Molecular characterisation of Australian *Coxiella burnetii* isolates

Gemma Anne Vincent

BSc (Hons) Biochemistry

This thesis is presented for the degree of Doctor of Philosophy of

Murdoch University

2013

Declaration

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

GEMMA VINCENT

Abstract

The intracellular bacterium *Coxiella burnetii* is the causative agent of Q fever, a human zoonotic disease with acute and chronic forms, which is most commonly associated with exposure to infected animals such as sheep, goats and cattle. Q fever occurs worldwide and is endemic in Australia with around 300 cases confirmed by laboratory testing each year. Although the first cases of the disease were first recognised in Australia in the 1930s, at the start of this study little was known about the molecular epidemiology of *C. burnetii* in this country. The work presented in this thesis provides the first extensive molecular analysis of the strains of the bacterium causing Q fever in Australia.

The small, pre-existing collection of seven Australian *C. burnetii* isolates was expanded to a larger collection comprising 43 isolates. The majority of the new isolates were obtained from serum samples taken from acute Q fever patients during the early stage of their disease in a two year period from 2010-2012. This demonstrated that the organisms remained viable in these specimens despite the absence of host cells, thus acute serum is a valuable source of *C. burnetii.* Attempted isolations of *C. burnetii* from kangaroo faeces and an Australian wombat tick were unsuccessful but bacterial DNA was obtained from these samples for further characterisation.

Several genotyping methods were used to characterise the Australian *C. burnetii* isolate collection at the molecular level. All the human isolates were found to contain the plasmid QpRS and were negative for the acute disease antigen A gene. Single nucleotide polymorphism typing also failed to discriminate between the human isolates but demonstrated that *C. burnetii* representing three different genotypes was present in the kangaroo faecal samples. Discrimination between the human isolates was only achieved using an extended panel of PCRs targeting the repetitive insertion sequence element

IS*1111* and multi-locus VNTR analysis. Both methods identified 14 genotypes, most of which were novel compared to the genotypes identified in characterised strains of *C. burnetii* from other countries and in combination the two methods determined 24 genotypes, providing an even greater discriminatory power. Many of the genotyping targets were not amplified from the bacterial DNA in the wombat tick leading to the conclusion that the organism present was a *Coxiella* species other than *C. burnetii.*

Overall, results showed that the Australian *C. burnetii* isolates are genetically closely related and unique to this country. The evaluation of different genotyping methods enabled the development of a set of guidelines that will reduce the cost and workload required to characterise new Australian isolates of this important pathogen.

Table of contents

Declaration i

Abstract ii

Table of contents iv

List of figures x

List of tables xiv

Acknowledgements xviii

Abbreviations xx

Dedication xxiii

1 Literature Review 1

1.1 Discovery of the causative agent of Q fever 1

1.2 Bacteriology of *Coxiella burnetii* 2

1.2.1 Classification 2

1.2.2 Phase variation 4

1.2.3 Intracellular survival and growth 5

1.2.3.1 Uptake by the host cell 5

1.2.3.2 Formation of the parasitophorous vacuole 6

1.2.3.3 Strategies for intracellular survival 9

1.2.3.4 *In vitro* growth 11

1.2.4 Developmental biology 13

1.2.4.1 Morphological variants 13

1.2.4.2 Developmental cycle 15

1.3 Human infection and disease 16

1.3.1 Routes of infection and persons at risk 16
1.3.2 Clinical manifestations 18
1.3.2.1 Acute Q fever 18
1.3.2.2 Chronic Q fever 19
1.3.2.3 Post Q fever fatigue syndrome (QFS) 20
1.3.3 Host immune response 20
1.3.4 Diagnosis 22
1.3.4.1 Serological methods 22
1.3.4.2 Polymerase chain reaction (PCR) 25
1.3.4.3 Isolation 26
1.3.5 Treatment of Q fever 27
1.3.6 Q fever vaccination 28
1.3.7 Epidemiology 30
1.3.8 Q fever outbreaks 32
1.4 *C. burnetii* in animals 34
1.4.1 Range of host species 34
1.4.2 Animal models for human disease 36
1.5 Genomics of *C. burnetii* 37
1.5.1 Whole genome sequencing 37
1.5.2 Plasmids 38
1.5.3 Genotyping 39
1.6 Aims of the current study 43
2 Materials and Methods 45
2.1 Bacterial strains and sequences 45
2.2 General molecular methods 46
2.2.1 DNA extraction 46
2.2.1.1 From clinical and tissue culture samples 46

