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# Prevalence and spatial distribution of *Coxiella burnetii* seropositivity in northern Australian beef cattle adjusted for diagnostic test uncertainty

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ABSTRACT

Q fever is a zoonotic disease caused by infection with *Coxiella burnetii* transmitted from animals including, but not limited to, cattle, sheep and goats. The infection in cattle is typically sub-clinical with some evidence suggesting associated reproductive loss. There is currently limited data on the true prevalence and distribution of coxiellosis in beef cattle across northern Australia. During this study, 2,012 sera samples from beef cattle managed on commercial farms located in Queensland and the Northern Territory were tested using an indirect immunofluorescent assay (IFA) for serological evidence of IgG antibodies against *C. burnetii*. Bayesian latent class models were used to estimate the true prevalence, adjusted for diagnostic test sensitivity and specificity and incorporating the hierarchical structure of the cattle within farms and regions. In this study, cattle in the Northern Territory had lower estimated true prevalence than cattle within most regions of Queensland with the exception of south-east Queensland. Results from this study have described the geographic distribution and estimated the true prevalence of antibodies to *C. burnetii* in a sample of extensively managed beef cattle located across the tropical grazing regions of northern Australia.

## 1. Introduction

*Coxiella burnetii* has been recognised globally as an important zoonotic infection by both human and animal health authorities (Eldin et al., 2016; OIE, 2018). The human disease is referred to as Q fever and the infection in animals as coxiellosis. However, many published texts refer to the infection in all species as Q fever. It is commonly reported that cattle, sheep and goats are the main sources of human Q fever (Eldin et al., 2016; Maurin and Raoult, 1999; Porter et al., 2011). Coxiellosis in cattle is often subclinical, however it has been associated with an increased prevalence of reproductive problems such as sporadic abortion, premature birth and birth of weak calves (Agerholm, 2013).

Human Q fever can occur as both acute and chronic forms with a range of clinical symptoms. There are reports that suggest clinical illness in Australia is different to that reported in other countries (Parker et al., 2006). In Australia, Q fever patients commonly present with a "flu like illness" including fever, headache, night sweats and fatigue (Eastwood et al., 2018; Gunaratnam et al., 2014). In southern Spain, Ontario and France signs of hepatitis are more common (Tissot Dupont et al., 1992); and in areas of Switzerland and Crete signs of atypical pneumonia are reported (Maurin and Raoult, 1999). Thus it is common in Australia for acute Q fever cases to be misdiagnosed as influenza and not immediately recognised or notified (Eastwood et al., 2018). A recent review by experts in the field has discouraged use of the term "chronic Q fever" as this oversimplifies the diagnosis and clinical implications; the term "persistent focalised *C. burnetii* infections" has been recommended (Eldin et al., 2016; Million & Raoult 2015). Persistent focalised *C. burnetii* infections can result in chronic hepatitis, gestational

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complications, paediatric osteomyelitis and endocarditis (Eldin et al., 2016; Parker et al., 2006).

In Australia, Q fever is a notifiable disease in humans with national case notifications that fluctuated between 317–868 cases per year between 1991 and 2018 (Australian Government, 2019). Australia consistently reports high annual notifications rates of Q fever, which are likely an underestimation of the true disease incidence (Gidding et al., 2020; Tozer, 2015). When comparing the 10-year average annual Q fever notification rates from 2009 to 2018, stratified by states and territories, there were marked differences between geographic regions of Australia. The state of Queensland reported an average 10-year rate of 4.3 cases per 100,000 population per year, New South Wales 2.5 and the remaining states reported rates of less than 1 case per 100,000 population per year (Australian Government, 2019). When comparing the states and territories of Australia, Queensland consistently reported the highest annual case notification rates over the last 20 years.

Although Q fever is listed by the OIE (World Organisation for Animal Health) as an important animal infection, it is not a nationally notifiable animal disease in Australia. Without current monitoring or surveillance of this infection in ruminants and with minimal incentive for research, the true prevalence and distribution of coxiellosis in Australian ruminant populations is unknown.

Beef cattle production is important to Australia's economy and represented approximately 20 % of the value of agricultural production in 2018−19, with a gross value of $AUD19.6 billion for cattle and calf production including live cattle exports ("Australian Bureau of Statistics, Australian Government," 2019). The northern Australian beef industry accounts for about 61 % of the total Australian beef cattle herd and ranges across an extensive remote geographic area. With approximately 42 % of the total Australian cattle population in Queensland (10.5 million head), 9% (2.2 million head) in the Northern Territory and 3% (0.8 million head) in Western Australia (north of the Tropic of Capricorn; Meat and Livestock Australia, 2019).

