positive vs. H ⁺ /K ⁺ -ATPase positive parietal cells per 30 μ m unit area in a 300 μ m wide column of ovine fundic mucosa for each group (control and 12 and 72h after transplantation of 10,000 adult <i>T. circumcincta</i>).	54
2.14	Numbers of TGF- α positive vs. H ⁺ /K ⁺ -ATPase positive parietal cells per 30 μ m unit area in a 300 μ m wide column of fundic mucosa of individual sheep for each group (control and 12 and 72h after transplantation of 10,000 adult <i>T. circumcincta</i>).	54
2.15	Fundic mucosa of uninfected control sheep - Parietal cell vacuolation.	54

2.16	Fundic mucosa of uninfected control sheep.	55
2.17	Fundic mucosa of sheep 12h after transplantation of 10,000 adult <i>T. circumcincta</i> .	55
2.18	Numbers of TGF- α positive, H ⁺ /K ⁺ -ATPase positive, and TGF- α positive vs. H ⁺ /K ⁺ -ATPase positive parietal cells per 30 μ m unit area in a 300 μ m wide column of ovine fundic mucosa before (control) and 12 and 72h after transplantation of 10,000 adult <i>T. circumcincta</i> .	55
2.19	Fundic mucosa of sheep 12h after transplantation of 10,000 adult <i>T. circumcincta</i> - Parietal cell vacuolation.	55
2.20	Fundic mucosa of sheep 12h after transplantation of 10,000 adult <i>T. circumcincta</i> .	55
2.21	Fundic mucosa of sheep 72h after transplantation of 10,000 adult <i>T. circumcincta</i> .	56
2.22	Fundic mucosa of sheep 72h after transplantation of 10,000 adult <i>T. circumcincta</i> - Parietal cell vacuolation.	56
2.23	Fundic mucosa of sheep 72h after transplantation of 10,000 adult <i>T. circumcincta</i> .	56
2.24	Fundic mucosa of sheep 72h after transplantation of 10,000 adult <i>T. circumcincta</i> .	56
2.25	Fundic mucosa of sheep 72h after transplantation of 10,000 adult <i>T. circumcincta</i> .	56
2.26	Mucosal thickness (μ m) and abomasal pH of individual sheep before (control) and 12 and 72 hours after transplantation of 10,000 adult <i>T. circumcincta</i> .	56

2.27	Thickness of the different regions (base-neck-pit) of fundic glands of individual sheep before (control), 12 and 72h after transplantation of 10,000 adult <i>T. circumcincta</i> .	57
3.1	Vacuolation in HeLa cells induced by <i>H. contortus</i> ES products (fresh)	79
3.2	Vacuolation in HeLa cells induced by <i>H. contortus</i> ES products (fresh).	79
3.3	Vacuolation in AGS cells induced by <i>H. contortus</i> ES products (fresh).	79
3.4	Vacuolation in HeLa and AGS cells induced by <i>H. contortus</i> ES products (after short-term freezing).	79
3.5	Effect of different storage conditions of <i>H. contortus</i> ES on its ability to induce vacuolation in HeLa cells.	80
3.6	Vacuolating ability of dilutions of <i>H. contortus</i> ES products on HeLa cells.	81
3.7	Time Course of vacuolation after exposure to <i>H. contortus</i> ES products to HeLa cells.	81
3.8	Reversibility of <i>H. contortus</i> ES-induced vacuolation in HeLa cells.	82
3.9	Vacuolation in HeLa cells induced by <i>H. contortus</i> ES which was harvested at different time points (successive incubation periods).	83
3.10	SDS-PAGE 5-20% of ES harvested at different time points (successive incubation periods).	83
3.11	2D BN/SDS-PAGE of ES harvested at different time points (successive incubation periods).	84
3.12	Effect of 1mM and 8mM ammonia in CEM and in ES on HeLa cells.	85

