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PRODUCTION ANIMALS

ORIGINAL ARTICLE

A cross-sectional survey of risk factors for the presence of *Coxiella burnetii* in Australian commercial dairy goat farms

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The largest Australian farm-based outbreak of Q fever originated from a dairy goat herd. We surveyed commercial dairy goat farms across Australia by testing bulk tank milk (BTM) samples using a commercial indirect enzyme-linked immunosorbent assay and two quantitative polymerase chain reactions (PCRs). Of the 66 commercial dairy goat herds on record, managers from 61 herds were contacted and 49 provided BTM samples. Five of the surveyed herds were positive on at least one of the diagnostic tests, thus herd-level apparent prevalence was 10% (95% confidence interval [CI] 4 to 22). True prevalence was estimated to be 3% (95% credible interval: 0 to 18). Herd managers completed a questionnaire on herd management, biosecurity and hygiene practices and risk factors were investigated using multivariable logistic regression. Herds with >900 milking does (the upper quartile) were more likely to be *Coxiella burnetii* positive (odds ratio = 6.75; 95% CI 1.65 to 27.7) compared with farms with ≤900 milking does. The odds of BTM positivity increased by a factor of 2.53 (95% CI 1.51 to 4.22) for each order of magnitude increase in the number of goats per acre. *C. burnetii* was not detected in samples from the majority of the Australian dairy goat herds suggesting there is an opportunity to protect the industry and contain this disease with strengthened biosecurity practices. Intensification appeared associated with an increased risk of positivity. Further investigation is required to discriminate the practices associated with an increased risk of introduction to disease-free herds, from practices associated with maintenance of *C. burnetii* infection in infected dairy goat herds.

Keywords Australia; *Coxiella burnetii*; dairy goat; prevalence; risk factor

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Coxiella burnetii is an intracellular gram-negative bacterium with a worldwide distribution, except for New Zealand. It is the causative agent of Q fever, a disease that is often asymptomatic, but mild influenza-like symptoms such as fever, fatigue and headache are frequently observed. It can also lead to a severe disease requiring hospitalization and occasionally, death.[1] Infected domestic small ruminants have been implicated as the primary zoonotic source of infection to humans.[2] In small ruminants, coxiellosis is associated with reproductive disorders such as abortion, stillbirths, delivery of weak offspring, reduced milk yields and mastitis.[3–5] *C. burnetii* is excreted by infected animals in their milk, urine, faeces and birthing products. Previous studies have identified the placenta and other birthing discharges to be important sources of environmental contamination and infection, with 1 g of placental tissue estimated to contain up to 1×10^9 *C. burnetii* organisms.[6]

Sheep and goats were the source of the 2007 to 2011 epidemic of Q fever in The Netherlands, which is the largest documented outbreak of Q fever in humans reported to date.[7–9] To contain the Dutch outbreak more than 50,000 small ruminants were culled.[7–9] Previous studies have identified the following risk factors for coxiellosis in goat herds: limited air flow in sheds, the presence of companion animals on farms, larger herd sizes, complex herd management systems, poor doe kidding hygiene and a history of reproductive disorders.[10–12]

Since 2014, there has been a gradual increase in the size of the dairy goat industry in Australia, coupled with intensification to meet market demands.[13–15] To date, the largest farm-based Q fever outbreak in Australia occurred in a dairy goat herd in Victoria in 2012 with 24 human cases in total.[16] A survey of 500 goats from 25 goat farms in Victoria undertaken in 2016 detected antibodies specific to *C. burnetii* in animals on 8% of farms.[17] Despite coxiellosis posing a clear zoonotic threat and negative impacts on milk production, data on its presence in goat herds across Australia remain scarce.[5] For this reason, it is necessary to first quantify the prevalence of coxiellosis at the herd level and secondly to identify characteristics that render herds more likely to be coxiellosis positive. This information will allow the managers of herds identified as coxiellosis-positive to take appropriate steps to control or eliminate the infection in their herds. For those identified as coxiellosis-negative, measures can be devised to reduce the likelihood of infection entering their herds.

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This cross-sectional study's aim was therefore to estimate the farm-level prevalence of *C. burnetii* among Australian commercial dairy goat farms and to identify physical and herd-level characteristics that increased the probability of infection being present.

Materials and methods

This report complies with the VET-STROBE guidelines.[18] This study was conducted as approved by the University of Melbourne Human Ethic Committee (ethics ID number: 1851934)

Study design, population and sampling strategy

For the most part commercial dairy goat farms in Australia are comprised of a single herd, that is, a group of animals under a common system of management. For this reason, throughout this paper, we use the term 'herd' and 'farm' interchangeably with 'herd' used when describing procedures or characteristics related to animals, and 'farm' for procedures or characteristics related to the physical facilities in which animals are kept.

