

JCU ePrints

This file is part of the following reference:

Lazzaroni, Sharon (2009) *Immune responses in Burkholderia pseudomallei* infection, relapse, reactivation and protection. Masters (Research) thesis, James Cook University.

Access to this file is available from:

<http://eprints.jcu.edu.au/11605>



**IMMUNE RESPONSES IN
BURKHOLDERIA PSEUDOMALLEI INFECTION,
RELAPSE, REACTIVATION AND PROTECTION**

**Thesis submitted by
Sharon Marie Lazzaroni, B. Med Lab Sci (JCU)
in February 2009**

**In fulfilment of the requirements for the
Degree of Masters by Research in Microbiology and Immunology
At the School of Veterinary and Biomedical Sciences,
James Cook University, Queensland, Australia**

DECLARATION

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 Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Sharon Marie Lazzaroni

January 2009

STATEMENT OF ACCESS

I, the undersigned, the author of this thesis, understand that James Cook University of North Queensland will make it available for use within the University Library and, by microfilm or other photographic means, allow access to users in other approved libraries. All users consulting this thesis will have to sign the following statement.

“In consulting with this thesis I agree not to copy or closely paraphrase it in whole or part without the written consent of the author, and to make proper written acknowledgment for any assistance which I have obtained from it.”

Beyond this, I do not wish to place any restriction on the access of this thesis.

Sharon Marie Lazzaroni

January 2009

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 Human* (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 A709, A987 and H1659).

Sharon Marie Lazzaroni

January 2009

ACKNOWLEDGEMENTS

So here we are.....4 years and 2 kids later, finally finishing one of the biggest challenges in my life....my thesis. There are many people who have contributed to this work that need to be mentioned. Firstly, DSTO (David Proll and Jane McAllister) for constructing the DNA vaccines that were used in this study and secondly a huge thanks to Helen Smith from QHPS for carrying out the PFGE when our machine decided to call it quits. And of course thanks to the blood donors who selflessly gave us blood to carry out our studies.

Of course this project would never have begun without the suggestion by Ketheesan and Brenda Govan that perhaps I should undertake a Masters degree. While sometimes I regretted the decision of taking it on, the feeling that I now have knowing that I have completed a project like this, was worth it. Thanks Ketheesan for pushing me to the point of a nervous breakdown. I know that you lost a lot of sleep over whether I would finish or not, but don't worry, I lost a lot of sleep too. And Brenda, you are the calm in the storm. Just when I was pushed just that little bit too far, you were the voice of reason. But at the same time, I knew you were pushing me just as hard as Ketheesan, although it was a gentler, more subtle approach. Thanks also for reading all my drafts. I know how much time you have put into my Masters and I am extremely grateful. Having supervisors who put in just as much time into the project as me is crazy, but I am eternally grateful and knowing that someone believed in me as much as you both did was sometimes all that kept me going when I lost belief in myself. Thanks also to Dr Robert Norton for your input into this project. I know that you also stressed out each time I fell pregnant wondering whether this project would get finished, so here it is.

And to Dr Jodie Morris....You have taught me everything I know. I will forever remember all the time we spent together measuring footpads, vaccinating mice and of course sitting in front of the FACS machine until midnight, hoping that there were enough cells. How painful those nights were!! Thanks also to Tasha Williams. Bleeding mouse tails and vaccinating mice was just not such a boring task with you there. I would say it was more of a social occasion. Thanks for making me laugh a lot!!! And to Davina Gorton, you have been my office mate since forever. Thanks for answering a lot of my questions and just being there for a chat when I was procrastinating. Huge thanks to the Techies, not only for autoclaving and doing all that fun stuff, but also for helping me procrastinate. I will miss the time spent in your office when I just didn't want to write or make any plugs. To the entire IDIRG team, past and present, YOU ROCK!!! What a team...everyone is there to help each other, laugh with each other and get through those dreaded lab meetings TOGETHER!!

Thanks to A/Prof Leigh Owens for your statistical help and Dr Jenny Elliman for optimising and running the qPCR. Thanks also to Scott Blyth for providing all the animals we needed.....even at short notice.

To the PFGE machine, thanks for making the last 5 years of my life miserable. I hope we never cross paths again.

