
**Application of a functional genomics approach to the identification of vaccine
subunits and diagnostic antigens for use in the control of swine dysentery**

by

Yong Song

M.D., M.Sc.

School of Veterinary and Biomedical Science

Murdoch University

Western Australia

This thesis is submitted for the degree of Doctor of Philosophy of Murdoch University

2007

Dedicated to my wife: Lulu Yin,
my daughters: Anya Song and Enya Song,
my parents: Guanghua Song and Xiaoyang Xu

DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

.....

Yong Song

ABSTRACT

The intestinal spirochaete *Brachyspira hyodysenteriae* is the causative agent of swine dysentery (SD), a diarrhoeal disease of pigs which has significant economic impact worldwide. Controlling SD remains problematic, particularly as there is no effective vaccine and there are few definitive diagnostic methods available. In this study, a partial genomic sequence of *B. hyodysenteriae* was screened *in silico*. A total of 19 putative open reading frames (ORFs) encoding outer-membrane proteins then were selected and these were subjected to a laboratory screening process. To select potential universal vaccines, a preliminary study was conducted using PCR to determine the distribution of the putative genes in 23 strains of *B. hyodysenteriae*. A total of 17 of the 19 ORFs were considered to be suitable for further testing as they were found to be present in the majority of strains investigated. After molecular cloning and protein expression and purification, of 19 cloned candidate molecules derived from 17 genes (one large gene was divided into two parts encoding N and C terminal proteins, respectively), 14 were expressed in *E. coli* and the recombinant proteins were successfully produced. A variety of sera from pigs naturally and experimentally infected with *B. hyodysenteriae* were tested for reactivity with the 14 recombinant proteins in an immunoblotting assay. Seven molecules from six genes reacted strongly with the tested sera, and therefore were selected and used to immunize mice. All these proteins generated a specific antibody response. Post-immunization sera raised against each recombinant protein had the capacity to agglutinate *B. hyodysenteriae* cells, and also recognized the cognate proteins of *B. hyodysenteriae* in cell extracts. Further sequencing analysis demonstrated that these molecules were highly conserved in the genomes of different *B.*

hyodysenteriae strains. Therefore, from the genomic-based study, the products of six genes were identified as promising candidates for vaccines or as diagnostic targets.

Four genes were expressed on a large scale, the products (NAV-H7, NAV-H17 C-terminal, NAV-H34 and NAV-H42) were combined into one vaccine, and then this preparation was used to immunise pigs that subsequently were challenged with *B. hyodysenteriae*. These antigens generate systemic and colonic antibody responses, and vaccination tended both to delay the onset of clinical signs and attenuate lesion development. Hence these recombinant proteins showed promise as components for further SD vaccines.

Recombinant proteins from the selected genes also were used as antigens in class-specific ELISAs used as serological assays for SD. Three antigens (NAV-H8, NAV-H42 and Bhlp29.7) were selected as good indicators of seroconversion in IgM ELISAs, and these were evaluated further using a large range of serum samples. The NAV-H8 IgM ELISA using a cut-off value 2.5 times the mean value of all negative pigs could be used as a herd test for SD, and both the NAV-H8 and NAV-H42 IgM ELISAs had potential for detecting exposure to *B. hyodysenteriae* at the pig level.

ACKNOWLEDGEMENTS

I would firstly like to express my gratitude to my principal supervisor, Professor David Hampson, for giving me this opportunity to undertake these studies and for his invaluable guidance, endless patience, support, and encouragement. As a supervisor of a student originating from non-English country, he spent considerable time and efforts in improving my English communication skills, which will certainly have great impact on my future research career.

My deep thanks go to Dr Tom La, whose excellent expertise, patience, kindness and friendship help me get through all the difficult stages of the project. The experimental components could have not been completed smoothly without his input and help. During the vaccine and diagnostic study, Tom generously provided the recombinant Bhlp29.7 as a control, which enriched the content of the research. I would also like to thank Dr Nyree Phillips for her assistance in bacterial culture, assistance with the pig trial and support in routine laboratory work. Also thanks to the other team members, Dr Kirsty Townsend, Abdolreza Movahedi and Arif Munshi for giving me advice, sharing experience and knowledge.

