Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

### Effects of Abomasal Nematode Parasites In Vivo and In Vitro

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

at Massey University, Palmerston North New Zealand

#### SABINE MAJA CHARLOTTE PRZEMECK

2003

## **Table of Contents**

Abstract	i
Acknowledgements	iii
List of Figures	vi
List of Tables	xv
List of Abbreviations	xvii
Introduction	xxii
Chapter 1: Literature Review	1
1.1 Lifecycle of abomasal Nematode Parasites	1
1.2 Abomasal Structure and Function	2
1.2.1 Antrum	2
1.2.1.1 Gland Structure and Cell Types	2
1.2.1.2 Secretion of Gastrin	3
1.2.2 Fundus	6
1.2.2.1 Gland Structure and Cell Types	6
1.2.2.2 Trophic agents	7
1.2.2.3 Secretion	10
1.2.2.3.1 Pepsinogen and the Chief Cell	10
1.2.2.3.2 Histamine and the ECL Cell	11
1.2.3 Acid Secretion and the Parietal Cell	12
1.2.3.1 Regulation of Acid Secretion	13
1.2.3.1.1 Acetylcholine	14
1.2.3.1.2 Gastrin	14
1.2.3.1.3 Histamine	15
1.2.3.2 Ultrastructure and the Secretory Process	16
1.3 Abomasal Parasitism	17
1.3.1 Histopathology	18
1.3.2 Pathophysiology	21
1.3.2.1 Hyperpepsinogenaemia	21
1.3.2.2 Hypoacidity	24

.

1.3.2.3 Hypergastrinaemia	26
1.3.2.4 Parasite Excretory/Secretory Products	29
1.3.2.5 Metabolic Disturbances	31
1.3.2.5.1 Loss of Appetite	31
1.3.2.5.2 Metabolism and Production	34
1.4 Host Factors in Abomasal Parasitism	36
1.4.1 Age and Nutritional Status	36
1.4.2 Innate and Acquired Immunity	37
1.4.3 Genetic Resistance, Resilience and Susceptibility	39
1.5 Conclusions	41
Chapter 2: Pathophysiological effects of infection with Ostertagia	
circumcincta in sheep selected for high fleeceweight	43
2.1 Introduction	43
2.2 Materials and Methods	46
2.2.1 Experimental Design	46
2.2.2 Animals	46
2.2.2.1 Housing and Feeding	46
2.2.2.2 Abomasal Cannulation	47
2.2.2.3 Necropsy	47
2.2.3 Experimental Sample Collection	48
2.2.4 Abomasal Contents	48
2.2.5 Blood Samples	49
2.2.5.1 Serum Pepsinogen	49
2.2.5.2 Serum Gastrin	49
2.2.5.3 Blood Eosinophils	49
2.2.5.4 Serum Antibody Titres	49
2.2.6 Abomasal Lymph Node Cytokine Profiles	50
2.2.7 Wool Follicles	50
2.2.7.1 Skin Biopsies	50
2.2.7.2 Paracortex in Wool Follicles	51
2.2.8 Parasitology	51

2.2.8.1 Parasite Cultures	51
2.2.8.2 Faecal Egg Counts	51
2.2.8.3 Worm Counts	52
2.2.9 Data Presentation and Statistical Analyses	52
2.3 Results: Experiment 1	53
2.3.1 Abomasal pH	53
2.3.2 Serum Gastrin	53
2.3.3 Serum Pepsinogen	54
2.3.4 Blood Eosinophils	55
2.3.5 Serum Antibody Titres	56
2.3.6 Faecal Egg Counts	56
2.3.7 Worm Counts	56
2.3.8 Wool Follicles	57
2.4 Results: Experiment 2	57
2.4.1 Abomasal pH	57
2.4.2 Serum Gastrin	58
2.4.3 Serum Pepsinogen	59
2.4.4 Blood Eosinophils	59
2.4.5 Faecal Egg Counts	60
2.4.6 Worm Counts	60
2.4.7 Food Intake	60
2.4.8 Wool Follicles	61
2.4.9 Cytokines	61
2.5 Discussion	62
2.5.1 Worm Burdens and FEC in HFW and Control Lambs	62
2.5.2 Are the Pathophysiological Effects of Abomasal Parasitism	
different in HFW and Control Lambs?	63
2.5.3 Are HFW Lambs Resilient?	66
2.5.4 What is the Basis of Resilience in the HFW Lambs?	68
2.5.4.1 Food Intake	69
2.5.4.2 Immune Response	70

2.5.4.3 Age and exposure to parasites	71
2.5.5 How do Abomasal Parasites inhibit the Parietal Cell?	73
Chapter 3: Histopathological changes during abomasal parasitism: Is	
the parietal cell a target?	75
3.1 Introduction	75
3.2 Materials and Methods	76
3.2.1 Animals	76
3.2.2 Abomasal pH and Serum Gastrin	77
3.2.3 Necropsy	78
3.2.4 Electron Microscopy	78
3.2.5 Light Microscopy	78
3.2.5.1 Tissue Preparation	78
3.2.5.2 Standard Stains	79
3.2.5.3 Immunohistochemistry	79
3.2.5.4 Eosinophil and TGF- $\alpha$ positive Parietal Cell Counts	80
3.2.5.5 TGF- $lpha$ negative Parietal Cell Counts	80
3.2.6 Statistical Analyses	80
3.3 Results	81
3.3.1 Group HFW	81
3.3.1.1 Abomasal Parameters	81
3.3.1.2 Light Microscopy	82
3.3.1.2.1 Day 8	82
3.3.1.2.2 Day 28	83
3.3.2 Group LI	83
3.3.2.1 Abomasal Parameters	83
3.3.2.2 Light Microscopy	84
3.3.2.2.1 Controls	84
3.3.2.2.2 Day 5	85
3.3.2.2.3 Day 10	86
3.3.2.2.4 Day 15	87
3.3.2.2.5 Day 20	88