Finally to all my family. To Dad, Mum, Chris, Kaz and Baz (plus the brother-in-laws) thanks for your continuous support throughout this drawn out process. Thanks Dad for reading the literature review. I too hope that there aren't any 'amateur' phagocytes and just 'professional' phagocytes. And of course to my husband, Steven, for trying to understand what on earth I have been doing for 4 years. I can only hope that you will read beyond this page. To my beautiful boys, Lucas and Jamie. You have taught me not only sleep deprivation but also patience. Thanks for making me laugh. I couldn't have imagined doing this without you all!!

PUBLICATIONS

Publications resulting from this Thesis:

1. McAllister J, Gauci P, Lazzaroni S, Barnes J, Ketheesan N and Proll D. 2007. Assessment of a DNA vaccine encoding *Burkholderia pseudomallei* bacterioferritin (technical report). *Defence Science and Technology Organisation*.
2. McAllister J, Lazzaroni S, Barnes J, Ketheesan N and Proll D. 2007. Development of DNA vaccines against *Burkholderia pseudomallei* (technical report). *Defence Science and Technology Organisation*.
3. Lazzaroni S M, Barnes J L, Williams N L, Govan B L, Norton R E, LaBrooy J T and Ketheesan N. 2008. Seropositivity to *Burkholderia pseudomallei* does not reflect the development of cell-mediated immunity. *Transactions of the Royal Society of Tropical Medicine and Hygiene (in press)*.

Presentations:

1. Lazzaroni S, Inglis T, Currie B, Ketheesan N, Norton R. 2004. The characterisation of *Burkholderia pseudomallei* isolates from the Townsville region using pulsed-field gel electrophoresis. 4th World Melioidosis Congress (poster presentation).
2. Lazzaroni S, Ketheesan N and Norton R. 2005. Molecular characterisation of *Burkholderia pseudomallei* isolates from Queensland. Australian Society for Microbiology Queensland Branch Meeting (oral presentation).
3. Lazzaroni S, Barnes J, Govan B, Norton R, Ketheesan N. 2006. Immune responses in melioidosis. Australian Institute of Medical Scientists 21st Annual Conference (oral presentation).
4. McAllister J, Lazzaroni S, Barnes J, Shahin S, Gauci P, Govan B, Ketheesan N, Proll D. 2006. Assessment of DNA vaccines against *Burkholderia pseudomallei*. Australian Society of Microbiology (poster presentation).

ABSTRACT

Burkholderia pseudomallei is the bacterium that causes the potentially fatal disease melioidosis. Endemic regions include South-east Asia and northern Australia. The immunopathogenesis of melioidosis is not fully understood as the bacterium can evade the host immune responses surviving within cells becoming apparent when the host is immunocompromised. Since *B. pseudomallei* is an intracellular organism, cell-mediated immune (CMI) responses are important in clearance and protection. The processes involved in the induction of a protective immune response is necessary to understand how the disease would progress and for developing vaccine strategies against *B. pseudomallei*. Therefore, the focus of the research in this thesis is to determine the extent of exposure to *B. pseudomallei* in northern Queensland and to characterise the CMI responses of seropositive healthy individuals from this endemic region. The immune responses involved in relapse and latent *B. pseudomallei* infection will also be determined by analysing the clonality of isolates from patients with recurrent melioidosis and developing a murine model of latency. The protective effect of DNA constructs of putative virulence factors will be studied in an experimental model of melioidosis.

Using the indirect haemagglutination assay, 1500 blood donors from northern Queensland were assessed for antibodies to *B. pseudomallei*. The results demonstrated 2.47% (n=37) of the population had an antibody response to *B. pseudomallei* with a titre of $\geq 1:40$. Using these seropositive donors, the Melioidosis IgG Rapid Cassette test was evaluated as a potential screening test for melioidosis. The results found only 32% were positive using this rapid test. This study demonstrated that individuals living in endemic regions may have been exposed to *B. pseudomallei* and consequently produced an antibody response. By characterising the CMI response of these seropositive individuals it will be possible to determine whether these individuals develop protective immunity against the bacterium.