The vaccine project involved substantial bioinformatic analysis. I am very grateful to the members in bioinformatics side, my associate supervisor, Professor Matthew Bellgard (Center for Comparative Genomics, Murdoch University) for providing a training platform and helpful advice in bioinformatics during my first year of PhD training, and to Dr David Dunn and Mr Yair Motro for their great efforts in performing genomic

mining, and help in using various computer algorithms and in coordination of the selection of suitable candidate genes for further analysis.

Many people in the State Agricultural Biotechnology Centre where I conducted most of my experiments should be acknowledged. My thanks are due to Dr David Berryman for his generous advice and assistance in using various laboratory equipments, Frances Brigg for running the sequencing, and Dr Rongchang Yang for being such a good friend.

This study was funded by Australian Research Council and Novartis Animal Vaccines (NAV) as the industry partner. Dr Ian Thompson from NAV was particularly supportive throughout the project.

Finally, I owe particular thanks to my parents and my dearest wife, Lulu Yin, and my lovely daughter, Anya Song. They always support me and are a source of pride for me over many years. Their faith, sustenance, understanding and companionship were the sources of my strength to pursue this dream.

ACHIEVEMENTS ASSOCIATED WITH THIS STUDY

Some of the genes identified during my PhD study have been granted provisional patents, and currently they are being applied for full patent protection. Two papers have been prepared, but only can be submitted once full patents are obtained.

The patented genes are six (NAV-H7, NAV-H8, NAV-H12, NAV-H17, NAV-H34, NAV-H42) for *B. hyodysenteriae*, PCT Patent No. 2005-903317 and two (NAV-P2 and NAV-P10) for *B. pilosicoli*, PCT Patent No. 2005-902418.

Publications prepared:

Identification and recombinant expression of candidate components for a swine dysentery vaccine.

Comparative evaluation of the usefulness of *B. hyodysenteriae* surface-associated antigens for serodiagnosis of swine dysentery.

Song, Y. and Hampson, D. J. (2006) Reverse vaccinology: application of functional genomics to *B. hyodysenteriae* and *B. pilosicoli* (Poster). *16th Annual Queenstown Molecular Biology Meeting*. Queenstown, New Zealand.

TABLE OF CONTENTS

TITLE PAGE.....	i
DEDICATION.....	ii
DECLARATION.....	iii
ABSTRACT.....	iv
ACKNOWLEDGEMENTS.....	vi
ACHIEVEMENTS ASSOCIATED WITH THIS STUDY.....	viii
TABLE OF CONTENTS.....	ix
LIST OF FIGURES.....	xviii
LIST OF TABLES.....	xxiv
ABBREVIATIONS.....	xxvi

CHAPTER ONE. LITERATURE REVIEW

1.1. FOREWORD.....	1
1.2. REVERSE VACCINOLOGY: A GENOMIC-BASED APPROACH FOR VACCINE DISCOVERY.....	2
1.2.1. Introduction.....	2
1.2.2. Classical vaccinology in the 20 th century.....	3
1.2.3. Reverse vaccinology.....	5
1.2.3.1. <i>In silico</i> analysis of genomes.....	6
1.2.3.1.1. Secreted or surface-exposed proteins.....	6
1.2.3.1.2. Virulence factors.....	7
1.2.3.2. Experimental strategies.....	8