3.3.2.2.6 Day 30	89
3.3.2.3 Electron Microscopy	90
3.3.2.3.1 Controls	90
3.3.2.3.2 Day 5	90
3.3.2.3.3 Day 10	90
3.3.3 Group AT	91
3.3.3.1 Abomasal Parameters	91
3.3.3.2 Light Microscopy	91
3.3.3.2.1 Controls	91
3.3.3.2.2 6 hours	91
3.3.3.2.3 12 hours	92
3.3.3.2.4 24 hours	92
3.3.3.2.5 72 hours	92
3.4 Discussion	93
3.4.1 Do the histological findings elucidate the mechanism of resilience	
of HFW sheep?	94
3.4.1.1 Tissue Eosinophilia	94
3.4.1.2 Mucosal Thickness	95
3.4.2 What is the mechanism underlying the development of	
hypoacidity in primary infections of the parasite-naïve host?	97
3.4.2.1 Is hypoacidity caused by inhibition of Parietal cells or their	
loss?	97
3.4.2.2 What is the role of the inflammatory process and of the	
Eosinophil in particular?	99
3.4.2.3 Is there a direct effect of parasites on the Parietal cell?	101
Chapter 4: Early changes within the abomasal mucosa following	
infection with Ostertagia circumcincta	103
4.1 Introduction	103
4.2 Materials and Methods	103
4.2.1 Animal	103
4.2.2 Histology	104

4.2.2.1 Tissue Preparation and Staining	104
4.2.2.2 Immunohistochemistry	104
4.2.2.3 Eosinophil and TGF- $\alpha$ positive Parietal Cell Counts	104
4.2.2.4 Light Microscopy	105
4.3 Results	105
4.3.1 $L_3$ in Folds	105
4.3.1.1 Uninfected Control Animals	105
4.3.1.2 Non-infested Fold Areas	106
4.3.1.3 $L_3$ at the surface	106
4.3.1.4 $L_3$ in the Pits and the Glands	106
4.3.1.5 Gland Base	107
4.3.2 $L_3$ in Tips of Folds	107
4.3.2.1 Controls	107
4.3.2.2 Infected 30-h Animal	107
4.4 Discussion	108
Chapter 5: In vitro vacuolating activity of nematode	
excretory/secretory products	111
5.1 Introduction	111
5.2 Materials and Methods	113
5.2.1 In vitro Effects of ES on Cultured Epithelial Cells	113
5.2.1.1 General Experimental Design	113
5.2.1.2 Cell Culture	113
5.2.1.3 Preparation of ES	113
5.2.1.4 Controls	114
5.2.1.5 Neutral Red (NR) Uptake Assay	115
5.2.1.6 Ammonia and Protein Determination in ES	115
5.2.1.7 Data Presentation	115
5.2.2 Probing Worm Genomic DNA for a VacA-like Gene	116
5.2.2.1 DNA Extraction	116
5.2.2.2 Restriction Digestion of Parasite Genomic DNA	117
5.2.2.3 VacA Probe	117

5.2.2.4 Southern Blotting	117
5.2.2.4.1 Gel	117
5.2.2.4.2 Southern Transfer	118
5.2.2.4.3 Probing the Blot	118
5.3 Results	119
5.3.1 Effects of Ammonia and ES on NR uptake by HeLa Cells	119
5.3.1.1 Ammonia Concentrations in ES	119
5.3.1.2 NR Uptake by HeLa Cells Incubated with NH4CI	119
5.3.1.3 NR Uptake by HeLa Cells Incubated with ES and NH₄CI	119
5.3.1.4 NR Uptake by HeLa Cells Incubated with ES	120
5.3.1.5 Effect of Storage on ES Potency	121
5.3.1.6 Protein Concentrations in ES	121
5.3.1.7 Effect of Media on NR Uptake	121
5.3.2 Probing Worm Genomic DNA for a VacA-like Protein	121
5.4 Discussion	122
5.4.1 Effect of Mixing Media	122
5.4.2 Is the Vacuolating Factor Ammonia?	123
5.4.3 Characteristics of ES Products	125
Chapter 6: General Discussion	127
References	131
Appendix 1: Parasitology	179
1.1 Larval Culture	179
1.2 Faecal Egg Counts	179
1.3 Total Worm Counts	180
1.4 Recovery of Adult Worms	180
Appendix 2: Assays	181
2.1 Ammonia Assay	181
2.2 Protein Microassay	181
2.3 Pepsinogen Assay	182
2.4 Gastrin Radioimmunoassays	183
2.4.1 Primary Ab 74	183

2.4.2 Primary Ab 1296	184
2.5 Serum Antibody Titres	186
2.5.1 Antigen preparation	186
2.5.2 ELISA Method	186
2.5.3 Immunoglobulin Isotypes	187
2.6 Abomasal Lymph Node Cytokine Profiles	188
2.6.1 Cell Culture	188
2.6.2 RNA extraction and reverse transcription	188
2.6.3 Primer design	189
2.6.4 Real time PCR and quantification of gene expression	189
Appendix 3: Cell Culture	191
3.1 Culture Conditions	191
3.2 Culture Medium	191
3.3 Passage of HeLa Cells	192
3.4 Seeding 96-well Microtitre Plates	193
3.5 Neutral Red (NR) Uptake Assay	194

#### Abstract

During abomasal parasitism, parietal cells are inhibited and may be lost, although the exact mechanism is not known. The present *in vivo* and *in vitro* experiments investigated whether the hypoacidity was linked to the host's inflammatory response and whether they are directly targeted by parasites.

Pathophysiological consequences of abomasal parasitism, particularly the effects on parietal cells were studied in different immunological settings in high-fleece weight lambs (HFW) and their unselected controls (C). Lambs were infected with a single dose of *Ostertagia circumcincta*  $L_3$  at the age of 6.5 months (Experiment 1) or 4.5 months (Experiment 2). The older lambs also received further doses of  $L_3$  weekly following primary infection. Lambs were killed on Day 94 or Day 8 or 28 respectively. Serum gastrin and pepsinogen concentrations, blood eosinophils, abomasal pH, paracortical cells in wool follicles (wool quality), FEC and worm burden were monitored. Antibody levels were measured in Experiment 1 and lymphocyte types in abomasal lymph nodes and food intake in Experiment 2.

