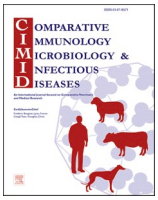
Comparative Immunology, Microbiology and Infectious Diseases 80 (2022) 101727

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid



Seropositivity to *Coxiella burnetii* in primiparous and multiparous ewes from southern Australia: A cross-sectional study

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ARTICLE INFO

Keywords:
Abortion
Coxiella burnetii reservoir
Lamb mortality
Lamb survival
Maiden ewe
Q-fever
Reproduction
Sheep

ABSTRACT

The role of infectious diseases including coxiellosis in causing poorer reproductive performance of primiparous ewes are not well studied. The aims of this study were to determine if natural exposure to *Coxiella burnetii* is widespread in breeding ewes and whether seropositivity is associated with poor reproductive performance of primiparous ewes. Seropositivity to *Coxiella burnetii* was 0.08% (CI95% 0.01, 0.36) in primiparous ewes and 0.36% (CI95% 0.07, 1.14) in mature ewes. *Coxiella burnetii* was not detected in aborted or stillborn lambs using qPCR. These findings suggest *C. burnetii* infection was unlikely to be an important contributor to abortion and perinatal mortalities observed for primiparous ewe flocks, and exposure to *C. burnetii* was not widespread in ewes on farms located over wide geographical region of southern Australia. Whilst ewes on these farms were not an important reservoir for *C. burnetii*, sporadic zoonotic transmission from sheep is reported and has public health implications.

1. Introduction

The reproductive performance of primiparous ewes is often lower than that observed for multiparous ewes [1–3]. Higher incidence of foetal or lamb mortality from pregnancy diagnosis to lamb marking has been reported for primiparous ewes compared to multiparous ewes [2–4]. However, the causes of losses that occur during this period are not well defined. A number of endemic diseases may cause abortion and poor viability of lambs in Australia [5,6]. It is not clear if infectious diseases are an important contributor to foetal and lamb mortality for primiparous ewes in Australia.

Coxiella burnetii is endemic in Australian livestock, including sheep, but prevalence and impact on sheep health is not well studied [7]. Infections in sheep can be asymptomatic, but in pregnant ewes can cause abortion, stillbirth and the birth of weak lambs that are less likely to survive [8–12]. The outcome of infection in the pregnant ewe may be influenced by strain virulence, severity of placental infection, and maternal and foetal immunity [13]. Abortions are more likely to occur during gestation following primary infection, with no lasting impacts on reproduction in subsequent pregnancies [10,14]. Therefore, younger ewes that are immunologically naïve are at risk of abortion if infection occurs during pregnancy.

Seroprevalence for *C. burnetii* in Australian sheep has been reported to range from 0% to 18.7% depending on the location, serological assay and cut-off values used [7,15–18]. However, most of these studies are either over 50 years old or localised to specific regions or single farms. Consequently, the prevalence of *C. burnetii* for the current Australian sheep population is poorly quantified and the impact of *C. burnetii* on the reproductive performance of Australian sheep has not been assessed.

Apart from impacts for sheep health and production, *C. burnetii* has important zoonotic implications. Australia has one of the highest rates of human Q-fever cases reported globally [19,20]. Livestock are considered to be an important reservoir for infection in humans [21,22]. Improved understanding of the role of sheep as a potential source of *C. burnetii*

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https://doi.org/10.1016/j.cimid.2021.101727
Received 11 August 2021; Received in revised form 23 November 2021; Accepted 24 November 2021
Available online 30 November 2021

infections will inform recommendations for managing Q-fever risk in susceptible people including occupational risks for farmers, veterinary staff and abattoir workers [23,24].

The aims of this study were to (i) determine if natural *C. burnetii* exposure is associated with poor reproductive performance of primiparous ewes in southern Australia, (ii) determine if natural exposure to *C. burnetii* is widespread in primiparous and multiparous ewes, and (iii) determine if ewes represent an important reservoir for *C. burnetii* infection in humans. We hypothesised that (i) *C. burnetii* infection and seropositivity is associated with foetal and lamb loss in primiparous ewes, and (ii) ewes will demonstrate seropositivity for *C. burnetii* indicating that ewes are reservoir for infection.

2. Methods

All procedures were conducted according to guidelines of the Australian Code of Practice for the Use of Animals for Scientific Purposes and were approved by the Murdoch University Animal Ethics Committee (R3004/17).

