

JCU ePrints

This file is part of the following reference:

Boon, Virginia (2007) *Factors responsible for the high rate of kidney disease in Indigenous Australians: a multifaceted approach focusing on streptococcal disease.* PhD thesis, James Cook University.

Access to this file is available from:

<http://eprints.jcu.edu.au/5463>



**Factors responsible for the high rate of kidney disease in
Indigenous Australians: a multifaceted approach focusing on
streptococcal disease**

Thesis submitted by

Virginia Anna Fay BOON

B. Pharm (School of Pharmacy, University of Queensland, 2001)

**B.Pharm (Hons) (School of Pharmacy and Molecular Sciences, James Cook University,
2002)**

October 2007

**for the degree of Doctor of Philosophy in the
School of Public Health, Tropical Medicine and Rehabilitation Sciences at James Cook
University**

STATEMENT OF ACCESS

I, the undersigned, the author of this work, understand that James Cook University will make it available for use within the University Library and, via the Australian Digital Thesis network, for use elsewhere.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and I do not wish to place any further restriction on access to this work.

Virginia Boon

STATEMENT OF SOURCES

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Virginia Boon

DECLARATION ON ETHICS

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the National Statement on Ethics Conduct in Research Involving Humans (1999), the Joint NHMRC/AVCC Statement and Guidelines on Research Practice (1997), the James Cook University Policy on Experimentation Ethics; Standard Practices and Guidelines (2001), and the James Cook University Statement and Guidelines on Research Practice (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval numbers A1037, A1017, A847-03, A1038, A1035, H2394) and The Townsville Hospital District Ethics Committee (03/04).

Virginia Boon 30/09/2007

STATEMENT ON CONTRIBUTION OF OTHERS

I would like to acknowledge the financial support of Kidney Health Australia for providing a Biomedical Scholarship to support the research. I would also like to acknowledge the financial support of the Queensland Government with the funding of a Smart State program grant.

Additionally, I would like to acknowledge the contribution of Dr Ted Donelan for collecting the canine samples in the two Indigenous communities and Dr George Kan and Loretta Woodford for collecting the blood for Chapter 8.

Virginia Boon 30/09/2007

ACKNOWLEDGMENTS

Firstly, I would like to thank my supervisors Rick Speare and Beverley Glass. Together they allowed me to explore my own research ideas and yet gave me the support to continue and complete this research. I would also like to thank George Kan for his support regarding the clinical research and Ted Donelan for facilitating the collection of animal swabs. I am also very grateful for the microbiological expertise provided by Annette Thomas and Judy Faulkner and the friendly staff at the Oonoonba Veterinary laboratories.

I am also very grateful to Jim Burnell and everyone in the annex for providing me with research space, laboratory advice and friendship. Thanks to everyone for putting up with those red plates and teaching me biochemistry! I would also like to thank Alan Baxter and the Baxter lab for helping me to conduct my animal experiments and particularly to Roby for always bleeding my mice for me.

I couldn't have finished this thesis if it wasn't for the constant support and motivation from Jim. This is his third PhD (his own, the house and now mine!) and I think he can't wait to be rid of it!

I would also like to thank the Boon clan, Mum, Dad, Syvi and John for supporting me through this PhD and even from the small beginnings of 'free choice'....

ABSTRACT

Indigenous communities in rural Australia have high rates of streptococcal disease including post-streptococcal glomerulonephritis (PSGN). The epidemiology of PSGN and streptococcal infection and the pathogenesis of streptococcal disease in these lower socio-economic communities is complex and not well understood. To gain an understanding of some aspects of this problem, this study tested several hypotheses. The major hypothesis underlying this project was that streptococci isolated from these communities will produce particular virulence factors (proteins) that will cause PSGN and that this can be demonstrated both in an animal model and in individuals with end-stage renal failure (ESRF). A second hypothesis was that dogs in these communities will play a role in the epidemiology of human streptococcal disease. These hypotheses gave rise to three objectives. Firstly; to determine if dogs residing in Indigenous communities carry streptococci that may be implicated in the high rate of streptococcal disease in the human residents of these communities. Secondly; to develop a murine model of PSGN to investigate the role of streptococcal inhibitor of complement mediated cell lysis (SIC) and the genetics of host in the pathogenesis of PSGN. Thirdly; to determine the association between elevated streptococcal antibody levels in patients to several streptococcal virulence factors and the presence of ESRF.

The first objective was achieved by sampling dogs residing in two geographically distinct Australian Indigenous rural communities to reduce the possibility of location bias. Both pharyngeal and skin swabs were obtained from the study animals. Utilising routine microbiological tests and culturing methods, presumptive streptococcal species were isolated. Following this, the isolate was identified to the species level with biochemical tests and Lancefield serological grouping was also performed. The study isolated streptococcal species of Lancefield's group A, B, C, D, G and L from the dogs. The specific species isolated were *Streptococcus orisratti* (Lancefield group A), *Streptococcus dysgalactiae* subsp. *equisimilis* (Lancefield's group A, C, G or L), *Streptococcus agalactiae* (Lancefield's group B), *Streptococcus dysgalactiae* subsp. *dysgalactiae* (Lancefield's group C), *Streptococcus equi* subsp. *equi* (Lancefield's group C), *Streptococcus canis* (Lancefield's group G), *Streptococcus bovis I* (Lancefield's group D), *Streptococcus minor* (no Lancefield's group identified) and *Streptococcus suis sp.* (no Lancefield's group identified). In this study the fact that the two species that carried the Lancefield's group A antigen were not biochemically identified as *Streptococcus pyogenes* highlights the need to assign species based on biochemical tests and not rely solely on serological grouping systems. The overall prevalence of pharyngeal and skin carriage (including the following genera's: *Streptococcus*, *Enterococcus*, *Lactococcus* and *Aerococcus*) was 47 and 10.8%, respectively.

