A Study of the Lawsonia intracellularis-induced porcine proliferative enteropathies.

by Alison Marie Collins

A thesis submitted for the degree of

Doctor of Philosophy

of the University of Sydney

Australia

August 2000

Department of Veterinary Clinical Sciences

Faculty of Veterinary Science

University of Sydney

Declaration

Apart from the acknowledgments and assistance stated in the text, this thesis represents the original work of the author. The investigations presented here have not been presented for any other degree or diploma at any other university.

Alison Collins
August 2000

Dedication

Dedicated to my mother

Coralie Collins and

to my partner in life

Andrew Craig,

whose strength and love sustain me.

Acknowledgments

Indulge me in the telling of a fable, of unknown source, to illustrate the support provided by my principal supervisor Associate Professor Robert Love.

It's a sunny day, and a rabbit is sitting outside her burrow, tapping on her typewriter. Along comes a fox, and asks the rabbit "What are you working on?" The rabbit replies "my thesis". The fox asks what it is about, and the rabbit responds "I am writing about how rabbits eat foxes". The fox appears incredulous, "That's ridiculous, any fool knows that rabbits don't eat foxes!" The rabbit motions to the fox to follow her into the burrow. After a few minutes the rabbit returns to the typewriter and resumes writing while gnawing on a bone.

Soon a wolf comes along and asks the hard working rabbit "What are you writing?" The rabbit replies, "I am doing a thesis on how rabbits eat wolves". The fox laughs loudly, "You don't expect to get such stuff published, do you?" The rabbit responds, "No problem, do you want to see why?" The rabbit and the wolf go down the burrow, and the rabbit returns by herself after a few minutes and goes back to typing.

Finally a bear comes along and asks, "What are you doing?" The rabbit replies, "I am doing a thesis on how rabbits eat bears". The surprised bear says "well that's absurd". The rabbit motions to the burrow and says "come into my home and I'll show you". As they enter the burrow, the rabbit introduces the bear to the lion.

The moral of this fable is that it doesn't matter how silly your thesis topic is, all that matters is who you have for a supervisor.

I would like to thank all of my supervisors, Dr Robert Love, Dr Ian Swift and Dr Steve McOrist for their support, encouragement and belief in me.

There are many people at the University of Sydney, La Trobe University and the Department of Agriculture at Bendigo who have guided, supported, helped and encouraged me along this journey. In particular I would like to thank Matt van Dijk

for his help and patience with all of the pig experiments, and Marilyn Jones for helping me get set up at Camden, and for both of their friendships.

I would like to acknowledge the help of all the academic staff at Camden and in particular Garry Cross, who provided inestimable help with computers and photography. My supervisor Dr Ian Swift, at La Trobe University, requires special thanks for guiding me through the upheaval of the closure of the Regional Veterinary Laboratories. Dr Barbara McDougall and Dr Robert Seviour also provided support and an excellent working environment.

There are many general staff that I would like to thank for their help, including Craig Kristo, Sandra Saville, Ron Henderson, Gina Attard, Jiri Tasler, and David Palmer at the University of Sydney. Dot, Peter, Sue and Lyn also helped at La Trobe University. The librarians also deserve a special mention for their help, including Judy Anson, Janine Maitland, Lauryl and Melanie.

I would also like to thank the postgraduate students and postdoctoral fellows who have provided excellent support and friendship, in particular Pam Megaw, Lun Li, Om Dhungyel, Javier Pozo, Imke Tammen, Anssi Tast, Shevahn Telfser and Jacques Soddell.

The tissue culture work would not have been possible without the excellent teaching of Rebecca Mackie and Steve McOrist at the University of Edinburgh. Thanks also to Dr Richard Whittington and Ian Marsh from NSW Agriculture at EMAI for their help in developing the hybridisation capture PCR method.