2.1. Animals and research sites

This cross-sectional study was conducted at 28 farms using 30 study flocks located in Western Australia ($n = 11$), South Australia ($n = 9$), and Victoria ($n = 10$) between 2018 and 2020 (Fig. 1; Supplementary File 1). Farms were selected based on convenience sampling, with eligibility for inclusion based on the farm having sufficient number of maiden ewes available for the study, capacity to monitor ewes and their progeny over the study period, and sheep genotype and management that were generally representative of standard commercial sheep farms in the region. Approximately two-hundred primiparous ewes at each farm were randomly selected at mating. All farms ran self-replacing flocks and ewes included in the study were managed according to standard farm practice.

2.2. Animal measurements and sample collection

Primiparous ewes were monitored between mating and lamb marking. Ewes were mated as either ewe lambs (7–10 months, $n = 19$ flocks) or primiparous yearling ewes (18–20 months, $n = 11$ flocks). Foetal mortality was determined via sequential transabdominal pregnancy ultrasounds at 62–101 days (scan 1) and 108–136 days (scan 2) from the start of mating. Lamb mortality between birth and marking were determined for each ewe based on birth type (single, twin or triplet), birth status (lambs dead or alive at lambing rounds) and survival status (lambs dead or alive) at marking which was approximately six weeks from the start of lambing. Ewe lactation status (lactating or non-lactating) was also determined at lamb marking.

Blood samples were collected from primiparous ewes by jugular venipuncture at pre-mating, scan 1, scan 2, pre-lambing and lamb marking. At each farm, 20 multiparous ewes aged three years or older that had been bred and reared on the same farm were also randomly selected for collection of blood samples. Timing of sampling relative to lambing and the reproductive outcome for pregnancy was not recorded for multiparous ewes (Supplementary File 2). Blood samples were collected into serum vacutainer tubes with clot activator and stored on ice. Blood samples were centrifuged at 4000 rpm for 10 min within 72 h of collection. Serum was then decanted into 2 mL low protein-binding polypropylene screw cap micro tubes and stored at - 20°C prior to serological testing.

2.3. Serology

Coxiella burnetii serology sample size was determined using expected seroprevalence 10% (based on an apparent prevalence ranging between 0% and 19% in previous Australian studies [7,15–18]). A sample size of 200 ewes was adequate to estimate true prevalence with CI95% based on assumed true prevalence 10% and precision 5% with test sensitivity 87% and specificity 98% [25]. Sample size required to detect disease was 27 ewes per flock assuming expected seroprevalence 10%, test sensitivity 87%, population size 200 ewes and CI 95%.