Tissue eosinophil and parietal cell counts, mucosal thickness and general histopathological changes in abomasal tissues from lambs in Experiment 2 were studied along with tissues collected from randomly bred lambs between five and 30 days after infection with *O. circumcincta*  $L_3$  or six to 72 hours after transplantation of adult *O. circumcincta*.

HFW lambs exhibited resilience, but its expression differed with age. Paracortical cells in wool follicles were lower and increased later in both experiments, yet FEC was higher in older HFW lambs. The rise in abomasal pH was delayed and serum pepsinogen concentrations were higher in older HFW lambs. Generally, blood eosinophils tended to be higher in controls and cytokine responses suggested a lower Th2 response in HFW lambs. In Experiment 2, tissue eosinophils were lower in HFW sheep on Day 8. Resilience in HFW lambs appeared to be based on a down-regulated inflammatory response and the expression of resilience, which was dependent on full immune responsiveness with age. Additional bases for resilience may be the ability to delay gastric hypoacidity and a higher food intake before and during parasitism as seen in the younger HFW lambs.

No conclusive answer could be given to the question of hypoacidity being secondary to inflammation, although there was a strong link between inflammation and raised abomasal pH associated with vacuolation and loss of parietal cells. Tissue eosinophils were correlated with abomasal pH in all lambs. Parietal cell loss coincided with hypoacidity and inflammation. Blood eosinophil levels did not correlate well with abomasal mucosal eosinohils. In tissues collected 30 hours after larval infection, eosinophils appeared chiefly in the tips of folds, but parietal cell numbers were not reduced.

Evidence for a direct effect of parasite excretory/secretory products on epithelial cells was obtained *in vitro* using the HeLa cell test system for vacuolating activity (neutral red uptake). Adult products were more potent than  $L_3$  chemicals.

These experiments support roles for both the host inflammatory response and parasite chemicals in inhibiting and damaging parietal cells.

#### Acknowledgements

I wish to thank the Institute of Food, Nutrition and Human Health and Institute of Veterinary, Animal and Biomedical Sciences for allowing me to use their facilities and for their administrative support throughout my Ph.D. I am also indebted to the latter for granting me the Joan Berry Fellowship in 1999, 2000 and 2001 as well as the Phyllis Irene Grey Fellowship in 2000, which helped considerably with my living costs during the duration of my studies. Generous funding from Meat New Zealand, C. Alma Baker Trust and E. & C. Thoms Bequest enabled the work to be undertaken. I wish to thank Dr A. Pernthaner and Dr I. Sutherland of AgResearch Ltd for performing the cytokine assays and Dr P. O'Toole for advice on molecular biology, Mr Richard Green of AgResearch Ltd, Wallaceville for assistance with the antibody assay and Dr D. Thomas for assistance with skin biopsies and histology. Antisera were kindly donated by Dr W. Hein, Dr J. Hansky and Dr. J. Walsh.

I am extremely thankful to my chief supervisor Assoc Prof Heather Simpson, whose patience I stretched on occasions to its absolute maximum. Always approachable and with the best for her students in mind she showed no tiredness to share her enthusiasm for research with me: "I had a thought while I was driving home last night..." became quickly the most dreaded phrase for me. Heather, many thanks for having me as a postgraduate student and for your good nature, support and encouragement throughout my studies. I also wish to thank Dr Ian Scott, who, first as postdoctoral fellow and later as one of my co supervisors, kept his calm and humour throughout all of my antics. His permission to use material as well as some of the data presented from the LI and AT experiments is gratefully acknowledged. Thank you very much for your support and encouragement during, and especially towards the end, of my studies. I am also indebted to Dr Gordon Reynolds for introducing me to sheep surgery and to Brett Guthrie, whose skills and calm were an irreplaceable asset during the days of surgery. The positive criticism and support from Dr Simon Brown is greatly appreciated and without his assistance I would be still a

headless chicken. Thank you very much for your kindness and willingness to help when it was needed most. Dr David Simcock, what shall I say? What had I done without his extraordinary sense of humour and perfect impersonations of Cartman during my years at Massey? Thank you so much for your invaluable help and kindness. Dr Kevin Pedley, the right man at the right place at the right Many thanks for your skilful help in the photograph section. time. The assistance of Mervin Birtles by taking some of my photographs is also greatly acknowledged. I want to thank Dr Mark Patchett and Dr Juliet Sutherland for their patience while introducing me to molecular biology techniques. Extra special thanks go to Juliet for sharing her experiences as a postdoc. Without the excellent technical assistance of Samera Khalaf, who spent countless hours especially hunting larvae and processing the histology, as well as Lois Taylor, who was a flexible allround help with fantastic catering skills, I could not have done all this work. The same has to be said about John Pedley, who came to my aid whenever technical equipment failed me as well as Barbara Addlington, who supplied me with countless parasites and books. Without sheep no thesis, so special thanks to the extremely helpful and friendly sheep crew: Debbie Chesterfield, Geoff Warren and Barry Parlane.

I wish to thank my fellow students and friends Stefanie Reinhardt, Lisa Walker, Heather Purnell, Alexandra Huber, Shu-Er Zeng, Zaid Mohamad, Alexander McLachlan and Janice Lloyd, without their friendship, moral support, resilience, humour and understanding lots of my days would have been a real drag! Keep in touch. To sum it up: The friendliness and kindness I have experienced was amazing. Thank you so much for it.

I am extremely grateful and utterly indebted to my parents without whose help my Ph.D. studies in New Zealand never could have taken place in the first instance. They not only supported me financially, but also believed in me when I did not. For my brothers Werner and Wolfgang a big hug for being so supportive and understanding. A big hug and special thanks go to my brother Gerhard, whose experience and understanding was an enormous reassurance. Perhaps I can help you one day? ;-) Silvia, favourite sister of mine, I can't thank you enough! Thanks Anci, for being such a source of funny craziness! Last but not least many thanks to my colleagues in Liverpool, especially to Mark for his patience and understanding and to Alice and Kevin for bringing Caffrey's to my attention, while finishing off this beast.