1.2.4.	Application of reverse vaccinology to vaccine discovery.....	11
1.2.5.	Functional genomics in vaccine discovery.....	14
1.2.6.	Comparative genome analysis in vaccine discovery.....	14
1.2.7.	Conclusions.....	15
2.2.	<i>BRACHYSPIRA HYODYSENTERIAE</i>: THE AETIOLOGICAL AGENT OF SWINE DYSENTERY.....	16
1.3.1.	General information on the Spirochaetes.....	16
1.3.2.	Description of the genus <i>Brachyspira</i>	18
1.3.3.	Swine dysentery.....	19
1.3.3.1.	Introduction.....	19
1.3.3.2.	Clinical signs.....	19
1.3.3.3.	Prevalence of swine dysentery.....	21
1.3.3.4.	Pathogenesis.....	22
1.3.4.	<i>B. hyodysenteriae</i>	24
1.3.4.1.	Characteristics of <i>B. hyodysenteriae</i>	24
1.3.4.2.	Molecular biology of <i>B. hyodysenteriae</i>	26
1.3.4.2.1.	Genome of <i>B. hyodysenteriae</i>	26
1.3.4.2.2.	RNA genes.....	27
1.3.4.2.3.	Flagellin genes.....	28
1.3.4.2.4.	Beta-haemolysin associated genes.....	30
1.3.4.2.5.	DNA gyrase gene.....	30
1.3.4.2.6.	<i>bhlp16</i> gene: encoding a 16 kDa lipoprotein.....	31
1.3.4.2.7.	<i>smpB</i> : encoding a novel outer membrane protein.....	32

1.3.4.2.8. <i>bhlp29.7</i> : encoding a 29.7 kDa outer envelope lipoprotein.....	33
1.3.4.2.9. The <i>blpGFEA</i> locus.....	34
1.3.4.2.10. <i>bhmp39</i> genes (formerly <i>vsp</i> genes): encoding variable surface proteins.....	35
1.3.4.2.11. NADH oxidase gene (<i>nox</i>).....	36
1.3.4.2.12. <i>VSH-1 svp38</i> gene.....	37
1.3.4.2.13. Iron metabolism related genes.....	37
1.3.4.2.14. <i>gap</i> gene.....	38
1.3.4.3. Virulence factors/traits associated with <i>B. hyodysenteriae</i>	39
1.3.4.3.1. Motility.....	39
1.3.4.3.2. Chemotaxis.....	40
1.3.4.3.3. Haemolysin / cytotoxin.....	41
1.3.4.3.4. Oxygen metabolism.....	42
1.3.4.3.5. Invasion.....	43
1.3.4.3.6. Lipopolysaccharide (LPS).....	44
1.3.4.3.7. Enterotoxin.....	45
1.3.5. Immunity.....	45
1.3.5.1. Humoral immunity.....	45
1.3.5.2. Cellular immunity.....	46
1.3.6. Detection of serum antibody.....	49
1.3.7. Vaccine development.....	50
1.3.7.1. Bacterin vaccines.....	50
1.3.7.2. Attenuated live vaccines.....	51
1.3.7.3. Protein-based vaccines.....	52

1.3.7.4.	DNA vaccine.....	53
1.3.7.5.	Summary.....	53
1.4.	AIMS AND OBJECTIVES OF THIS PROJECT.....	54
CHAPTER TWO. IDENTIFICATION AND RECOMBINANT EXPRESSION OF CANDIDATE COMPONENTS FOR A SWINE DYSENTERY VACCINE		
2.1.	INTRODUCTION.....	56
2.2.	MATERIALS AND METHODS.....	58
2.2.1.	Spirochaete strains.....	58
2.2.2.	Animals.....	58
2.2.3.	<i>In silico</i> analysis.....	60
2.2.4.	Gene distribution study by PCR.....	62
2.2.4.1.	Primer design.....	62
2.2.4.2.	PCR amplification.....	64
2.2.5.	Recombinant DNA techniques.....	64
2.2.5.1.	Molecular cloning.....	64
2.2.5.1.1.	Preparation of <i>E. coli</i> plasmid DNA.....	64
2.2.5.1.2.	Preparation of competent cells (<i>E. coli</i> strain JM109).....	65
2.2.5.1.3.	PCR for preparing inserts.....	66
2.2.5.1.4.	PCR product purification.....	69
2.2.5.1.5.	DNA quantification.....	69
2.2.5.1.6.	Restriction digestion.....	70
2.2.5.1.7.	Ligation of DNA fragments.....	70
2.2.5.1.8.	Transformation of competent cells.....	70