2.2.1.2 From kangaroo faeces 47

2.2.1.3 From ticks and their eggs 49

2.2.2 Real-time PCR detection and quantitation of *C. burnetii* DNA 49

2.2.2.1 Preparation of a standard curve for quantitation 52

2.2.3 Whole genome amplification of extracted DNA 53

2.2.4 Agarose gel electrophoresis 53

2.2.5 DNA sequencing 54

2.3 Isolation of *C. burnetii* in Vero cells 56

2.3.1 Maintenance of the Vero cell line 56

2.3.2 Isolation of *C. burnetii* 57

2.3.3 Harvest of isolates 58

2.4 Isolation of *C. burnetii* in mice 58

2.5 Axenic culture of *C. burnetii* 59

2.5.1 Preparation of ACCM-2 59

2.5.2 Growth of *C. burnetii* in ACCM-2 59

2.6 Genotyping methods 61

2.6.1 Determination of plasmid type 61

2.6.2 Characterisation of the acute disease antigen A gene region 62

2.6.3 Dual probe single nucleotide polymorphism (SNP) analysis 65

2.6.4 SNP analysis using melt-mismatch amplification mutation assays (MAMAs) . 67

2.6.4.1 Development of additional melt-MAMA assays for genomic group IV 70

2.6.4.2 Amplification and sequencing of MST spacers 75

2.6.5 Repetitive element PCRs 76

2.6.6 Extended repetitive element PCR panels 78

2.6.6.1 IS*1111*-10 78

2.6.6.2 IS*1111*-32 79

2.6.7 Multi-locus VNTR analysis (MLVA) 81

2.6.8 Calculation of discrimination index 84

3 Creation of an Australian collection of *C. burnetii* isolates ... 86

3.1 Introduction ... 86

3.2 Isolation from acute Q fever patient serum ... 88

3.2.1 Sample selection ... 88

3.2.2 Results of attempted isolations ... 89

3.2.3 Stability of viable organisms in serum ... 92

3.3 Isolation from other clinical specimens ... 94

3.4 Isolation from environmental samples ... 95

3.4.1 Kangaroo faeces ... 95

3.4.2 Tick specimens ... 97

3.5 Evaluation of axenic culture for the isolation of *C. burnetii* ... 100

3.5.1 Axenic culture of *C. burnetii* Nine Mile Clone 4 ... 100

3.5.2 Attempted isolation from clinical samples in ACCM-2 ... 101

3.5.3 Growth of selected Australian isolates in ACCM-2 ... 102

3.6 Characteristics of the isolate collection ... 105

3.6.1 Patient demographics and clinical manifestations ... 106

3.6.2 Geographical location ... 108

3.7 Discussion ... 110

3.7.1 Acute serum is a valuable source of viable *C. burnetii* ... 110

3.7.2 Isolation from other sources ... 116

3.7.3 ACCM-2 may not be suitable for isolation of *C. burnetii* from Australian clinical samples ... 122

3.7.4 The Australian *C. burnetii* isolate collection ... 127

3.7.5 Conclusions ... 130

4 Determination of plasmid type and characterisation of the *adaA* gene region ... 131

4.1 Introduction ... 131

4.2 Determination of plasmid type **134**

4.3 Characterisation of the *adaA* gene region **135**

4.4 Discussion **143**

4.4.1 The plasmid QpRS is present in all the Australian isolates 143

4.4.2 A novel Q154 *adaA* gene deletion type is present in the Australian isolates 144

4.4.3 Conclusions 149

5 Genotyping based on single nucleotide polymorphisms (SNPs) 150

5.1 Introduction **150**

5.2 Dual probe SNP typing **155**

5.2.1 Optimisation of probe concentrations 156

5.2.2 Results of the dual probe SNP assays performed using the optimised probe concentrations 158

5.3 Melt-MAMA SNP typing **164**

5.3.1 Results from the published assays 164

5.3.2 Development of new melt-MAMAs for genomic group IV 169

5.3.3 Results of genomic group IV melt-MAMAs 176

5.4 Discussion **181**

5.4.1 SNP genotyping by dual probe SNP analysis 181

5.4.2 SNP genotyping by melt-MAMA SNP analysis 188

5.4.3 Conclusions 193

***6* Genotyping based on the insertion sequence element IS*1111* 195**

6.1 Introduction **195**

6.2 IS*1111*-4 panel of repetitive element PCRs **198**

6.2.1 Determination of the limit of detection 198

6.2.2 Design of nested primers 200

6.2.3 Results of IS*1111*-4 repetitive element PCRs performed on the Australian *C. burnetii* isolates ... 204

6.3 IS*1111*-10 panel of repetitive element PCRs ... 207

6.4 IS*1111*-32 panel of repetitive element PCRs ... 209