# List of figures

	Facing page
<b>Figure 1.1.</b> Principal physiological and inflammatory regulators of gastrin secretion by the G cell.	4
Figure 1.2. The structure of the pit-gland unit in the fundic mucosa.	6
<b>Figure 1.3</b> . Trophic agents and their proposed site of action in the fundic pit-gland unit.	7
Figure 1.4. Regulation of pepsinogen secretion by the chief cell.	10
Figure 1.5. Regulation of histamine secretion by the enterochromaffin-like (ECL) cell.	12
Figure 1.6. Regulation of acid secretion by the parietal cell.	14
Figure 1.7. Overview of the cellular control of acid secretion.	15
<b>Figure 1.8.</b> Principal cytokines secreted by Th1 and Th2 CD4 <sup>+</sup> cells.	39
<b>Figure 2.1.</b> Abomasal pH (mean $\pm$ SEM) in high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> and at Days 35, 42, 49, 56, 63 and 70 with 10,000 L <sub>3</sub> <i>O. circumcincta</i> .	53
<b>Figure 2.2.</b> Abomasal pH (mean $\pm$ SEM) in high fleece weight (HFW) and control (C) lambs on Days 1-15 after infection on Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> .	53
<b>Figure 2.3.</b> Abomasal pH 1 hour before, and 3 hours after, feeding in individual high fleece weight (HFW) and control (C) lambs which were infected on Day 0 with 50,000 $L_3$ <i>O. circumcincta.</i>	53
<b>Figure 2.4.</b> Abomasal pH and serum gastrin concentrations in individual lambs after infection at Day 0 with 50,000 $L_3$ <i>O. circumcincta</i> and at Days 35, 42, 49, 56, 63 and 70 with 10,000 $L_3$ <i>O. circumcincta</i> .	53
<b>Figure 2.5.</b> Serum gastrin concentration (mean $\pm$ SEM) in high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> and at Days 35, 42, 49,	53

56, 63 and 70 with 10,000  $L_3$  O. circumcincta.

<b>Figure 2.6.</b> Serum pepsinogen concentration (mean $\pm$ SEM) in high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> and at Days 35, 42, 49, 56, 63 and 70 with 10,000 L <sub>3</sub> <i>O. circumcincta</i> .	54
<b>Figure 2.7.</b> Serum pepsinogen concentration in individual lambs after infection at Day 0 with 50,000 $L_3$ <i>O. circumcincta</i> and at Days 35, 42, 49, 56, 63 and 70 with 10,000 $L_3$ <i>O. circumcincta</i>	54
<b>Figure 2.8.</b> Blood eosinophil counts (mean $\pm$ SEM) in high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> and at Days 35, 42, 49, 56, 63 and 70 with 10,000 L <sub>3</sub> <i>O. circumcincta</i> .	55
<b>Figure 2.9.</b> Blood eosinophil counts in individual lambs after infection at Day 0 with 50,000 $L_3$ <i>O. circumcincta</i> and at Days 35, 42, 49, 56, 63 and 70 with 10,000 $L_3$ <i>O. circumcincta</i> .	55
<b>Figure 2.10.</b> IgG1, IgM and total immunoglobulins specific for adult and $L_3$ <i>O. circumcincta</i> in individual lambs after infection at Day 0 with 50,000 $L_3$ <i>O. circumcincta</i> and at Days 35, 42, 49, 56, 63 and 70 with 10,000 $L_3$ <i>O. circumcincta</i> .	56
<b>Figure 2.11.</b> Faecal egg counts in individual lambs after infection at Day 0 with 50,000 $L_3$ <i>O. circumcincta</i> and at Days 35, 42, 49, 56, 63 and 70 with 10,000 $L_3$ <i>O. circumcincta</i> .	56
<b>Figure 2.12.</b> Faecal egg counts (mean $\pm$ SEM) in high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> and at Days 35, 42, 49, 56, 63 and 70 with 10,000 L <sub>3</sub> <i>O. circumcincta</i> .	56
<b>Figure 2.13.</b> Percentage of wool follicles containing paracortex (mean $\pm$ SEM) in high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> and at Days 35, 42, 49, 56, 63 and 70 with 10,000 L <sub>3</sub> <i>O. circumcincta</i> .	57
<b>Figure 2.14.</b> Percentage of wool follicles containing paracortex in individual lambs after infection at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> and at Days 35, 42, 49, 56, 63 and 70 with 10,000 L <sub>3</sub> <i>O. circumcincta</i> .	57

<b>Figure 2.15.</b> Abomasal pH (mean $\pm$ SEM) in high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta.</i>	57
<b>Figure 2.16.</b> Abomasal pH in individual lambs after infection at Day 0 with 50,000 $L_3$ <i>O. circumcincta</i> .	57
<b>Figure 2.17.</b> Abomasal pH in individual lambs after infection at Day 0 with 50,000 $L_3$ <i>O. circumcincta.</i>	57
<b>Figure 2.18.</b> Serum gastrin concentration (mean $\pm$ SEM) in high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta.</i>	58
<b>Figure 2.19.</b> Serum gastrin concentration in individual lambs after infection at Day 0 with 50,000 $L_3$ <i>O. circumcincta</i> .	58
<b>Figure 2.20.</b> Abomasal pH and serum gastrin concentration in individual lambs after infection at Day 0 with 50,000 $L_3$ <i>O. circumcincta.</i>	58
<b>Figure 2.21.</b> Serum pepsinogen concentration (mean $\pm$ SEM) in high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> .	59
<b>Figure 2.22.</b> Serum pepsinogen concentration in individual lambs after infection at Day 0 with 50,000 $L_3$ <i>O. circumcincta.</i>	59
<b>Figure 2.23.</b> Blood eosinophil counts (mean $\pm$ SEM) in high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta.</i>	59
<b>Figure 2.24.</b> Blood eosinophil counts in individual lambs after infection at Day 0 with 50,000 $L_3$ <i>O. circumcincta.</i>	59
<b>Figure 2.25.</b> Faecal egg counts in individual lambs after infection at Day 0 with 50,000 $L_3$ <i>O. circumcincta</i> .	60
<b>Figure 2.26.</b> Faecal egg counts (mean $\pm$ SEM) in high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> .	60
<b>Figure 2.27.</b> Relationship between number of eggs <i>in utero</i> per female worm (mean $\pm$ SEM) and number of female worms in both high fleece weight (HFW) and control lambs, which were	
intected with 50,000 $L_3$ O. circumcincta and killed on Day 28 after infection.	60