Furthermore, in the current study, ten strains of large-colony forming beta-haemolytic *S. dysgalactiae* subsp. *equisimilis* were identified from the pharynx of dogs which were taxonomically identical to 'human strains'. This species of Lancefield group C and G are important human pathogens that share virulence factors with *S. pyogenes* and pharyngeal carriage of this bacterium in Australian Indigenous communities is high. Therefore, molecular studies were undertaken on the dog isolates to determine if they share virulence factors with the 'human' strains. Specifically, bacterial isolates of Lancefield's group A, B, C, G or L were included in the molecular study and an isolate of *Streptococcus minor* which is a newly recognised species. To determine if these bacteria possessed virulence factors which have been identified in *S. pyogenes* of pathogenic origin, the isolates were screened by polymerase chain reaction using oligonucleotides that were specific for *S. pyogenes* virulence genes. The gene for the M or M-like protein was found in isolates of *S. canis*, *S. dysgalactiae* subsp. *equisimilis* and *S. equi* subsp. *equi* at a prevalence of 23.1, 90 and 100%, respectively. Tested isolates of *S. agalactiae*, *S. canis*, *S. dysgalactiae* subsp. *equisimilis* and *S. orisratti* harboured the streptokinase (ska) gene at prevalence rates of 62-100%. None of the dog isolates tested harboured a gene encoding the streptococcal C5a peptidase protein. The gene encoding streptococcal pyrogenic exotoxin type G was found in 80% of *S. dysgalactiae* subsp. *equisimilis* strains and 100% of *S. minor* strains. Ten percent of *S. dysgalactiae* subsp. *equisimilis* strains carried a gene encoding the fibronectin binding protein.

This aspect of the study found that genes for various virulence factors considered significant to human health were present in the dog isolates at varying prevalence rates. This study showed that dogs residing in Australian Indigenous communities, where socioeconomic factors such as overcrowded housing conditions and poor water quality exist, carry streptococci which may mediate human disease. Furthermore, these findings suggest that in these populations where humans and animals live in close proximity, gene-transfers have occurred between traditional human and animal streptococcal populations.

The findings of this study are highly significant and support the hypothesis that streptococci carried by dogs are important in the epidemiology of streptococcal disease in Indigenous residents of rural communities. This project has provided the justification for integrated epidemiological studies of humans and dogs in these communities to further test the hypothesis.

To investigate the pathogenesis of PSGN a murine model for PSGN was developed using the congenic variant (NOD.C-*Hc*) of the non-obese diabetic (NOD) mouse and the streptococcal virulence protein SIC. We found that the production of complement component 5 was a genetic factor that was involved in the development of PSGN. Furthermore, using this murine model for PSGN, we showed that the production of SIC protein by nephritic strains of *S. pyogenes* is related to the capacity of the strain to induce nephritis.

Finally, to investigate whether streptococci played a role in ESRF, ELISA tests were developed to detect antibodies against virulence factors in human serum and used to screen patients on haemodialysis. A significantly higher proportion of patients compared to controls were seropositive to SIC ($P=0.018$), CRS ($P=0.0098$) and DRS ($P=0.0003$). Regression analysis showed seroreactivity to DRS ($R^2=0.85$, $P=0.001$) predicted the development of ESRF. These results suggest that these ESRF patients were exposed to strains of *S. pyogenes* that secrete SIC, CRS and DRS and may have a pathological significance. No significant difference was observed between Indigenous patients and the non-Indigenous patients.

The studies described in this thesis have resulted in significant advances in our understanding of streptococcal disease, particularly as it relates to renal disease in Indigenous communities in rural Australia. The project has contributed significantly to knowledge in the three study topics. Firstly, carriage of streptococci by dogs has been demonstrated. Furthermore, the horizontal transfer of genes for virulence factors present in human streptococcal strains to canine streptococci is a major advance. It highlights the necessity to consider dogs in the epidemiology of streptococcal disease in Indigenous communities. Secondly, the rodent model for PSGN appears to be arguably the best developed so far and will allow testing of more virulence factors. Finally, the demonstration of an association between ESRF and antibody levels to particular virulence factors supports the hypothesis that chronic streptococcal infection plays a role in this disease and justifies further studies to test the hypothesis, even antibiotic prophylaxis to prevent progression of PSGN.

STATEMENT OF ACCESS	i
DECLARATION	i
DECLARATION ON ETHICS	ii
STATEMENT ON CONTRIBUTION OF OTHERS	ii
ACKNOWLEDGMENTS	iii
ABSTRACT	iv
LIST OF FIGURES	xvii
LIST OF TABLES	xxii
CHAPTER 1	1
General Introduction	1
CHAPTER 2	5
Review of Literature	5
2.1 Introduction	5
2.1 History of Streptococcal Research	5
2.2 <i>Streptococcus pyogenes</i> (group A streptococcus)	8
2.2.1 Microbiology.....	8
2.2.2 Streptococcal disease.....	8
2.2.3 Classification of <i>Streptococcus pyogenes</i>	9
2.3 Aetiology of Glomerulonephritis	11
2.4 Post-streptococcal Glomerulonephritis	14
2.4.1 Clinical Presentation and Diagnosis.....	14
2.4.2 Treatment and Prognosis	17
2.5 Epidemiology of Post-Streptococcal Glomerulonephritis	17
2.6 Prevention of Post-streptococcal Glomerulonephritis	24
2.6.1 Prophylactic Antibiotic Therapy	24
2.6.2 Scabies Treatment	25
2.6.3 Development of a <i>Streptococcus pyogenes</i> Vaccine Candidate.....	26

2.6.4	Phage Therapy.....	28
2.6.5	Replacement Therapy.....	29
2.6.6	Host Immunomodulation.....	29
2.7	Economic Burden of Post-streptococcal Glomerulonephritis.....	30
2.8	Pathogenesis of Post-streptococcal Glomerulonephritis	31
2.8.1	Host Factors	32
2.8.2	<i>Streptococcus pyogenes</i> Virulence Factors	36
	M Protein	38
	C5a Peptidase.....	39
	Streptococcal Inhibitor of Complement.....	40
	Streptokinase.....	43
	Hyaluronate Capsule.....	44
	Streptococcal Pyrogenic Exotoxin Type B.....	45
	Nephritis-Associated Plasmin Receptor	46
	Pre-absorbing Antigen	47
2.8.3	Prophage Encoded Virulence Factors	48
2.8.4	Animal Models of Acute Post-streptococcal Glomerulonephritis.....	49
2.9	Future Research.....	50
	CHAPTER 3	51
3.1	Introduction.....	51
3.2	Microbiological Methods.....	51
3.2.1	Origin of Bacterial Strains.....	51
3.2.2	Storage and Propagation of Bacterial Strains.....	52
3.2.3	Spectrophotometric Analysis and Estimation of <i>Escherichia coli</i> Cell Density	52
3.2.4	Preparation of Competent <i>Escherichia coli</i> Cells	53
	3.2.4.1 Chemically Competent <i>Escherichia coli</i> Cells	53
	3.2.4.2 Electrocompetent <i>Escherichia coli</i> Cells.....	53
3.2.5	Transformation of Competent <i>Escherichia coli</i> Cells.....	54
	3.2.5.1 Chemically Competent Cells	54
	3.2.5.2 Electrocompetent Cells.....	54
3.3	Deoxyribonucleic Acid Methods	54