This was a cross-sectional study of commercial dairy herds in Australia. Commercial dairy goat herds were defined as goat herds that were licensed by their respective state dairy food safety organizations to sell dairy goat milk or milk-containing products to the public and to those manufacturing dairy goat milk products for human consumption. The total number of dairy goat herds in Australia was previously estimated to be 68.[19] While dairy licensing boards and state government animal health authorities maintain lists of dairy goat herds in their jurisdiction, these organizations were not able to share a list of contacts due to confidentiality reasons. For this reason, dairy goat enterprises were identified by several means: web search engines, social media, local news, word of mouth and obtaining lists of commercial and smallholder producers from the dairy goat herd book of Australia.[20] A sampling frame was developed listing the name of the dairy goat farm, its physical address and contact details (including email address). At the start of the study in May 2018, the listed contact person for each farm was invited by email to participate in the study. In accordance with the approved human research ethics protocol, a printed plain language statement was attached to provide project information. An AUD 50 supermarket gift card was offered as an incentive. Following provision of written consent to take part in the study each of the participating farms were visited to collect a bulk tank milk (BTM) sample and to provide the herd manager with the opportunity to complete a questionnaire (as detailed below).

Sample size calculations were carried out to determine the appropriate number of herds to sample to estimate the herd-level prevalence of coxiellosis to within 5% of the true population value, assumed to be 15%. Imperfect diagnostic test sensitivity and specificity was accounted for following the methodology of Humphry et al.,[21] using the contributed epiR package[22] in R.[23] Based on previous estimates,[24] we assumed that for herds that were coxiellosis positive the within-herd prevalence approximated 15%. A finite population correction factor was applied because the calculated crude sample size (n = 265) was in excess of the estimated population of 68 commercial dairy goat farms in Australia.[25] This returned an estimated minimum sample size of 55 herds to meet the estimation requirements.

Although the minimum sample size was estimated based on estimated prevalence, a power analysis was undertaken to ascertain the minimum detectable odds ratios using the package 'epiR' (Stevenson et al., 2018).[22] For this, we assumed the prevalence of coxiellosis was 5% in the unexposed group and 15% in exposed group, and estimated power for a range of sample sizes and minimum detectable odds ratios.

BTM collection and testing

At the time of each farm interview a 50 mL sample of BTM was collected in a sterile single-use plastic container either by the herd manager or by the member of the research team visiting the farm. The protocol for milk sampling is described in supplementary material S1. The milk sample was immediately refrigerated at 4°C. Where sampling was carried out by the herd manager the milk sample was express posted to the research team at the University of Melbourne. Upon arrival at the University of Melbourne, BTM samples were labelled and stored in a −20°C freezer until testing.

Quantitative PCR

The total milk DNA was extracted from duplicate aliquots of 200 μL from each BTM sample with the HiYield™ Genomic DNA Mini Kit (Real Biotech Corporation, Taiwan) using a protocol previously validated for extracting DNA from milk.[26] Phosphate-buffered saline was used as a negative extraction control, while a known concentration of extracted *Listeria innocua* genomic DNA was used as an extraction control. A quantitative polymerase chain reaction (qPCR) targeting a 62 bp DNA fragment from the *lin02483* gene for *L. innocua* was used to validate the DNA extraction process, and if no inhibition was detected, each DNA extract was tested twice with each qPCR, and each BTM specimen considered positive by qPCR only if both *IS*1111 and *com1* qPCR returned positive results and replicates were in agreement (see supplementary material S2, Figure S2.1 and Table S2.2). First, we ran the qPCR for detecting the presence of the *IS*1111 gene of *C. burnetii* as described by Lockhart et al.[26] For a given test replicate, if a cycle threshold (Ct) value was obtained and it was ≤35 cycles, the replicate was considered positive to *IS*1111 qPCR; and if no Ct value was obtained or the Ct value was >35 cycles, the replicate was considered negative to qPCR overall and no further qPCR testing was conducted. If an extract replicate was positive to *IS*1111 qPCR, the extract was tested next for the *com1* gene of *C. burnetii*. If a test replicates yielded a Ct value ≤38 cycles, the replicate was considered positive to qPCR overall; and if no Ct value was obtained on the *com1* qPCR or the Ct value was >38 cycles, the replicate was considered negative to qPCR overall.

Antibody detection

The presence of IgG against phase 1 and phase 2 *C. burnetii* antigens in BTM samples was tested using the indirect enzyme-linked immunosorbent assay (ELISA) IDEXX Q fever (*C. burnetii*) antibody test kit (IDEXX Laboratories, Switzerland). The test was performed on 1:5 diluted milk whey obtained by BTM sample centrifugation for IgG against phase 1 and phase 2 *C. burnetii* according to the

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manufacturer's instructions. Positive and negative controls were included in the kit and they were each tested in duplicate.

The optical density (OD) was measured using a standard photometer at 450 nm wavelength and the sample-to-positive ratio (S/P) was calculated for each tested BTM sample as:

$$S/P = \frac{OD_{Sample} - OD_{Negative\ control}}{OD_{Positive\ control} - OD_{Negative\ control}},$$

Samples with a S/P ratio <30% were considered negative and >40% positive. Samples with S/P ratios that fell outside of these ranges were declared inconclusive and the sample was retested. If the result was again inconclusive the sample was declared negative. A herd was then declared *C. burnetii* positive if either the BTM qPCR or ELISA were positive.