3.13	SDS-PAGE 5-20% of ES fractions.	86
3.14	SDS-PAGE 5-20% of fractions of protein standards.	86
3.15	SDS-PAGE 5-20% of ES fractions (Triton-X-100- and β -Mercaptoethanol-treated).	87
3.16	Detection of prostaglandins and lipids in <i>H. contortus</i> ES products via SDS-PAGE 20%.	88
3.17	Detection of prostaglandins and lipids in <i>H. contortus</i> ES products via TLC.	89
3.18	Vacuolating ability of prostaglandin standards [0.5mg/ml] in HeLa cells.	90
3.19	Vacuolating ability of prostaglandin standards [0.5mg/ml] in AGS cells.	90
3.20	Vacuolating ability of extracted lipid spots from TLC in HeLa cells.	90
3.21	Effect of protease and phosphatase inhibitors on HeLa and AGS cells.	90
3.22	SDS-PAGE 5-20% of proteinase K digested control (CEM) and ES products.	91
3.23	Vacuolation in HeLa cells induced by heat-treated <i>H. contortus</i> ES.	92
3.24	Vacuolation in HeLa cells induced by acid-treated <i>H. contortus</i> ES.	93
4.1	ES-induced detachment of HeLa cells 24h after exposure to <i>H. contortus</i> ES products.	107
4.2	ES-induced detachment of HeLa cells 48h after exposure to <i>H. contortus</i> ES products.	107

4.3	ES-induced detachment of AGS cells 24h after exposure to <i>H. contortus</i> ES products.	107
4.4	Detachment of HeLa cells - Control incubations vs. exposure to <i>H. contortus</i> ES.	108
4.5	ES-induced detachment of HeLa cells 24h after exposure to <i>H. contortus</i> ES products - apoptotic processes.	108
4.6	ES-induced detachment of HeLa cells 48h after exposure to <i>H. contortus</i> ES products - apoptotic processes.	108
4.7	ES-induced detachment of AGS cells 24h after exposure to <i>H. contortus</i> ES products - apoptotic processes.	108
4.8	Cell Detachment due to exposure to <i>H. contortus</i> ES products - Quantification.	109
5.1	Transwell® system.	119
5.2	Set-up for TEER measurements of cell monolayers growing on transwells with the volt-ohm meter.	120
5.3	TEER of CaCo-2 cell monolayers after 6 and 24h exposure to ES preparations of <i>H. contortus</i> adults (n=12) and corresponding controls (n=17).	121
5.4	Effect of <i>H. contortus</i> ES on the tight junctional structure of CaCo-2 cell monolayers.	122
5.5	Effect of <i>T. circumcincta</i> ES on the tight junctional structure of CaCo-2 cell monolayers.	122
5.6	Effect of <i>H. contortus</i> infection on the tight junctional structure of abomasal mucosa.	123

5.7	Effect of <i>H. contortus</i> infection on the tight junctional structure of abomasal mucosa - close-up.	123
IV.1	Bradford assay standard curve with BSA.	205
IV.2	Ammonia assay standard curve.	206

List of Tables

	Facing page
1.1	Activators and inhibitors of pepsinogen secretion. 6
1.2	Possible functions of PDZ containing proteins of the tight junction plaque. 19
1.3	Non-PDZ containing proteins of the tight junction plaque, including interaction partners and possible functions. 19
2.1	Number of larvae (mean \pm SEM, n=10) recovered from abomasal contents, fundus and antrum from a sheep 24h p.i. with 200,000 labelled <i>H. contortus</i> L ₃ . 49
2.2	Numbers of TGF- α and H ⁺ /K ⁺ -ATPase positive parietal cells (mean \pm SEM, n=2) in fundic tissue of sheep before (uninfected control, Ct) or 12 and 72h after the transplantation of 10,000 adult <i>T. circumcincta</i> . 54
2.3	Mucosal thickness (fundus) and abomasal pH (mean \pm SEM) in sheep before (uninfected control, Ct) or 12 and 72h after the transplantation of 10,000 adult <i>T. circumcincta</i> . 56
3.1	Scheme of incubation periods for successive ES samples with the same worm batch per experiment for three experiments. 70
3.2	Molecular weight ranges of ES fractions generated with Vivaspin centrifugal concentrators. 72
3.3	Summary of the different ES applications on HeLa and AGS cells. 78