3.3.1	Genomic Deoxyribonucleic Acid	54
3.3.2	Plasmid Propagation and Extraction	55
3.3.3	Polymerase Chain Reaction	55
3.3.4	Agarose Gel Electrophoresis	55
3.3.5	Molecular Weight Markers	56
3.3.6	Deoxyribonucleic Acid Digestion with Restriction Enzymes	56
3.3.7	Deoxyribonucleic Acid Ligations	56
3.3.8	Determination of Deoxyribonucleic Acid Concentration.....	56
3.3.9	Ethanol Precipitation.....	57
3.3.10	Automated Sequencing	57
3.4	Protein Methods.....	57
3.4.1	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis	57
3.4.1.1	Gel Preparation	57
3.4.1.2	Sample Preparation and Gel Electrophoresis.....	58
3.4.1.3	Drying of Protein Gels.....	58
3.4.2	Induction of Protein Expression	58
3.4.3	Native Protein Purification.....	59
3.4.4	Denatured Protein Purification.....	59
3.4.5	Production of Antibodies	60
3.4.6	Western Blot Analysis of Polyacrylamide Gels	60
3.4.7	Determination of Protein Concentration	60
3.5	Animal Experimentation	61
3.5.1	Experimental Animals.....	61
3.5.2	Histology	61
3.5.2.1	Immunopathological Staining.....	61
3.5.3	Animal Ethics.....	62
3.6	Human Experimentation	63
3.6.1	Patients	63
3.6.2	Human Ethics	63

3.7	Statistical Analysis of Data	63
CHAPTER 4		64
The Carriage of Streptococci by Dogs in Indigenous Communities		64
4.1	Introduction.....	64
4.2	Materials and Methods.....	66
4.2.1	Positive Control.....	66
4.2.2	Sampling Procedures.....	66
4.2.3	Bacterial Isolation	67
4.2.4	Species Identification	67
4.2.5	Lancefield Serology	68
4.2.6	16S rRNA Sequence Analysis.....	68
4.2.7	Determination of Animal Health.....	70
4.3	Results.....	71
4.3.1	Sample Demographics.....	71
4.3.2	Bacterial Species and Lancefield Serogroup.....	75
4.3.3	Lancefield Serology	80
4.3.4	Results of 16S rRNA Sequencing	80
4.3.5	Association between Animal Health and the Carriage of Streptococci	83
4.4	Discussion	84
4.5	Conclusion	90
CHAPTER 5		92
The Molecular Epidemiology of Lancefield Strains A, B, C, G and L of Canine Origin		92
5.1	Introduction.....	92
5.2	Materials and Methods.....	93
5.2.1	Positive Controls	93
5.2.2	Bacterial Strains	94
5.2.3	DNA Extraction.....	94

5.2.4	<i>Emm</i> Sequence Typing.....	94
5.2.5	Polymerase Chain Reaction	96
5.2.6	Determination of Deoxyribonucleic Acid Sequence.....	96
5.2.6	Database Searches and Sequence Analysis.....	96
5.2.7	Nucleotide Sequence Accession Numbers.....	96
5.3	Results.....	97
5.3.1	Prevalence of Virulence Factors amongst Isolates.....	97
5.3.2	<i>emm</i> and <i>emm</i> -like Types among Canine Isolates Recovered from Dogs in Australian Aboriginal and Torres Strait Islander Communities.....	99
5.3.3	Nucleotide Sequence of the <i>emm</i> -like Gene from Strain 37MS β (<i>stC37.0</i>)	100
5.3.4	Canine Strains of Streptococci have Deoxyribonucleic Acid Homologous to Several Group A Streptococcal Virulence Factors.....	104
5.3.5	Animal strains of Streptococci Carry the Streptokinase Gene	111
5.4	Discussion	113
5.5	Conclusion	116
CHAPTER 6	118
	The Development of a Murine Model for Post-streptococcal Glomerulonephritis	118
6.1	Introduction.....	118
6.2	Materials and Methods.....	120
6.2.1	Bacterial Strains and Growth	120
6.2.2	Preparation of Antigen	120
6.2.3	Injection with Killed <i>Streptococcus pyogenes</i>	121
6.2.3.1	Experiment 1 – Duration Experiment in NOD/Lt Mice	121
6.2.3.2	Experiment 2 – PSGN induction with Different Genetic Backgrounds	121
6.2.3.3	Experiment 3 – Dose Optimisation of <i>Streptococcus pyogenes</i> in NOD.C- <i>Hc</i> Mice	121
6.2.4	Plasma and Tissue Collection	122
6.2.5	Morphometry and Histopathology	122
6.2.5.1	Experiment 1.....	122
6.2.5.2	Experiments 2 and 3	122

6.2.6	Immunohistopathology.....	123
6.2.8	Plasma Protein Levels.....	123
6.2.9	Plasma Antibodies.....	123
6.3	Results.....	124
6.3.1	Experiment 1- Duration Experiment in NOD/Lt Mice.....	124
6.3.1.1	Complications.....	124
6.3.1.2	Morphological Findings.....	124
6.3.1.3	Immunological Response.....	129
6.3.1.4	Progress of Disease.....	129
6.4.1	Experiment 2 - PSGN Induction with Different Genetic Backgrounds	130
6.4.1.1	Complications.....	130
6.4.1.2	Morphological Findings.....	130
6.4.1.3	Plasma Protein Levels.....	135
6.4.1.4	Immunological Response.....	135
6.4.1.5	Progression of Disease.....	136
6.4.2	Experiment 3 - Dose Optimisation of <i>Streptococcus pyogenes</i> in NOD.C-Hc Mice	136
6.4.2.1	Complications.....	136
6.4.2.2	Morphological findings	136
6.4.2.3	Plasma Protein Levels.....	137
6.4.2.4	Progression of Disease.....	137
6.5	Discussion	137
	CHAPTER 7	142
	Investigation into the Role of SIC protein in a Murine Model of Post-Streptococcal Glomerulonephritis.....	142
7.1	Introduction.....	142
7.2	Materials and Methods.....	144
7.2.1	Bacterial Strains and Growth	144
7.2.2	Preparation of Antigen	144
7.2.3	Injections with Killed <i>Streptococcus pyogenes</i>	144
7.3	Results.....	145
7.3.1	Complications	145