viii

<b>Figure 2.28.</b> Daily food intake (mean $\pm$ SEM) in high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O</i> , <i>circumcincta</i> .	60
<b>Figure 2.29.</b> Daily food intake in individual lambs after infection at Day 0 with 50,000 $L_3$ <i>O. circumcincta</i> .	60
<b>Figure 2.30.</b> Percentage of wool follicles containing paracortex (mean $\pm$ SEM) in high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta.</i>	61
<b>Figure 2.31.</b> Percentage of wool follicles containing paracortex in individual lambs after infection at Day 0 with 50,000 $L_3$ O. <i>circumcincta</i> .	61
<b>Figure 3.1.</b> Mucosal thickness ( $\mu$ m) and numbers of TGF- $\alpha$ -positive parietal cells and eosinophils per 258 $\mu$ m wide column of fundic tissue in individual high fleece weight (HFW) and control (C) sheep which were infected on Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> and killed on Day 8 or Day 28.	81
<b>Figure 3.2.</b> Fundic mucosa of a control (C) sheep, 8 days after infection with 50,000 $L_3$ <i>O. circumcincta</i> .	83
<b>Figure 3.3.</b> Fundic mucosa of a control (C) sheep, 8 days after infection with 50,000 $L_3$ <i>O. circumcincta</i> .	83
<b>Figure 3.4.</b> Fundic mucosa of a control (C) sheep, 8 days after infection with 50,000 $L_3$ <i>O. circumcincta</i> .	83
<b>Figure 3.5.</b> Fundic mucosa of a control (C) sheep, 28 days after infection with 50,000 $L_3$ <i>O. circumcincta</i> .	83
<b>Figure 3.6.</b> Fundic mucosa of a control (C) sheep, 28 days after infection with 50,000 $L_3$ <i>O. circumcincta</i> .	83
<b>Figure 3.7.</b> Abomasal pH at necropsy, mucosal thickness ( $\mu$ m) and numbers of TGF- $\alpha$ -positive parietal cells and eosinophils per 258 $\mu$ m wide column of fundic mucosa of individual sheep before (control, CT) and 5, 10, 15, 20 or 30 days after infection with 35,000 L <sub>3</sub> <i>O. circumcincta.</i>	83
<b>Figure 3.8.</b> Serum gastrin concentration in Sheep #50 and the group mean (mean $\pm$ SEM) for all sheep surviving on each day after infection on Day 0 with 35,000 L <sub>3</sub> <i>O. circumcincta.</i>	83

ix

Figure 3.9a and b. Fundic mucosa of an uninfected control sheep from the larval infection experiment, showing the distribution of TGF- $\alpha$  positive parietal cells within the pits and glands. 84 **Figure 3.10.** Number of TGF- $\alpha$  stained parietal cells (mean  $\pm$ SEM) per unit area in 258 wide columns of fundic mucosa of groups of sheep before and 5, 10, 15, 20 or 30 days after infection with 35,000 L<sub>3</sub> O.circumcincta. 84 **Figure 3.11.** Number of TGF- $\alpha$  stained parietal cells (mean  $\pm$ SEM) per unit area in 258 wide columns of fundic mucosa of individual sheep before and 5, 10, 15, 20 or 30 days after infection with 35,000 L<sub>3</sub> O.circumcincta. 84 Figure 3.12. Fundic mucosa of an uninfected control sheep from the larval infection experiment, showing the distribution of TGF- $\alpha$  positive parietal cells within the pits and glands in the tip of the fold. 85 Figure 3.13. Fundic mucosa of an uninfected control sheep from the larval infection experiment. 85 Figure 3.14. Fundic mucosa at mid-gland level of an uninfected control sheep from the larval infection experiment. 85 Figure 3.15. Fundic mucosa at the level of the gland base of an uninfected control sheep from the larval infection experiment. 85 Figure 3.16. Fundic mucosa of sheep 31. 5 days after infection with 35,000 L<sub>3</sub> O. circumcincta. 85 **Figure 3.17.** Fundic mucosa of a sheep, 5 days after infection with 35,000 L<sub>3</sub> O. circumcincta. 85 Figure 3.18. Fundic mucosa of a sheep, 5 days after infection with  $35,000 L_3 O.$  circumcincta. 86 Figure 3.19a and b. Fundic mucosa of a sheep, 5 days after infection with 35,000 L<sub>3</sub> O. circumcincta. 86 Figure 3.20. Fundic mucosa of a sheep, 10 days after infection

**Figure 3.21.** Fundic mucosa of a sheep, 10 days after infection with  $35,000 L_3 O.$  *circumcincta*.

with 35,000 L<sub>3</sub> O. circumcincta.

Х

87

87

<b>Figure 3.22.</b> Fundic mucosa of a sheep, 10 days after infection with $35,000 L_3 O.$ <i>circumcincta</i> .	87
<b>Figure 3.23.</b> Fundic mucosa of a sheep, 10 days after infection with 35,000 $L_3$ <i>O. circumcincta</i> .87	87
<b>Figure 3.24.</b> Fundic mucosa of a sheep, 10 days after infection with $35,000 L_3 O$ . <i>circumcincta</i> .	87
<b>Figure 3.25a and b.</b> Fundic mucosa of a sheep, 10 days after infection with 35,000 $L_3$ O. <i>circumcincta</i> .	87
<b>Figure 3.26.</b> Fundic mucosa of a sheep, 15 days after infection with $35,000 L_3 O.$ <i>circumcincta</i> .	88
<b>Figure 3.27.</b> Fundic mucosa of a sheep, 15 days after infection with $35,000 L_3 O.$ circumcincta.	88
<b>Figure 3.28.</b> Fundic mucosa of a sheep, 15 days after infection with $35,000 L_3 O.$ <i>circumcincta</i> .	88
<b>Figure 3.29.</b> Fundic mucosa of a sheep, 20 days after infection with $35,000 L_3 O.$ <i>circumcincta</i> .	88
<b>Figure 3.30.</b> Fundic mucosa of a sheep, 20 days after infection with $35,000 L_3 O$ . <i>circumcincta</i> .	89
<b>Figure 3.32.</b> Fundic mucosa of sheep 32, 30 days after infection with 35,000 $L_3$ <i>O. circumcincta</i> .	89
<b>Figure 3.33a and b.</b> Parietal cells from uninfected control sheep from the larval infection experiment, with what appear to be true secretory canaliculi consisting of numerous microvillar extensions of the plasmalemma.	90
Figure 3.34a and b. Parietal cell from a sheep, 5 days after infection with 35,000 $L_3$ O. circumcincta.	90
<b>Figure 3.35.</b> Parietal cell from a sheep, 10 days after infection with $35,000 L_3 O$ . <i>circumcincta</i> .	90
<b>Figure 3.36.</b> Parietal cell from a sheep, 10 days after infection with $35,000 L_3 O$ . <i>circumcincta</i> .	90
<b>Figure 3.37.</b> Parietal cell from a sheep, 10 days after infection with $35,000 L_3 O.$ <i>circumcincta</i> .	90
<b>Figure 3.38.</b> Parietal cell from a sheep, 10 days after infection with $35,000 L_3 O.$ circumcincta.	90