Quantitative data on coxiellosis in cattle in Australia is limited. Between 1954 and 2018, southern states of Australia reported an animal-level prevalence of antibodies to *C. burnetii* in cattle of less than 1% (Cronin, 2015; Forbes et al., 1954; Hore and Kovesdy, 1972; Tan, 2018). Only two publications were identified that reported *C. burnetii* prevalence in cattle in northern Australia. Firstly, using complement fixation testing, cows (n = 330) from 11 dairy herds in north Queensland were found to have 0.0 % seropositive (Pitt 1997). However, testing of beef cattle from a sample of farms across Queensland using an in-house enzyme-linked immunosorbent assay (ELISA), found *C. burnetii* IgG seropositivity of 16.8 % (95 % CI 16.7, 16.8 %; n = 1835) at the animal level, with 78.5 % of farms having at least one positive animal (Cooper et al., 2011). This estimate was higher than other Australian studies and higher than reports for beef cattle internationally. However, the diagnostic sensitivity and specificity of the ELISA test used was not determined, and thus what has been reported is the apparent prevalence of *C. burnetii* seropositivity. As most serological test methods are imperfect, test results should be adjusted to estimate true rather than apparent prevalence to avoid biased and overly confident estimates (Dohoo et al., 2009).

The objectives of this study were to investigate the prevalence of antibodies specific for *C. burnetii* in beef cattle from breeding herds across northern Australia and estimate the true prevalence and spatial distribution at that time point (during 2011). Based on differences in human notification rates between regions, it was hypothesised that there may be an associated regional difference in cattle *C. burnetii* seropositivity between cattle farmed in the Northern Territory and regions of Queensland.

## 2. Materials and methods

### 2.1. Ethics statements

Animal ethics for this study were approved by the University of Queensland Animal Ethics Committee: SVS/115/11/MLA (NF) and ANRFA/SVS/100/16.

### 2.2. Study design

A cross-sectional study design, using previously collected samples, was used to estimate the seroprevalence of *C. burnetii* in a sample of beef cows from farms in Queensland and the Northern Territory, Australia. A total of 2,012 sera samples, collected between December 2010 and December 2011, were tested for anti-*C. burnetii* IgG. Blood samples were collected as part of a Meat and Livestock Australia-funded epidemiological study investigating factors affecting the reproductive performance of beef cattle in northern Australia: Northern Australian Beef Fertility project: CashCow (McCosker et al., 2020; McGowan et al., 2014).

### 2.3. Farm selection criteria and blood sampling protocol from previous study

The target population for the CashCow project was all commercial beef breeding farms within Queensland, Northern Territory and northern Western Australia. The source population for the project included those beef breeding farms with high quality cattle handling facilities and that conducted annual pregnancy testing of all females by experienced cattle veterinarians. Selected cattle farms represented a convenience sample of the source population across all regions of interest where the owner/manager was committed to collection of all required animal and farm data over the study period (2008–2011; McGowan et al., 2014). Seventy-eight farms with approximately 56,000 electronically identified breeding females managed in approximately 165 management groups were enrolled in the study.

One component of the CashCow project involved cross-sectional blood sampling of management groups of cattle to determine the prevalence of infectious diseases that may affect reproductive performance (McCosker et al., 2020). Sample size estimates were based on a systematic random sample designed to achieve 90 % confidence to detect a design prevalence of 50 % and achieve precision ranging from 15 to 30%. The result was a design intention to collect a blood sample from every 10$^{th}$ animal in a management group during a scheduled time when animals were due to be yarded for husbandry and data collection purposes (McGowan et al., 2014). Given the expected mob sizes for extensively run commercial beef farms across northern Australia, this resulted in between 15 and 30 blood samples collected from participating management groups. Blood samples were collected by coccygeal venepuncture, into 9 ml plain collection tubes. Sera were decanted approximately 24 h after collection and shipped either frozen or chilled to the University of Queensland (UQ) for storage. Samples were stored frozen at −20 °C at UQ until laboratory testing. During the CashCow project, serum samples underwent a maximum of three freeze/thaw cycles prior to *C. burnetii* testing that was performed in 2017.

For this current study, all remaining serum samples from the 2011 blood sampling, having sufficient volume to perform laboratory testing were utilised. Of the original 78 farms enrolled, 60 farms were included in this study; Fig. 1 shows the geographical location of the 60 farms.

### 2.4. Serological methods

The sera were tested for IgG antibodies specific for *C. burnetii* using an indirect immunofluorescent assay (IFA) modified and validated for use in cattle (Wood et al., 2019). The IFA slides were produced in-house, coated with *C. burnetii* phase I and/or phase II antigen as previously

