The Detection of *Coxiella burnetii* (Q fever) in Clinical and Environmental Samples

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Declaration

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

Michelle Lockhart

Abstract

The zoonotic intracellular bacterium *Coxiella burnetii* is the cause of the human disease Q fever. *Coxiella burnetii* can be shed by infected animals, can survive harsh environments and has been shown to persist within the human host. The detection and isolation of this bacterium is difficult due to its intracellular nature. In order to detect minimal concentrations of this bacterium in various clinical and environmental samples, highly sensitive assays were needed. A duplex real-time polymerase chain reaction (qPCR) assay was developed to detect *C. burnetii* DNA (targeting the *Com1* gene and the *IS1111a* gene). This assay was then tested on a variety of environmental and clinical sample types.

Samples (such as water, soil, aerosols, blood and bone marrow) were spiked with *C. burnetii* (either living cell cultures or formalin killed cells) to determine the optimal method for extracting and detecting *C. burnetii* DNA. The silica column method followed by qPCR assay of the *Com1* gene was shown to have a sensitivity of approximately 1100 copies/litre in water, 1900 copies/kg in soil, 870 copies/litre in milk, and seven copies/litre of air. When the same technique was applied to clinical samples the silica column method proved to be the most effective in purifying DNA from the small cell variant of *C. burnetii* and effectively removed potential PCR inhibitors from mock clinical samples of blood, plasma, serum and bone marrow. However, because the qPCR cannot differentiate between viable and non-viable *C. burnetii* DNA it was important to establish a sensitive assay for the detection of viable *C. burnetii* in order to investigate persistent infections and to obtain isolates of the bacteria from cases of Q fever for further studies.

As isolation of *Coxiella* can be achieved using cell culture or animal inoculation these methods were compared for their sensitivity for *C. burnetii* detection. Vero and DH82 cell lines were the most sensitive for cell culture isolation of the Arandale and Henzerling isolates of *C. burnetii* respectively. When cell culture was compared to PCR and inoculation of severely combined immunodeficient (SCID) mice it was found that inoculation of SCID mice followed by euthanasia (at day 42) and removal and analysis of the spleen was the most sensitive method for the detection of viable *C. burnetii*.

It has recently been hypothesised that genetic differences between isolates of *C. burnetii* are responsible for differences in pathogenicity and disease outcomes. Hence the differences between Australian isolates were investigated. Seven new Australian isolates of *C. burnetii* were genetically analysed by conventional PCR of insertion sequences and detection of the acute disease antigen A (*adaA*) gene. Six Australian isolates of *C. burnetii* were placed in geno-group III but were negative for the *adaA* gene. One new Australian isolate (Poowong) was placed in geno-group II and was positive for the *adaA* gene. The Poowong isolate was from a seronegative asymptomatic patient, with bacteraemia detected by PCR in four initial samples as well as all 12 blood samples taken over a one month period. Through sequencing of 468bp of the *ankyrin* gene (*ankH* sequenced in triplicate) it was shown that the Poowong isolate had two base pair differences compared to the Henzerling isolate (also geno-group II) and the Nine Mile isolate (geno-group I). This demonstrates that the Poowong isolate can be distinguished from the other isolates within the laboratory.

The optimal methods of detection as determined in this study were used to analyse and evaluate clinical specimens. Blood samples (serum, plasma and peripheral blood mononuclear cells) from 12 patients infected during an outbreak of Q fever in Newport UK in 2002 were examined. Cell culture of the peripheral blood mononuclear cells (PBMC) demonstrated that no viable *C. burnetii* cells were present. In contrast, six of the spleens from SCID mice inoculated with the PBMCs were positive for *C. burnetii* DNA (by *Com1* qPCR) and six were positive for *C. burnetii* antigen (by IFA). However, only two were positive for both. This suggests that in some patients low numbers of viable *C. burnetii* cells persist and in others *C. burnetii* persist as non-viable antigen.

In conclusion, this study demonstrated sensitive and specific optimal methods for the detection of *C. burnetii* in clinical and environmental samples, the optimal method for isolation of *C. burnetii*, the application of these methods on a number of clinical samples and the characterisation of seven new isolates, including an isolate from a highly unusual asymptomatic case that is genetically unique from the others. This study has also shown that the pathogenesis of *C. burnetii* infection in humans and the effect of genetic differences in isolates on pathogenesis are far from adequately understood. The optimal methods of detection, isolation and grouping determined in this study will have an effect on future studies and will allow a greater understanding of *C. burnetii* and its persistence, both in the environment and in Q fever infections.

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Co-Authored Manuscripts

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Abbreviations

-	Negative
+	Positive
A	Adenine
ACT	Australian Capital Territory
AQIS	Australian Quarantine and Inspection Service
C	Cytosine
CFS	Chronic Fatigue syndrome
CPE	Cytopathic effect
Ct	Cycling threshold
DD	Detection dose
DNA	Deoxyribonucleic acid
DPI	Death Post Infection
EDTA	Ethylene-Diamine-Tetra-Acetic acid
ELISA	Enzyme Linked Immunosorbent Assay
FITC	Fluorescent-Labeled anti-human Conjugate
G	Guanine
H&E	Hematoxylin and Eosin
HBSS	Hanks' Balanced Salt Solution
HEPES	4-(2-HydroxyEthyl)-1-PiperazineEthaneSulfonic acid
ID	Infectious Dose
IF	Immunofluorescence
IFA	IF assay
IgA	Immunoglobulin A

IgG	Immunoglobulin G
IgM	Immunoglobulin M
IRS	Infrequent Restriction Site
IS	Insertion Sequence
LCD	Low Cost and Density
LCN	Light Cycler Nested
LCV	Large Cell Variant
LD	Lethal Dose
LPS	Lipopolysaccharide
MLVA	Multiple-Locus VNTR Analysis
MST	Multi-Spacer Sequence Typing
ND	Not Done
NP	No Plasmid - plasmid sequence integrated into genome
NSW	New South Wales
NT	Northern Teritory
NTC	No Template Control
OD	Optical Density
OMP	Outer Membrane Protein
PAGE	Poly Acrylamide Gel Electrophoresis
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PC1	Physical Containment level 1
PC2	Physical Containment level 2
PC3	Physical Containment level 3
PCR	Polymerase Chain Reaction
PFGE	Pulse Field Gel Electrophoresis

QFS	Post Q Fever Fatigue Syndrome
QLD	Queensland
qPCR	Quantitative Real-Time PCR
RBC	Red Blood Cell
RBCL	RBC Lysis buffer
RFLP	Restriction Fragment Length Polymorphism
RPMI	Roswell Park Memorial Institute media
rRNA	Ribosomal Ribonucleic acid
SA	South Australia
SCID	Severe Combined Immuno-Deficient mice
SCV	Small Cell Variant
SDS	Sodium Dodecyl Sulfate
T	Thymine
TCID	Tissue Culture Infectious Dose
UK	United Kingdom
USA	United States of America
VIC	Victoria
VNTR	Variable Number Tandem Repeats
WA	Western Australia