xi

<b>Figure 3.39.</b> Abomasal pH at necropsy, mucosal thickness ( $\mu$ m) and numbers of TGF- $\alpha$ -positive parietal cells and eosinophils per 258 $\mu$ m wide column of fundic mucosa of individual sheep before (control, CT) or 6, 12, 24 or 72 hours after the transplantation of 10,000 adult <i>O. circumcincta</i> .	91
<b>Figure 3.40.</b> Numbers of TGF- $\alpha$ -positive parietal cells per unit area in a 258µm wide column of fundic mucosa (mean $\pm$ SEM) in sheep before, (control) or 6, 12, 24 or 72 hours after, the transplantation of 10,000 adult <i>O. circumcincta</i> .	91
<b>Figure 3.41.</b> Numbers of TGF- $\alpha$ -positive parietal cells per unit area in a 258µm wide column of fundic mucosa of individual sheep before (control, CT) or 6, 12, 24 or 72 hours after the transplantation of 10,000 adult <i>O. circumcincta</i> .	91
<b>Figure 3.42.</b> Fundic mucosa of a sheep, 12 h after the transplant of approximately 10,000 adult <i>O. circumcincta</i> .	92
<b>Figure 3.43.</b> Fundic mucosa of a sheep, 24 h after the transplant of approximately 10,000 adult <i>O. circumcincta</i> .	92
<b>Figure 4.1.</b> Fundic mucosa from a lamb, 30h after infection with $25,000 L_3 O.$ <i>circumcincta.</i>	106
<b>Figure 4.2</b> Fundic mucosa from a lamb, 30h after infection with $25,000 L_3 O.$ circumcincta.	106
<b>Figure 4.3.</b> Number of cells staining positively for TGF- $\alpha$ (a) or eosinophils (b) in infested and non-infested areas of the main portion and in the tip of the fundic folds of a sheep infected with 25,000 L3 <i>O circumcincta</i> .	106
<b>Figure 4.4.</b> Fundic mucosa from a lamb, 30h after infection with $25,000 L_3 O$ . <i>circumcincta</i> .	106
<b>Figure 4.5.</b> Fundic mucosa from a lamb, 30h after infection with $25,000 L_3 O.$ circumcincta.	106
<b>Figure 4.6.</b> Fundic mucosa from a lamb, 30h after infection with $25,000 L_3 O.$ circumcincta.	106
<b>Figure 4.7.</b> Fundic mucosa from a lamb, 30h after infection with $25,000 L_3 O.$ circumcincta.	106
<b>Figure 4.8.</b> Fundic mucosa from a lamb, 30h after infection with $25,000 L_3 O$ . <i>circumcincta</i> .	107
<b>Figure 4.9.</b> Fundic mucosa from a lamb, 30h after infection with $25,000 L_3 O.$ <i>circumcincta</i> .	107

xii

<b>Figure 4.10.</b> Fundic mucosa from a lamb, 30h after infection with 25,000 $L_3$ <i>O. circumcincta</i> .	108
<b>Figure 4.11.</b> Fundic mucosa from a lamb, 30h after infection with 25,000 $L_3$ <i>O. circumcincta</i> .	108
Figure 5.1. Effect of exposure to a series of ammonium chloride concentrations (3.125mM, 6.25mM, 12.5mM, 25mM and 50mM) for 24 hours on neutral red (NR) uptake by HeLa cells.	119
<b>Figure 5.2.</b> The effect of exposure for 24 hours to <i>H. contortus</i> $L_3$ excretory/secretory products (a) HL1c or (b) HL1e, with or without added 6.25 mM NH <sub>4</sub> Cl, on Neutral Red (NR) uptake by HeLa cells (mean $\pm$ SEM), relative to that in Complete Essential Medium.	119
<b>Figure 5.3.</b> The effect of exposure for 24 hours to adult <i>O. circumcincta</i> excretory/secretory products, incubates OAD1 (2000h ml <sup>-1</sup> ) and OAD 2A (375 h ml <sup>-1</sup> and 187.5h ml <sup>-1</sup> ) at different concentrations in the presence of up to 20mM NH <sub>4</sub> Cl on Neutral Red (NR) uptake by HeLa cells (mean $\pm$ SEM), relative to that in Complete Essential Medium.	119
<b>Figure 5.4.</b> The effect of excretory/secretory products of adult <i>Ostertagia circumcincta</i> (a), <i>Ostertagia circumcincta</i> $L_3$ , which had been incubated for 24h (b) and <i>Haemonchus contortus</i> $L_3$ , which had been incubated for 24h, except HL4 (144h) and HL5 (168h) (c and d) on Neutral red uptake by HeLa cells after exposure for 24 hours.	120
<b>Figure 5.5.</b> The effect of exposure for 24 hours to $L_3$ <i>O.</i> <i>circumcincta</i> excretory/secretory products which had been stored under different conditions on Neutral Red (NR) uptake by HeLa cells (mean $\pm$ SEM), relative to that in Complete Essential Medium.	121
<b>Figure 5.6.</b> Rate of protein appearance in incubations with varying concentrations of <i>H. contortus</i> $L_3$ or <i>O. circumcincta</i> $L_3$ or adult worms.	121
<b>Figure 5.7.</b> Effect on Neutral Red (NR) uptake by HeLa cells of exposure to CEM, combinations of CEM and RPMI, CEM and RPMI-inc and a 10kDa fraction of RPMI-inc and CEM, for 24 hours.	121
Figure 5.8. Genomic <i>O. circumcincta</i> DNA run on a 0.7% argarose gel. From top to bottom: DNA ladder, EcoRI digestedDNA with rest RNA, empty and EcoRI digested DNA free from RNA, HindIII digested DNA free from RNA.	121