6.5 Discussion ... 222

6.5.1 Genomic group assignment by the IS*1111*-4 repetitive element PCRs and associated algorithm ... 222

6.5.2 The IS*1111*-10 panel does not provide further discrimination between the Australian *C. burnetii* isolates ... 227

6.5.3 Improved discrimination between the Australian isolates by an extended panel of repetitive element PCRs ... 229

6.5.4 Conclusions ... 234

7 Genotyping based on variable number of tandem repeat (VNTR) loci .. 236

7.1 Introduction ... 236

7.2 Determination of MLVA amplicon size ... 238

7.2.1 Agarose gel electrophoresis of Panel 1 amplicons ... 238

7.2.2 Capillary electrophoresis of Panel 1 and 2 amplicons ... 239

7.3 Analysis of loci refractory to amplification ... 242

7.3.1 ms23 ... 242

7.3.2 ms24 ... 245

7.4 Results from MLVA of the Australian *C. burnetii* isolates ... 245

7.5 Concordance of MLVA and IS*1111*-31 genotyping ... 257

7.6 Discussion ... 260

7.6.1 Validation and appropriate controls are required for accurate MLVA results ... 260

7.6.2 MLVA provides good discrimination between the Australian *C. burnetii* isolates ... 265

7.6.3 Conclusions 270

8 Final discussion 271

8.1 Main outcomes 271

8.2 Guidelines for genotyping new Australian isolates 277

8.3 Future work 281

8.4 Concluding remarks 282

Appendices 283

A Details of the Australian *C. burnetii* isolates 283

B *adaA* gene deletion region sequences 285

C MST genotype profiles 286

D MLVA amplicon sizes 287

Bibliography 291

List of figures

Figure 1.1 Development of the parasitophorous vacuole (PV) *7*

Figure 1.2 L929 mouse fibroblast cell infected with C. burnetii *9*

Figure 1.3 Electron micrograph of C. burnetii *small and large cell variants* *14*

Figure 1.4 Kinetics of the serological response and bacteraemia during acute Q fever infection *23*

Figure 1.5 Number of Q fever notifications in Australia, 1991-2012 ..... *31*

Figure 2.1 Principle of creating an alternate allele-specific positive control from C. burnetii *Nine Mile DNA* *73*

Figure 2.2 Algorithm for determination of C. burnetii *genomic group from the results of the IS*1111-*4 panel of repetitive element PCRs* *78*

Figure 3.1 Graph to show the time at which C. burnetii *DNA was first detected in the positive isolation flasks* 91
Figure 3.2 Graph to show the distribution of sample storage time and isolation outcome 92
Figure 3.3 Growth of the C. burnetii *Nine Mile Clone 4 strain in axenic culture* 101
Figure 3.4 Map to show the geographical location of the Australian C. burnetii *isolates* 108
Figure 3.5 Close up map of New South Wales showing the distribution of the Australian C. burnetii *isolates in this region* 109
Figure 3.6 Total number of Q fever notifications by state, 2008-2012 130
Figure 4.1 Phylogenetic relationship of C. burnetii *isolates determined by analysis of the* adaA *gene region* 133
Figure 4.2 Example of plasmid QpRS PCR results 134
Figure 4.3 Example of melt curves from the adaA *PCRs* 136
Figure 4.4 Diagram of the Q154 adaA *gene deletion region* 138
Figure 4.5 Example of results from adaA *gene deletion region PCRs* 138
Figure 4.6 Phylogenetic relationship of the Australian C. burnetii *isolates to other* C. burnetii *isolates based on the* adaA *gene deletion region* 142
Figure 5.1 The principle of dual probe SNP analysis 152
Figure 5.2 Phylogenetic tree of MST genotypes with branches defined by melt-MAMAs 154
Figure 5.3 Allelic discrimination plot showing the results of the probe optimisation reactions for SNP assay 2287 157
Figure 5.4 Example allelic discrimination plot for SNP assay 7726 158
Figure 5.5 Example amplification plot for SNP assay 769 159
Figure 5.6 Agarose gel electrophoresis of SNP assay 769 reactions 160
Figure 5.7 Example sequence of SNP assay 769 amplicon 161
Figure 5.8 Difference matrix of SNP genotypes and inferred dendrogram 163
Figure 5.9 Examples of melt curves from melt-MAMAs targeting branches Br.IV.011 and Br.IV/VI 165