Questionnaire design

The literature was reviewed to explore dairy goat farm- and herd-level characteristics associated with *C. burnetii* positivity.[11, 12] Characteristics included herd size and type, the presence or absence of animal introductions into the herd, the presence or absence of a history of reproductive disorders in the herd, animal housing facilities, biosecurity and hygiene measures (including vaccination status of those working on the farm) (Table 1). Based on this review, a questionnaire was developed (supplementary material S3). The questionnaire was piloted with three representatives of commercial dairy goat farms before being distributed to participants. The form of the questionnaire distributed to participants depended on their preferences: that is, for those preferring paper-based questionnaires either posted to them or brought to the farm for completion, others received a link to the questionnaire via

Table 1. Explanatory variables analyzed for associations with *Coxiella burnetii* bulk milk tank positivity in a cross-sectional study of coxiellosis in commercial Australian dairy goat herds, 2018–2019

Variable group	Variable (units)
Farm demographics	Number of goats on farm; number of sheep on the farm; number of cattle on farm; number of horses on farm; number of dogs on farm; number of cats on farm; number of alpacas on farm; number of chicken on farm; number of ducks on farm; other animals on the farm that had not been listed[a]; any animal introduction during last 24 months[a]; number of goats introduced during last 24 months; number of sheep introduced during last 24 months; number of cattle introduced during last 24 months; number of horses introduced during last 24 months; number of dogs introduced during last 24 months; number of cats introduced during last 24 months; number of alpacas introduced during last 24 months; number of chicken introduced during last 24 months; number of ducks introduced during last 24 months; species and number of other animal introductions to the farm during last 24 months; goats housed outdoors[a]; shared boundary with other livestock farms,[a] sighting free-roaming livestock,[a] sighting free-roaming wildlife,[a] the approximate size (in acres) of farm, the size of the fields (in acres) available for your goats, farm income last year[b]
Farm management	Veterinarian contacted,[c] veterinarian visited and clinically examined goats,[c] internal parasite treatment,[c] external parasite treatment,[c] antibiotic applied to goat herd,[c] handwashing before working,[d] change into clean cloth before working,[d] change into new shoes before working,[d] ask visitor to disinfect or change shoes on arrival,[d] limited visitor access to goats,[d] handwashing after working,[d] change into clean cloth after working,[d] change to new shoes after working,[d] disinfect by footbath,[d] where are the cloth laundered,[e] vaccination status of on-farm personal for Q fever,[f] number of adults living on the property, number of adults working on the property, number of adults on farm vaccinated for Q fever, number of adult living on farm screened positive for Q fever, visitor regulation for proof of vaccination,[g] how goat manure is disposed
Herd management	Goat breed, number of male goats (>12 month), number of female goats (>12 month), number of kids (≤12 month), ticks seen on goat,[a] specific kidding season(s),[a] starting and ending month of each kidding season, number of goats mated in each kidding season, number of goats pregnant in each kidding season, does that deliver kid in each kidding season, shed cleaning before kidding,[h] bedding material replacement before kidding,[d] feed container and milk feeder cleaning before kidding,[d] places where goats give birth,[i] kidding place accessibility of other animals during kidding,[a] shed cleaning when one batch of herd finished kidding[a]; shed cleaning frequency after kidding season,[j] method of cleaning kidding place, kind of bedding material and its origin, disposal of bedding material, history of reproductive disorders[a]

[a] Binary outcome (1 = yes, 0 = no).
[b] Ordinal variable (1 = under $50,000, 2 = $50,000 to $99,999, 3 = $100,000 to $249,999, 4 = $250,000 to $499,999, 5 = over $ 500,000, 6 = uncertain, 7 = I would rather not answer).
[c] Ordinal variables (1 = weekly, 2 = monthly, 3 = every 2 to 11 months, 4 = 1 to 2 times yearly, 5 = not in the last 2 years).
[d] Ordinal variables (1 = never, 2 = rarely, 3 = sometimes, 4 = often, 5 = always, 6 = not applicable). shed cleaning before kidding.
[e] Ordinal variable (1 = onsite by the farm, 2 = offsite by workers, 3 = other, that is, we wash ours on farm, while father and mother-in-law wash theirs on their farm).
[f] Ordinal variable (1 = unsure, 2 = none vaccinated/immune, 3 = some vaccinated/immune, 4 = all vaccinated/immune, 4 = I prefer not to say).
[g] Ordinal variable (1 = not required, 2 = proof of vaccination will be asked but not mandatory, 3 = proof of vaccination is required and mandatory, 4 = not sure, 5 = I prefer not to say, 6 = other, that is, for biosecurity reasons visitors are not allowed into the area where the goats are, except for our vet).
[h] Ordinal variable (1 = indoors in a kidding shed, 2 = outdoors, 3 = other, that is, depending on weather, goat chose their kidding place etc.).
[i] Ordinal variable (1 = every day, 2 = every week, 3 = every month, 4 = at the end of kidding season, 5 = never).