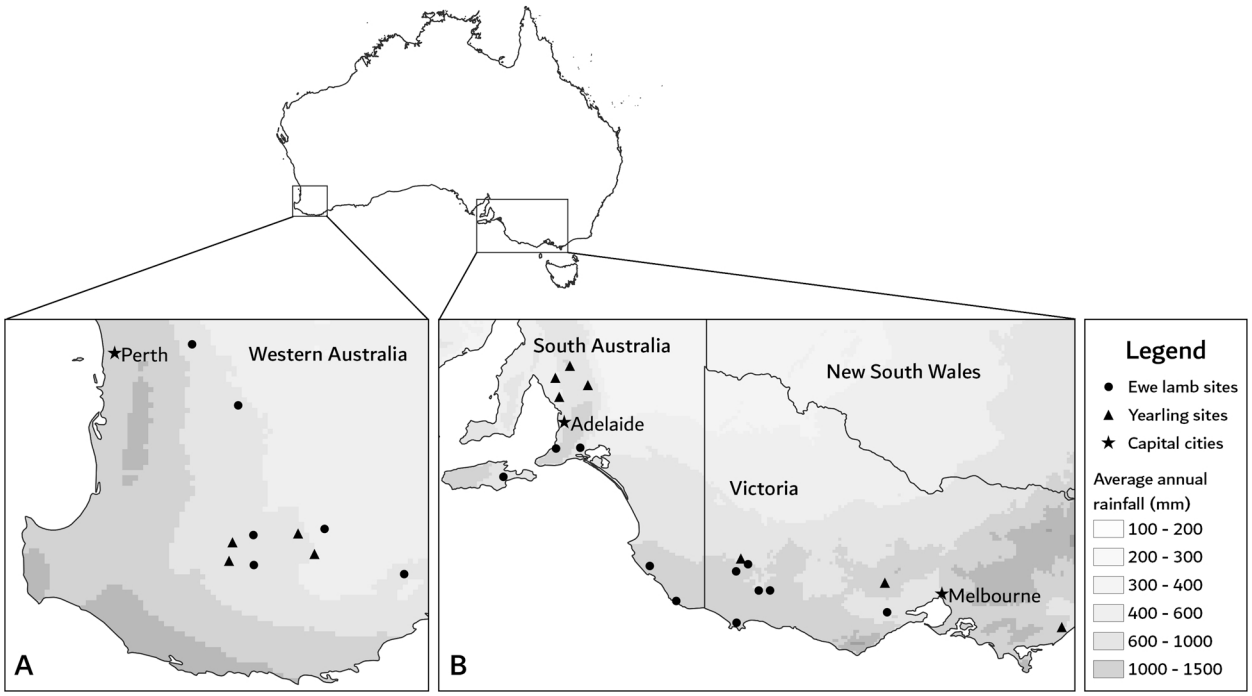

Fig. 1. : Location of sheep farms sampled in Western Australia (Map A), and South Australia and Victoria (Map B). Average annual rainfall data sourced from Australian Government Bureau of Meteorology [57].

A sub-sample of at least 40 primiparous ewes from each study flock were selected for *C. burnetii* serology (Supplementary File 1). Where possible, selection was based on ewes that were identified as pregnant at scan 1 but failed to successfully rear a lamb. This included ewes that aborted as well as ewes for which lamb mortality occurred in the perinatal period. Ewes that had reared single or twin lambs were included for flocks with less than 40 ewes that failed to rear a lamb (Supplementary File 1). Blood samples collected from primiparous ewes at lamb marking were used for serological screening except where not available, in which case samples from the latest available timepoint were screened instead. All blood samples collected from the multiparous ewes ($n = 20$ per farm) were used for serological screening (Supplementary File 2).

Anti-*C. burnetii* IgG serology were determined by VETPATH Laboratories (Perth, Western Australia) using a commercial indirect ELISA kit (ID Screen Q-Fever Indirect Multispecies, ID Vet, France) according to the manufacturer's instructions. The results were read at 450 nm using a Multiskan FC, Thermo Scientific spectrophotometer. Each plate included positive and negative internal controls. Optical density (OD) values were expressed as the mean percentage of sample/positive (S/P) values, as recommended by the manufacturer:

$$\text{S/P value} = (OD_{sample} - OD_{negative\cdot control})/(OD_{positive\ control} - OD_{negative\cdot control})$$

Serum samples were classified as positive (S/P value ≥50), doubtful (S/P value 40 to <50) or negative (S/P value <40) according to the manufacturer's recommendation. Lurier, et al. [25] reported this indirect ELISA kit to have median sensitivity 86.9% (95% credible interval (CrI95%) 71.2%, 93.6%) and specificity 98.5% (CrI95% 97.3%, 99.4%) for sheep.

For cases that returned positive or doubtful results using indirect ELISA, samples were re-tested with the same indirect ELISA as describe above, plus a complement fixation test (CFT) to determine the end-point titre. The CFTs were performed on serum by Department of Primary Industry and Regional Development Diagnostic Laboratory Service using methodology previously described by Ellis and Barton [26]. For the CFT, a serum tyre of 1:8 or higher was considered positive [26]. Samples that returned one 'positive' result for either test were considered positive. Both laboratories used are NATA (National Association of Testing Authorities) accredited under ISO 17025 for veterinary testing.

2.4. Aborted and stillborn tissues

Tissue samples from aborted ($n = 2$) or stillborn ($n = 33$) lambs recovered from a subset of seven flocks of primiparous ewes (Flocks 1, 2, 3, 7, 11, 14, 16) from six farms in Western Australia were submitted to the Department of Primary Industry and Regional Development Diagnostic Laboratory Services (Perth, Western Australia). Tissue samples were screened for *C. burnetii* using qPCR as previously described in more detail by [27].

2.5. Statistical analyses

Lamb mortality was calculated based on the number of foetuses identified at scan 1 and the number of lambs marked. Lamb mortality was classified as 'abortion' based on foetal loss between scan 1 and scan 2 (and validated with lambing records and ewe lactation status). Apparent *C. burnetii* seropositivity proportion was calculated using number positive samples as a proportion of samples tested, with 95% confidence interval was determined using Jeffreys method [28]. Seropositivity proportion for the ewe age categories were compared using a two sample z-test to compare sample proportions (2-tailed) with *P*-value < 0.05 accepted as significant [29].