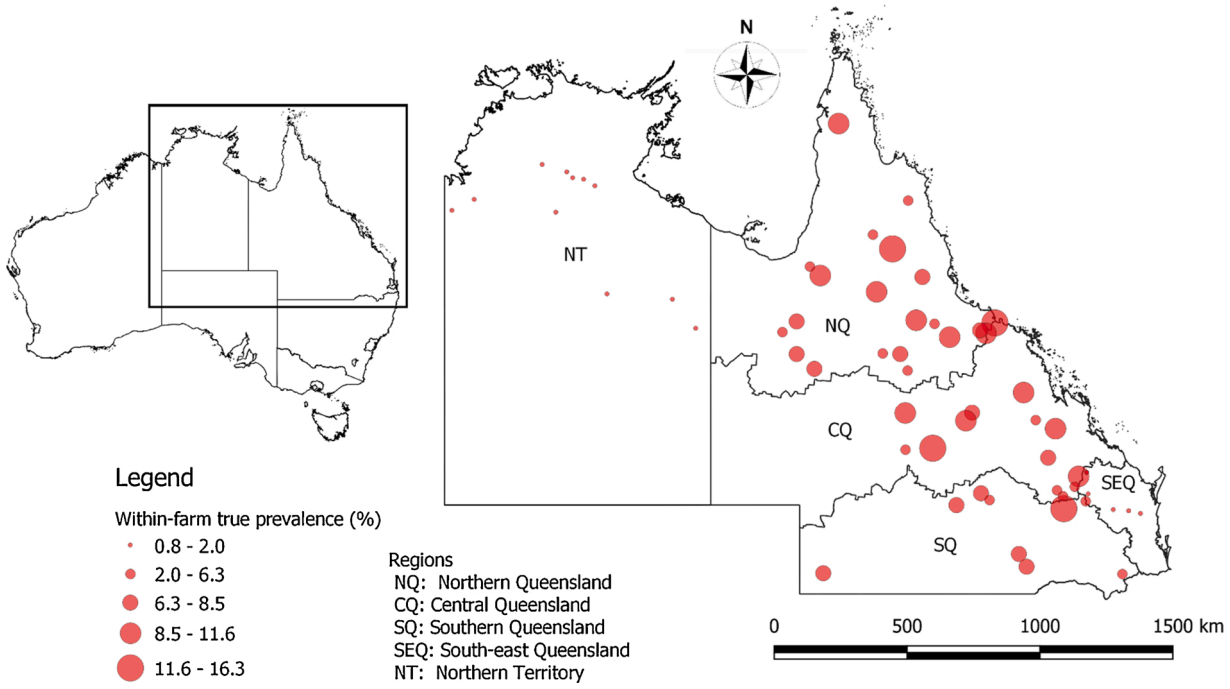


**Fig. 1.** Geographical distribution of participating cattle properties from northern Australia (n = 60) with serum samples tested for *C. burnetii* specific IgG. This map of the Northern Territory and Queensland shows the distribution of within-farm true prevalence estimates. Point values are the median posterior true prevalence estimates.

described, with best estimates of the diagnostic sensitivity (DSe) and specificity (DSp) of this assay being, 73.6 % (95 % Credible Interval (CrI) 61.1, 85.9) and 98.2 % (95 % CrI 95.1, 99.7), respectively (Wood et al., 2019). The IFA test parameters were estimated using Bayesian latent class methods of comparing two tests across four separate cattle populations in the absence of a gold standard reference test. It was assumed that the DSe and DSp of the two tests were constant across the four populations. Detailed methods of the IFA test optimisation and validation have been reported by Wood et al., 2019.

For this study, all test sera were initially screened at a dilution of 1:160, on slides with a combined phase I and phase II antigen coating as this was identified as the optimal screening dilution for bovine sera (Wood et al., 2019). Test sera that tested positive at this screening dilution were then titered out to endpoint on separate phase I and phase II slides. Sera were considered positive if they showed reactivity to either phase I or phase II antigen at the 1:160 cut-off dilution. The IFA DSe and DSp was previously estimated using the same test method (Wood et al., 2019).

Briefly, IFA slides, test sera and reagents were brought to room temperature before use. Serum samples were diluted with 2% casein-phosphate-buffered saline (PBS) to minimise non-specific binding. Diluted test sera were placed on the slides in duplicate and incubated in a humidity chamber for a 30 min incubation period at 37 °C; if the serum contained specific *C. burnetii* antibodies, they adhered to the antigen during this time. The slides were washed in 10 % PBS for 5 min, three times and allowed to air dry. Anti-bovine IgG-FITC conjugate, diluted in 0.05 % Evans blue dye, was added and incubated in a humidity chamber for 30 min at 37 °C. If IgG antibody-antigen complexes were adhered on the slide, the FITC conjugate would attach to it during this incubation period. The slides were again washed in 10 % PBS for 5 min, three times and allowed to air dry. Coverslips were mounted to slides with mounting media. An immunofluorescent microscope (Nikon Eclipse E600) was used to view the slides at 40x magnification and then with oil immersion at 100x magnification. If the test serum contained IgG antibodies against *C. burnetii*, there was an apple green fluorescence indicating a positive result.

### 2.5. Statistical analysis

#### 2.5.1. Apparent prevalence

Crude diagnostic test results were reported for the serological testing as an apparent prevalence (AP); equal to the percentage of test-positive animals from the total samples tested. Results were calculated at the individual animal level for state, region and farms with Wilson-Score 95 % Confidence Intervals (CI). Farm-level AP was also presented with 95 % Wilson-Score CI; a farm was considered positive if one or more individual animals on the farm were test positive.