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Table 2. Questions used to create binary variable 'biosecurity awareness (on farm)' in a cross-sectional study of coxiellosis in commercial Australian dairy goat herds, 2018–2019

Biosecurity awareness related questions	Always n (%)	Often n (%)	Sometimes n (%)	Rarely n (%)	Never n (%)
How often do you					
Wash your hands[a] before handling goats	21 (43)	12 (24)	6 (12)	6 (12)	4 (8)
Change your clothes before handling goats	9 (18)	7 (14)	12 (24)	9 (18)	13 (27)
Change your shoes before handling goats	7 (14)	6 (12)	10 (20)	10 (20)	16 (33)
Wash your hands[a] after handling goats	37 (76)	9 (18)	2 (4)	1 (2)	0
Change your clothes after handling goats	14 (29)	6 (12)	8 (16)	13 (26)	8 (16)
Change your shoes after handling goats	14 (29)	6 (12)	9 (18)	10 (20)	10 (20)
Disinfect your shoes using a footbath after handling goats	5 (10)	3 (6)	1 (2)	8 (16)	32 (65)
Practice used	**Yes n (%)**	**No n (%)**			
Composted/burned manure	24 (49)	25 (51)			
Wash cloth on farm	21 (43)	28 (57)			
Fully immune to Q fever	16 (33)	33 (67)			

[a] With soap and water (or equivalent).

email based on a version generated in REDcap[27] electronic data capture tools hosted at The University of Melbourne.

Farmers' responses about their farm's biosecurity practices were recorded by their responses to 10 questions related to on-farm biosecurity. These 10 questions were based on the National Farm Biosecurity Reference Manual,[28] with seven questions related to generic farm hygiene practices and three questions related to practices of direct relevance to Q fever biosecurity (Table 2). Farms were classified as having 'higher biosecurity practices' if the respondent answered ('always', 'often' or 'sometimes') to at least five of the seven generic hygiene questions and to all three Q fever relevant questions. Otherwise, the farm was classified as having 'lower biosecurity practices'. A binary variable 'farm biosecurity practices' was constructed based on the biosecurity classifications of farms. Data were entered into REDCap[27] for collation and storage and were analysed using Stata v.14.1.[29]

Survey data analysis

Herds were classified as *C. burnetii* positive if their BTM specimen tested positive on either qPCR or the ELISA. Apparent herd-level prevalence of coxiellosis was reported using exact binomial 95% confidence intervals (CIs), and true herd-level prevalence was estimated by a stochastic approach considering the overall sensitivity and specificity of the testing strategy through the combined distributions of the individual test characteristics (see details and R code in supplementary material S4).

The association between each candidate risk factor and herd-level *C. burnetii* positivity was assessed using multivariable logistic regression. Responses to closed, semi-closed and open-ended questions were re-coded into dichotomous variables for the purpose of regression analysis. The questionnaire responses were collapsed into the following 15 candidate risk factors to construct a putative causal diagram: herd size, indoor infrastructure, tick infestation, stocking density, indoor kidding, kidding pen accessibility for other animals, postkidding cleaning, livestock farm next door, free roaming stock, pest and wildlife, visitor restrictions, new stock introduced, farm staff size, companion animal on site and multiple kidding seasons. The variables 'kidding pen accessibility' and 'postkidding cleaning' were excluded because they had more than 10% values missing. Stocking density was calculated as the count of goats on the farm divided by the area accessible to the goats (in acres). A directed acyclic graph (DAG) was developed based on the available literature (Figure 1), face validated with two commercial dairy goat farmers and used to guide the model building. Explanations of the rationale for each causal link are provided in supplementary material S5. DAGitty was used to create the DAG and separately inform and assess minimal sufficient adjustment sets for each risk factor of interest.[30] Logistic regression models were constructed and analysed using the Stata command 'survey' logistic regression, applying the finite populate correction as well as accounting for lack of independence in the data arising from some farms comprised of more than one herd.[29] Exact logistic regression was used to estimate the median unbiased odds ratio wherever quasi-complete separation of the data was encountered.[31]

Results

Survey participants and response rate

Data were collected from June 2018 to December 2019 targeting all commercial dairy goat farms across Australia. A flow diagram detailing the recruitment and exclusion process as well as the distribution of farms by Australian State is shown in Figure 2. Out of the 68 farms in our sampling frame, nine farms had ceased their commercial activities by the time this study was carried out. Seven new farms were recruited into the population producing a total of 66 farms