Lymphocyte proliferation assays and flow cytometry were used to characterise the CMI response in seropositive individuals (n=8). Lymphocytes were isolated and stimulated

with *B. pseudomallei* antigen (BpLy1) the proliferative response was assessed, as well as activation of T cell subsets and cytokine production. Results demonstrated no significant differences in the proliferative response of lymphocytes in seropositive individuals when compared to seronegative individuals. There were no significant differences observed in CD4+ and CD8+ populations, interferon (IFN)- γ or interleukin (IL)-4 production. These results did not demonstrate an induction of a CMI response following stimulation with BpLy1. Although these seropositive individuals may have produced an antibody response to *B. pseudomallei*, the exposure was not sufficient to produce a CMI response.

Clonality of *B. pseudomallei* isolates from patients who had relapsed with melioidosis was determined using pulsed-field gel electrophoresis (PFGE). Fragment patterns produced following PFGE demonstrated clonal isolates were responsible for each relapse episode in four patients. One patient who had relapsed with melioidosis nine times over a period of eight years was due to the same *B. pseudomallei* isolate recrudescing rather than infection with a different isolate. These results suggest that the bacterium is able to evade the immune responses and cause continual episodes of melioidosis, therefore, treatment of the disease may need to be reassessed.

The development of a latent model of melioidosis involved C57BL/6 mice being infected with a low dose of a low virulence strain of *B. pseudomallei* (NCTC13179). The mice were allowed to clear the infection over a period of 30 days with bacterial load, delayed-type hypersensitivity (DTH) and lymphocyte proliferation responses assessed at days 10, 20 and 30. These results demonstrated that a CMI response had been produced with significant differences seen in the DTH response and proliferative response of lymphocytes following stimulation with BpLy1. There was also an apparent clearance of *B. pseudomallei*, with negative culture from spleen and liver of infected animals. Mice were then immunosuppressed with dexamethasone and monitored for survival, DTH and lymphocyte proliferation responses and bacterial load. Following immunosuppression, there was a significantly reduced immune response demonstrated by the DTH response. The proliferative response of lymphocytes in infected, immunosuppressed mice was not reflective of observations seen *in vivo* with no

differences observed in proliferation of lymphocytes, following stimulation with BpLy1, in infected, immunosuppressed mice compared to infected, non-immunosuppressed mice. Interestingly, results from bacterial loads demonstrated that by bacterial culture, *B. pseudomallei* was not present, however using real-time PCR (qPCR) *B. pseudomallei* DNA was detected in the spleens of both groups of mice. There was apparent reactivation of *B. pseudomallei* infection with a significant decrease in survival of infected mice that had been immunosuppressed and infected mice that had not been immunosuppressed. This aspect of the investigation demonstrates the initial development of an animal model of latent melioidosis.

Potential DNA vaccine constructs were evaluated using a BALB/c mouse model of experimental *B. pseudomallei* infection to determine their protective potential. The DNA vaccine constructs were developed against selective putative virulence factors of *B. pseudomallei*. Mice were vaccinated at three time points using a gene gun and subsequently challenged with a low virulence strain of *B. pseudomallei* (NCTC13179). Mice were monitored for survival and bacterial load. There were no significant differences seen in survival of mice vaccinated with different DNA constructs. When a combination of DNA constructs or CpG, an immunostimulator, was added to the vaccination regime, no significant differences were seen in survival of mice in these groups when compared to control mice. Splenic bacterial loads were not reduced in vaccinated groups when compared to control mice.

The results of this present study have provided further knowledge into host-pathogen interactions of *B. pseudomallei*. There is evidence that although there is exposure to *B. pseudomallei* in northern Queensland, detected by assessing antibody production, this exposure does not produce a CMI response which is essential in protection against the bacterium. Initial studies in determining whether *B. pseudomallei* persists within the host has been modelled in a murine model of latency. To produce a successful vaccine to protect against *B. pseudomallei* infection, further understanding of the immune responses and interaction of the bacterium is required.