#### 2.5.2. Bayesian latent class models

Bayesian hierarchical latent class modelling (LCM) was utilised to estimate the true prevalence (TP) of *C. burnetii* seropositivity based on this sample of beef cattle within farms ($TP_{farm}$) and within regions ($TP_{region}$), with the unit of interest being the individual animal. The test samples used in this study had a hierarchical data structure, with cattle clustered in farms and farms clustered within regions. Due to this complex data structure, statistical approaches accounting for the clustering were required. The true prevalence model was developed to account for the data structure and included random effects for region and for farms nested in the regions. The IFA serological test is not a perfect test; therefore the DSe and DSp of the IFA were also accounted for in the model using the formula to estimate TP as a function of AP (Rogan and Gladen, 1978).

$$AP_{ij} = DSe \times TP_{ij} + (1 - TP_{ij}) \times (1 - DSp)$$

Where, $AP_{ij}$ is the apparent prevalence, i = 1, …, 60 is the $i^{th}$ farm and j = 1, …, 5 is the $j^{th}$ region. Let $y_{ij}$ represent counts of positive sera for farm i in region j, then it can be shown that the counts follow a binomial distribution as:

$y_{ij} \sim dbinom(AP_{ij}, n_{ij})$

Random effects for farms ($u_{ij}$) nested in regions (i.e., $w_j$) and the regions themselves ($w_j$) were included in the model to address the data structure of the sampling method in this study. Hence, the true prevalence ($TP_{ij}$) could be expressed with a logit link as follows:

$$\text{logit}(TP_{ij}) = \beta_0 + w_j + u_{ij}$$

Where $TP_{ij}$ is the true prevalence for the $i^{th}$ farm in the $j^{th}$ region, $u_{ij}$ and $w_j$ represent the random effects of the farm and region, respectively, and:

$\beta_0 \sim$ dnorm (0, 0.1)

$u_{ij} \sim$ dnorm(wj, $1/\sigma^2_u$)

wj $\sim$ dnorm(0, $1/\sigma^2_w$)

Uniform hyper-priors were assumed for the region and farm random effects, (Christensen et al., 2010) i.e.,

$\sigma_u \sim$ dunif(0,1)

$\sigma_w \sim$ dunif(0,1)

The true prevalence of *C. burnetii* seropositivity in each farm within each region could be inferred according to the random effects as follows:

$$\pi_{ij} = \frac{\exp(\beta_0 + w_j * region_j + u_{ij} * property_{ij})}{1 + \exp(\beta_0 + w_j * region_j + u_{ij} * property_{ij})}$$

And the true prevalence of *C. burnetii* seropositivity in each region could be calculated as follows:

$$\pi_j = \frac{\exp(\beta_0 + w_j * \text{region}_j)}{1 + \exp(\beta_0 + w_j * \text{region}_j)}$$

Informed priors for DSe and DSp of the IFA were incorporated into the model using unimodal beta distributions based on published diagnostic test parameters (Supplementary material; Table S-1; Wood et al., 2019). Unimodal beta distributions were elicited from the "epi.betabuster" function implemented within the "epiR" library (Stevenson, 2017) in R ("R: A language and environment for statistical computing," 2019). Sensitivity analysis was performed by re-running the model with alternative priors for IFA DSe and with hyper-priors for the random effects ($\sigma_u$ and $\sigma_w$) of dunif(0, $b$) for $b$ = 1.5, 2 and 2.5 (Supplementary material; Table S-2).

The Bayesian hierarchical true prevalence model was coded in OpenBUGS (version 3.2.3, rev 1012). See supplementary materials for example data and R code from these analyses. Bayesian inferences were based on the joint posterior distribution, approximated using the computer software JAGS (version 4.3.0, citation), implemented with R2jags package (Yu-Sung and Masanao, 2015) in the R statistical package. This implementation makes use of a Markov Chain Monte Carlo (MCMC) sampling algorithm to acquire Monte Carlo (MC) samples from the posterior distribution (Paul et al., 2014). The MCMC model was initiated with three independent chains and run for 250 000 iterations, burning the first 20 000 (Kruschke, 2015). Model diagnostic was assessed using visual methods by looking at the history of the three chains of the MCMC. Convergence was also assessed using the Gelman-Rubin plots and the Raftery-Lewis measures which is one at convergence. Autocorrelation and effective sample size were also obtained (Kruschke, 2015).

Posterior estimates were used as $TP_{farm}$ estimates for the 60 cattle farms located within distinct regional areas of northern Australia. $TP_{region}$ were calculated from model outputs for each of the five geographical regions specified in the model: north Queensland, central Queensland, southern Queensland, south-east Queensland and the Northern Territory. $TP_{region}$ should be interpreted as the predicted true prevalence of *C. burnetii* exposure on a typical farm selected within that specific region. All true prevalence estimates were reported as the median and 95 % CrIs of the posterior distribution. The Queensland $TP_{region}$ estimates were compared with the Northern Territory $TP_{region}$.