3.4	Time scheme of viability of worms in successive ES samples from experiment I and II.	82
3.5	Protein concentrations of ES samples from successive incubation periods.	83
3.6	Effects of fractions generated in the presence of Triton-X-100 (1%)/ β -Mercaptoethanol (0.1%) (in CEM; single applications) and Triton-X-100 and β -Mercaptoethanol itself at different concentrations (in CEM; double applications) on HeLa cells.	87
3.7	Rf-values of prostaglandin standards and lipids present in <i>H. contortus</i> ES (solvent system ethylacetate : acetic acid 98:2).	89
5.1	Selection of diseases related to dysfunctional tight junctions.	116
5.2	TEER values (mean \pm SEM) of CaCo-2 cell monolayers before (0h) and after 6 and 24h exposure to ES preparations of <i>H. contortus</i> adults (n=12) and corresponding controls (n=17).	121

List of Abbreviations

#	Number
$[Ca^{2+}]_i$	Intracellular Ca^{2+} concentration
$[cAMP]_i$	Intracellular cAMP concentration
~	Approximately
μ g	Microgram
μ l	Microlitre
μ m	Micrometre
μ M	Micromolar
1D	1-dimensional
2D	2-dimensional
3T3	Fibroblasts isolated from mouse embryo
7H6	Tight junction associated antigen
<i>A. caninum</i>	<i>Ancylostoma caninum</i>
AA	Aminoacid
Ac	<i>A. caninum</i>
ACase	Adenylate cyclase
ACh	Acetylcholine
ADP	Adenosine diphosphate
AEBSF	4-(2-Aminoethyl)-benzenesulfonyl fluoride
AF-6	ALL-1 fusion partner from chromosome 6, also called afadin
AGS	Human gastric adenocarcinoma cell line derived from the stomach
Akt	Serine-threonine protein kinase
Amot	Angiomotin
AP	Adaptor protein
aPKC	Atypical protein kinase C
APR	Aspartic protease
AQ	Aquaporin

AR	Amphiregulin
ASH1	Absent, small, or homeotic-like (<i>Drosophila</i>), transcription factor
ASIP	Atypical PKC isotype specific interacting protein
ATP	Adenosin triphosphate
ATPase	Enzyme that can bind and hydrolyse ATP to ADP and P _i
<i>B. malayi</i>	<i>Brugia malayi</i>
Bcl	B cell lymphoma
biotin-XX	Biotinylated
BN-PAGE	Blue native-polyacrylamide gel electrophoresis
BSA	Bovine serum albumin
BSL	<i>Bandieraea simplicifolia</i> lectin
C1q	Complement component 1, subcomponent q
Ca ²⁺	Calcium ion
CaCl ₂	Calcium chloride
CaCo-2	Human epithelial colonic adenocarcinoma cell line
CagA	<i>H. pylori</i> virulence factor encoded by cytotoxin-associated gene A
CaM	Calmodulin
CAM	Cell adhesion molecule
CaMK	Calmodulin kinase
cAMP	Adenosine 3', 5'-cyclic monophosphate
CAR	Coxsackie virus and adenovirus receptor
CaSR	Calcium-sensing receptor
CBL	Cathepsin B-like cystein protease
CCK	Cholecystokinin
CCK _B	Cholecystokinin B/gastrin receptor
CD95	Cluster of differentiation, added numbers are standing for distinct Antigenes, death receptor, also called Fas, TNF receptor superfamily, member 6
Cdc42	Cell division cycle 42, small GTPase, belonging to the subfamily of Rho GTPases, superfamily Ras GTPases
cDNA	Complementary DNA
CEM	Complete essential medium