7.3.2	Morphological Findings	145
7.3.2.1	Grade of glomerulonephritis.....	145
7.3.2.2	Grade of pathology	148
7.3.2.3	Deposition of IgG, C3 and Streptococcal inhibitor of Complement Mediated Cell Lysis Protein.....	148
7.3.3	Immunological Response	149
7.3.4	Progression of Disease	149
7.3.5	Plasma Protein Levels.....	150
CHAPTER 8		153
Antibodies against Streptococcal Virulence Factors in Patients with End-Stage Renal Failure.....		153
8.1	Introduction.....	153
8.2	Materials and Methods.....	154
8.2.1	Patients and Controls.....	154
8.2.2	Streptococcal Strains.....	155
8.2.3	Deoxyribonucleic Acid Isolation.....	155
8.2.4	Immunostaining Western blots.....	157
8.2.5	Optimisation of Indirect Enzyme Linked Immunosorbent Assay	157
8.2.6	Screening for Streptococcal Antibodies using Indirect ELISA.....	159
8.2.7	Statistical Methods	160
8.3	Results.....	160
8.3.1	Expression of Purified Streptococcal Antigens.....	160
8.3.2	Antibodies against Streptococcal Proteins	161
8.3.3	Development of an ELISA to Screen for Anti-Streptococcal Antibodies.....	163
8.3.4	Patient Follow-up	166
8.3.5	Absorbance Reading Obtained for each Streptococcal Recombinant Protein .	166
8.3.6	Presence of Streptococcal Antibodies in Human Serum Samples	167
8.3.7	Data Analysed According to Diabetic Status	171
8.3.8	Relationship between Antibodies and Experimental Group	171

8.3.9	Correlations between Antibody levels	174
8.3.10	Validation of ELISA	174
8.4	Discussion	174
8.5	Conclusion	176
CHAPTER 9	178
General Discussion	178
LITERATURE CITED	183
APPENDIX 1 - Media	220
Sheep Blood Agar	220
Luria Burtani Agar	220
Luria Burtani Broth	220
Todd Hewitt Yeast Broth	220
10 % Glycerol	221
L9 Minimal Agar	221
SOB medium	221
SOC medium	222
APPENDIX 2 – Molecular biology chemicals	223
50 mM NaOH	223
1M Tris-HCl/7mM EDTA pH 8	223
0.85% w/v NaCl	223
TE Buffer	223
Mutanolysin 3000 units/ml	223
Hyaluronidase (30mg/ml)	223
Tris-acetate (TAE) Buffer (50X)	223
Ethidium Bromide Stock Solution	224
Agarose Gel Loading Buffer (6X)	224
Sodium Acetate	224
APPENDIX 3 – Protein chemistry chemicals	225

Isopropyl B-D Thiogalactopyranoside (IPTG) Solution	225
SDS-Page Gel (12%) Separating Gel	225
Tris-HCl Buffer (1M) pH 8.8	225
10% SDS Solution	225
10% APS Solution	225
SDS-PAGE Buffer (2X)	225
Stacking Gel 4%	226
Running Buffer (5X)	226
Coomassie Blue Staining Solution	226
Destaining Solution	226
Gel Drying Solution.....	226
Lysis Buffer	226
Wash Buffer.....	227
Elution Buffer	227
Denaturing Buffer.....	227
Buffer B	227
Buffer C	227
Buffer D	227
Transfer Buffer	227
Lysozyme.....	227
APPENDIX 4 – Immunology methods chemicals.....	228
4% Paraformaldehyde Solution	228
Solutions for Positive Charged Slide.....	228
Phosphate Buffered Saline (PBS).....	228
0.3% H ₂ O ₂ in 70% methanol/TBS	228
Tris Buffered Saline 10X Solution (TBS)	228
Diaminobenzidine Histochemistry Substrate	228
APPENDIX 5 – Microbiological data.....	229

APPENDIX 6 – Enzyme linked immunosorbent assay chemicals and data	233
Carbonate Coating Buffer.....	233
APPENDIX 7 – Papers and Presentations	234
Papers	234
Presentations.....	234

LIST OF FIGURES

Figure 2.1. Typical haemolysis reactions on a sheep blood agar plate, designated gamma – no haemolysis (A), alpha – incomplete haemolysis (B), beta – complete haemolysis (C) (photo by Virginia Boon).....	5
Figure 2.2. Culture of <i>Streptococcus pyogenes</i> on 5% blood agar plate (photo by Virginia Boon).....	8
Figure 2.3. Schematic diagram depicting the pathways by which immune deposits mediate glomerular injury (adapted from Couser <i>et al.</i> 1998).....	12
Figure 2.4. Comparison of a healthy glomerulus (left) with the glomerulus in PSGN (right). The healthy glomerulus has thin and delicate capillary loops and normal tubules whilst the diseased glomerulus is hypercellular, with occluded and lobulated capillaries (Churg and Sobin 1982).....	15
Figure 2.5. Immunofluorescence staining showing deposits of complement 3 in the capillary loops. The deposits are granular and bumpy due to the focal nature of the deposition (Churg and Sobin 1982).....	16
Figure 2.6. Immune deposits seen as subepithelial ‘electron humps’ above the basement membrane and below the epithelial cell (Churg and Sobin 1982). Note: The yellow arrow denotes the subepithelial electron dense deposits or ‘humps’, the pink arrow denotes the cytoplasm of an endothelial cell and the orange arrow denotes the basement membrane.....	16
Figure 2.7. Schematic diagram relating group A streptococcal infection with post-infection sequelae.....	18
Figure 2.8. Factors affecting skin disease in Aboriginal communities (adapted from Currie and Carapetis 2000). Note: GAS = <i>Streptococcus pyogenes</i> , group A streptococcus; APSGN = acute post-streptococcal glomerulonephritis; ARF = acute rheumatic fever.	20
Figure 2.9. Rates of non-diabetic ESRF in Saskatchewan, 1981 to 1990, among registered indigenous and non-indigenous people by age group (Dyck and Tan 1998). Note: Shaded boxes represent the indigenous population while the non-shaded boxes represent the non-indigenous population.	22
Figure 2.10. Incidence rates (per million population) of end-stage renal failure in Canada during 1981-1996 (observed) and 1997-2005 (projected) among people without diabetes mellitus (left) and those with diabetes (right) (Schaubel <i>et al.</i> 1999).....	23
Figure 2.11. Different activation pathways of the complement system that converge on a common terminal pathway (Mathieson 1998). Note: (MAC: membrane attack complex)	35
Figure 2.12. Growth phase dependent transcription of <i>mga</i> , RofA-like proteins (RALPs) and <i>rgg/ropB</i> and their influence on <i>Streptococcus pyogenes</i> virulence factor expression (Kreikemeyer <i>et al.</i> 2003) Note: Boxes contain virulence genes either upregulated (up arrow) or downregulated (down arrow) by <i>Mga</i> (red), <i>Nra/RofA</i> (blue) and <i>Rgg/RopB</i> (green). Autoregulated regulons are identified by an asterix. MSCRAMMs refer to microbial surface components recognizing adhesive matrix molecules.	38
Figure 2.13. Proportion of the population with a history of PSGN and the presence or absence of antibodies to CRS and DRS (Sriprakash <i>et al.</i> 2002). The data represent sera from 112 subjects with recorded PSGN history and 86 subjects who had no recorded history of PSGN; +ve and -ve represent sera with and without reactions to the antigens, CRS or DRS. *, Statistically significant ($P=0.009$). Note: SIC – streptococcal inhibitor of complement mediated cell lysis, CRS – closely related to SIC, or DRS – distantly related to SIC.....	42
Figure 2.14. Schematic representation of proposed mechanism for PSGN (Yamakami <i>et al.</i> 2000). Note: C, complement; \bar{C} , activated complement; CIC, circulating immune complex; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GAP-Ab, anti-	