For regions with low prevalence estimates (median $TP_{region} < 5\%$), the likelihood of *C. burnetii* disease freedom was inferred as the probability of *C. burnetii* seropositivity being less than a pre-specified design prevalence ($\theta$). Two values of this design prevalence were tested (5% and 1%) using the Boolean step() function. The step () function created a Boolean value for each simulations in which the $TP < \theta$. The mean value of a Boolean node is a probability; hence the Monte-Carlo estimate of P (TP < θ) represented the probability of the prevalence being less than the specified design prevalence for each region under observation.

#### 2.5.3. Spatial visualisation

Enrolled beef cattle farms were geo-referenced with latitude and longitude at the time of the original study; each farm was then referenced with a code for "region" within Queensland, according to farm geo-referenced points from open-access Australian government regional boundary maps. Maps were produced to visualise the distribution of true prevalence model output estimates as point data ($TP_{farm}$). All maps were created using software QGIS 2.18.26 ("QGIS Geographic Information System," 2019).

## 3. Results

### 3.1. Laboratory testing of bovine samples

Approximately 80 % (n = 1,602) of the cattle tested were located in Queensland and 20 % (n = 410) in the Northern Territory. The median number of sera tested per farm was 31 with a range of 4–112 (interquartile range 17–41). A descriptive summary including a breakdown of the number of farms per region are shown in Table 1.

### 3.2. IFA serological test results

Overall, 5.2 % (95 %CI 4.3, 6.2 ; n = 104/2,012) of test sera returned a positive result using the IFA test at the 1:160 cut-off for either phase I or phase 2 IgG antibodies against *C. burnetii*. From the test sera, 4.4 % (95 %CI 3.6, 5.4; n = 89/2,012) were positive for phase 1, 3.5 % (95 %CI 2.8, 4.4; n = 70/2,012) were positive for phase 2 and 2.7 % (95 %CI 2.1, 3.5; n = 55/2,012) were positive for both phase 1 and phase 2. Serum was considered positive if it was IFA positive for either phase 1 or phase 2 or both phases.

Apparent prevalence of anti-*C. burnetii* IgG antibodies varied between cattle in Queensland and the Northern Territory; 6.4 % (95 %CI 5.3, 7.7) of individual sera from Queensland cattle were test positive, whereas 0.5 % (95 %CI 0.1, 1.8) of individual sera from Northern Territory cattle were test positive. From Queensland, 61.2 % (95 %CI 47.2, 73.6; n = 30/49) of the farms tested, had at least one test positive animal identified. From the Northern Territory, 18.2 % (95 %CI 5.1, 47.7; n = 2/11) of farms tested, had at least one animal IFA positive.

A descriptive summary of the IFA test results and AP at the animal and farm level, stratified by state/territory and geographic regions are shown in Table 1. Overall, 53.3 % (32/60) of farms had evidence of IgG antibodies to *C. burnetii*. From the positive farms, the average within-farm AP was 8.7 % with a range of 0.9 %–22.2 %.

### 3.3. Bayesian latent class modelling

True prevalence estimates from the Bayesian hierarchical LCM are presented as a caterpillar plot (Fig. 2) and as geo-referenced data points on a map of Queensland and the Northern Territory (Fig. 1). The caterpillar plot indicates the $TP_{farm}$ as a median posterior prevalence estimate for each farm with 95 % CrI. The wide CrIs noted may be due to small sample sizes and relatively uninformed priors; they indicate a moderate amount of uncertainty within the estimates. For georeferenced data-points on the map, the median estimates are displayed as percentage positive without CrIs, for better visualisation. When mapped, the distribution of Queensland $TP_{farm}$ did not suggest areas of clustering. The median $TP_{farm}$ estimates within Queensland ranged from 1.6%–16.3 %. However, the 95 % credible intervals of these estimates are very wide and the highest upper limit reached 35.8 %. Farms sampled in the Northern Territory returned median $TP_{farm}$ estimates ranging from 0.8 % to 1.2 %. Much more variance in within-farm true prevalence is apparent in farms sampled from Queensland.

Regional predicted true prevalence estimates indicate that cattle in this study, from the Northern Territory had lower $TP_{region}$ of *C. burnetii* seropositivity than cattle from all regions of Queensland except south-east Queensland. Cattle in this study originating from northern,