At the start of this journey Dr Frank Allison inspired me to question and expand my universe. Thank you for the instillation of ideas and the time spent teaching me. I would also like to thank Dr Parry Monckton for supervising me at the early stages of this project and the Pig Research and Development Corporation for providing a 2 year scholarship. I would also like to thank Denise Millar for her wise words when I first started my career in science.

On a personal note I'd like to thank my family, Coralie, Kevin, Mark, Dianne and Kim. It is a pleasure sharing my life with you. I also owe a big thank you to my friends Jill Hall, John Golding, Silvana and Brian and Carleen Cullinane.

The best is saved for last. Thank you Andrew for your constant love.

Summary

The porcine proliferative enteropathies (PPE) are a group of diseases ranging from intestinal adenomatosis (PIA), a chronic condition causing reduced growth rates in post weaning pigs, to the often fatal proliferative haemorrhagic enteropathy (PHE), resulting in intestinal haemorrhage. PHE predominantly occurs in older and heavier pigs than the chronic disease PIA. This thesis examined whether the age when susceptible pigs are infected affects the clinical response to *L.intracellularis* infection.

The characteristic pathologic lesion of PPE is the abnormal proliferation of crypt epithelial cells in the ileum and colon. Closely associated with this proliferation is the presence of an obligately intracellular bacterium, *Lawsonia intracellularis*. Characterisation of *L.intracellularis* was performed in *in-vitro* co-cultures of *L.intracellularis* extracted from PHE-affected mucosa. The efficacy of antimicrobials to inhibit the growth of *L.intracellularis in-vitro* was evaluated and compared with isolates cultured in the United Kingdom. The results were analysed with respect to medication strategies currently used to control PPE in piggeries.

PPE occurs in virtually all piggery management systems, including newly developed systems that are aimed at improving the herd health, such as segregated early weaning and multiple site production. PPE is currently controlled in Australia with the routine addition of antimicrobials in pig feed, in particular olaquindox. Recommendations to reduce the use of feed-based antibiotics in Australia require the development of alternate strategies to control diseases such as PPE.

Sequential outbreaks of PHE reported in minimal disease herds suggested that pigs could develop immunity to disease. An experimental model of *L.intracellularis* infection was developed in this thesis to demonstrate that immunity to re-infection with *L.intracellularis* could be developed. Infection was monitored by detection of faecal shedding of *L.intracellularis* and serum IgG antibodies against *L.intracellularis*.

Two in-feed antimicrobial strategies were analysed in this thesis for their ability to induce the development of immunity to *L.intracellularis*, while avoiding clinical signs of disease. The first strategy evaluated the use of low levels of in-feed antimicrobials to allow subclinical infection and the development of immunity. The second strategy evaluated the use of high levels of in-feed antimicrobials to terminate infection two weeks after exposure to *L.intracellularis*.

Gaining a greater understanding of how *L.intracellularis* infection is spread both within and between piggeries will enable the development of management strategies to control the spread of infection. This thesis examined the possibility that other species in contact with pigs and piggeries such as rats, mice and birds may transmit infection to pigs. The transmission of infection between pigs via the faecal/oral route was also examined, as was the survival and infectivity of *L.intracellularis* over time.

Ultimately this thesis aimed to understand the pattern of *L.intracellularis* infection and the survival and transmission of *L.intracellularis* in order to develop effective control measures for PPE, especially in minimal disease herds.

Publications

The following is a list of papers published in journals or proceedings of conferences that are related to the work presented in this thesis.

Collins, A.M., Love, R.J., Pozo, J., Smith, S.H. and McOrist, S. (2000). Studies on the ex vivo survival of *Lawsonia intracellularis*. *Swine Health and Production*, 8 (5):(in press).

Collins, A.M., Love, R.J., Jasni, S. and McOrist, S. (1999). Attempted infection of mice, rats and chickens by porcine strains of *Lawsonia intracellularis*. *Aust Vet.J.* 77:25-27.