2.2.5.1.9. Screening of recombinant clones by PCR.....	71
2.2.5.1.10. Sequencing of the insert.....	71
2.2.5.2. Recombinant protein expression.....	71
2.2.5.2.1. Growth of standard <i>E. coli</i> expression cultures.....	72
2.2.5.2.2. Sodium dodecyl sulphate – polyacrylamide gel electrophoresis.....	72
2.2.5.2.3. Western blot analysis.....	73
2.2.5.3. Protein purification.....	74
2.2.5.3.1. Determination of target protein solubility.....	74
2.2.5.3.2. Purification methods.....	75
2.2.5.3.2.1. Protein purification under denaturing conditions.....	75
2.2.5.3.2.2. Protein purification under native conditions.....	76
2.2.5.3.3. Coomassie Brilliant Blue Staining.....	76
2.2.5.3.4. Protein quantification.....	77
2.2.5.3.5. Calculation of the recombinant proteins expression level.....	77
2.2.6. Immunogenicity analysis by Western blot analysis.....	78
2.2.6.1. Preparation of sera.....	78
2.2.6.2. Western blot analysis of serum.....	78
2.2.7. Sequencing and analysis.....	79
2.2.7.1. Primer design and PCR.....	79
2.2.7.2. Sequencing reaction.....	80
2.2.7.3. Post-reaction purification.....	81
2.2.7.4. Alignment of sequences.....	81
2.2.8. <i>In vivo</i> immunogenicity test.....	82

2.2.8.1.	Treatment of recombinant proteins: Dialysis and freeze drying...	82
2.2.8.2.	Immunisation of mice.....	82
2.2.8.3.	Western blotting of recombinant proteins and cell envelopes of <i>B. hyodysenteriae</i> using sera from immunized mice.....	83
2.2.9.	Bacterial agglutination assay.....	84
2.3.	RESULTS	85
2.3.1.	Functional annotation of ORF products.....	85
2.3.2.	Preliminary analysis of conservation of vaccine candidates among <i>B. hyodysenteriae</i> strains	91
2.3.3.	Antigen cloning.....	91
2.3.4.	Expression and purification of recombinant proteins in <i>E. coli</i>	95
2.3.5.	Antigenicity of the selected <i>in vitro</i> recombinant proteins.....	103
2.3.6.	Analysis of antigenic conservation among <i>B. hyodysenteriae</i> strains.....	110
2.3.7.	Bacterial agglutination.....	110
2.4.	DISCUSSION	112
CHAPTER THREE. THE USE OF RECOMBINANT OUTER MEMBRANE PROTEINS IN A PROTECTION STUDY AGAINST SWINE DYSENTERY IN PIGS		
3.1.	INTRODUCTION	119
3.2.	MATERIALS AND METHODS	120
3.2.1.	Permissions.....	121
3.2.2.	Animals and diet.....	121
3.2.3.	Bacterial strains and culture conditions.....	123

3.2.4.	Generation of the recombinant proteins for vaccination.....	123
3.2.5.	Immunization and challenge of pigs.....	124
3.2.6.	Pig weights.....	124
3.2.7.	Observation of clinical signs.....	124
3.2.8.	Sample collection.....	125
3.2.8.1.	Swab samples and detection of <i>B. hyodysenteriae</i>	125
3.2.8.2.	Serum samples.....	126
3.2.9.	Necropsy.....	126
3.2.10.	ELISA for serum antibodies.....	127
3.2.11.	ELISA for colonic IgA and IgG.....	128
3.2.12.	Western blot analysis of serum IgG and colonic IgA.....	128
3.2.13.	Data analysis.....	129
3.2.14.	Statistic analysis.....	129
3.3.	RESULTS.....	130
3.3.1.	Product of recombinant protein.....	130
3.3.2.	Evaluation of weights.....	131
3.3.3.	Health status of pigs pre-challenge.....	133
3.3.4.	Systemic antibody responses.....	133
3.3.5.	Local antibody response.....	147
3.3.6.	Faecal excretion of <i>B. hyodysenteriae</i>	150
3.3.7.	Development of clinical signs, and lesions at post-mortem.....	153
3.4.	DISCUSSION.....	157

CHAPTER FOUR. DEVELOPMENT OF ELISA ASSAYS FOR DETECTING SEROLOGICAL EVIDENCE OF SWINE DYSENTERY INFECTION USING RECOMBINANT OUTER MEMBRANE PROTEINS OF *B. HYODYSENTERIAE*