**Table 1**
Descriptive summary of crude IFA results for *Coxiella burnetii* IgG seropositivity in bovine serum.

| | | Animal level | | | Property level | | |
|---|---|---|---|---|---|---|---|
| | | No. of positive samples | Total no. samples | Percentage positive (95 % CI) | No. of positive properties | Total no. properties | Percentage positive (95 % CI) |
| **Overall** | Northern Australia | 104 | 2012 | 5.2 % (3.9, 6.8) | 32 | 60 | 53.3 % (40.9, 65.4) |
| **State** | Queensland | 102 | 1602 | 6.4 % (4.9, 8.2) | 30 | 49 | 61.2 % (60.1, 61.5) |
| | Northern Territory | 2 | 410 | 0.5 % (0.1, 1.8) | 2 | 11 | 18.2 % (14.6, 22.0) |
| **Region** | Northern Queensland | 41 | 606 | 6.8 % (5.0, 9.1) | 12 | 20 | 60.0 % (58.8, 60.8) |
| | Central Queensland | 44 | 628 | 7.0 % (5.2, 9.2) | 10 | 14 | 71.4 % (69.7, 71.7) |
| | Southern Queensland | 16 | 240 | 6.7 % (4.1, 10.6) | 7 | 9 | 77.8 % (74.9, 77.2) |
| | South-east Queensland | 1 | 128 | 0.8 % (0.1, 4.3) | 1 | 6 | 16.7 % (9.9, 23.7) |
| | Northern Territory | 2 | 410 | 0.5 % (0.1, 1.8) | 2 | 11 | 18.2 % (14.6, 22.0) |

KEY: CI, Confidence Interval.

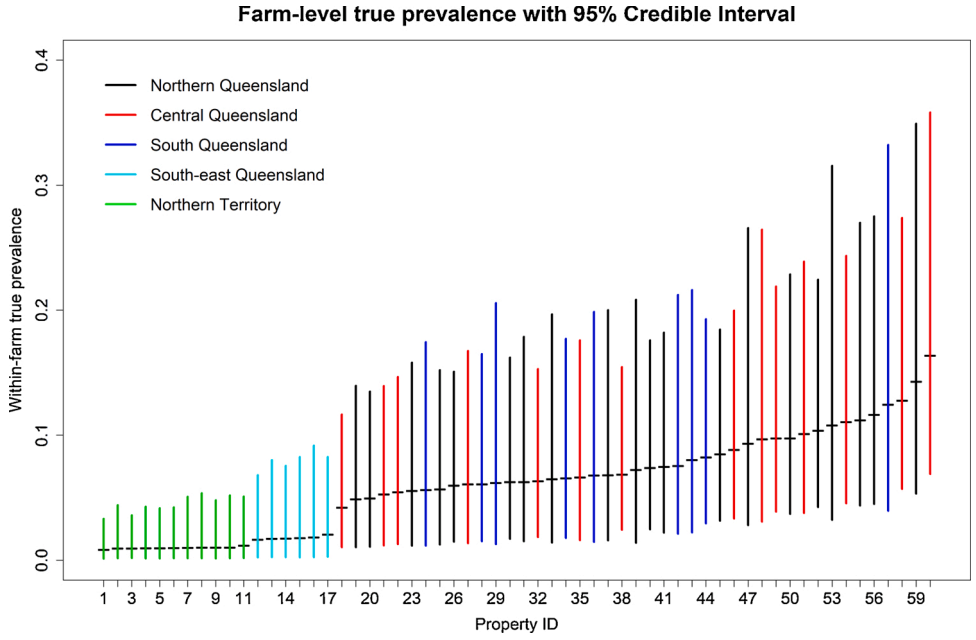


**Fig. 2.** Caterpillar plot of model estimates for farm-level true prevalence as a proportion; the point estimate is the median of the posterior distribution of predicted prevalence and the solid vertical lines indicate 95 % Credible Intervals. Colours of the solid vertical lines represent regions within northern Australia where the properties are located.

central and southern Queensland had very similar $TP_{region}$ (Table 2) and the south-east region of Queensland has the lowest regional estimate for the state of Queensland.

For samples from the Northern Territory, the probabilities that the posterior estimate of true prevalence is less than design prevalence (5% and 1%) are 0.99 and 0.48, respectively. For samples from south-east Queensland the probabilities are 0.94 and 0.19 for the same design prevalence (5% and 1%), respectively. Model diagnostics were all satisfactory; supplementary material S1. Changes in the model outputs from the sensitivity analyses were robust to the use of different priors (supplementary material; Table S-2).

## 4. Discussion

This study revealed that the apparent prevalence or unadjusted seroprevalence of *C. burnetii* in a large sample of beef cattle from Northern Australia (the Northern Territory and Queensland), using an IFA, validated for use in cattle, was 5.2 % (95 % CI 4.3, 6.2). When stratified by territory/state, the apparent prevalence of cattle sampled from the Northern Territory was 0.5 % (95 % CI 0.1, 1.8) and cattle sampled from Queensland was 6.4 % (95 % CI 5.3, 7.7). Although the majority of published prevalence studies investigating *C. burnetii* seropositivity in cattle have focused on dairy cattle, some international publications included beef cattle. It was reported that beef cattle going to slaughter in Denmark had an AP of 4.5 % (95 % CI 3.2, 6.3) using a commercial ELISA Q fever kit (Paul et al., 2014). These crude serological test results are similar to reports from beef cattle in other countries with reported APs ranging from 1.7 %–6.6 % (Alvarez et al., 2012; Lyoo et al., 2017; McCaughey et al., 2010; Ruiz-Fons et al., 2010).