CGRP	Calcitonin gene-related peptide
CHO	Chinese hamster ovary epithelial cell line
Cl ⁻	Chloride ion
cm	Centimetre
cm ²	Square centimetre
CMC	Critical micelle concentration
Conc.	Concentration
CO ₂	Carbon dioxide
CP	Cysteine protease
CRP	C-reactive protein
Ct	Control
d	Day
<i>D. pteronyssinus</i>	<i>Dermatophagoides pteronyssinus</i>
D10	DMEM medium with 10% FBS
DAG	Diacylglycerol
DBA	<i>Dolichos biflorus</i> agglutinin (horse gram)
Der p 1	Allergen 1 from <i>D. pteronyssinus</i>
DLG	Discs-large protein in <i>Drosophila</i>
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
DNA	Desoxyribonucleinacid
<i>E. caproni</i>	<i>Echinostoma caproni</i>
<i>E. coli</i>	<i>Escherichia coli</i>
<i>E. histolytica</i>	<i>Entamoeba histolytica</i>
e.p.g.	Eggs per gram
EC	Enterochromaffin
E-cadherin	Cadherin in epithelial cells
ECL	Enterochromaffin-like
ECL1	Extracellular loop 1 of claudin
EDTA	Ethylenediaminetetraacetic acid
EEA1	Early endosome antigen 1
EGF	Epidermal growth factor

ERK	Extracellular signal-regulated protein kinase
ES	Excretory/Secretory (products)
ESAM	Endothelial cell selective adhesion molecule
EVOM	Epithelial volt-ohm meter
<i>F. hepatica</i>	<i>Fasciola hepatica</i>
F12-Hams	Originally developed to support growth of several clones of Chinese hamster ovary (CHO) cells
F-actin	Filamentous actin, filaments can be dissociated in their globular subunits (G-Actin)
FAK	Focal adhesion kinase
Fas	Death receptor, also called CD95, TNF receptor superfamily, member 6
FBS	Fetal bovine serum
FEC	Faecal egg count
g	Gram
g	Gravitational force
G protein	Guanine nucleotide binding protein
GAP	GTPase activating proteins
Gd ³⁺	Gadolinium ion
GDH	Glutamate dehydrogenase
GEF	Guanine nucleotide exchange factor
GEF-H1	Guanine nucleotide exchange factor H1
Gi	Guanine nucleotide binding protein, inhibitory
GIP	Gastric inhibitory peptide
Gq	Guanine nucleotide binding protein, q polypeptide, regulating PLC
GRP	Gastrin-releasing peptide
Gs	Guanine nucleotide binding protein, stimulatory
GTP	Guanosine triphosphate
GTPase	Enzyme that can bind and hydrolyse GTP to GDP (guanosine diphosphate) and P _i (inorganic phosphate)
h	Hour
H&E	Hematoxylin and eosin
<i>H. contortus</i>	<i>Haemonchus contortus</i>

<i>H. pylori</i>	<i>Helicobacter pylori</i>
H.c.	<i>Haemonchus contortus</i> infected
H ⁺	Hydrogen ion/proton
H ⁺ /K ⁺ -ATPase	Hydrogen/Potassium-ATPase, proton pump
H11	Hidden antigen of <i>H. contortus</i>
H ₂ /H ₃	Histamine receptor
H ₂ CO ₃	Carbonic acid
H ₂ O	Water
Hb	Hemoglobin
HB-EGF	Heparin binding-epidermal growth factor-like growth factor
HBSS	Hank's balanced salt solution
HCl	Hydrochloric acid
HCO ₃ ⁻	Hydrogen carbonate/bicarbonate ion
HDC	Histidine decarboxylase
HeLa cells	Derived from Henrietta Lacks, cell line of her cervical cancer
Hep2 cells	Originally thought to be derived from human epidermoid larynx carcinoma but actually derived via HeLa cell contamination
HES5	Hairy and enhancer-of-split homologue, basic helix-loop-helix protein
H-gal-GP	<i>Haemonchus</i> galactose-containing glycoprotein complex
HGF	Hepatocyte growth factor
HAT29-D4	Human colon adenocarcinoma cell line HAT 29, D4 is a clonal cell line of these
IEC-6	Epithelial cell line from newborn rat intestine
IFN-γ	Interferon-γ
Ig	Immunoglobulin
IgA1 protease	Secreted protease from <i>Neisseria gonorrhoeae</i> which inactivates human IgA1
IGF	Insulin-like growth factor
IgG ₁	Immunoglobulin G subclass 1
IL	Interleukin
ILK	Integrin linked kinase
INT407	Intestinal epithelial cell line from human embryo