GAPDH antibody; Pl, plasmin; Plr, nephritis-associated plasmin receptor; Plr-Ab, anti-Plr antibody; SK, streptokinase.....	47
Figure 2.15. The prophage content of <i>Streptococcus pyogenes</i> strains belonging to serotypes M1 and M18 (Boyd and Brussow 2002) Note: A. The insert in the center shows a dot-plot of the M1 vs. M18 DNA sequence. The prophages causing interruptions were numbered one to five and this numbering was also used in the outer circle.	48
Figure 4.1. Map of Australia showing location of community 1 (pink star) and community 2 (yellow star).	67
Figure 4.2. Isolates of streptococcal bacteria from dogs in Cape York Aboriginal and Torres Strait Islander community 1 (n=23). Note: Percentage is prevalence of the bacterium in the sample.	76
Figure 4.3. Isolation of streptococcal bacteria from dogs in Northern Territory Aboriginal Community 2 (n=60). Note: Percentage is prevalence of the bacterium in the sample.	77
Figure 4.4. Number of isolates of streptococci from dogs residing in two Aboriginal and Torres Strait Islander communities in geographically distinct areas (n=83) grouped using Lancefield serology. Note: This does not include bacterial species identified in either the <i>Aerococcus</i> or <i>Lactococcus</i> genus.....	80
Figure 4.5. Phylogenetic tree (phylogram) showing the distance matrix of canine isolates 16S sequences compared with published streptococcal species 16S sequences constructed using the neighbour-joining method with the ClustalW program. Note: distance is shown after the name of isolate. Isolates from this study are coded in Appendix 5. The following species are not from this study: <i>Streptococcus porcinus</i> (ATCC 43138, accession no. AB002523), <i>Enterococcus casseliflavus</i> (accession no. AF039899), <i>Streptococcus gallolyticus</i> (accession no. DQ232525), <i>Streptococcus sinensis</i> (accession no. EF371928), <i>Streptococcus mitis</i> (ATCC 903, accession no. AY281078), <i>Streptococcus iniae</i> (accession no. AQ985468), GGS (<i>Streptococcus dysgalactiae</i> , streptococcus sp. 'group G', ATCC 27961, accession no. AB002517), <i>Streptococcus sanguinis</i> (accession no. CP000387), <i>Streptococcus suis</i> (accession no. AF009509), <i>Streptococcus orisratti</i> (accession no. AF124350), <i>Streptococcus dysgalactiae</i> * (<i>Streptococcus dysgalactiae</i> subsp. <i>equisimilis</i> , ATCC 35666, accession no. AB096755), <i>Streptococcus anginosus</i> (accession no. AY691536), <i>Streptococcus oral</i> (<i>Streptococcus</i> sp. oral clone, accession no. AF432136), <i>Streptococcus pyogenes</i> (MGAS 6180, accession no. CP000056), <i>Streptococcus minor</i> (accession no. AY232833), <i>S. minor2</i> (accession no. AY232832), <i>Streptococcus canis</i> (accession no. AJ413203), <i>S. canis2</i> (accession no. DQ303184), <i>S. dysgalactiae</i> (accession no. AB102730), <i>Streptococcus macedonicus</i> (accession no. AF459431), <i>S. dysgalactiae2</i> (accession no. AB002497), <i>Streptococcus agalactiae</i> (accession no. AE014210), <i>S. canis3</i> (accession no. AJ413205), <i>Streptococcus ratti</i> (ATCC 19645, accession no. AJ42020), <i>Enterococcus gallinarum</i> (accession no. AJ301833), <i>Streptococcus uberis</i> (accession no. AB023576), <i>Streptococcus equinus</i> (accession no. DQ148956), <i>Enterococcus gallinaceus</i> (accession no. AJ307888), <i>Streptococcus bovis</i> (accession no. AF429762).....	82
Figure 4.6. Skin Condition Score versus Purina Body Condition Score for dogs in community 2.	84
Figure 5.1. The DNA sequence of <i>stC37.0</i> and the corresponding deduced amino acid sequence. Note: >>>=start codon, SP= signal peptide, repeat regions are designated by B1, B2 and B3, and conserved C repeat regions are designated by C1, C2 and C3. ...	101
Figure 5.3. Agarose gel (1%) showing the amplified PCR products for fibronectin-binding protein. Note: Lane M=1 kb DNA ladder (Promega, Australia), 2, 7 and 12 show amplified product of approximately 750 bp encoding a multi-drug resistance protein and lane 16 shows an amplified product of approximately 1500 bp encoding a fibronectin-binding protein from <i>S. dysgalactiae</i> subsp. <i>equisimilis</i> . The other lanes show negative reactions.	104

