Serological evidence of *Coxiella burnetii* infection in beef cattle in Queensland

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**Background** Queensland has the highest incidence of Q fever in Australia. The aim of this study was to undertake a cross-sectional seroprevalence survey of *Coxiella burnetii*, the causative agent of Q fever, in beef cattle in Queensland.

**Methods** Serum samples were tested by ELISA for both phase II and phase I antigens of the organism using an Australian isolate. Blood samples were collected at an abattoir that processes beef cattle originating from northern and north-western Queensland, in addition to blood samples taken from beef cattle across Queensland as part of a second survey.

**Results** Seropositivity was 16.8% (95% confidence interval 16.7–16.8%).

**Conclusion** Evidence of *C. burnetii* infection in beef cattle has public health implications for occupational exposure of primary producers and veterinarians and for the proximity of beef cattle properties to residential areas in regional Queensland. This study is the first known investigation of *C. burnetii* seroprevalence in beef cattle in Queensland and the first known use of an Australian *C. burnetii* isolate for screening using both phase II and phase I antigens.

**Keywords** Australia; beef cattle; *Coxiella burnetii*; Q fever; Queensland; serology

**Abbreviations** CI, confidence interval; ddH2O, double-distilled water; ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; LPS, lipopolysaccharide; OD, optical density; PBS, phosphate-buffered saline

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Q fever is a worldwide zoonosis caused by *Coxiella burnetii*, an obligate intracellular bacterium.¹ In animals the infection is referred to as coxiellosis. The organism is ubiquitous, with a geographical spread extending worldwide, with the exception of New Zealand.² *Coxiella burnetii* exists in two antigenically different phases, which are characterised by transition of lipopolysaccharide (LPS) from a smooth form with full-length O-side chains in phase I to a rough form with truncated O-side chains in phase II. In humans, differences in the titres of immunoglobulin (Ig) subsets to phase I or II antigens form the basis for diagnosis of acute (antibodies to phase II antigens) or chronic (antibodies to phase I antigens) Q fever.³ It has been demonstrated that animals infected with *C. burnetii* produce antibodies to both phase I and phase II antigens.³ Most commercial animal Q fever enzyme-linked immunosorbent assays (ELISAs) consist of a mixture of phase I and phase II antigens.

The domestic reservoir, consisting of cattle, sheep and goats, is considered the major source of transmission of *C. burnetii* infection to humans in Australia. *Coxiella burnetii* is thought to be initially transmitted to livestock via ticks, which form part of the natural transmission cycle of the organism in wildlife. Kangaroos⁴,⁵ and bandicoots⁶ have been identified as potential reservoirs of Q fever in Australia. Human infection is usually acquired by inhalation of contaminated material from infected animals. Less commonly, infection may result from consumption of infected animal products, such as unpasteurised milk. Very rarely, it may result via sexual transmission following exposure to infected body fluids.⁷ Those most at risk of Q fever infection include individuals with occupational contact with reservoir animals, particularly those in the meat and livestock industries, veterinarians and laboratory workers.¹ The organism is capable of long-term environmental persistence, it being suggested but not conclusively shown that this persistence may be in a spore-like form.⁸ As a result of environmental contamination, a number of outbreaks of Q fever are reported to have occurred following strong winds blowing through areas where livestock are bred, held or transported.⁹,¹⁰

Since 1934, when sporadic cases of fever with a typhoid-like presentation became apparent to medical practitioners in Brisbane, outbreaks of Q fever in Australia have been predominantly associated with abattoirs.¹⁰ In a recent series of cases over a 20-year period in southern Australia, 92% of the 111 cases of Q fever were among abattoir workers.¹¹ In North Queensland, a study of Q fever cases during 1994–2006, found that 22% of acute and 33% of chronic Q fever patients reported exposure to cattle.¹² This trend extends internationally, where an occupation in the meat-processing industries has been associated with increased risk of Q fever infection.¹³

Infections in animals are usually subclinical, although infection can be associated with abortions and reproductive disorders.¹⁴–¹⁶ Seropositivity is believed to be seasonal in livestock, particularly sheep, as serological studies indicate the presence of recurring annual cycles of antibody prevalence in response to *C. burnetii* exposure during lambing.¹⁶ More recent studies in dairy cattle indicate that seropositivity is related to changes in endocrine patterns during gestation.¹⁷ However, these studies were performed overseas and no comparable data are available for Australia.