A Bayesian hierarchical LCM was developed to provide more robust prevalence estimates in order to compare coxiellosis seropositivity from this sample of cattle across five distinct regions of northern Australia (the Northern Territory, northern Queensland, central Queensland, southern Queensland and south-east Queensland). The model was designed to account for the IFA test DSe and DSp and incorporate the hierarchical structure of the data into the analysis. From the posterior outputs of the final model, the predicted probability of *C. burnetii* seropositivity in a typical beef cattle farm from the Northern Territory was

**Table 2**
Predicted regional true prevalence estimates and 95 % Credible Intervals, derived from the Bayesian hierarchical latent class model.

| Regions | Estimated true prevalence | 95 % Credible Interval | Design prevalence | |
|---|---|---|---|---|
| | | | 1% | 5% |
| Northern Queensland | 7.30 % | (3.8, 13.6) | | |
| Central Queensland | 7.60 % | (4.0, 14.2) | | |
| Southern Queensland | 6.80 % | (2.9, 14.9) | | |
| South-east Queensland | 1.90 % | (0.4, 6.2) | 0.19[a] | 0.94[a] |
| Northern Territory | 1.00 % | (0.3, 3.3) | 0.48[a] | 0.99[a] |

Regional true prevalence estimate can be interpreted as the predicted prevalence of *C. burnetii* exposure on a typical farm within that specific region. The point estimate is the median of the posterior distribution with the 2.5 % and 97.5 % percentiles presented as 95 % credible intervals.

[a] Posterior probabilities of estimated true prevalence being less than a specified design prevalence.

1.0 % (95 % CrI 0.3, 3.3). This was lower than 3 out of the 4 regions within Queensland. However, from the data presented, it cannot be concluded that the Northern Territory cattle are free of disease. Nonetheless, we estimated that there was a >95 % probability that these cattle have a true prevalence of <5%. A diagnostic test with higher sensitivity and using a study design specific for this purpose would be required in order to contribute towards demonstration of regional freedom of disease.

This study is the first to report any prevalence estimates for anti-*C. burnetii* IgG antibodies in cattle from the Northern Territory. We noticed from national Q fever surveillance data that human case notifications have historically been different between regions of Queensland and the Northern Territory. Prior to 2002, the Northern Territory had never reported a case of Q fever. From 2002–2018, the annual notification rates fluctuated from 0 to 2.4 cases/100,000 with an average annual rate of 0.8 cases/100,000 and very low case numbers. The Queensland data from the same time-period shows an average annual notification rate of 4.7 cases/100,000; with significantly higher notification rates prior to the National Australian Q Fever Management Program (Palmer et al., 2007). Results from this study support the hypothesis that cattle managed in the Northern Territory have a lower true prevalence of antibodies against *C. burnetii* than cattle managed in most regions of Queensland. Although the south-east Queensland region was an exception. While this pattern is interesting, it does not imply causality between beef cattle coxiellosis and human Q fever cases. As *C. burnetii* is known to survive in the environment and is able to transmit between many reservoir animals, this pattern may suggest less *C. burnetii* in the general environment in the Northern Territory, or that the conditions do not favour the persistence and spread of the bacterium. In this respect, beef cattle seropositivity may function as a sentinel marker for *C. burnetii* within this regional ecosystem.

Within the cattle sampled from Queensland, the predicted prevalence of *C. burnetii* IgG positivity in a typical farm within three broad regions: northern, central, southern, was similar. However, cattle from the south-east Queensland region had the lowest $TP_{region}$ within the state of Queensland. This region had the smallest number of farms enrolled and small sample sizes within farms; which may be indicative of less farming in the region and smaller farm sizes. In general, there was a lot of variation in $TP_{farm}$ estimates for Queensland cattle farms. In general, most beef farming across northern Australia follow an all-year round calving pattern without any strong seasonal patterns of pregnancy and calving (McCosker et al., 2020). Transmission of coxiellosis may be influenced by parturition, as *C. burnetii* is known to replicate in the placenta and be excreted in high numbers through birthing fluids (Agerholm, 2013). Although beyond the scope of this analysis, the influence of farming density, farming practices and seasonal patterns on regional prevalence could provide insights into protective factors from coxiellosis. An in-depth analysis of risk factors for coxiellosis in beef cattle of northern Australia would be beneficial to explore in further research.

Within Australia, only one preliminary study from Victoria investigated the regional TP of *C. burnetii* seropositivity in beef and dairy cattle taking into account the performance of the diagnostic test used (Tan, 2018). From Goulburn Valley, Victoria, cattle had a TP 0.0 % (CrI 0.0, 0.0 %) (n = 278) and from Gippsland, Victoria 0.4 % (CrI 0.0, 3.5 %) (n = 247). True prevalence estimates were calculated using similar Bayesian methods as described here, however without the additional multilevel modelling to account for clustering of animals within farms. The TP results reported from Victorian cattle was very similar to the cattle from the Northern Territory and south-east Queensland in this study. Most Queensland regions, however, had higher results than Victorian regions. Therefore, cattle in Queensland may truly have a higher level seropositivity specific to *C. burnetii* than cattle in southern states as suggested from a previous study (Cooper et al., 2011).