- Figure 5.4. The nucleotide sequence encoding a fibronectin-binding protein from a canine isolate of *S. dysgalactiae* subsp. *equisimilis* and the deduced amino acid sequence. Note: The amino acid sequence highlighted in blue depicts the signal sequence of the propeptide, and the two fibronectin-binding repeat domains are highlighted in yellow. 105
- Figure 5.5. Alignment of the amino acid sequences corresponding to fibronectin-binding protein. Note: A=Fibronectin-binding protein from *streptococcus* group G (accession no. U31115, Kline *et al.* 1996), B=Fibronectin-binding protein F from Streptococcus group G (accession no. AAB06623, Kline *et al.* 1996), C=Fibronectin-binding protein 1 from *S. pyogenes* NS1042 (accession no. CAC87682.1, Towers *et al.* 2003), D=Fibronectin-binding protein from *S. pyogenes* DSM207 (accession no. CAA48133, Talay *et al.* 1996), E=Fibronectin-binding protein from *S. dysgalactiae* subsp. *equisimilis* 39MP (this study). 106
- Figure 5.6. Alignment of the encoding amino acid sequence of several strains. Note: A=SPEG of *S. dysgalactiae* subsp. *equisimilis* (accession no. AJ294849, Sachse *et al.* 2002), B=SPEG of 74MP α^{MIN} , C= SPEG of GAS strain M1 SF370 (accession number AE004092, Ferretti *et al.* 2001), D=SPEG of *Streptococcus dysgalactiae* subsp. *dysgalactiae* spegg7 (accession number AB105086), E=SPEG of 36MS β^{EQU} , F=SPEG of 37MS β^{EQU} , G= SPEG of 74MP β^{EQU} , H= SPEG of 11MP β^{EQU} , I= SPEG of 61MP β^{EQU} , J= SPEG of 34MP β^{EQU} and K= SPEG of 93MP β^{EQU} 108
- Figure 5.7. Comparison of nucleotide sequence corresponding to the *scpB* gene from a canine and human (accession no. CP000114, Tettelin *et al.* 2005) *S. agalactiae* isolate. Note: nucleotide changes are shown in red. 110
- Figure 5.8. Rooted phylogenetic tree based on the β -domain-encoding region of *skc*, *skcg* and *ska*. Note: *=isolates derived from this study, *ska21* (accession no. AY234261, Kalia and Bessen 2004), *ska38* (accession no. AY234262, Kalia and Bessen 2004), *skcg09* from *S. dysgalactiae* subsp. *equisimilis* strain 4951G (accession no. AY234242, Kalia and Bessen 2004), *skc* from *S. dysgalactiae* subsp. *equisimilis* H46A (accession no. K02986.1, Malke *et al.* 1985), *skg* from group G streptococcus (accession no. X13400, Walter *et al.* 1989), *ska54* (accession no. AY234273, Kalia and Bessen 2004), *ska37* (accession no. AY234300, Kalia and Bessen 2004), *skc* from *S. dysgalactiae* subsp. *equisimilis* 89-272 (accession no. AF104300, Caballero *et al.* 1999), *skc* from *S. uberis* (accession no. AJ006413, Johnsen 1998). 111
- Figure 5.9. Alignment of the amino acid sequence of the β -domain of streptokinase from several streptococcal strains. Note: A=*Streptococcus agalactiae* (this study), B=*Streptococcus canis* (this study), C=*Streptococcus dysgalactiae* subsp. *equisimilis* *skcg16* allele (accession no. AY234249), D=*Streptococcus pyogenes* *ska37* (accession no. AY234300), E=*S. pyogenes* *ska54* (accession no. AY234273), F=*S. pyogenes* *ska21* (accession no. AY234261), G=*S. equisimilis* (this study), H=*S. equisimilis*, group C streptococci, equine isolate *ESk* (accession no. AF104301), I=*Streptococcus uberis* NCTC 3858, group C streptococci, bovine isolate (accession no. AJ006413). 112
- Figure 6. 1. Kidney sections of glomeruli, stained with hematoxylin-eosin. A) Group 3 (control) mouse, B) Group 2 (injected control) mouse. C & D) Group 1 (*Streptococcus pyogenes* injected) mouse. Note: the increased cellularity, and occlusion of capillaries in C, The occlusion of capillaries and extracapillary cellular proliferation in D. Original magnification x1000. 126
- Figure 6. 2. Kidney sections of glomeruli, stained with hematoxylin-eosin and periodic acid Schiff. A) Group 3 (control) mouse, B) Group 2 (injected control) mouse. C, D, E and F) Group 1 (*Streptococcus pyogenes* injected) mouse. Note: The thickening of the basement membrane in C, the lobulation in D, and the increased cellularity, the occlusion of capillaries and lobulation of the glomerular tuft in E and F. In E and F there is also slight extracapillary proliferation (crescent formation). Original magnification x1000. 127
- Figure 6.3. Immunofluorescent staining for C3 in kidney sections of glomeruli. A) Group 2 (injected control) mouse, B) Group 1 (*Streptococcus pyogenes* injected) mouse. This

glomerulus was regarded as positive for C3 deposition since an arrangement of fine and coarse granular deposits could be detected in the mesangium equivalent to the so-called 'mesangial pattern. Original magnification x1000.....	128
Figure 6.4. Immunofluorescent staining for IgG in kidney sections of glomeruli. A) Group 2 (injected control) mouse, B) Group 1 (<i>Streptococcus pyogenes</i> injected) mouse. Original magnification x1000.	128
Figure 6.5. Immunoperoxidase staining for SIC in kidney sections of glomeruli. A) Group 2 (injected control) mouse, B, C & D) Group 1 (<i>Streptococcus pyogenes</i> injected) mouse. Note the deposition of SIC protein in the mesangium, basement membrane and the presence of glomerular deposits of SIC protein which appear to distort configuration of the capillary loop in B. Deposition of SIC protein in the walls of an arteriole muscular artery. D shows deposition of SIC protein in tubules of the renal cortex. Original magnification x1000 (A-C), x100 (D).....	129
Figure 6.6. Diffuse glomerular hypercellularity in NOD.C- <i>Hc</i> animal injected with killed <i>Streptococcus pyogenes</i> . Note: Periodic Acid Schiff stain, 100X magnification.	130
Figure 6.7. Illustrative examples of immunofluorescence grading scores (IgG staining) from animals injected with PBS (A, -), <i>Streptococcus pyogenes</i> (B, +/-; C, +; D, ++; E, +++).	133
Figure 6.8. Plasma protein levels of mice injected with <i>Streptococcus pyogenes</i> for each congenic strain ($n=4$). Note: The final experimental point was day 21 or the day of sacrifice for the animal, for the NOD.C- <i>Hc</i> strain, the final data point is $n=3$. A statistically significant difference exists between day 0 and 21 for NOD/Lt ($P<0.05$, $q=4.5$), for NOD.C- <i>Hc</i> between day 0 and 21 ($P<0.01$, $q=9.3$), day 7 and 21 ($P<0.001$, $q=10.2$), for NOD.H2 ^d between days 0 and 7 ($P<0.05$, $q=5.2$), 0 and 21 ($P<0.001$, $q=20$), 7 and 21 ($P<0.001$, $q=15$) and for NOD.H2 ^b between day 7 and 21 ($P<0.05$, $q=4.6$). No significant difference was found for any of the control congenic strains over the experimental period.....	135
Figure 7.1. Glomeruli from a mouse in group 2 (PBS injected) showing healthy glomeruli with no evidence of hypercellularity (Periodic acid Schiff and haematoxylin stain, 40x).	146
Figure 7.2. Kidney section from mouse in A) group 2, negative for hypercellularity (PBS injected), B) group 3, positive for diffuse hypercellularity (grade IV) (<i>sic</i> ⁺) and C) group 4, positive for diffuse hypercellularity (grade III) (<i>sic</i> ⁻). (Haematoxylin and eosin stain, 20x objective).	147
Table 7.3. Assignment of scores for pathological changes in experimental groups	148
Figure 7.3. A) Glomerulus from mouse in group 4 (<i>sic</i> ⁻) exhibiting lobulation and occlusion, B) glomerulus from mouse in group 3 (<i>sic</i> ⁺) exhibiting crescent formation and occlusion of capillaries.....	148
Figure 7.4. Immunohistochemistry result for anti-SIC staining. Note: A) glomerulus from group 3 animal (injected with wild type AP1 strain, <i>sic</i> ⁺); showing positive staining for SIC protein deposition, B) glomerulus from group 4 animal (injected with isogenic mutant strain, <i>sic</i> ⁻ ; showing no staining for the SIC protein.....	149
Figure 8.1. Schematic diagram representing indirect ELISA. Note: HRP=horseradish peroxidase, ABTS=.2,2'-azino-di-3-ethylbenzthiazoline-6-sulphonic acid.	158
Figure 8.2. Schematic representing checkerboard titration conducted to optimise indirect ELISA. Note: This figure represents the positive control's serum, antigen diluted in ELISA diluent (TropBio, Townsville, Australia) and Sarstedt (Crown Scientific) 96-well flat-bottom polystyrene plates.	159
Figure 8.3. The expression of several recombinant streptococcal proteins on a Coomassie stained SDS-PAGE gel. The antigens run in each lane are: 1 is streptokinase, 2 is streptococcal pyrogenic exotoxin type B, 3 is thioredoxin, 4 is CRS, 5 is DRS, and 6 is SIC. Note: the arrows denote the purified protein band of interest, SIC: streptococcal inhibitor of complement, CRS: closely related to SIC and DRS: distantly related to SIC, protein ladder used was #SM0431 (Fermentas, Australia).	161