IP ₃	Inositol 1,4,5-triphosphate
IPEC-1	Intestinal epithelial cell line from neonatal piglet
IRD-98	Epithelial cell line from rat fetus intestine
I κ B	Inhibitor of NF κ B
JAM	Junctional adhesion molecule
JEAP	Junction enriched and associated protein, Angiotensin-like protein 1
K ⁺	Potassium ion
KCL	Potassium chloride
kDa	Kilodalton
kg	Kilogram
KGF	Keratinocyte growth factor
KH ₂ PO ₄	Potassium dihydrogen phosphate
l	Litre
L/L ₁ /L ₃ /L ₄	Larva/first stage larva/third stage larva/fourth stage larva
LAMP1	Lysosome-associated membrane protein 1
Lasp-1	Includes a LIM domain (<i>Lin11</i> , <i>Isl-1</i> , and <i>Mec-3</i>) in the NH ₂ -terminal region followed by two actin-binding repeats (R1, R2), and an Src homology 3 (SH3) domain in the COOH-terminal region
LEA	<i>Lycopersicon esculentum</i> agglutinin
LIM	Domain including <i>Lin11</i> , <i>Isl-1</i> , and <i>Mec-3</i>
M	Molar
M ₃	Muscarinic acetylcholine receptor
MAGI	Membrane-associated guanyl kinase inverted proteins
MAPK	Mitogen-activated protein kinase
MASCOT	MAGI-associated coiled-coil tight junction protein, Angiotensin-like protein 2
max.	Maximum
MDCK	Madin-Darby canine kidney epithelial cell line
MEM	Minimum essential medium
MEP	Metalloprotease
mg	Milligram
Mg ²⁺	Magnesium ion

MIDAS	Metal ion dependent adhesion site
min	Minute
ml	Millilitre
MLC	Myosin light chain
MLCK	Myosin light chain kinase
mM	Millimolar
mm	Millimetre
mm ²	Square millimetre
MMP	Matrix metalloprotease
Mn ²⁺	Manganese ion
m-Numb	Mammalian Numb (notch inhibitor)
mOsM	Milliosmol
mRNA	Messenger ribonucleic acid
MUPP-1	Multi PDZ domain protein 1
MW	Molecular weight
n	Number/quantity
<i>N. americanus</i>	<i>Necator americanus</i>
<i>N. brasiliensis</i>	<i>Nippostrongylus brasiliensis</i>
Na ⁺	Sodium ion
Na ⁺ /K ⁺ -ATPase	Sodium/Potassium pump
Na ₂ HPO ₄	Disodium hydrogen orthophosphate
NaCl	Sodium chloride
NaHCO ₃	Sodium bicarbonate
NaOCl	Sodium hypochlorite
NaOH	Sodiumhydroxide
neg.	Negative
NFκB	Nuclear factor κB
ng	Nanogram
NH ₄ Cl	Ammonium chloride
nm	Nanometre
No.	Number

Notch	Transmembrane receptor, notch gene discovered in <i>Drosophila melanogaster</i> with notches in their wings
NR	Neutral Red
<i>O. leptospicularis</i>	<i>Ostertagia leptospicularis</i>
<i>O. ostertagi</i>	<i>Ostertagia ostertagi</i>
<i>O. sinensis</i>	<i>Ovomermis sinensis</i>
<i>O. volvulus</i>	<i>Onchocerca volvulus</i>
OH ⁻	Hydroxide ion
OMV	Outer membrane vesicles
p	Passage
p	Probability
p.i.	Past infection
p120CAS	p120 catenin isoform
p130CAS	v-Crk (oncogene product of avian sarcoma virus CT10) associated tyrosine kinase substrate
p120ctn	Catenin in adherens junctions
PACAP	Pituitary adenylyl cyclase-activating peptide
PALS-1	Protein associated with Lin-7
PAR	Partitioning defective protein (interacting partner aPKC)
PAR	Protease activated receptor
PATJ	PALS-1 associated tight junction
PBS	Phosphate buffered saline
PC	Parietal cell
PDZ	Binding domain first described in the proteins PSD-95, DLG and ZO-1
PEPI	Parasite pepsinogen-like aspartic protease
PG	Prostaglandin
P _i	Inorganic phosphate
PI3	Phosphatidylinositol 3
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
PNA	Peanut agglutinin