The statistical model presented in this study incorporates both imperfections of the diagnostic test used and the hierarchical data structure of the cattle populations into the final TP estimates. The structure of this model may be useful to provide assistance with infectious disease prevalence estimates in future studies. It could also be utilised during passive surveillance of *C. burnetii* (or other infections) within animal populations, thus enabling a more accurate interpretation of serological test results from serum banks or samples collected for other purposes. Statistical methods applied in this study may enable improved comparisons between *C. burnetii* prevalence estimates both within and between regions / countries and allow further analysis into putative risk factors of coxiellosis in cattle in Australia.

For this current study, there were no samples available for IFA testing from beef cattle located in Western Australia. Although, one previous publication has reported the AP of *C. burnetii* from beef cattle in Western Australia, the specific geographical location of sampling within the state was not specified (Banazis et al., 2010). Cattle in that study (n = 329) had a seroprevalence of 0.6 % using an IDEXX CHEKiT Q Fever ELISA (IDEXX Laboratories Inc., Switzerland) and 7.9 % tested PCR positive from urine and faecal samples. Banazis et al., 2010 reported *C. burnetii* PCR results much higher than cattle seroprevalence, which may indicate that bacterial shedding in urine and faeces was higher than seropositivity. However, there is also a possibility that samples collected in that study had an increased chance of *C. burnetii* contamination from the environment thus leading to higher DNA detection.

There are several limitations to the present study. Firstly, it should be noted that the samples tested during this study might not be representative of the broader regions of northern Australia due to the structure of sampling; therefore the external validity of the regional true prevalence estimates may be limited. Although the farms enrolled in the original CashCow study were stratified to ensure a balanced sample across major beef-cattle breeding regions, it did not constitute a random sampling method from a complete sample frame. Selection of farmers that would be enthusiastic and committed to the project may have led to selection bias, and the farms enrolled in the original study may represent the better-managed farms from northern Australia. Secondly, only serum samples from 60 of the 78 farms were available to be tested for anti-*C. burnetii* IgG. Some samples collected in the initial study did not have sufficient volume of serum remaining for *C. burnetii* testing. Therefore, there are some discrepancies between original sample size estimates and final serum sample numbers tested. For the final analysis, the clustering of smaller management groups of cattle within farms was not incorporated into the model as not all farms had multiple groups; however, some farms had several management groups. Hence, all IFA serum results were aggregated at the farm level. Although we do not think these factors would bias the *C. burnetii* prevalence results in any particular

direction, these factors are acknowledged to ensure awareness of potential limitations with respect to any inferences made from the true prevalence estimates.

We also acknowledge that the presence of IgG antibodies in serum may not be a reliable indicator of active *C. burnetii* infection in cattle. Although serology can be useful to detect past exposure or recent infection, it is not known exactly how long anti-*C. burnetii* immunoglobulins remain detectable in serum of cattle (Natale et al., 2012). Therefore, interpretation of anti-*C. burnetii* IgG presence should be performed cautiously. There are recent publications investigating different phase specific serological patterns to identify *C. burnetii* infection status and shedding patterns in cattle, however, interpretation is still uncertain (Lucchese et al., 2015). Although the IFA test used here provided phase-specific serological results, the current Bayesian model was run using the case definition of positive for phase 1 and/or phase 2 to indicate an IFA positive serum result.

In this study, sera were tested using an IFA method validated for use in cattle specifically across eastern Australia and New Zealand. Estimates of the IFA performance were previously published, assuming constant test DSe and DSp across different populations and levels of prevalence. The IFA was chosen for this study mainly for economic reasons and secondly because the diagnostic test accuracy of the test had been estimated and published. Firstly, when testing large numbers of cattle sera, the IFA has lower consumable costs than the IDEXX ELISA kit. Although the IFA may have a lower sensitivity than the ELISA, as with any imperfect test, if the diagnostic sensitivity and specificity have been appropriately characterised for the study population, which they have here (Wood et al., 2019), then estimates can be adjusted appropriately.

Results from this study have provided baseline true prevalence estimates and described the geographic distribution of *C. burnetii* seropositivity in a sample of extensively-farmed beef cattle from areas of northern Australia (Queensland and the Northern Territory). Further representative sampling of cattle across broad regions of Australia for *C. burnetii* testing, followed by in-depth geo-statistical and spatiotemporal analyses are warranted and should help to investigate putative risk factors for coxiellosis such as livestock density, wildlife density, environmental conditions (including rainfall, humidity and wind) and animal movements.

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## Declaration of Competing Interest

The authors report no declarations of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2021.105282.

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