4.1. INTRODUCTION.....	160
4.2. MATERIALS AND METHODS.....	162
4.2.1. Serum samples.....	162
4.2.2. Antigen coating.....	164
4.2.3. ELISA assays for determining total specific IgG and IgM.....	165
4.2.4. Optimization of the protein and serum concentration in NAV-H8 and NAV-H42 IgM ELISAs.....	165
4.2.5. Expression of ELISA antibody titres and determination of cut-off values.....	166
4.2.6. Statistical analysis.....	167
4.3. RESULTS.....	168
4.3.1. Standardisation of ELISA titres.....	168
4.3.2. IgG response.....	169
4.3.3. IgM response.....	170
4.3.4. Combination of different antigens for IgM reactions.....	174
4.3.5. Specificity at pig level in the small scale testing stage.....	174
4.3.6. Optimisation of protein concentration.....	175
4.3.7. Optimisation of serum concentration.....	178
4.3.8. Further large scale evaluation of NAV-H8, NAV-H42 and Bhlp29.7 in IgM ELISA.....	178

4.3.8.1.	Specificities at the herd and pig levels with the NAV-H8, NAV-H42 and Bhlp29.7 IgM ELISAs in large scale testing.....	178
4.3.8.2.	Sensitivity at the herd level and detection ratio at the pig level.....	180
4.3.8.3.	Comparison of overall antibody titres between the negative and positive herds.....	186
4.3.8.4.	Comparison of ELISA values among and within each herd and distribution of antibody titres in negative samples.....	187
4.3.8.5.	Association of the three ELISA systems.....	190
4.3.9.	Reproducibility.....	193
4.4.	DISCUSSION.....	193
 CHAPTER FIVE. GENERAL DISCUSSION		
5.1.	INTRODUCTION.....	203
5.2.	BIOINFORMATIC ANALYSIS.....	203
5.3.	DISTRIBUTION OF PUTATIVE ORFS.....	204
5.4.	GENE CLONING AND PREPARATION OF RECOMBINANT PROTEINS.....	205
5.5.	IMMUNOGENICITY SCREENING AND SEQUENCE ANALYSIS.....	206
5.6.	PROTECTION STUDY IN PIGS.....	208
5.7.	DEVELOPMENT OF A SEROLOGICAL ASSAY FOR SD.....	211
5.8.	SUMMARY.....	213
	REFERENCES.....	215
	APPENDIX A.....	251
	APPENDIX B.....	261

LIST OF FIGURES

1.1.	Conventional approach to vaccine development.	4
1.2.	Schematic representation of the “reverse vaccinology” genome-based approach to vaccine development.	6
1.3	<i>Neisseria meningitidis</i> serogroup B as an example of reverse vaccinology, demonstrating how complete microbial genome sequence data can accelerate vaccine development.	12
1.4	16S rRNA dendrogram of the spirochaetes, demonstrating phylogenetic relationships between representatives of each genera.	17
1.5	Transmission electron micrograph of <i>B. hyodysenteriae</i> B78 ^T type strain showing a blunt cell end and 12 periplasmic flagella (six originating at each cell end).	24
1.6	Genetic map of <i>B. hyodysenteriae</i> .	27
2.1	The SignalP output for NAV-H7 as an example of a secretion signal sequence.	86
2.2	NAV-H26 shown as an example of transmembrane regions predicted by TMpred.	87
2.3	Distribution study by PCR on NAV-H46.	93
2.4	Example of agarose gel electrophoresis of PCR products obtained whilst selecting positive colonies containing NAV-49.	94
2.5	Recombinant protein expression detected by Western blot using anti-Histidine-tagged mouse serum.	96
2.6	Western blot analysis of NAV-H42 expression using anti-Histidine-tagged mouse serum.	97

LIST OF FIGURES (Continued)