- Figure 8.4. Immunoblots of streptococcal antigens. The antigens run on each lanes: 1, 6 and 11 are CRS, 2, 7 and 12 are SIC, 3, 8 and 13 are DRS, 4, 9 and 14 are streptokinase (ska), and 5, 10 and 15 are streptococcal pyrogenic exotoxin type B (speB) in its zymogen form. Tetra-his antibody was used as a positive control for the detection of ska and speB, polyclonal rabbit SIC antiserum was used to detect CRS and SIC, and anti-DRS rabbit polyclonal antibodies were used to detect DRS. Note: the arrows denote the reaction of the antibody with the protein of interest, SIC: streptococcal inhibitor of complement, CRS: closely related to SIC and DRS: distantly related to SIC, *low titres of this patient were equal to the negative control in the ELISA (no serum) and thus were classified as negative, protein ladder used was #SM0431 (Fermentas, Australia)..... 162
- Figure 8.5. Western blot of streptokinase and positive control serum. Note: Lane 1=denatured recombinant streptokinase, 2=pre-stained molecular weight marker (SM0671, Fementas, Australia) and 3=ative streptokinase from group C streptococci. 163
- Figure 8.6. Comparison of coating buffers for optimisation of indirect ELISA. Note: PC=positive control, NC=negative control, PBS=phosphate buffered saline, (1)=carbonate coating buffer, (2)=PBS, (3)=ELISA diluent, DRS antigen used at a concentration of 80 µg/ml. 164
- Figure 8.7. Comparison of antigen and serum in the optimisation of an indirect ELISA for positive control serum. Note: PC=positive control serum, No antigen = the plate was coated with ELISA diluent, mcg=micrograms. The results presented are for the DRS antigen, however similar curves were obtained for each antigen. The plates used were Sarstedt 96-well U bottom polystyrene plates (Crown Scientific) and the antigen was diluted in ELISA diluent. Note: DRS= distantly related to streptococcal inhibitor of complement protein..... 165
- Figure 8.8. Comparison of antigen and serum in the optimisation of an indirect ELISA for negative control serum. Note: NC=negative control serum, No antigen = the plate was coated with ELISA diluent, mcg=micrograms. The results presented are for the DRS antigen; however similar curves were obtained for each antigen. The plates used were Sarstedt 96-well U bottom polystyrene plates (Crown Scientific) and the antigen was diluted in ELISA diluent. Note: DRS= distantly related to streptococcal inhibitor of complement protein..... 166
- Figure 8.10. Graph showing mean antibody levels obtained for each streptococcal protein. Note: error bars represent standard error of the mean, (*) denotes a statistically significant difference from controls ($P < 0.05$), Ska1=recombinant streptokinase protein from M1 strain, DRS=distantly related to SIC, CRS=closely related to SIC, SIC=streptococcal inhibitor of complement protein and SpeB=streptococcal pyrogenic exotoxin type B in its inactive zymogen form. 168
- Figure 8.11. Scatterplots of mean optical density (+SD) measured by indirect ELISA analysed according to patient groups. Note: SD=standard deviation, DRS=distantly related to SIC, CRS=closely related to SIC, SIC=streptococcal inhibitor of complement, SpeB=streptococcal pyrogenic exotoxin type B. The significant results of statistical analyses of the mean serological responses are shown (calculated using Tamhane's post-hoc test). Solid horizontal lines represent the means of each category and dotted horizontal lines show the cut-off for seropositivity (three times the overall mean of the lowest quartile)..... 171
- Figure 8.12. Model showing regression coefficients between constructs being antibody levels to 5 streptococcal antigens (Ska, DRS, CRS, SpeB and SIC) and being an Indigenous ESRF patient. Note: SKA=streptokinase, DRS=distantly related to SIC, CRS=closely related to SIC, SPEB=streptococcal pyrogenic exotoxin type B in its zymogen form, SIC=streptococcal inhibitor of complement mediated cell lysis, Patient= end-stage renal failure patient being treated with haemodialysis, Indigenous=Torres Strait Islander and/or Aboriginal. 173