TABLE OF CONTENTS

ABSTRACT.....	iv
LIST OF TABLES.....	xi
LIST OF FIGURES.....	xii
LIST OF ABBREVIATIONS.....	xiv
CHAPTER 1: INTRODUCTION.....	1
CHAPTER 2: LITERATURE REVIEW.....	5
2.1 INTRODUCTION.....	5
2.2 EPIDEMIOLOGY.....	5
2.2.1 <i>Epidemiology of melioidosis in Australia</i>	8
2.2.2 <i>Ecology of B. pseudomallei</i>	9
2.2.3 <i>Seroprevalence of melioidosis</i>	10
2.2.4 <i>Characterisation of B. pseudomallei isolates and clonality</i>	11
2.2.5 <i>Disease association and clonality of B. pseudomallei isolates</i>	14
2.3 ROUTE OF INFECTION.....	15
2.4 HOST RISK FACTORS.....	16
2.5 PATHOGENESIS AND ASSOCIATED VIRULENCE FACTORS.....	17
2.6 IMMUNE RESPONSES TO <i>B. PSEUDOMALLEI</i>	19
2.7 INNATE IMMUNE RESPONSE.....	19
2.7.1 <i>Toll-like receptors (TLRs) in B. pseudomallei infection</i>	22
2.7.2 <i>The role of cytokines in innate immunity</i>	22
2.7.3 <i>The role of dendritic cells</i>	25
2.8 ADAPTIVE IMMUNE RESPONSE.....	27
2.8.1 <i>Antibody response to B. pseudomallei</i>	27
2.8.2 <i>CMI response to B. pseudomallei</i>	28
2.9 CLINICAL PRESENTATION.....	31
2.9.1 <i>Acute infection</i>	32
2.9.2 <i>Chronic infection</i>	33
2.9.3 <i>Relapse</i>	33
2.9.4 <i>Latent/subclinical infection</i>	36
2.10 BACTERIOLOGY AND ISOLATION.....	36
2.11 DIAGNOSIS OF <i>B. PSEUDOMALLEI</i>	37
2.11.1 <i>Immunological diagnostic assays</i>	38
2.11.2 <i>Molecular diagnosis of B. pseudomallei</i>	40
2.12 TREATMENT.....	41
2.13 VACCINES.....	42
CHAPTER 3: GENERAL MATERIALS AND METHODS.....	45
3.1 BACTERIAL ISOLATES.....	45
3.1.1 <i>Handling Category 3 pathogen, B. pseudomallei</i>	45
3.1.2 <i>Origin of B. pseudomallei isolates</i>	45
3.1.3 <i>Preparation of B. pseudomallei lysate (BpLy1)</i>	45
3.2 EXPERIMENTAL ANIMALS.....	46
3.2.1 <i>Ethics approval</i>	46
3.2.2 <i>Maintenance of animals</i>	46
3.2.3 <i>Preparation and delivery of bacteria</i>	47
3.2.4 <i>Bacterial loads on animal organs</i>	47
3.2.5 <i>Lymphocyte proliferation assay</i>	47

CHAPTER 4: DETERMINATION OF THE SEROPREVALENCE TO <i>B. PSEUDOMALLEI</i> IN THE NORTHERN QUEENSLAND POPULATION.....	49
4.1 INTRODUCTION.....	49
4.2 MATERIALS AND METHODS.....	52
4.2.1 <i>Subjects</i>	52
4.2.2 <i>Antigen preparation</i>	52
4.2.3 <i>Indirect haemagglutination assay</i>	52
4.2.4 <i>Melioidosis IgG Rapid Cassette Test</i>	53
4.3 RESULTS	
4.3.1 <i>Serum antibody levels</i>	54
4.4 DISCUSSION.....	55
CHAPTER 5: CHARACTERISATION OF CELL-MEDIATED IMMUNE RESPONSE IN SEROPOSITIVE DONORS.....	60
5.1 INTRODUCTION.....	60
5.2 MATERIALS AND METHODS.....	61
5.2.1 <i>Subjects</i>	61
5.2.2 <i>Lymphocyte proliferation assay</i>	62
5.2.3 <i>Intracellular cytokine staining</i>	62
5.2.4 <i>Flourescent-activated cell scanning (FACS) analysis</i>	63
5.2.5 <i>Statistical analysis</i>	63
5.3 RESULTS.....	64
5.3.1 <i>Lymphocyte proliferation</i>	64
5.3.2 <i>FACS analysis of lymphocytes from donors</i>	64
5.4 DISCUSSION.....	69
CHAPTER 6: RELAPSE AND LATENCY IN <i>B. PSEUDOMALLEI</i> INFECTION.....	73
6.1 INTRODUCTION.....	73
6.2 MATERIALS AND METHODS.....	75
6.2.1 <i>Characterisation of B. pseudomallei isolates from patients with recurrent melioidosis</i>	75
6.2.1.1 <i>Collection and recovery of B. pseudomallei isolates from patients</i>	75
6.2.1.2 <i>Preparation of plugs for PFGE</i>	76
6.2.1.3 <i>Washing and enzymatic treatment</i>	77
6.2.1.4 <i>Preparation of gel for PFGE</i>	77
6.2.1.5 <i>Loading and electrophoresis</i>	78
6.2.1.6 <i>Staining of agarose gel</i>	78
6.2.2 <i>Murine model of latent melioidosis</i>	78
6.2.2.1 <i>Infection of mice</i>	78
6.2.2.2 <i>Delayed-type hypersensitivity (DTH) response to B. pseudomallei</i>	78
6.2.2.3 <i>Bacterial loads in mice</i>	78
6.2.2.4 <i>Lymphocyte proliferation assays</i>	79
6.2.2.5 <i>Storage of organs and extraction of DNA for qPCR</i>	79
6.2.2.6 <i>Immunosuppression of mice</i>	79
6.2.2.7 <i>Real-time PCR</i>	80
6.2.2.8 <i>Statistical analysis</i>	80
6.3 RESULTS.....	80
6.3.1 <i>Clonality of isolates from patients with recurrent melioidosis</i>	80
6.3.2 <i>DTH responses following infection with B. pseudomallei</i>	82
6.3.3 <i>Lymphocyte proliferation following infection with B. pseudomallei</i>	82
6.3.4 <i>Bacterial loads following infection with B. pseudomallei</i>	83