pos.	Positive
PP	Pancreatic polypeptide
PP2A	Protein phosphatase 2A
Proteinase K	Broad specificity serine protease from <i>Engyodontium album</i> , it was called Proteinase K because it is able to digest native keratin
PSD-95	Post-synaptic density protein 95
PSN	Penicillin-Streptomycin-Neomycin
PTEN	Phosphatase and tensin homologue
PYK2	Protein tyrosin kinase 2, FAK homologue
PYY	Peptide YY
Rab	Rab family of small G proteins, role in vesicular transport
Rac	Ras-related C3 botulinum toxin substrate, small GTPase, belonging to the subfamily of Rho GTPases, superfamily Ras GTPases
Rap	Small GTPase, belonging to the superfamily of Ras GTP
Ras	Small GTPases, from rat sarcoma
RCA	<i>Ricinus communis</i> agglutinin
Reg	Regenerating gene product
Rf	Retention factor
Rho	Ras homologue gene family
RhoA	Ras homologue gene family, member A, small GTPase, belonging to the subfamily of Rho GTPases, superfamily Ras GTPases
RIC	Epithelial cell line from adult rabbit intestine
Rich-1	Cdc42 GAP
RNA	Ribonucleic acid
rpm	Rounds per minute
RPTP β	Receptor protein tyrosine phosphatase beta
RT	Room temperature
s	Second
<i>S. mansoni</i>	<i>Schistosoma mansoni</i>
Sat	Secreted autotransporter toxin
SBA	Soybean agglutinin
SDS	Sodium dodecylsulfate

SDS-PAGE	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
Serpin	Serine protease inhibitor
SH	Sheath
SH3	Src homology region 3
SMC	Surface mucous cell
SNARE	Soluble N-ethylmaleimide-sensitive factor attachment protein
SOD	Superoxide dismutase
SPATE	Serine protease autotransporters of <i>Enterobacteriaceae</i>
spp.	Species pluralis
SS	Somatostatin
SS2	Somatostatin receptor
STA	<i>Solanum tuberosum</i> agglutinin
<i>T. brucei</i>	<i>Trypanosoma brucei</i>
<i>T. circumcincta</i>	<i>Teladorsagia circumcincta</i>
<i>T. colubriformis</i>	<i>Trichostrongylus colubriformis</i>
<i>T. cruzi</i>	<i>Trypanosoma cruzi</i>
<i>T. muris</i>	<i>Trichuris muris</i>
<i>T. spiralis</i>	<i>Trichinella spiralis</i>
<i>T. suis</i>	<i>Trichuris suis</i>
<i>T. vitrinus</i>	<i>Trichostrongylus vitrinus</i>
T75	Tissue/cell culture flask 75cm ²
TEER	Transepithelial resistance
TEMED	N,N,N',N'-Tetramethylethylenediamine
TFF	Trefoil factor
TGF	Transforming growth factor
Tiam-1	T-lymphoma invasion and metastasis, Rac specific GEF
TJ	Tight junction
TLC	Thin-layer chromatography
TNF	Tumor necrosis factor
tPA	Tissue type plasminogen activator
tSNARE	SNARE protein on the target membrane
Tuba	Cdc42 specific GEF