Collins, A.M., McOrist, S., van Dijk, M. and Love, R.J. The development of immunity to *Lawsonia intracellularis* in weaned pigs. *Proceedings of the Seventh Biennial Conference of the Australasian Pig Science Association*; Adelaide; 1999.

Collins, A.M., McOrist, S., van Dijk, M. and Love, R.J. Effect of age on clinical disease associated with *Lawsonia intracellularis* infection. . *Proceedings of the Seventh Biennial Conference of the Australasian Pig Science Association*; Adelaide; 1999.

Collins, A.M., Swift, I. and Monckton, R.P. (1996) Replication of Australian porcine isolates of Ileal symbiont intracellularis in tissue culture. *Veterinary Microbiology*, 49:249-55.

Collins, A.M., Swift, I. and Monckton, R.P. (1995). Replication of Ileal symbiont intracellularis in rat enterocyte cells. *Australian Microbiologist* 16(4): 29.

Collins, A.M., Monckton, R.P., Hasse, D. and Swift, I. (1994). Culture of Ileal symbiont intracellularis. *Asian-Pacific J. Molecular Biology and Biotechnology*, 2(3): 269.

Monckton, R.P., Hasse, D., **Collins, A.M.**, McCormick, B.M., and Moses, E.K. (1994). Ileal symbiont intracellularis associated with porcine proliferative enteropathies. *Proceedings of the 13th International Pig Veterinary Society*, Bangkok, Thailand.

Monckton, R.P., Hasse, D., **Collins, A.M.**, Morrow, C.J. and Moses, E.K. (1993). Campylobacter-like organisms (CLO) associated with porcine proliferative enteropathies. *Aust. Microbiologist* 14: 53.

Table of Contents

Abbreviations

ASP250	100g/t chlortetracycline, 100g/t sulphadimidine and 50g/t penicillin
bp	base pairs
BSA	Bovine serum albumin
CTC	Chlortetracycline
Da	Dalton
DEPEX	Gurrs' DEPEX mounting medium
DMEM	Dulbeccos Modified Eagles medium
dNTPs	deoxyribonucleoside triphosphates
EDTA	Ethylene diamine tetra acetic acid
FCS	Foetal calf serum
FITC	Fluorescein isothiocyanate
HC PCR	Hybridisation capture polymerase chain reaction
H&E	Haematoxylin and Eosin
HEPES	N-2-hydroxylethylpiperazine-N'-2-ethanesulfonic acid
HIC	Heavily infected cell
IEC	Ileal enterocyte cells from rat
IFAT	Indirect Fluorescent Antibody Test
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IP	Intra-peritoneal
IPX	Immunoperoxidase
LP	Lamina Propria
LPS	Lipopolysaccharide
MHC	Major Histocompatability Complex
MIC	Minimum inhibitory concentration
MLN	Mesenteric Lymph Node
NCTC	National Collection of Type Cultures
PBS	Phosphate buffered saline

PCR	Polymerase chain reaction
PE	Proliferative Enteropathy
PHE	Proliferative Haemorrhagic Enteropathy
pi	post inoculation
pi #2	post secondary inoculation
pi #3	post third inoculation
PIA	Porcine Intestinal Adenomatosis
PP	Peyer's patches
PPE	Porcine Proliferative Enteropathy
ppi	post primary inoculation
ppm	parts per million
PUC19/HpaII	PUC 19 plasmid digested with the restriction enzyme HpaII
rRNA	ribosomal RNA
SDS	Sodium dodecyl sulphate
SEW	Segregated early weaning
SPF	Specific pathogen free
SPG	Sucrose potassium glutamate
SSC	Sodium chloride, sodium citrate
TAE	Tris acetate buffer
Taq	*Thermus aquaticus*
TBE	Tris, Boric acid buffer
TE	10mM Tris, 1mM EDTA (pH 8.0)
Tris	Tris (hydroxymethyl) aminomethane