6.3.5	<i>DTH responses following immunosuppression</i>	83
6.3.6	<i>Lymphocyte proliferation following immunosuppression</i>	84
6.3.7	<i>Bacterial loads following immunosuppression</i>	85
6.3.8	<i>Monitoring survival following immunosuppression</i>	85
6.4	DISCUSSION.....	87
CHAPTER 7: EVALUATION OF DNA VACCINES FOR PROTECTION AGAINST EXPERIMENTAL <i>B. PSEUDOMALLEI</i> INFECTION		92
7.1	INTRODUCTION.....	92
7.2	MATERIALS AND METHODS.....	94
7.2.1	<i>Preparation of candidate vaccines</i>	94
7.2.2	<i>Vaccination studies</i>	94
7.2.3	<i>Challenging with <i>B. pseudomallei</i> following vaccination</i>	95
7.2.4	<i>Monitoring mice for survival following challenge with <i>B. pseudomallei</i></i>	96
7.2.5	<i>Bacterial loads following challenge with <i>B. pseudomallei</i></i>	96
7.2.6	<i>Statistical analysis</i>	96
7.3	RESULTS.....	97
7.3.1	<i>Survival of vaccinated mice</i>	97
7.3.2	<i>Bacterial loads</i>	97
7.4	DISCUSSION.....	99
CHAPTER 8: GENERAL DISCUSSION		105
REFERENCES		112
APPENDIX 1: SOLUTIONS AND REAGENTS		127
APPENDIX 2: FACS DATA FOR CHARACTERISATION OF CMI RESPONSES IN SEROPOSITIVE INDIVIDUALS		132

LIST OF TABLES

Table 2.1	Cell-associated virulence factors of <i>B. pseudomallei</i>	18
Table 2.2	Secretory virulence determinants of <i>B. pseudomallei</i>	19
Table 2.3	Clinical presentations of melioidosis.....	32
Table 2.4	Characterisation of <i>B. pseudomallei</i> isolates from relapse patients...	35
Table 4.1	Exposure of <i>B. pseudomallei</i> as assessed by indirect haemagglutination assay.....	55
Table 5.1	Combinations of monoclonal antibodies used in this study.....	63
Table 6.1	<i>B. pseudomallei</i> isolates cultured from patients with recurrent melioidosis.....	76
Table 6.2	Bacterial loads and qPCR following immunosuppression of mice...	86
Table 7.1	Oligonucleotides used for amplification of <i>B. pseudomallei</i> gene targets.....	95

