Development of tools for surveillance of *Coxiella burnetii* in domestic ruminants and Australian marsupials and their waste

Presented By

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Declaration

The experiments in this thesis constitute work carried out by the candidate unless otherwise stated. The thesis is less than 100,000 words in length, exclusive of tables, figures, bibliography and appendices, and complies with the stipulations set out for the degree of Doctor of Philosophy by Murdoch University.

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Finally, I’d like to thank my friends and family who have graciously accepted my moods and general lack of availability. This is especially true of Cynthia who has only known me as a PhD student and perhaps doesn’t know what she’s been missing.
Aims

1. To develop and apply quantitative polymerase chain reaction tools for the detection of *Coxiella burnetii* in waste from livestock production industries.

2. To develop a quantitative assay to assess the efficacy of disinfectants against *Coxiella burnetii* in liquid waste from livestock production.

3. To develop molecular tools to determine the viability of *Coxiella burnetii* recovered from wastes of livestock production.

4. To develop an enzyme-linked immunosorbent assay for the detection of anti-*Coxiella burnetii* antibodies in Australian marsupials.
Thesis abstract

The aim of this study was to develop improved methods to detect viable Coxiella burnetii in wastes from livestock production. The impetus for this work arose because there is a significant risk of infection for humans attributed to contact with waste products from the livestock production industry. This situation is further compounded by the lack of suitable tools to detect viable C. burnetii in these wastes. In addition, effective disinfection strategies for livestock wastes are also required to reduce the risk of infection with C. burnetii for individuals that come into contact with these waste products.

A quantitative real-time PCR system (qPCR) with high sensitivity and specificity was developed to detect the C. burnetii in environmental samples associated with domestic ruminants and native Australian marsupials. Different detection chemistries and procedures were evaluated based on their sensitivity, specificity and reproducibility. Overall it was found that the TaqMan PCR targeting the IS1111a locus provided the most sensitive and reproducible test. The Geneworks PowerSoil™ DNA isolation kit provided the best compromise between reproducibility and recovery of DNA from livestock wastes. When combined, the IS1111a TaqMan qPCR and Geneworks PowerSoil DNA Extraction Kit provided a test which was capable of detecting as few as two C. burnetii genome equivalents in 0.2g of soil or faeces.

Coxiella burnetii has been shown to display extreme resistance to environmental exposure. Therefore, assessment of the viability of the organism in environmental matrices is more useful for risk assessment programs than detection of DNA alone. A quantitative reverse transcriptase PCR was developed
that was able to detect viable *C. burnetii* cells in soil. The sensitivity of the assay was enhanced by heat-treating the soil samples prior to extraction of RNA.

The factor most often associated with transfer of *C. burnetii* to humans is exposure to livestock or their waste. Therefore, decontamination of waste from livestock production industries is a key factor in preventing outbreaks of Q fever. A system was developed to determine the efficacy of various disinfectant treatments against the environmental pathogen *C. burnetii*. Treatments evaluated included sodium hypochlorite, ozone, ultraviolet light, peracetic acid (PAA), and Virkon S®. Sodium hypochlorite at a concentration of 0.1 mM reduced the infectivity of *C. burnetii* by over 92% while treatment with the same sodium hypochlorite concentration in wastewater showed significantly reduced efficacy. Despite this reduced potency, sodium hypochlorite is still useful for control of *C. burnetii* in the liquid waste of animal production.

Commercially available ELISA and CFT assays exist for ruminants but there are no immunological tests available for detecting *C. burnetii* in marsupials even though Australian marsupials are known to be susceptible to *C. burnetii*. An indirect ELISA for detecting anti-*Coxiella* antibodies in kangaroos was developed. Paired serum and faecal samples were taken from 379 ruminants from Western Australia and the serum was tested with a commercially available ELISA and the complement fixation test while the faeces was tested using the qPCR developed during this study. Paired serum and faecal samples were taken from 343 kangaroos from WA and were tested with the antibody-ELISA developed during this study and by qPCR. A very low prevalence of anti-*Coxiella* antibodies was observed in the ruminants sampled and results from immunological tests correlated poorly with qPCR data. The development of an ELISA for use with kangaroo serum was problematic because of the lack of reference sera from
animals known to be infected with *C. burnetii*. Despite this results from the ELISA developed suggested that the apparent seroprevalence in the WA animals surveyed was approximately 34%. Results from testing kangaroo faeces with the qPCR correlated poorly with the results from the antibody-ELISA. These data suggest that kangaroos may be a significant reservoir of *C. burnetii* in Western Australia and due to cohabitation of kangaroos and domestic ruminants, may provide a link between the wildlife and domestic cycles of *C. burnetii*. 
## Abbreviations

- `<` less than
- `>` more than
- `≤` less than or equal to
- `≥` more than or equal to
- `±` plus or minus
- `%` percent
- `μ (prefix)` micro \( (10^{-6}) \)
- `p (prefix)` pico \( (10^{-9}) \)
- `°C` degrees Celsius
- `ABTS` 2,2’-azino-di-(3-ethylbenzylhiazoline-6-sulfonate)
- `CFT` complement-fixation test
- `CT` cycle threshold
- `DMEM` Dulbecco’s modified eagle’s medium
- `DMSO` dimethyl sulfoxide
- `EDTA` ethylenediamine-tetra acetic acid, tri-potassium salt
- `ELISA` enzyme-linked immunosorbent assay
- `et al.` and others
- `FCS` foetal calf serum

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<table>
<thead>
<tr>
<th>Symbol</th>
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<tbody>
<tr>
<td>$g$</td>
<td>unit of gravitational field</td>
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<tr>
<td>HP</td>
<td>highly pure</td>
</tr>
<tr>
<td>HRP</td>
<td>horseradish peroxidase</td>
</tr>
<tr>
<td>IFAT</td>
<td>indirect fluorescent antibody test</td>
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<tr>
<td>L</td>
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</tr>
<tr>
<td>LCV</td>
<td>large cell variant</td>
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<tr>
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<td>lippopolysaccharide</td>
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<td>molar concentration</td>
</tr>
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</tr>
<tr>
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</tr>
<tr>
<td>NaOCl</td>
<td>sodium hypochlorite</td>
</tr>
<tr>
<td>NT</td>
<td>no treatment</td>
</tr>
<tr>
<td>NTC</td>
<td>no template control</td>
</tr>
<tr>
<td>OD</td>
<td>optical density</td>
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<td>Description</td>
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<td>-------------</td>
</tr>
<tr>
<td>PAA</td>
<td>peracetic acid</td>
</tr>
<tr>
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</tr>
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<td>qPCR</td>
<td>quantitative polymerase chain reaction</td>
</tr>
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<td>SCV</td>
<td>small cell variant</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SDC</td>
<td>small dense cell</td>
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<td>Tris (hydroxymethyl) methylamine EDTA</td>
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<tr>
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</tr>
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