2.7	Three recombinant proteins (NAV-H7, NAV-H8 and NAV-H17 C-terminal) shown in a SDS-PAGE gel for determination of target solubility.	98
2.8	Denaturing purification of heterologously expressed NAV-H18.	99
2.9	Purification of NAV-H8 under native conditions.	100
2.10	SDS-PAGE analysis of recombinant proteins purified from <i>E. coli</i> , resolved in a 10% PAGE gel and stained with Commassie blue.	101
2.11	Western blot showing reactivity of all 14 selected candidate proteins with convalescent serum from a pig recovered from SD.	104
2.12	Western blot analysis of NAV-H12 recombinant protein, performed using sera from pigs naturally or experimentally infected with SD, or immunized with <i>Brachyspira</i> spp. bacterins.	105
2.13	Western blot analysis of NAV-H42 recombinant protein, performed using sera from pigs naturally or experimentally infected with SD, or immunized with <i>Brachyspira</i> spp. bacterins	106
2.14	Western blot reactivity of mouse serum raised against recombinant His ₆ -NAV-H7 with a whole cell preparation of <i>B. hyodysenteriae</i> WA1 and NAV-H7 recombinant protein.	108
2.15	Western blot reactivity of mouse serum raised against recombinant His ₆ -NAV-H17 C-terminal with a whole cell preparation of <i>B. hyodysenteriae</i> WA1 and NAV-H17 C-terminal fusion protein.	109
3.1	The author conducting the pig experiment in the Isolation Animal House at Murdoch University.	122

LIST OF FIGURES (Continued)

- 3.2 10% SDS – polyacrylamide gel electrophoresis of purified recombinant proteins of *B. hyodysenteriae*, followed by Coomassie blue R-250 staining. 130
- 3.3 Comparison of the mean body weights in the three groups from the beginning to the end of the trial. 131
- 3.4 Comparison of the whole-cell IgM reactivity and body weights in non-vaccinated pigs. 132
- 3.5 Systemic IgG antibody titres in ELISA against NAV-H7 in individual pigs from the control and experimental vaccine groups. 134
- 3.6 Systemic IgG antibody titres in ELISA against NAV-H17 C-terminal in individual pigs from the control and experimental vaccine groups. 135
- 3.7 Systemic IgG antibody titres in ELISA against NAV-H34 in individual pigs from the control and experimental vaccine groups. 135
- 3.8 Systemic IgG antibody titres in ELISA against NAV-H42 in individual pigs from the control and experimental vaccine groups. 136
- 3.9 Systemic IgG antibody titres in ELISA against His₆-Bhlp29.7 in individual pigs from the control and Bhlp29.7 vaccinated groups. 136
- 3.10 Systemic IgG antibody titres in ELISA against *B. hyodysenteriae* whole-cell extracts in individual pigs from the control, Bhlp29.7 and experimental vaccine groups. 137
- 3.11 Systemic IgM antibody titres in ELISA against NAV-H7 in individual pigs from the control and experimental vaccine groups. 139
- 3.12 Systemic IgM antibody titres in ELISA against NAV-H17 C-terminal in individual pigs from the control and experimental vaccine groups. 139

LIST OF FIGURES (Continued)

- 3.13 Systemic IgM antibody titres in ELISA against NAV-H34 in individual pigs 140
from the control and experimental vaccine groups.
- 3.14 Systemic IgM antibody titres in ELISA against NAV-H42 in individual pigs 140
from the control and experimental vaccine groups.
- 3.15 Systemic IgG antibody titres in ELISA against His₆-Bhlp29.7 in individual 141
pigs from the control and Bhlp29.7 vaccinated groups.
- 3.16 Systemic IgM antibody titres in ELISA against *B. hyodysenteriae* whole-cell 141
extracts in individual pigs from the control, Bhlp29.7 and experimental
vaccine groups.
- 3.17 Examples of comparison of systemic antibody responses against vaccine 144
antigens or whole-cell (WC) antigens in the non-vaccinated pigs at
slaughter.
- 3.18 Western blot reactivity of serum from pigs in the experimental vaccine 145
group to a mixture of the four recombinant proteins.
- 3.19 Western blot reactivity of serum from pigs in the non-vaccinated control 146
group to whole-cell preparation of *B. hyodysenteriae* WA1.
- 3.20 Comparison of colonic IgA response in ELISA against recombinant proteins 147
and the whole-cell preparation in the control and experimental vaccine
groups at slaughter.
- 3.21 Comparison of colonic IgG response in ELISA against recombinant proteins 148
and the whole-cell preparation in the control and experimental vaccine
groups at slaughter.