LIST OF FIGURES

Figure 2.1	Global distribution of melioidosis.....	7
Figure 2.2	Activation and role of DCs.....	26
Figure 4.1	Example of Melioidosis IgG Rapid ICT Cassette tests.....	54
Figure 4.2	Positive IHA titres of $\geq 1:40$	55
Figure 5.1	Proliferation of lymphocytes from seropositive and seronegative donors in a) response to BpLy1 and b) in response to BpLy1 and PPD.....	65
Figure 5.2	Percentage difference between BpLy1 stimulated and unstimulated a) CD3+/CD4+ cells and b) CD3+/CD8+ cells.....	66
Figure 5.3	Percentage difference between BpLy1 stimulated and unstimulated a) activated CD4+ cells producing IL-4 and b) activated CD4+ cells producing IFN- γ cells.....	67
Figure 5.4	Percentage difference between BpLy1 stimulated and unstimulated a) activated CD8+ cells producing IL-4 and b) activated CD8+ cells producing IFN- γ cells.....	68
Figure 6.1	Example of PFGE image of isolates culture from patient A with recurrent melioidosis.....	81
Figure 6.2	Example of PFGE image of isolates culture from patients D, E & F with recurrent melioidosis.....	82
Figure 6.3	DTH responses at days 10, 20 and 30 following low dose infection with <i>B. pseudomallei</i>	83
Figure 6.4	Lymphocyte proliferaton responses at days 10, 20 and 30 following low dose infection with <i>B. pseudomallei</i>	84
Figure 6.5	DTH responses at day 60 post infection following immunosuppression of mice.....	85
Figure 6.6	Lymphocyte proliferaton at day 60 post infection with <i>B. pseudomallei</i> following immunosuppression with dexamethasone.....	86

Figure 6.7	Survival of infected mice following immunosuppression with dexamethasone.....	87
Figure 7.1	Survival rates of vaccinated BALB/c mice following challenge with NCTC 13179 at a dose of a) 3X LD ₅₀ , b) 2.5X LD ₅₀ and c) 2X LD ₅₀	98
Figure 7.2	Day two splenic bacterial loads of vaccinated BALB/c mice challenged with NCTC 13179 at a dose of a) 2.5X LD ₅₀ and b) 3X LD ₅₀	99

LIST OF ABBREVIATIONS

APC – antigen presenting cell	NO – nitric oxide
BpLy1 – <i>B. pseudomallei</i> lysate (NCTC13179)	OD – optical density
BSA – bovine serum albumin	ODN – oligodeoxynucleotides
CDC – Centre of Disease Control and Prevention	PAMP – pathogen-associated molecular patterns
CFA – culture filtrate antigen	PBS – phosphate buffered saline
cfu – colony forming units	PC – physical containment
CMI – cell-mediated immunity	PCR – polymerase chain reaction
CO ₂ – carbon dioxide	PFGE – pulsed field gel electrophoresis
Con A – concavalin A	PHA – phytohaemagglutinin
Da – dalton	PMA – phorbol myristate acetate
DC – dendritic cell	PMED – particle-mediated epidermal delivery
DNA – deoxyribonucleic acid	PMNL – polymorphonuclear leukocytes
EDTA – ethylene diamine tetraacetic acid	PPD – purified protein derivative
ELISA – enzyme linked immunosorbent assays	PS - polysaccharide
FACS – fluorescent-activated cell sorting	qPCR – real-time PCR
FBS – foetal bovine serum	RAPD – random amplified polymorphic DNA
ICT – immunochromatographic test	RNA – ribonucleic acid
ID – intradermal	rRNA – ribosomal RNA
IFA – immunofluorescent antibody assays	<i>sc</i> – subcutaneous
IFN – interferon	SI – stimulation index
Ig – immunoglobulin	TE – Tris-EDTA
IHA – indirect haemagglutination assay	TBE – Tris-Boric-EDTA
IL - interleukin	TES – Tris-EDTA sarcosine
<i>ip</i> – intraperitoneal	Th – T helper
<i>in</i> – intranasal	TLRs – toll-like receptors
iNOS – inducible nitric oxide synthase	TNF – tumour necrosis factor
<i>iv</i> – intravenous	TTSS – type III secretion system
kb – kilobases	
kDa – kilodalton	
LPS – lipopolysaccharide	
MAb – monoclonal antibodies	
MEE – multilocus enzyme electrophoresis	
MLST – multilocus sequence testing	
MNL – mononuclear leukocytes	
mRNA – messenger RNA	
NF – nuclear transcription factor	
NK – natural killer	