xiii

**Fig. 5.9.** Radiographic film after exposure to a radioactively probed Southern blot of genomic *O. circumcincta* DNA for 3 days. From left to right: 5µg of undigested, 5µg of EcoRI digested DNA, 5µg of HindIII digested genomic worm DNA; 10ng, 1ng and 10ng of vacA PCR product.

122

### List of tables

	Facing page
<b>Table 2.1.</b> Pre-infection values for abomasal pH, serum gastrinand pepsinogen and blood eosinophil content for parasite-naïvelambs used in Experiments 1 and 2.	52
<b>Table 2.2.</b> Worm counts (L <sub>3</sub> , L <sub>4</sub> , immature adults (I.A.), adult female worms and adult male worms) in high fleece weight (HFW) and control lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> and at Days 35, 42, 49, 56, 63 and 70 with 10,000 L <sub>3</sub> <i>O. circumcincta</i> and killed on Day 94 after infection.	56
<b>Table 2.3.</b> Worm counts (L <sub>3</sub> , L <sub>4</sub> , immature adults (I.A.), adult female worms, adult male worms, eggs <i>in utero</i> per female worm (mean $\pm$ SEM) and total worms) in high fleece weight (HFW) and control lambs, which were infected with 50,000 L <sub>3</sub> <i>O. circumcincta</i> and killed either on Day 8 or Day 28 after infection.	60
<b>Table 2.4.</b> Relative cytokine gene expression (mean $\pm$ SEM and median) by abomasal lymph nodes collected from high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> and killed on Day 8.	61
<b>Table 2.5.</b> Relative cytokine gene expression (mean $\pm$ SEM and median) by abomasal lymph nodes collected from high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> and killed on Day 28.	61
<b>Table 2.6.</b> Relative cytokine gene expression by abomasal lymph nodes collected from individual high fleece weight (HFW) and control (C) lambs which were infected at Day 0 with 50,000 $L_3 O.$ <i>circumcincta</i> and killed on Days 8 or 28.	61
<b>Table 3.1.</b> Parietal cells stained with TGF- $\alpha$ antibody, mucosal eosinophils and mucosal thickness ( $\mu$ m) (mean ± SEM) in fundic tissue from high fleece weight (HFW) and control (C) sheep which were infected on Day 0 with 50,000 L <sub>3</sub> <i>O. circumcincta</i> and killed on Day 8 or Day 28.	81
<b>Table 3.2.</b> Abomasal pH, parietal cells stained with TGF- $\alpha$ antibody, mucosal eosinophils and mucosal thickness ( $\mu$ m) (mean ± SEM) in fundic tissue from sheep (Group LI) which were infected on Day 0 with 35,000 L <sub>3</sub> <i>O. circumcincta</i> and killed on Days 5, 10, 15, 20 or 30.	83

**Table 3.3** Percentage of total parietal cell numbers (mean  $\pm$  SEM) which were TGF- $\alpha$  negative, but counterstained with Haematoxylin and Eosin, in fundic mucosa of individual sheep (Group LI) which were infected on Day 0 with 35,000 L<sub>3</sub> *O. circumcincta* and killed on Days 5, 10, 15, 20 or 30.

**Table 3.4** Numbers of TGF- $\alpha$ -positive parietal cells (PC) and eosinophils in a 258µm wide column of fundic mucosa, abomasal pH at necropsy and mucosal thickness ( $\mu$ m) (mean ± SEM) in sheep before, (control, CT) or 6, 12, 24 or 72 hours after, the transplantation of 10,000 adult *O. circumcincta.* 

**Table 3.5.** Percentage of total parietal cells which stained with Haematoxylin and Eosin, but not TGF- $\alpha$  (mean ± SEM) from individual sheep sampled before, (control, CT) or 6, 12, 24 or 72 hours after, the transplantation of 10,000 adult *O. circumcincta*. The total parietal cell number was obtained from the total of TGF- $\alpha$ -positive and TGF- $\alpha$ -negative parietal cells.

**Table 4.1.** Numbers of TGF- $\alpha$  stained parietal cells and eosinophils (mean  $\pm$  SEM) per 258µm wide column of fundic tissue in infested and non-infested areas of the main portion and in the tip of the fundic folds of a sheep killed 30 hours after infection with 25,000 L<sub>3</sub> *O. circumcincta*.

**Table 5.1.** Components of the three media MEM (10x), RPMI-1640 and EBSS. RPMI-1640 contained in addition Glutathione (reduced) 0.001g/l, L-Asparagine (anhydrous) 0.05g/l, L-Aspartic acid 0.02g/l, L-Glumatic acid 0.02g/l, L-Glutamine 0.3g/l, Glycine 0.01g/l, Hydroxy-L-Proline 0.02g/l, D-Biotin (traces), p-Amino Benzoic Acid 0.001g/l and Vitamin B12 (traces).