LIST OF FIGURES (Continued)

- 3.22 Comparison of colonic antibody response in ELISA against Bhlp29.7 and 148
the whole-cell preparation in the control and Bhlp29.7 vaccinated groups
at slaughter.
- 3.23 Examples of comparison of colonic antibody responses against vaccine 149
antigens or whole-cell (WC) antigens in the non-vaccinated pigs at
slaughter.
- 3.24 A lack of macroscopic lesions in the colon of an experimental pig. 153
- 3.25 Pathological changes in pig R4 from the control group, showing severe and 154
extensive mucohaemorrhagic colitis extending from the caecum to the
rectum.
- 3.26 Pathological changes in pig OR3 from the vaccine group. 154
- 3.27 Histological section of the colon of a *B. hyodysenteriae* –infected pig, 155
showing infiltration of a large number of inflammatory cells in the lamina
propria.
- 4.1 Representation of the relationship between the observed antibody titres of 168
the positive control serum sample obtained from serial dilution in a NAV-
H34 IgM ELISA and their corresponding concentration units.
- 4.2 Evaluation of varying concentration of recombinant NAV-H8 (A) and 176
NAV-H42 (B) at a single serum dilution of 1:200 in an IgM ELISA.
- 4.3 Optimisation of serum concentration in NAV-H8 (A) and NAV-H42 (B) 177
IgM ELISAs at the concentrations of 25ng/well and 12.5ng/well.

LIST OF FIGURES (Continued)

- 4.4 Diagnostic sensitivity and specificity at herd level using the third cut-off method (2.5 times the mean of all the negative sera) in the NAV-H8 IgM ELISA. 182
- 4.5 Diagnostic and detection sensitivity and specificity in the different groups using the NAV-H8 IgM ELISA. 183
- 4.6 Diagnostic and detection sensitivity and specificity in the different groups using the NAV-H42 IgM ELISA. 184
- 4.7 Diagnostic and detection sensitivity and specificity in the different groups using the Bhlp29.7 IgM ELISA. 185
- 4.8 Comparison of antibody titres between sera from the negative and positive herds using NAV-H8, NAV-H42 and Bhlp29.7 IgM ELISAs. 186
- 4.9 Antibody profiles in the different groups using the NAV-H8 (A), NAV-H42 (B) and Bhlp29.7 (C) IgM ELISAs. 188
- 4.10 Distribution of ELISA values using NAV-H8 (A), NAV-H42 (B) and Bhlp29.7 (C) IgM ELISAs in the five negative herds. 189
- 4.11 Correlation of reactivity of NAV-H8 and NAV-H42 IgM ELISAs for all sera, and as positive and negative subgroups. 190
- 4.12 Correlation of reactivity of NAV-H8 and Bhlp29.7 IgM ELISAs for all sera, and as positive and negative subgroups. 191
- 4.13 Correlation of reactivity of Bhlp29.7 and NAV-H42 IgM ELISAs for all sera, and as positive and negative subgroups. 192

LIST OF TABLES

1.1	Comparison of conventional and genomic approaches to vaccine development.	10
1.2	Examples of application of reverse vaccinology to bacterial pathogens.	13
1.3	Biochemical reaction scheme for <i>Brachyspira</i> species.	20
2.1	Strains of <i>B. hyodysenteriae</i> used for the genomic study. All originated from pigs.	59
2.2	Primers used for gene distribution studies in <i>B. hyodysenteriae</i> .	62
2.3	Primers and cloning sites used in gene cloning.	66
2.4	Flanking primers used for sequencing in <i>B. hyodysenteriae</i> .	80
2.5	Characterisation of products of selected candidate genes identified by bioinformatics analysis	88
2.6	<i>B. hyodysenteriae</i> gene distribution by PCR.	92
2.7	Molecular weights of native and recombinant proteins and expression level in the pTrcHisA <i>E. coli</i> expression vectors of 14 <i>B. hyodysenteriae</i> genes and gene fragments.	102
2.8	Results of the reactivity of purified candidate antigens with a range of sera from healthy or naturally and experimentally infected pigs.	107
2.9	Selected ORF candidates used in the sequencing study.	110
2.10	The highest dilution of mouse serum raised against each recombinant protein that agglutinated <i>B. hyodysenteriae</i> cells.	111
3.1	Typical analysis of commercial diet.	122
3.2	Means and standard deviation of group IgG titres (OD values) to different ELISA antigens at four sampling times during the experiment.	138