In epidemiological studies during the past 6 years, the seroprevalence of *C. burnetii* in cattle populations has varied according to geographical location: 6.2% in Northern Ireland,¹⁸ 87.9% in Albania,¹⁹ 10.75% in Iran,²⁰ 14.3% in the Central African Republic,²¹ 24% in Newfoundland²² and Cyprus²³ and 25.6% in Korea.²⁴ Previous serological investigations of the prevalence of *C. burnetii* infection in Australian cattle
demonstrated it was not common in beef cattle in Western Australia\(^4\) and South Australia,\(^3\) dairy cattle in Victoria\(^6\) or beef and dairy cattle in New South Wales,\(^2\) with seroprevalence in all four studies found to be >1%. Although the incidence of Q fever in humans is much higher in Queensland compared with the other states,\(^7\) no record of serological surveys performed on beef cattle in Queensland has been found.

Queensland has the largest beef cattle herd in Australia and is also the largest producer and exporter of beef cattle.\(^2\) Beef cattle production represents the largest agricultural industry in the state and was valued at A$3.4 billion in 2007–08.\(^3\) The Queensland beef cattle herd is estimated to be 12.2 million head, 96% of which is managed on 14,568 specialised beef cattle properties.\(^2\)

To determine the risk of microbial infection, the sources of potential infection and routes of transmission must be identified. Consequences associated with a lack of such knowledge were highlighted when an outbreak of Q fever occurred at an Australian abattoir slaughtering feral goats.\(^3\) Poor understanding of both the transmission of \(C. burnetii\) and the potential for goats as a reservoir resulted in a failure to use methods of reducing the production of aerosols. The capacity to identify such potential sources of Q fever infection in Australia, particularly in the tropical north, is compromised by the lack of data regarding reservoir populations. The aims of this study were to undertake a serological survey to establish the prevalence of \(C. burnetii\) infection in beef cattle in Queensland and to determine if seropositivity varied seasonally.

**Materials and methods**

**Bovine blood collection**

Blood samples were obtained immediately following slaughter of beef cattle at an abattoir in the Townsville Shire District of Northern Queensland. The catchment area of this abattoir includes the regions of northern, far-northern and north-western Queensland, as well as the Northern Territory. The abattoir has a daily processing capacity of 900 beasts. Samples (n = 730) were collected during 2006–07 on four separate occasions, approximately 6 months apart, with 150–200 samples collected randomly on each occasion. Samples were stratified by cattle property to ensure a representative spectrum of the northern Queensland beef industry was covered, with animals originating from 17 different cattle properties in 8 districts. Only properties with greater than 10 samples were included in the statistical analyses.

A further set of samples was obtained from a collection of randomly selected samples (n = 1344) taken from breeders and heifers originating from 46 different Queensland beef cattle properties in 24 districts during 2008–09. A statistically valid sample size was calculated for each property based on the total number of cattle present in each mob. Serum was separated from each blood sample by centrifugation and stored at −20°C.

**Preparation of ELISA antigens**

Antigen was prepared according to a protocol described elsewhere.\(^3\) Both phase I and phase II \(C. burnetii\) antigens were produced using an Australian \(C. burnetii\) isolate (Cumberland strain). This isolate was obtained from the Australian Rickettsial Reference Laboratory, Geelong, Victoria, and came from a patient who contracted Q fever through contact with beef cattle. Phase II \(C. burnetii\) was obtained by serial passage in Vero cell culture to a total of 15 passages. Cell suspensions and supernatants were pooled and centrifuged at 1000g at 20°C for 10 min. Supernatants were discarded and the cells resuspended in phosphate-buffered saline (PBS), pH 7.4. Host cells were disrupted by sonication and bacteria were separated from cell debris by centrifugation at 550g at 20°C for 10 min. The resultant supernatant was layered over 25% sucrose and centrifuged at 4500g at 20°C for 20 min. The supernatant was discarded and the \(C. burnetii\) resuspended in PBS, pH 7.4. The bacteria were inactivated with 1% formalin for 48 h at 4°C. Inactivated \(C. burnetii\) pellets were pelleted at 10,000g at 20°C for 10 min and washed three times in sterile double-distilled water (ddH\(_2\)O). The inactivated cells were resuspended in sterile ddH\(_2\)O, after which they were referred to as whole cell antigen.