106

113

84

91

91

# List of abbreviations

hð	Microgram
μM	Micromolar
μm	Micrometre
Ab	Antibody
ACh	Acetylcholine
ALN	Abomasal Lymph Node
ANOVA	Analysis of Variance
AP	Aminopyrine
AR	Amphiregulin
AT	Adult Transplant
ATV	Antibiotic-Trypsin-Versene solution
BSA	Bovine Serum Albumin
С	Control flock
cAMP	Cyclic Adenosine Monophosphate
ССК	Cholecystokinin
CCK-A/B	Cholecystokinin Receptor type A or B
CEM	Complete Essential Medium
CNS	Central Nervous System
CGRP	Calcitonin Gene Related Peptide
conA	Concavalin A
DAG	Diacyl Glycerol
e.p.g.	eggs per gram
EBSS	Earle's Balanced Salt Solution
EC	Enterochromaffin

ECL	Enterochromaffin-like
EDTA	di-sodium Ethylene Diamine Tetra-Acetate
EGF	Epidermal Growth Factor
ES	Excretory/Secretory products
FBS	Fetal Bovine Serum
FEC	Faecal Egg Count
g	Gram
g	Gravitational force
Gal	Galanin
GIP	Gastric Inhibitory Peptide
Gm-CSF	Granulocyte-macrophage Colony-Stimulating Factor
GRP	Gastrin Releasing Peptide
GRPR1	Gastrin Releasing Peptide Receptor type 1
h	Hour
H2	Histamine receptor type 2
H&E	Haematoxalin & Eosin
H. pylori	Helicobacter pylori
H.contortus	Haemonchus contortus
HB-EGF	Heparin Binding-Epidermal Growth Factor
HBSS	Hank's Balanced Salt Solution
HDC	Histidine Decarboxylase
HFW	High Fleece Weight flock
I.A.	Immature Adult
i.m.	intramuscular
i.v.	intravenous
IFN-γ	Interferon-gamma

lg	Immunoglobulin
IL	Interleukin
IP <sub>3</sub>	Inositol 1,4,5-triphosphate
kg	Kilogram
ł	Litre
L <sub>3</sub>	Third-stage larva
L4	Fourth-stage larva
L <sub>5</sub>	Fifth stage larva
LI	Larval infection
LT	lymphotoxin
М	Molar
МЗ	Muscarinic receptor type 3
МЕМ	Minimal Essential Medium
mg	Milligram
min	Minute
ml	Millilitre
mm	Millimetre
ММС	Mucosal Mast Cell
mRNA	Messenger Ribonucleic Acid
MWCO	Molecular Weight Cut-Off
n	Number
N. brasiliensis	Nippostrongylus brasiliensis
NA	Noradrenalin
NHE2	Sodium/Hydrogen Exchanger 2
NK	Neurokinin
nm	Nanometers
NR	Neutral Red

O. circumcincta	Ostertagia circumcincta
O. leptospicularis	Ostertagia. Ieptospicularis
O. ostertagi	Ostertagia ostertagi
OD	Optical Density
P follicle	Primary follicle
p.i.	Post infection
PACAP	Pituitary Adenylate Cyclase-Activating Peptide
PAC-R1	Pituitary Adenylate Cyclase-Activating Peptide Receptor type 1
PAS	Periodic Acid Schiff
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PG	Prostaglandin
PHI	Peptide Histidine Isoleucine
РКА	Protein kinase A
РКС	Protein kinase C
PLC	PhospholipaseC
PP	Pancreatic Polypeptide
PSN	Penicillin-Streptomycin-Neomycin
reg	Regenerating gene
RO water	Distilled water
S follicle	Secondary follicle
S.C.	subcutaneous
SD	Standard Deviation
SEM	Standard Error of the Mean
SP	Substance P
SPSS	Statistic Package for Social Scientists

SST	Somatostatin
SST2	Somatostatin receptor subtype 1
T. axei	Trichostronglus axei
T. colubriformis	Trichostronglus colubriformis
TAE	Tris Acetate EDTA
TGF-α	Transforming Growth Factor-alpha
Th	T helper cell
TNF-α	Tumor Necrosis Factor-alpha
v/v	volume per volume
VIP	Vasoactive Intestinal Polypeptide
w/v	weight per volume

### Introduction

The agricultural industry, especially pastoral farming, plays an important role in the New Zealand economy. Since livestock are almost exclusively kept on pasture in New Zealand, parasites, especially internal helminths, have a significant impact on the outcome of the sheep and beef and dairy industries.

The alarming number of reports of drench resistance, the increasing interest in organic farming and the recognition of the long-term impact of agricultural chemicals on the environment are all incentives to develop alternative strategies to the current chemical anthelmintics. High concentrations of anthelmintics are excreted in the faeces onto the pasture where free-living nematodes and other soil and pasture dwellers are also being affected by highly potent chemicals. Further, there is a significant incidence of anthelmintic resistance amongst the common nematodes infecting ruminants in New Zealand, as is the case elsewhere in the world. This alone has highlighted the need to supplement current drenches with other measures to minimise the use of these chemicals to slow the rate of development of resistance.

Alternative strategies to drenching include better management to minimise larval intake, exploiting the genetic variation in susceptibility of animals to parasites and to their impact on productivity, the use of plants containing natural anthelmintics as fodder, trapping the free-living stages of nematodes by fungi and the development of vaccines against the parasites. Another alternative would be biological control by interfering with parasite establishment, metabolism or reproduction. This approach will depend on a much more detailed understanding of the host-parasite interaction, parasite biology and the pathophysiological basis for resistance, resilience and susceptibility of hosts.

The objective of the present experiments was to study the responses of sheep of different genetic backgrounds to infection with abomasal parasites in order to learn more about the communication between the parasite and the host tissues and the relative importance of host inflammatory responses and the excretory/secretory products of the worms in causing abomasal dysfunction. The effects of infection with *Ostertagia circumcincta* or *Haemonchus contortus* in outbred sheep are well described: inhibition of gastric acid secretion, hypersecretion of gastrin, increased levels of circulating pepsinogen, loss of functioning parietal cells, inflammation characterised by large numbers of eosinophils and mucous cell hyperplasia in the infected abomasum. What is still unknown is whether the parasites actively inhibit acid secretion to promote their survival, if so, how do they do it and could this be a target for biological control of abomasal nematodes.

Alternatively, it may be more practical to breed for resistant or resilient hosts to reduce the impact of the parasites with a minimum of chemical intervention. Whereas it has been established that resistance has an immunological basis, there seems to be no information on the physiological basis for resilience to parasitism. If resistance and resilience are not opposite extremes of the same phenomenon, then selection may provide a way for the incorporation of both traits into a breeding programme. The experiments described in this thesis are also aimed at establishing the underlying reasons for different host responses to abomasal parasites and from these studies also to gain a better understanding of the pathophysiological processes initiated by the parasites.