LIST OF TABLES (Continued)

3.3	Means and standard deviation of group IgM titres (OD values) to different ELISA antigens at four sampling times during the experiment.	142
3.4	Pearson correlation between each recombinant antigen and whole-cell ELISA reactivity in control pigs at post-mortem.	143
3.5	Colonisation results for pigs in the three groups. Colonisation was determined by culture of faeces and PCR of the growth on plates.	151
3.6	Clinical signs, post-mortem culture results and colonic lesions in the experimental pigs.	156
4.1	Sources of field serum samples used in this study.	164
4.2	Comparative results for reactivities of positive (B series) and negative test sera to recombinant antigens and whole cells of <i>B. hyodysenteriae</i> in IgG ELISAs.	170
4.3	Comparative results for reactivities of test sera to recombinant antigens and whole cells of <i>B. hyodysenteriae</i> in IgM ELISA.	171
4.4	IgG and IgM ELISA results for the investigated antigens tested alone and in different combinations (IgM) with 120 individual sera.	172
4.5	Comparative results for reactivities of test sera to different combination of recombinant antigens of <i>B. hyodysenteriae</i> in IgM ELISA.	175
4.6	Summarized results for reactivities of 707 sera to recombinant NAV-H8, NAV-H42 and Bhlp29.7 in IgM ELISA using cut-off values of 3SD and 2SD above the mean.	181

ABBREVIATIONS AND SYMBOLS LIST

amino acid	aa
ammonium persulphate	APS
alkaline phosphatase	AP
Australian Genome Research Facility	AGRF
base pair	bp
bovine serum albumin	BSA
Center for Comparative Genomics	CCG
centimeter	cm
comparative genomic hybridization	CGH
concentration unit	CU
confidence intervals	CI
Degree Celsius	°C
deoxyribonucleic acid	DNA
distilled H ₂ O	dH ₂ O
<i>Escherichia coli</i>	<i>E. coli</i>
enzyme-linked immunosorbent assay	ELISA
fluorescence-activated cell sorter	FACS
genetic horizontal transfer	GHT
immobilized-metal affinity chromatography	IMAC
immunoglobulin	Ig
intramuscular	im
leukocyte migration inhibition agarose test	LMAT
interleukin	IL

ABBREVIATIONS AND SYMBOLS LIST (Continued)

kilobase/s	Kb
kilodalton	kDa
kilogram/s	kg
lipopolysaccharide	LPS
Luria Broth	LB
megabases/s	Mb
melting point	T _m
meter/s	m
micro	μ
microgram/s	μg
micrometer/s	μm
micromolar	μM
millilitre/s	ml
millimolar	mM
minute/s	m
NADH oxidase	<i>Nox</i>
nanogram/s	ng
nanometer/s	nm
deoxyribonucleotide triphosphate	dNTP
open reading frame	ORF
optical density	OD
outer membrane proteins	OMP _s
pathogenicity islands	PAIS

ABBREVIATIONS AND SYMBOLS LIST (Continued)

percent	%
percentage guanosine and cytosine	% G+C
periplasmic flagella	FP
phosphate buffered saline	PBS
plaque forming unit	pfu
polymerase chain reaction	PCR
reverse transcription	RT
revolution per minute	rpm
ribonucleic acid	RNA
second/s	s
sodium dodecyl sulphate	SDS
SDS-polyacrylamide gel electrophoresis	SDS-PAGE
swine dysentery	SD
tris-buffered saline	TBS
trypticase soy broth	TSB
tumor necrosis factor	TNF
ultra violet	UV
unit/s	u
volts	v
volume : volume ratio	v/v
weight : volume ratio	w/v