Figure 5.10 Example of melt curves from the melt-MAMA targeting branch Br.I.001 .... *166*

Figure 5.11 Example of amplification plots from the Br.I.00I melt-MAMA performed as non-competitive reactions *167*

Figure 5.12 Expanded view of genomic group IV to show the branches targeted by the novel melt-MAMAs *170*

Figure 5.13 Predicted melt curves from in silico *melting of amplicons from the novel genomic group IV melt-MAMAs* *171*

Figure 5.14 Alignment of the Br.IV.005 synthetic control and Nine Mile Clone 4 sequences 172

Figure 5.15 Examples of melt curves from primer concentration optimisation reactions *174*

Figure 5.16 Example of melt curves from amplification cycle optimisation reactions............ *175*

Figure 5.17 Results from the assay targeting Br.IV.007 performed using DNA from the B. auruginans *tick homogenate* *177*

Figure 5.18 Sequence alignment of the MST alleles for the Cox18 locus *179*

Figure 5.19 Phylogenetic tree of MST genotypes showing the likely position of the novel genotype identified in the Australian C. burnetii *isolates* *180*

Figure 5.20 Multiple alignment of the whole genome sequences of five strains of C. burnetii *using Mauve* *184*

Figure 5.21 Definition of homoplasy *190*

Figure 6.1 Agarose gel electrophoresis of IS1111-4 repetitive element PCRs *198*

Figure 6.2 Probit regression analysis of overall IS1111-4 repetitive *element PCR results*... *200*

*Figure 6.3 Agarose gel electrophoresis of the second round IS*1111*-4 repetitive element PCRs* *201*

*Figure 6.4 Probit analysis of overall results for the IS*1111*-4 repetitive element PCRs in which a second round of amplification was performed using the nested primers* *203*

Figure 6.5 Agarose gel electrophoresis of repetitive element PCRs IS8, IS27, IS30, IS32, IS36 and IS37 .... *208*

Figure 6.6 Results of repetitive element Multiplex assays 1-4 performed on five dilutions of Henzerling and AuQ19 DNA *210*

Figure 6.7 Agarose gel electrophoresis of individual IS25 reactions *211*

Figure 6.8 Examples of results from repetitive element Multiplex assays 1-4 performed on the Australian C. burnetii *isolates* *213*

Figure 6.9 Results of the repetitive element Multiplex assays 1-4 performed on DNA from the B. auruginans *tick and five representative Australian* C. burnetii *isolates* *217*

*Figure 6.10 Dendrogram of IS*1111-*31 genotypes amongst the Australian* C. burnetii *isolates and whole genome sequenced strains* *219*

*Figure 6.11 Map of Eastern Australia showing the geographical distribution of the IS*1111-*31 genotypes* *220*

*Figure 6.12 Close up map of north-east New South Wales showing the distribution of the IS*1111-*31 genotypes in this region* *221*

Figure 6.13 Region of the C. burnetii *Nine Mile genome containing the IS14 insertion sequence element* *224*

Figure 6.14 Genomic group IV clade of the phylogenetic tree determined by the melt-MAMA SNP analysis *226*

Figure 7.1 Agarose gel electrophoresis of VNTR locus ms20 amplicons *239*

Figure 7.2 Sequences of the ms23 locus region in C. burnetii *strains Nine Mile and CbuK* ... *243*

Figure 7.3 Agarose gel electrophoresis of reactions in which the ms23 locus was amplified with the new primer set *244*

Figure 7.4 Multiple alignment of VNTR locus ms21 sequences from C. burnetii *Nine Mile and three Australian isolates* *250*

Figure 7.5 Multiple alignment of sequences of the VNTR locus ms36 from four Australian C. burnetii *isolates* *251*