Phase I antigen was produced via animal passage in A/J strain mice, followed by a single passage in the yolk sac of embryonated chicken eggs. Mice were inoculated with \(1 \times 10^8\) \(C. burnetii\) LP and maintained for 7 days prior to euthanasia by CO\(_2\) asphyxiation. The spleens were harvested for bacterial extraction as described for phase II antigen. Animal passage was carried out under approval of the James Cook University Animal Ethics Committee under PC3 conditions (A1139). Bacteria were separated from egg yolk and formalin-inactivated as described for phase II antigen.

Phase I and II antigenicity was confirmed by complement block titration using commercial anti-\(C. burnetii\) phase I and I control sera and antigens (Virion/Serion, Germany). The commercially available phase I and II \(C. burnetii\) antigen and control sera were initially used for comparison and quality control of the prepared antigen. Absorbance values for control sera against commercial antigen were comparable to those obtained with control sera against in-house prepared ELISA antigen. The ELISA was initially optimised and validated using serum from C57BI/6 mice experimentally infected with \(C. burnetii\) and PBS-inoculated negative controls, carried out under approval of the James Cook University Animal Ethics Committee under PC3 conditions (A1139).

**Bovine screening ELISA**

The screening ELISA used an indirect design of separately coating the wells of NUNC\(^\text{TM}\) Maxisorp plates with the phase I and phase II antigens. Plates were coated with antigen at 50 μg/mL and incubated at room temperature overnight in a humidified chamber. The plates were then blocked and stabilised with 100 μL of post-coating buffer (Trop-Bio, QLD, Australia), incubated at room temperature for 2 h, before the supernatant was removed and the plates dried. Plates were then sealed for later use. Coated plates were incubated with 50 μL aliquots of the animal serum to be tested at 1/100 dilutions and left to incubate at 37°C for 1 h. The plates were washed with PBS and 0.05% Tween 20 and then 50 μL 1/1,000 rabbit anti-bovine Ig-horseradish peroxidase conjugate (Serotec, NSW, Australia) was added to each well and left to incubate at 37°C for 1 h. The plates were washed again and 100 μL of ABTS was added to each well and left to incubate at 37°C for 30 min. The optical density (OD) at 414–494 nm was then determined using a Multiskan EX (Labsystems) plate reader. Initially, 30 samples were randomly selected and tested as described. Five high-reacting samples and five low-reacting samples were each pooled and used as the positive and...
negative control sera respectively. The ratio of the sample OD to positive control OD (S/P%) was calculated for each sample: S/P% = (OD sample – OD negative control) ÷ (OD positive control – OD negative control) × 100. An S/P% < 30% was considered to be negative; between 30% and 50% was considered to be weak positive; between 50% and 70% was considered to be positive; >70% was considered to be strongly positive. Sera with an S/P% > 50% to either or both phase II and phase I antigens were considered to be positive.

**Statistical analyses**

ANOVA was performed on seasonality data to determine whether differences in beef cow seropositivity at various times of year were statistically significant.

### Results

**Screening of bovine sera from abattoir**

Of the 720 abattoir samples collected, 67 (9.3%, 95% confidence interval (CI) 9.29–9.32%) and 34 (4.7%, 95% CI 4.70–4.73%) were positive for antibodies to phase II or phase I C. burnetii antigen, respectively. Of these, 28 (38.9%) were positive for both phases, with 82.3% of beef cattle positive for phase II and phase I also positive for phase I. A total of 73 (10.1%, 95% CI 10.1–10.2%) samples were seropositive for either or both antigenic phases. A further breakdown of seropositivity is shown in Table 1. Positive samples were returned from 40% of the properties. Approximately 70% of the phase II and 80% of the phase I positive samples came from a single property in the Townsville Shire, recording a high seroprevalence of 26.7% and 13.7% for phase II and phase I, respectively.

**Screening of bovine sera from breeders and heifers**

Of the 1344 samples tested, 134 (9.9%, 95% CI 9.9–10.0%) and 145 (10.7%, 95% CI 10.7–10.8%) were positive for antibodies to phase II or phase I C. burnetii antigen, respectively. Of these, 28 (26.9%) were positive for both phases, with 82.3% of beef cattle positive for phase II and phase I also positive for phase I. A total of 72 (5.4%) and 84 (6.2%) samples were seropositive for either or both antigenic phases. A further breakdown of seropositivity is shown in Table 1. Positive samples were returned from 78.2% of the properties. Seropositivity ranged from 0.0% to 65.4% for antibodies to phase II antigen and from 0.0% to 46.7% for antibodies to phase I antigen.