Tyr	Tyrosine
UEA	<i>Ulex europaeus</i> agglutinin (gorse)
uPA	Urokinase plasminogen activator
UV	Ultraviolet
V	Volt
v/v	Volume per volume
VacA	Vacuolating cytotoxin from <i>H. pylori</i>
VAMP	Vesicle-associated membrane protein
VASP	Vasodilator stimulated phosphoprotein
Vero cells	Kidney epithelial cells from African green monkey, Vero derived from “Verda Reno”, meaning “green” and “kidney” in esperanto
VIP	Vasoactive intestinal peptide
VMAT-2	Vesicular monoamine transporter of subtype 2
vSNARE	SNARE protein on the vesicle membrane
V-type ATPase	Vacuolar-type ATPase
w/v	Weight per volume
Yes	Tyrosine kinase
ZAK	Sterile alpha motif and leucine zipper containing kinase AZK, which is also known as ZAK
ZO	Zonula occludens
ZONAB	ZO-1 associated nucleic acid binding protein
Ω	Ohm, SI unit for electric resistance

Introduction

Infections with gastrointestinal nematodes, particularly trichostrongylids, in domestic ruminants are an important economic burden worldwide (Holmes, 1985; Fox, 1997; Miller and Horohov, 2006). A major factor in the pathogenesis of abomasal parasitism of sheep (McLeay *et al.*, 1973; Anderson *et al.*, 1976) and cattle (Fox *et al.*, 1989a; b) is a depression in voluntary food intake (anorexia) that leads to decreased weight gain (Holmes, 1985; 1993; Parkins and Holmes, 1989) as well as diarrhoea, malabsorption (Koski and Scott, 2001) and increased protein loss due to a more permeable epithelium, which all in turn increases susceptibility to infection (van Houtert and Sykes, 1996; Scrimshaw and SanGiovanni, 1997). In particular, infections with the blood-feeding nematode *Haemonchus contortus* are concerning, as they additionally cause considerable anaemia in infected animals (Parkins and Holmes, 1989). Adding to the production loss are costs for anthelmintics, veterinary care and death of infected animals. Around 29% of the animal health market are anti-parasitic drugs, worth US\$5.2 billion in 2007 (International Federation for Animal Health, 2007).

Parasite control relies on anthelmintic treatment, although alternative strategies also exist to cope with increasing anthelmintic resistance, including grazing management, breeding management, nutrition supplementation, use of plants containing natural anthelmintics and vaccine development (Coop and Kyriazakis, 1999; Newton and Munn, 1999; Koski and Scott, 2001; 2003; Bakker *et al.*, 2004; Miller and Horohov, 2006; Pomroy and Adlington, 2006). Anthelmintic resistance is widespread, even multiple drug resistant parasites have been reported worldwide (Kaplan, 2004; Wolstenholme *et al.*, 2004; von Samson-Himmelstjerna, 2006; Kaminski *et al.*, 2009). Although a new class of anthelmintics has been recently discovered and monepantel as the first component was launched earlier this year (Kaminski *et al.*, 2008; 2009), there is still need for the improvement of already existing alternative strategies and the development of new ones.

An alternative control method would be an antiparasitic vaccine. Attention has focused on using antigens present in the excretory/secretory (ES) products, as these are often recognised by immune animals (Schallig and Leeuwen, 1997; Schallig *et al.*, 1997; Bakker *et al.*, 2004) and may also be a source of ongoing antigenic stimulation to maintain immunity. ES products contain numerous proteins and glycoproteins with functions including depression of host immunity and probably also in initiating the host immune response and pathology.

The role of parasite ES products in abomasal pathophysiology and histopathology has been previously examined (Rhoads and Fetterer, 1996; Scott and McKellar, 1998; Lawton *et al.*, 2002; Merkelbach *et al.*, 2002; Haag *et al.*, 2005; Huber *et al.*, 2005; Przemeczek *et al.*, 2005), but it is still unclear whether ES products act at least in part directly on abomasal cells or also indirectly through the inflammatory response. The aim of this study was to further investigate the host-parasite interactions with the main focus on the role of ES products. Particular interest was on the effects on parietal cells leading to impaired acid secretion. Tissue samples of infected and control animals were used as well as different cell lines as model systems.