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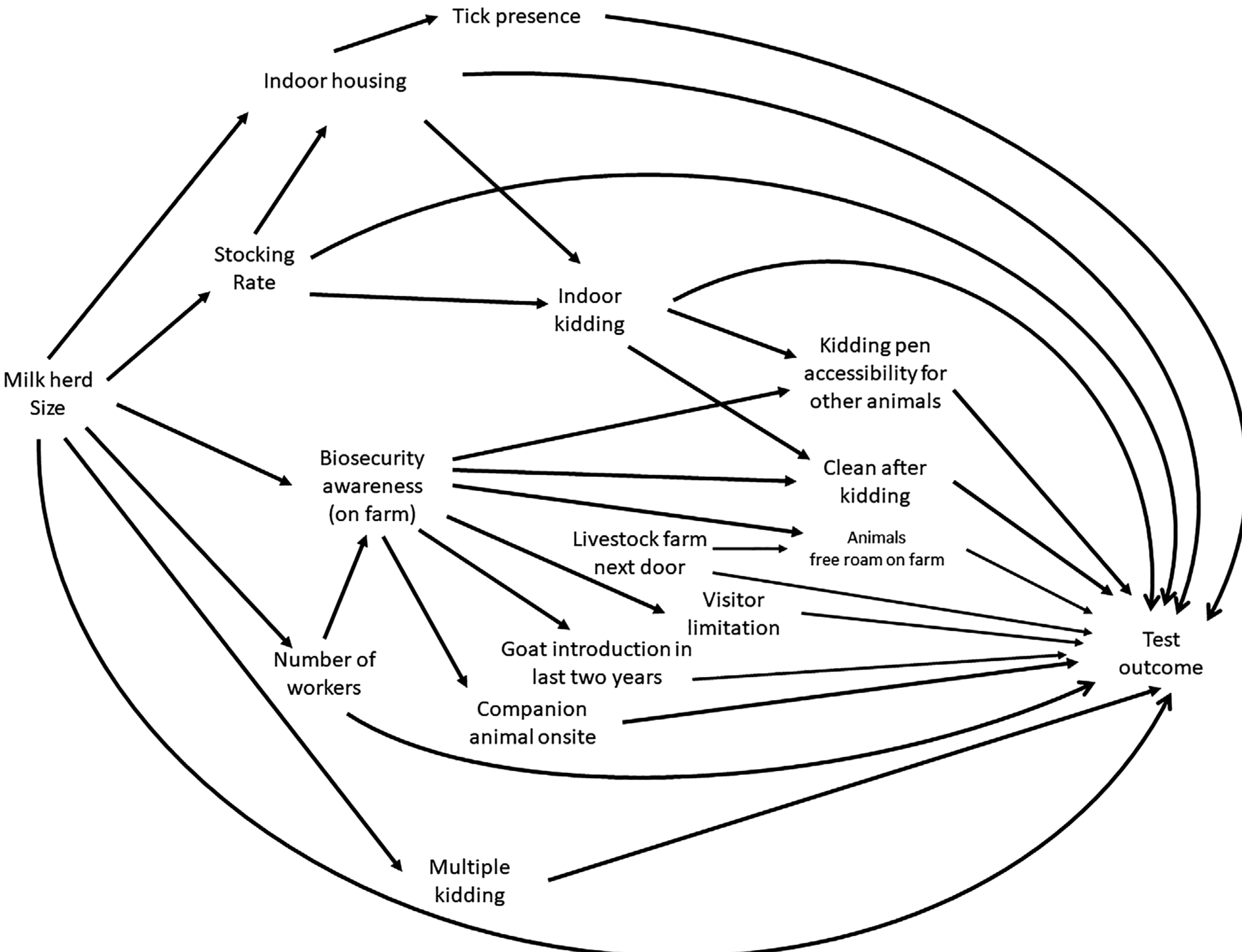

Figure 1. Directed acyclic graph showing putative causal paths linking explanatory variables to the bulk tank milk sample test outcome in a cross-sectional study of coxiellosis in commercial Australian dairy goat herds, 2018–2019. A detailed description of each of the links in the above plot is provided in supplementary materials S5.

eligible for sampling. We were unable to contact herd managers from 14 farms and three herd managers ceased to communicate following our initial invitation to take part in the study. This left a total of 49 of the total population of 66 farms that comprised the study population, a participation proportion of 74%.

Prevalence of C. burnetii *and minimum detectable odds ratios*

Of the 49 participating farms, three farms were positive to both ELISA and qPCR and two were ELISA positive but qPCR negative. The overall apparent herd-level *C. burnetii* positivity was 10% (95% CI 4 to 22) and true prevalence was 3% (95% credible interval: 0 to 18). Four of the positive farms were located in Victoria and one was in Queensland.

For a sample size of 49, with 80% power and 95% confidence, the minimum detectable odds ratio was estimated to be 4.6. This study had 50% power for detecting an odds ratio of 3 with 95% confidence.

Herd and farm-level characteristics

The number of goats kept on the 49 study population farms was highly skewed, ranging from 20 to 8,000 goats with a median of 167 goats (median of 114 does, ranging from 12 to 5,000). Male goats were kept on 82% of farms. Of the 49 study population farms, three did not keep any kids on farm. Study population farms ranged in area from 2.5 to 1,100 acres, with half being less than 99 acres. The number of goats per acre varied markedly with 75% of the farms having stocking densities of ≤50 goats per acre and 9 farms having >100 goats per acre. Four out of 49 study population farms held exclusively goats and all of these were large enterprises managing more than 2,000 does. Dogs were the species kept most often among the rest of the study population farms. Other species kept included chickens, cats, cattle and horses, as detailed in Table 3 along with descriptive statistics for the other continuously distributed variables.