**Overall seropositivity in cattle**

Overall seropositivity to phase II and phase I C. burnetii antigens in beef cattle sampled (n = 1835) in Queensland was 10.0% (95% CI 9.9–10.0%) and 9.2% (95% CI 9.20–9.22%), respectively. Seropositivity to either or both antigenic phases of C. burnetii was 16.8% (95% CI 16.7–16.8%). The percentage of samples positive for both phase II and phase I differed between the two sample sets, with abattoir samples having a higher percentage of antibodies to both antigens compared with the breeder and heifer samples. Samples were obtained from 58 mobs on 56 beef cattle properties located in 24 districts across Queensland. These districts covered most of the statistical divisions of Queensland, with the exception of the south-east corner. Seropositivity varied both between and within many of the districts and regions sampled (Table 2). The greatest variation was observed in the Fitzroy, Central West and Mackay regions.

**Seasonality of seropositivity**

Cattle seropositivity to both antigenic phases of C. burnetii at the time of pregnancy testing was lower in the earlier months of the year (March/April), higher mid-year (May/June) and increasingly higher in the later months of the year (August/September) (Figure 1). Similar trends were seen for seropositivity to both phase II and phase I antigens (Figure 1).

### Discussion

The current study demonstrated that the seroprevalence of C. burnetii in the bovine population sampled was approximately 10.0% (95% CI 9.9–10.0%) for phase II and 9.2% (95% CI 9.20–9.22%) for phase I, with an overall seropositivity of 16.8% (95% CI 16.7–16.8%). Although this is within the range of reported seroprevalences of C. burnetii in cattle in other parts of the world, it is still considered high. In this study, antibodies to both phase II and phase I C. burnetii antigens were detected by ELISA. This is the first known study to use an Australian C. burnetii isolate as a source of antigen in an ELISA. A recent Australian study conducted in Western Australia used a commercial Q fever ELISA developed in Europe (IDEXX CHEKIQ Q fever ELISA) and reported very low numbers of positive samples in beef cattle and sheep samples, despite relatively high numbers of positive samples being detected in kangaroo samples from the same areas. Differences in antigen production, such as the use of whole cell antigen, lysates or chemical extracts, may account for the variation in ELISA results. Also, it is possible that there are antigenic differences between C. burnetii isolates from Europe and Australia that account for the variations in ELISA results.

<table>
<thead>
<tr>
<th></th>
<th>S/P% (negative control)</th>
<th>S/P% (positive control)</th>
<th>S/P% (sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative</strong></td>
<td>(S/P% &lt; 30%)</td>
<td>(S/P% 30–50%)</td>
<td>(S/P% &gt; 70%)</td>
</tr>
<tr>
<td>Phase II</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Phase I</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Abattoir (n = 720)</td>
<td>653 (90.7)</td>
<td>686 (95.3)</td>
<td>43 (6.0)</td>
</tr>
<tr>
<td>Breeders and heifers (n = 1344)</td>
<td>1210 (90.0)</td>
<td>1199 (89.2)</td>
<td>62 (4.6)</td>
</tr>
<tr>
<td><strong>Weakly positive</strong></td>
<td>(S/P% 30–50%)</td>
<td>(S/P% 50–70%)</td>
<td>(S/P% &gt; 70%)</td>
</tr>
<tr>
<td>Phase II</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Phase I</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td><strong>Positive</strong></td>
<td>(S/P% 50–70%)</td>
<td>(S/P% &gt; 70%)</td>
<td></td>
</tr>
<tr>
<td>Phase II</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Phase I</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
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<tr>
<td><strong>Strongly positive</strong></td>
<td>(S/P% &gt; 70%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase II</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Phase I</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
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</tbody>
</table>

S/P%, ratio of the sample’s optical density (OD) to positive control OD, calculated for each sample: S/P% = (OD sample – OD negative control) ÷ (OD positive control – OD negative control) × 100.

Table 1. Breakdown of seropositivity in abattoir samples and samples collected from breeders and heifers
In serological studies of Q fever, high antibody titres to phase II antigen indicate acute infection and high antibody titres to phase I indicate chronic infection. The significance of antibody development to each antigenic phase of *C. burnetii* in animal infections has not been fully established. However, some studies have suggested that the presence of antibodies to phase II antigen in animal sera indicates recent infection. Serological tests for the presence of antibodies against *C. burnetii* in animals are unable to determine whether an animal is actively shedding the organism. Animals can remain seropositive for long periods after the initial infection has been cleared and some can seroconvert without shedding *C. burnetii*. Alternatively, animals may begin to shed the organism prior to the production of antibodies and some infected animals never demonstrate seroconversion. In this study, abattoir samples had a higher percentage of antibodies to both antigens compared with the breeder and heifer samples. The significance of this observation could not be determined in this study and further investigation is required to determine whether seropositivity to both antigenic phases in beef cattle is indicative of recent or chronic infection with *C. burnetii*.