Figure 7.6 Minimum spanning tree of MLVA genotypes of the Australian C. burnetii *isolates and seven whole genome sequenced strains* *253*

Figure 7.7 Map of Eastern Australia showing the distribution of the MLVA genotypes *255*

Figure 7.8 Close up map of north-east New South Wales showing the distribution of MLVA genotypes in this region *256*

Figure 7.9 Comparison of the Australian C. burnetii *isolate clustering by the MLVA and IS*1111-*31 genotyping methods* *259*

Figure 8.1 Relative power of published genotyping methods to discriminate between the Australian C. burnetii *isolates* *272*

List of tables

Table 1.1 Range of host species of C. burnetii *and examples of seroprevalence* *34*

Table 1.2 Published methods used for genotyping strains of C. burnetii......... *40*

Table 1.3 Hunter-Gaston discrimination indices of selected C. burnetii *genotyping schemes* *42*

Table 2.1 C. burnetii *strains and whole genome sequences used in this study* *45*

Table 2.2 Real-time PCR assays for the detection of C. burnetii *DNA* *51*

Table 2.3 Recommended template DNA quantities for sequencing reactions *55*

Table 2.4 Formulation of the axenic medium ACCM-2 .... *60*

Table 2.5 Sequences of the primers used for determination of plasmid type......... *62*

Table 2.6 Sequences of primers used for amplification and sequencing of the adaA *gene region* *63*

Table 2.7 Sequences of the oligonucleotides used for dual probe SNP analysis......... *66*

Table 2.8 Sequences of primers and assay information for melt-MAMA SNP genotyping *68*

Table 2.9 Sequences of primers for novel genomic group IV melt-MAMA assays *71*

Table 2.10 Sequences of primers used to create alternate allele-specific synthetic controls for the novel genomic group IV melt-MAMA assays *74*

Table 2.11 Sequences of the primers used to amplify and sequence the relevant MST spacers *75*

*Table 2.12 Sequences of the primers used for the IS*1111-*4 panel of repetitive element PCRs* *77*

*Table 2.13 Sequences of primers used for the IS*1111-*10 panel of repetitive element PCRs*.... *79*

*Table 2.14 Sequences of primers used for the IS*1111-*32 panel of repetitive element PCRs*.... *80*

Table 2.15 Sequences of the primers used for MLVA *82*

Table 3.1 Results of attempted isolation of C. burnetii *from acute Q fever patient sera*........ *89*

Table 3.2 Differences in the proportions of positive isolations between different groups of sera........ *90*

Table 3.3 Estimated coefficients of a Poisson regression to assess the effect of storage time on the rate of positivity *93*

Table 3.4 Results of attempted isolation of C. burnetii from a range of clinical specimens *94*

Table 3.5 Results of PCRs performed on DNA extracted from kangaroo faecal samples........ *96*

Table 3.6 Results of attempted isolation of C. burnetii *from kangaroo faeces* *97*

Table 3.7 Results of attempted isolation of C. burnetii *from* B. auruginans *ticks and tick eggs* *99*

Table 3.8 Summary of attempted isolation of C. burnetii *from clinical samples using axenic culture in ACCM-2*........ *102*

Table 3.9 Attempted axenic culture of the Australian C. burnetii *isolate AuQ16 from frozen stock*........ *103*

Table 3.10 Attempted axenic growth of six Australian C. burnetii *isolates* *104*

Table 3.11 Attempted axenic growth of six Australian C. burnetii *isolates in pre-conditioned ACCM-2*........ *105*

Table 3.12 Demographics of the patients from whom isolates were obtained *106*

Table 3.13 Clinical manifestations of acute Q fever patients from whom isolates were obtained........ *107*

Table 4.1 Determination of plasmid type in the Australian C. burnetii *isolates and PCR positive DNA extracts* *135*

Table 4.2 Determination of adaA *gene presence in the Australian* C. burnetii *isolates and Com1 PCR positive DNA extracts* *137*

Table 4.3 Results of the adaA *gene deletion region PCRs performed on the Australian* C. burnetii *isolates and Com1 PCR positive DNA from a* B. auruginans *tick* *139*

Table 4.4 Differential bases of the Q154 adaA *gene deletion type in the subgroups A, B_1 and B_2, and nine Australian* C. burnetii *isolates* *140*