New goats had been introduced during the past 2 years in 61% of the study population farms (Table 4). In terms of housing, 34 of the

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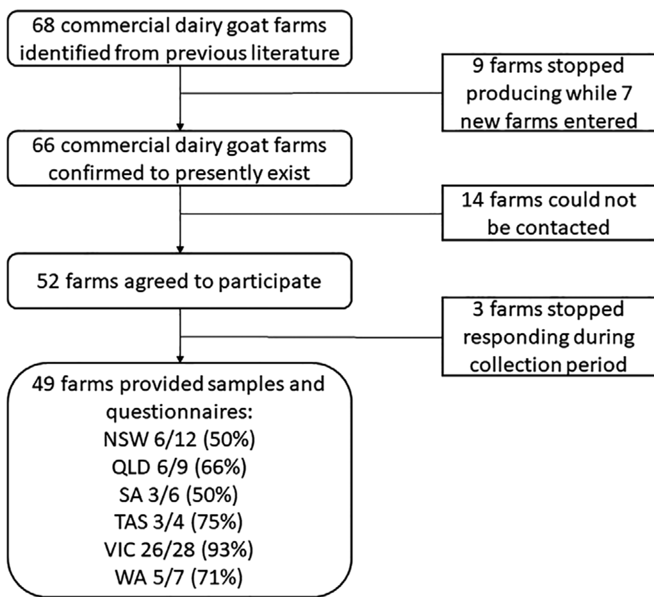

Figure 2. **Recruitment process and the distribution of farms by state, in a cross-sectional study of coxiellosis in commercial Australian dairy goat herds, 2018–2019.**

49 farms kept their goats outside at all times of the year whilst on 15 farms goats were exclusively housed indoors. Most dairy goat farms had another livestock farm adjacent to their farm (n = 42). It was very common for herd managers to observe animals (domestic or wildlife) free-roaming onto their farms (n = 32).

Only four of the 49 study population herd managers reported seeing ticks on their goats. BTM samples from all of these four farms tested negative. Of the 49 study population herd managers, 59% reported that their herd had only one kidding season each year. The remainder had two or more kidding seasons or all-year around kidding. In terms of kidding management, pregnant does kidded outside on 23 of the 49 study population farms while 17 farms had an indoor kidding shed. The other nine herd managers reported that the place of kidding (inside or outside) was dependent on the weather. A little over one-half of the 49 study population herd managers (59%) reported that during kidding periods other animals can gain access to the kidding pen. Most (90%) reported no increase in the incidence of reproductive disorders in the past 2 years. Six of the 49 study population herd managers recalled the presence of reproductive disorders in the last 2 years, of whom three assumed that these were due to nutritional problems.

Factors associated with C. burnetii *positivity*

Crude associations between each of the hypothesized risk factors and herd-level *C. burnetii* positivity are presented in Table 4. After adjustment for confounding only two of the hypothesized risk factors were identified as associated with *C. burnetii* positivity: the size of the milking herd and stocking rate. Farms with >900 does had a 6.75 (95% CI 1.65 to 27.7) fold increase in the odds of being

Table 3. **Descriptive statistics of continuous variables in a cross-sectional study of coxiellosis in commercial Australian dairy goat herds, 2018–2019**

Variable	Valid entries n	Mean	Median	Range
Total number of goats	49	782	167	20–8,000
Male goats (>1 year)[a]	40	11	5	1–50
Female goats (>1 year)	49	556	114	12–5,000
Kids (≤1 year)[a]	46	229	66	1–3,000
Other livestock on the farm[a]				
Sheep	13	52	22	4–200
Cattle	19	45	14	2–400
Horses	16	4	3	1–10
Alpacas	6	3	2	1–8
Poultry kept on the farm[a]				
Chickens	29	6,909	10	1–200,000
Ducks	10	10	7	2–20
Companion animals kept on the farm[a]				
Dogs	37	4	2	1–15
Cats	24	3	2	1–12
Alpacas	6	3	2	1–8
Farm size (acres)	49	195	99	2.5–1,100
Stocking density (head/acre)	49	135	5	1–2000
Adult people living on the farm	49	3	2	0–10
Adults working on the farm	47	4	2	0–25

[a] Summary statistics only for the farms with any animals of this class.