Levels of seropositivity to *C. burnetii* antigens in the beef cattle tested varied according to the time of year. Seasonality of antibody levels has also been demonstrated in sheep in North America in association with lambing. This effect is unlikely to be seen in beef cattle because year-round breeding occurs in most areas. However, as large quantities of *C. burnetii* can be shed during parturition it may be possible for infection of other species to occur, particularly in areas where primary production of both cattle and sheep is concentrated. In Queensland, lambing is unlikely to be a factor in the seasonality of antibody levels observed in cattle, because of the similarity in antibody level trends in beef cattle in both the northern and southern regions of Queensland despite the differences in lambing season between the regions. Generally, in the areas south of the central-west region lambing occurs during July–September, whereas in the areas north of the central-west, lambing occurs during February–April. The rise in seropositivity to *C. burnetii* in the later months of the year may be related to the increase in tick populations. In Queensland, ticks are predominately in the nymphal phase during winter, with the emergence of adults and increase in tick numbers occurring during spring.

In conclusion, the ELISA technique developed in this study enabled large numbers of animal sera to be screened at a relatively low cost per sample. As there are no published studies on the exposure of animals to *C. burnetii* in this region, this investigation is a step towards understanding the epidemiology of Q fever in Queensland. The evidence of *C. burnetii* infection in beef cattle may have public health implications for occupational exposure of veterinarians and primary producers and for the proximity of beef cattle properties to residential areas in regional Queensland. Further investigation of additional potential

### Table 2. Beef cattle seropositivity in various statistical divisions of Queensland, Australia

<table>
<thead>
<tr>
<th>Region</th>
<th>Cattle (n)</th>
<th>Samples (n)</th>
<th>Seropositivity (%) (±95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phase II</td>
</tr>
<tr>
<td>Far North Queensland</td>
<td>756,400</td>
<td>96</td>
<td>26.4 (17.5–35.3)</td>
</tr>
<tr>
<td>Northern Queensland</td>
<td>1,073,600</td>
<td>539</td>
<td>12.1 (12.0–12.1)</td>
</tr>
<tr>
<td>North-west Queensland</td>
<td>1,915,400</td>
<td>350</td>
<td>9.4 (9.4–9.5)</td>
</tr>
<tr>
<td>Mackay</td>
<td>1,232,200</td>
<td>30</td>
<td>0.0 (0.0–0.4)</td>
</tr>
<tr>
<td>Central-west Queensland</td>
<td>1,012,600</td>
<td>88</td>
<td>9.1 (9.0–9.3)</td>
</tr>
<tr>
<td>Fitzroy</td>
<td>2,354,600</td>
<td>55</td>
<td>5.5 (5.4–5.7)</td>
</tr>
<tr>
<td>Wide Bay/Burnett</td>
<td>939,400</td>
<td>245</td>
<td>4.9 (4.8–4.9)</td>
</tr>
<tr>
<td>Darling Downs</td>
<td>1,390,800</td>
<td>240</td>
<td>10.0 (9.9–10.1)</td>
</tr>
<tr>
<td>South-west Queensland</td>
<td>1,146,800</td>
<td>155</td>
<td>2.6 (2.5–2.6)</td>
</tr>
<tr>
<td>Total</td>
<td>11,821,800</td>
<td>1835</td>
<td>10.0 (10.0–10.0)</td>
</tr>
</tbody>
</table>

CI, confidence interval.

Figure 1. Seasonality of seropositivity to *Coxiella burnetii* antigens in beef cattle in Queensland. Seropositivity to either or both phase II and phase I antigens of *C. burnetii* in various months of the year is shown. Horizontal bars represent mean for each period and vertical represent standard error of the mean for each period. Statistically significant differences were found between March/April and May/June (P < 0.10), March/April and July/August (P < 0.10) and March/April and September/October (P < 0.05).
reservoirs, such as other livestock, companion animals and wildlife, is needed to determine their role in Q fever.

Acknowledgments

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References