LIST OF TABLES

Table 2.1. Complications of <i>Streptococcus pyogenes</i> illness (Bisno and Stevens 1996, Bisno <i>et al.</i> 1997).....	9
Table 2.2. The various types of glomerulonephritis.....	11
Table 2.3. Case definition for acute post-streptococcal glomerulonephritis (Streeton <i>et al.</i> 1995, Muscatello <i>et al.</i> 2001).....	14
Table 3.1. The strain number, study number and source of isolation of <i>Streptococcus pyogenes</i> isolates used in this study.....	51
Table 4.1. Demographic parameters of dogs examined in the Cape York Peninsula (community 1 - pilot study).	71
Table 4.2. Demographic parameters of dogs examined in the Northern Territory (community 2) and bacterial isolation.....	72
Table 4.3. Bacterial isolates according to species identification and Lancefield's serogroup.	73
Table 4.4. A summary of the prevalence of bacterial carriage by dogs residing in each community. Note: includes the following genera: <i>Streptococcus</i> , <i>Enterococcus</i> , <i>Lactococcus</i> and <i>Aerococcus</i>	79
Table 4.5. Grading systems used to classify canine populations in community 2.	80
Table 5.1. The overall prevalence rate for group A streptococcal virulence factors in dogs strains.....	95
Table 5.2. Epidemiological properties and molecular analysis of canine isolates.....	97
Table 5.3. <i>emm</i> and <i>emm</i> -like types distributed among canine strains.....	98
Table 5.4. The primer sequences used to detect virulence factors.....	100
Table 6.1. Experimental groups in congenic strain experiment.....	12121
Table 6.2. Glomerular reactivity after immunisation schedule.....	1244
Table 6.3. Immunohistochemical and histopathological findings in kidneys of mice	12525
Table 6.4. P values between congenic strains for glomerular cell numbers of animals injected with <i>Streptococcus pyogenes</i>	131
Table 6.5. Post-streptococcal glomerulonephritis in NOD congenic mice.....	13131
Table 6.6. Statistical significance between congenic strains for grade of glomerulonephritis using the Mann-Whitney U test.	132
Table 6.7. Statistical significance (mean difference) between congenic strains for grade of pathological changes using the Mann-Whitney U test.....	133
Table 6.8. Immunofluorescence results for each congenic strain.....	134
Table 6.9. P values and mean difference for C3 immunofluorescence between congenic strains injected with <i>Streptococcus pyogenes</i>	134
Table 6.10. Post-streptococcal glomerulonephritis in NOD.C-Hc congenic mice utilising differing dosages of <i>Streptococcus pyogenes</i>	136
Table 6.11. Immunofluorescence studies for the deposition of C3 and IgG at different dosages.....	137
Table 7.1. Experimental design and groups utilised.....	145
Table 7.2. Assignment of scores for glomerulonephritis in experimental groups.....	146
Table 7.3. Assignment of scores for pathological changes in experimental groups.....	148
Table 7.4. Immunofluorescence results for each experimental group.....	149
Table 7.5. Plasma protein levels \pm standard deviation (mg/ml) over the experimental period.	150
Table 8.1. Primers used in this study to amplify streptococcal genes.....	156
Table A5.1. Individual bacterial species isolated from dogs in from both the Northern Territory and Cape York Peninsula Indigenous Communities.....	229

LIST OF ABBREVIATIONS

°C	degrees Celsius
A ₂₆₀	absorbance at 260 nm
A ₂₈₀	absorbance at 280 nm
A ₃₂₀	absorbance at 320 nm
A ₄₁₄	absorbance at 414 nm
A ₆₀₀	absorbance at 600 nm
ABS	Australian Bureau of Statistics
ABTS	2,2'-azino-di(ethylbenzthiazoline-6-sulphonic acid)
Anti-DNase B	anti-deoxyribonuclease B
ARF	acute rheumatic fever
ASO	antistreptolysin O
ATCC	American Type Culture Collection
AUD	Australia
BCA	bicinchoninic acid
BCS	Purina body condition score
BLIS	bacteriocin-like inhibitory substance
BLAST	Basic Local Alignment Search Tool
C1	complement component 1
C3	complement component 3
C5	complement component 5
CDC	Centers for Disease Control and Prevention
CFA	complete Freund's adjuvant
CRS	closely related to SIC
DAB	3,3'-diaminobenzidine tetrahydrochloride
DNA	Deoxyribonucleic acid
DPX	di-n-butylphthalate polystyrene xylene
DRS	distantly related to SIC
EDTA	ethylenediamine tetraacetic acid
ELISA	enzyme linked immunosorbent assay
ESRF	end stage renal failure
ET-1	endothelin-1
G	Gauge
<i>g</i>	gravity
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GAS	group A Streptococcus

GBM	glomerular basement membrane
GBS	group B Streptococcus
GCS	group C Streptococcus
GGs	group G Streptococcus
GFR	glomerular filtration rate
HBA	horse blood agar
HRP	horseradish peroxidase
IDDM	insulin-dependent diabetes mellitus
IFA	incomplete Freund's adjuvant
IPTG	Isopropyl β -D-1-thiogalactopyranoside
JCU	James Cook University
kb	kilobase
KW	Kruskal Wallis statistic
LB	Luria Burtani
LPC	lipidic polylysine core
M	molar
MAC	membrane attack complex
Mga	multiple gene regulator
MHC	major histocompatibility complex
mM	millimolar
mmol	millimole
MSCRAMMS	microbial surface components recognizing adhesive matrix molecules
mw	molecular weight
NAP1r	nephritis-associated plasmin receptor
NATSISS	National Aboriginal and Torres Strait Islander Survey
NOD	non obese diabetic
NSW	New South Wales
NT	Northern Territory
OF	opacity factor
ORF	open reading frame
PA-Ag	pre-absorbing antigen
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pmp	per million population
PSGN	post streptococcal glomerulonephritis
PTSAgs	pyrogenic toxin superantigens

PVDF	polyvinylidene fluoride
RALP	RofA-like protein
RAPD	random amplification of polymorphic DNA
RFLP	restriction fragment length polymorphism
RFR	renal functional reserve
Rgg/RopB	transcriptional regulator
RHD	rheumatic heart disease
rpm	revolutions per minute
rSIC	recombinant SIC
rRNA	16S ribosomal ribonucleic acid
SalA	salivaricin A
SBA	sheep blood agar
SCPA	C5a Peptidase
SCS	skin condition score
SD	standard deviation
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	structural equational modeling
SIC	streptococcal inhibitor of complement mediated cell lysis
Ska	streptokinase
SLE	systemic lupus erythematosus
SpeA	superantigens
SpeB	streptococcal pyrogenic exotoxin type B
SpeG	streptococcal pyrogenic exotoxin type G
SPSS	statistical package of social sciences
TAE	tris/acetate/EDTA
TBS	tris buffered saline
TE	tris/EDTA
TH	Todd Hewitt
THY	Todd Hewitt Yeast
US	United States
UV	ultraviolet
V	volts
VRE	vancomycin resistant enterococci
WHO	World Health Organization