Table 5.1 Matrix to show the combinations tested to determine the optimal probe concentrations *156*

Table 5.2 Optimised probe concentrations for dual probe SNP assays *157*

Table 5.3 Results of dual probe SNP typing for the Australian C. burnetii *isolates and PCR positive DNA extracts* *161*

Table 5.4 Results of the melt-MAMA SNP assays performed on the Australian C. burnetii *isolates and DNA from the* B. auruginans *tick* *168*

Table 5.5 Ratios of primer concentrations tested during validation of novel genomic group IV melt-MAMAs *173*

Table 5.6 Optimised reaction conditions for the novel genomic group IV melt-MAMAs *175*

Table 5.7 Results of the genomic group IV melt-MAMAs for the Australian C. burnetii *isolates and DNA from the* B. auruginans *tick* *176*

Table 5.8 Assignment of MST alleles from the sequences of three MST spacers *179*

*Table 6.1 Results of IS*1111-*4 repetitive element PCRs performed on eight dilutions of* C. burnetii *Nine Mile Clone 4 DNA* *199*

*Table 6.2 Results of IS*1111-*4 repetitive elements PCRs in which a second round of amplification was performed with the nested primers* *202*

*Table 6.3 Results of the IS*1111-*4 repetitive element PCRs performed on DNA from the Australian* C. burnetii *isolates* *205*

*Table 6.4 IS*1111-*4 repetitive element PCRs performed* in silico *on* C. burnetii *whole genome sequences*........ 207

Table 6.5 Results of the repetitive element PCRs IS8, IS27, IS30, IS32, IS36 and IS37 performed on the Australian C. burnetii *isolates and* B. auruginans *tick DNA extract*........ 208

Table 6.6 Approximate limit of detection for Multiplex assays 1-4........ 210

*Table 6.7 Genotypes determined by the IS*1111-*31 panel of repetitive element PCRs*........ 214

Table 6.8 Assignment of the Australian C. burnetii *isolates to an IS*1111-*31 genotype based on results of the repetitive element PCRs*........ 215

Table 6.9 Hunter-Gaston discrimination indices (HGDI) of the repetitive element PCR panels........ 216

Table 7.1 Comparison of the published and experimentally determined sizes of the VNTR loci from C. burnetii *Nine Mile*........ 241

Table 7.2 MLVA genotypes identified from the C. burnetii *whole genome sequences and the Australian isolates of* C. burnetii........ 247

Table 7.3 Assignment of the Australian C. burnetii *isolates to a MLVA genotype*........ 248

Table 7.4 Hunter-Gaston discrimination indices (HGDIs) for each MLVA locus and the overall MLVA typing method........ 249

Table 7.5 Number of repeat units in the VNTR loci amplified from the B. auruginans *tick homogenate DNA extract determined by amplicon sizing and sequencing*........ 252

Table 7.6 Comparison of genotypes assigned to the Australian C. burnetii *isolates by the MLVA and IS*1111-*31 typing methods*........ 257

Table 8.1 Recommendations for genotyping new Australian C. burnetii *isolates*........ 278

Acknowledgements

The work presented in this thesis was carried out in full at the Australian Rickettsial Reference Laboratory (ARRL). I am very grateful to my supervisors John Stenos and Stephen Graves from the laboratory for agreeing to financially support an expensive international student, valuable scientific input throughout the course of the project and their amazingly quick turnaround of drafts of my thesis. Thanks are also due to my university supervisor Stan Fenwick for helping to organise a Murdoch University Partnership Scholarship, without which this PhD would not have been possible and for signing off all the necessary paperwork over the last three years.

Other members of the ARRL made important contributions to the project. Particular thanks are owed to Aminul Islam who performed all the animal work and to Chelsea Nguyen and Hazizul Hussain-Yusef who performed the diagnostic serology and PCR testing on the clinical and tick samples used for the attempted isolations.