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Table 4. Putative causal diagram-guided logistic regression analysis outputs for variables associated with *Coxiella burnetii* bulk milk tank positivity in a cross-sectional study of coxiellosis in commercial Australian dairy goat herds, 2018–2019

Variable	No. positive/n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)[a]	Adjustment set (total effect)
Average milking herd size				
>900	3/13 (23)	6.75 (1.08, 42.01)	6.75 (1.08, 42.01)	NA[b]
≤900	2/36 (6)	Reference	Reference	
$\log_{10}$ (stocking rate)[b]	-	2.94 (1.52, 5.67)	2.53 (1.51, 4.23)	Milking herd size
Number of workers on farm				
>5	4/22 (18)	5.78 (0.90, 37.29)	3.33 (0.62,18.00)	Milking herd size
≤5	1/27 (4)	Reference	Reference	
Housing				
Indoor	4/15 (27)	12.0 (1.83, 78.59)	5.10 (0.65, 39.76)	Milking herd size
Outdoor	1/34 (3)	1	1	Stocking rate
Kidding				
Indoor only	4/17 (24)	9.54 (1.47, 62.07)	1.03 (0.20, 5.22)	Indoor housing,
Outdoor	1/32 (3)	1	1	Stocking rate
Tick presence[c]				
Yes	0/4	1.64 (0, 15.59)	1.89 (0, 27.39)	Indoor housing
No	5/45	1	1	
Livestock next door[c]				
Yes	5/42 (12)	1.18 (0.14, inf)	1.18 (0.14, inf)	NA
No	0/7 (0)	Reference	Reference	
Animals free roaming[c]				
Yes	3/35 (9)	0.56 (0.21, 1.54)	0.61 (0.05, 9.58)	Livestock, companion animal
No	2/14 (17)	Reference	Reference	Presence, indoor kidding
New goat introductions in the last 2 years				
Yes	3/30 (10)	0.94 (0.27, 3.27)	1.12 (0.26, 4.75)	Companion animal presence,
No	2/19 (11)	Reference	Reference	Indoor kidding
Companion animals on site				
Yes	2/39 (5)	0.13 (0.04, 0.37)	0.22 (0.02, 2.58)	Indoor kidding, animals free
No	3/10 (30)	Reference	Reference	Roaming
Multiple kidding seasons per year				
Multiple	3/22 (14)	1.97 (0.33, 11.88)	1.10 (0.27, 4.54)	Milking herd size
Single	2/27 (7)	Reference	Reference	
Biosecurity awareness				
High	0/1 (0)	8.80 (0, 4.343.2)	29.14 (0, 1136.57)	Milking herd size, number of
Low	5/43 (10)	Reference	Reference	Workers on farm
Visitor limitation				
Yes	3/23 (13)	0.67 (0.19, 2.33)	0.44 (0.14, 1.40)	Milking herd size, number of
No	2/22 (9)	Reference	Reference	Workers on farm

[a] These effect estimates, their odds ratio and confidence intervals represent total effect based on minimum adjustment sets as informed by the directed acyclic graph presented in Figure 2.
[b] Variable not categorized as it demonstrated a linear association once log transformed. Stocking density calculated as the count of goats on the farm divided by the acres accessible to the goats.
[c] Exact logistic regression was used for this variable due to quasi-complete separation of the data.
CI, confidence interval; NA, not applicable; OR, odds ratio.

C. burnetii positive compared with farms with ≤900 does. For each order of magnitude increase in the number of goats per acre, the odds of BTM positivity increased by a factor of 2.53 (95% CI 1.51 to 4.22).

Discussion

To the best of the authors' knowledge this is the first study that has quantified the herd-level prevalence of *C. burnetii* among

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commercial dairy goat farms in Australia. The true herd-level prevalence of *C. burnetii* in commercial dairy goat herds in Australia was 3% (95% CI 0 to 18%). This herd-level prevalence estimate is consistent with previous estimates including a recent study only in Victoria that found herd-level seroprevalence of 8% in farms holding goats.[17]

Out of the five herds that were *C. burnetii* positive, three tested positive with both PCR and ELISA suggesting the presence of ongoing active infections.[32] Two other herds were only positive to the ELISA which indicates that while does in the herd were likely to have been exposed to *C. burnetii* there were few (if any) actively shedding does at the time of sampling or other factors may have reduced the amount of target DNA in the milk to a concentration below the limit of detection of the PCR assays used. In a previous study on a known infected farm,[33] BTM ELISA results were constantly positive, while PCR results alternated between positive and negative suggesting that the concentrations of *C. burnetii* DNA present were close to the limit of detection of the assay on such samples. We therefore considered the two PCR negative / ELISA positive farms to be PCR false negatives.

The method of targeting the *IS*1111 gene in qPCR is very sensitive as 7 to 120 copies of *IS*1111 exist within the *C. burnetii* genome. It is therefore used to test for the presence of the bacterium with high sensitivity.[34] However, *IS*1111 is not specific for the *C. burnetii* genome as this sequence can also be found in *Coxiella*-like organisms.[35, 36] The single *com1* gene from *C. burnetii* is very specific and highly conserved and therefore can be used to confirm *IS*1111 findings.[37] Although unlikely, a small number of farms could be infected by a strain of *C. burnetii* which is missing the *IS*1111 transposon and therefore test negative by our qPCR protocols. In most cases, these farms should be detected as positive by ELISA.

In this study, two related risk factors were identified as being associated with herd *C. burnetii* positivity: milking herd size and stocking rate. The association between the size of the milking herd and *C. burnetii* positivity has previously been reported in studies of goats.[10–12] Intensively managed herds are more susceptible to infectious disease due to high rates of contact between animals and risks of exposure to higher levels of environmental contamination. High biosecurity levels need to be maintained to prevent disease and the odds of pathogen introduction and transmission within herds can be increased in such settings. Nevertheless, one BTM study in 2015 investigating the prevalence of and risk factors for *C. burnetii* in dairy herds in Iran[38] reported a contrasting result. In the Iranian study 163 cattle BTM samples were tested by qPCR using the *IS*1111 gene finding that herds with less than 80 animals had a higher risk of being *C. burnetii* positive compared with larger herds. This is likely to be explained by differences in the way large herds are managed in Iran, in particular their use of a nonseasonal calving systems and use of dedicated calving facilities which result in (presumably) lower levels of environmental contamination. Another explanation is the dilution effect. In smaller herds with animals shedding *C. burnetii*, there may be a higher concentration of genomic DNA compared to larger herds.

The following characteristics were previously found to be associated with *C. burnetii* positivity in dairy goat farms: the presence of companion animals on site, the presence of low herd manager biosecurity awareness, the presence of ticks on goats and housing and kidding indoors. That none of these factors were identified as being associated with *C. burnetii* positivity in this study may be due to a lack of statistical power to identify risk factors where the strength of the association was weak. Three out of the five positive herds had previously been identified as being *C. burnetii* positive and had implemented biosecurity regulations including a prohibition of allowing companion animals on site. Most of the 49 study population herd managers (39 of 49) reported having at least one dog or cat present on farm. Tick presence was not associated with infections likely because the presence of ticks was only reported by a small number of the study population herd managers. Additional possible explanations for the lack of detection of an association in the present study with the presence of companion animals on farms may be because a high proportion of such animals were likely to have been desexed, less risk has been associated with nonperiparturient dogs and cats.[39, 40] Also, it was noted in interview comments that companion animals were mostly kept indoors on many of the farms.

A previous study from the USA found that housing of goats indoors was not associated with *C. burnetii* positivity.[41] In this study, indoor kidding was hypothesized to be a potential risk factor for *C. burnetii* positivity as does that kid indoors have a greater chance of contacting *C. burnetii* shed by other infected goats kidding in the same environment. A previous Canadian study by Meadows *et al.* found that the risk of *C. burnetii* positivity was lower in those herds where does kid outdoors.[10]The response rate for this study was 74%, so we can likely assume that the results presented in this study are valid for the population of 68 commercial dairy goat herds across Australia from 2018 to 2019. We did not achieve the target sample size, confidence intervals were therefore wider than desired, and in some respects, the study was underpowered. However, we had enough power to detect risk factors strongly associated with farm positivity (50% power to detect odds ratios ≥3).

We constructed a DAG to guide the data analysis. Ideally the DAG should be constructed to inform the questionnaire design process, otherwise some risk factors could be omitted in the data collection process. However, as we did a comprehensive literature review before constructing the questionnaire and no further risk factors were identified in piloting; it is unlikely that any major risk factors were missing.

The number of commercial dairy goat farms across Australia fluctuates from year to year and at any given time it is difficult to determine the exact number of farms in operation. Nonrecruitment analysis could be one means of attempting to address this issue; however, this was not possible in this study because nonparticipant could not be contacted. Although almost all of the herd managers that could be contacted were interested in taking part in this study, it is still possible that some did not want to know their farm's Q fever status for fear of consequences if their herd was found to be positive. Some of the farms recruited have more than one kidding season each year, and chances of detecting infected farms would have been higher if multiple BTM samples were collected to ensure sampling close to each kidding season. However, resources were limited, as was opportunity to repeatedly sample these farms. In a follow-up

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risk assessment, we have repeated sampling on farms that tested negative in this analysis, none have been subsequently found to be positive [data not shown].

Conclusions

This study found five *C. burnetii* positive farms based on BTM samples collected from 49 commercial dairy goat farms in Australia between 2018 and 2019, equivalent to a herd-level apparent prevalence of 10% and estimated true prevalence of 3%. Large herds (>900 does) and higher stocking densities were associated with an increased risk of being *C. burnetii* positive. Given the relatively low prevalence of *C. burnetii* positivity among commercial dairy goat herds it would be beneficial for herds that were negative for *C. burnetii* to focus on strategies to prevent introduction of *C. burnetii* into their herds through risk assessment, implementation of specific biosecurity measures and establishing effective, ongoing surveillance plans to promptly detect incursions of infection if they occur. Further research could also focus on *C. burnetii* positive herds, such as designing cohort studies to investigate animal-level risk factors for coxiellosis as well as establishing which control measures minimize the risk of *C. burnetii* outbreaks and transmission to *C. burnetii* negative herds.

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Conflicts of interest and sources of funding

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Supporting information

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Data S1 Supporting information.

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