Several other people are deserving of individual acknowledgment for their assistance and input into my research. Thanks go to Jenny Robson from Sullivan Nicolaides Pathology in Brisbane for providing several of the clinical samples that yielded *C. burnetii* isolates, to Ian Beveridge from the University of Melbourne Vet School for helping me with tick speciation and to Steve Lane from Barwon Health for his assistance with the statistics in Chapters 3 and 6. The genotyping work was greatly enhanced by the extended panel of repetitive element PCR assays used in Chapter 6, which was kindly shared ahead of its publication by Dimitrios Frangoulidis from the Bundeswehr Institute of Microbiology in Munich. The amplicon sizing for the MLVA genotyping in Chapter 7 was carried out at two other laboratories. Thanks to Richard Vipond for allowing me to return to the Porton

Down laboratories of Microbiological Services, Public Health England and to Jennie Latham for helping me with the laboratory work and also for performing SNP analysis on a selection of my isolates using MGB labelled probes. Andrew Szentirmay from Gene Target Solutions was very generous in allowing me to use his Fragment Analyzer CE System in Sydney and I am grateful to him for running my samples through the machine and sharing the software with me so I could perform my own data analysis back in Geelong.

On a more personal level I would like to say a huge thank you to my family, especially Mum, Dad and Sarah. I know it was hard for you all when I decided to move to Australia but you have always been incredibly supportive of my decision and I know how proud you all are of what I have achieved out here. Thanks to my friends in the UK for encouragement over Skype and to my new friends in Australia for all the extra-curricular activities that have helped me to keep my sanity.

Finally, the biggest thanks of all are due to Andy Hill; for being the reason for the Australian adventure, for providing stress relief on the days where it all seemed impossible, for making sure I ventured out of the thesis cocoon occasionally and for being there for me every step of the way. I cannot thank you enough for everything you have done for me; we can have our lives back now.

Abbreviations

°C	degrees celcius
<	less than
>	more than
μ (prefix)	micro (10^{-6})
ACCM	acidified citrate cysteine medium
ACT	Australian Capital Territory
adaA	acute disease antigen A
ARRL	Australian Rickettsial Reference Laboratory
AT	adenine-thymine
AUD	Australian dollar
BHQ	black hole quencher
BLAST	basic local alignment search tool
bp	base pair
CFS	chronic fatigue syndrome
com1	*Coxiella* outer membrane protein 1
CI	confidence interval
cm	centimetre
C_T	threshold cycle
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
dUTP	deoxyuridine triphosphate
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FAM	6-carboxyfluorescein
g	gram
g	gravitational acceleration
GC	guanine-cytosine
GE	genome equivalent
HEPA	high efficiency particulate air
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesufonic acid
HGDI	Hunter-Gaston discrimination index
IFA	immunofluorescence assay
IFN-γ	interferon-γ

IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IL	interleukin
IPS	integrated plasmid homologous sequence
iPCR	immuno-polymerase chain reaction
IS	insertion sequence
km	kilometre
l	litre
LCV	large cell variant
LOD	limit of detection
LPS	lipopolysaccharide
m	metre
M	molar
m (prefix)	milli (10^{-3})
MAMA	mismatch amplification mutation assay
MGB	minor groove binder
MLVA	multi-locus VNTR analysis
MOI	multiplicity of infection
mol	moles
MST	multi-spacer sequencing typing
MST	minimum spanning tree
n (prefix)	nano (10^{-9})
n/a	not applicable
NFQ	non-fluorescent quencher
NSW	New South Wales
NT	Northern Territory
NTC	no template control
ORF	open reading frame
p (prefix)	pico (10^{-12})
p.i.	post-inoculation
PBS	phosphate buffered saline
PC3	physical containment level 3
PCR	polymerase chain reaction
PV	parasitophorous vacuole
QFS	post Q fever fatigue syndrome

QLD	Queensland
qPCR	quantitative real-time PCR
RFLP	restriction fragment length polymorphism
ROS	reactive oxygen species
ROX	6-carboxy-X-rhodamine
rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute
rRNA	ribosomal ribonucleic acid
s	seconds
SA	South Australia
SCID	severe combined immunodeficient
SCV	small cell variant
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SNP	single nucleotide polymorphism
T4SS	type IV secretion system
TAE	tris-acetic EDTA
TAS	Tasmania
T_m	melting temperature
Tris	trisaminomethane
UDG	uracil DNA glycosylase
UK	United Kingdom
UPGMA	unweighted pair group method with arithmetic mean
USA	United States of America
v/v	volume per volume
VIC	Victoria
VNTR	variable number of tandem repeats
w/v	weight per volume
WA	Western Australia

Dedication

In loving memory of my granny, who sadly did not live to see me finish but she would have been very proud of her Dr Gem.

Joyce Kallmeier

1923-2012