The true *C. burnetii* seropositivity proportion and 95% credible intervals (95% CrI) was estimated using Bayesian inference, considering the sensitivity and specificity and their 95% CrI derived from Lurier, et al. [25] as beta-pert distribution for priors [30].

3. Results

3.1. Reproductive performance of primiparous ewes

Reproductive performance for primiparous ewes are described in more detail by [31]. Briefly, foetal and lamb mortality between scan 1 and lamb marking for primiparous ewe lambs was 36% (1567/4351 foetuses identified at scan 1; range 14–71%) and for primiparous yearlings was 29% (582/2103 foetuses identified at scan 1; range 20–53%). Abortion (foetal loss between scan 1 and scan 2) was detected in 14/19 primiparous ewe lamb flocks and 6/11 primiparous yearling flocks. Abortion was detected in 5.2% (155/2968) ewes for primiparous ewe lamb flocks, ranging 0–50.0% across flocks. For primiparous yearling flocks, abortion was detected in 0.8% (16/1886) ewes, ranging 0–4.4% across flocks.

3.2. Coxiella burnetii serology

Apparent and true seropositivity to *C. burnetii* for ewe age categories are shown in Table 1. Apparent seropositivity to *C. burnetii* was 0.08% (CI95% 0.01%, 0.36%) in primiparous ewes and 0.36% (CI95% 0.07%, 1.14%) in mature ewes. Seropositivity to *C. burnetii* did not differ between primiparous ewes and mature multiparous ewes ($P = 0.174$), nor between primiparous yearlings and ewe lambs ($P = 0.165$).

Farm-level seropositivity to *C. burnetii* (detected in at least one animal) was 10.7% (3/28) farms. Within-flock seropositivity for the three flocks where seropositivity was detected ranged 2.5%–5.0% (Supplementary file 1). The three flocks with seropositivity to *C. burnetii* were located in Western Australia, South Australia and Victoria (Supplementary file 1).

All three samples with seropositivity detected using indirect ELISA were negative for *C. burnetii* by CFT (Supplementary File 3).

3.2.1. Molecular detection of C. burnetii in tissues from aborted and stillborn lambs

Coxiella burnetii was not detected by qPCR in tissue samples from aborted ($n = 2$) or stillborn lambs ($n = 33$) recovered from primiparous ewes on the subset of seven flocks in Western Australia [27].

4. Discussion

There was no evidence to implicate *C. burnetii* as an important contributor to abortion or perinatal lamb mortality in 30 primiparous ewe flocks located across southern Australia. The very low *C. burnetii* seropositivity was consistent with the absence of detection of *C. burnetii* in tissues from aborted or stillborn lambs from a subset of farms. These findings are consistent with recent reviews of veterinary laboratory investigations that reported coxiellosis to be an uncommon diagnosis in Australian sheep abortion investigations [5,6]. *Coxiella burnetii* control programmes such as routine vaccination of breeding ewes are not warranted for sheep farms in southern Australia in the absence of further evidence that coxiellosis is contributing to lamb mortality. Nonetheless, *C. burnetii* should continue to be included in sheep abortion and perinatal mortality investigation protocols due to the sporadic nature of disease and important zoonotic implications.

This is the most geographically widespread serological study for *C. burnetii* in Australian sheep. Very low seropositivity to *C. burnetii* was consistent with previous studies from Western Australia [18] and Victoria [7] that reported individual seroprevalence ranging from 0% to 4.1%, and flock-level seroprevalence ranging from 0% to 17.6%. Our study did not include sheep flocks from New South Wales, Queensland or Tasmania. New South Wales and Queensland have the highest rates of human Q-fever reported in Australia [20]. The most recent studies reporting *C. burnetii* prevalence in sheep from New South Wales and Queensland are considerably dated and involve either single farms [15, 16] or abattoir surveys [17]. Increased incidence of local acquisition of

Table 1
Apparent *C. burnetii* seropositivity and estimated true seropositivity using indirect ELISA for primiparous ewes mated as ewe lambs (approximately one-year-old at sampling) or yearlings (approximately two-years old at sampling) and mature multiparous ewes (aged 3 years or older) from 28 Australian farms.

	Ewes sampled				
	Flocks (n)	Individual ewes (n)	Seropositive samples (n)	Apparent seropositivity % (CI95%)	Estimated true seropositivity % (CrI95%)
Primiparous ewes					
Ewe lambs	19	839	0	0 (0, 0.30)	0.1 (0.0, 0.4)
Yearling	11	440	1	0.23 (0.02, 1.06)	0.3 (0.0, 1.1)
Mature ewes	28	558	2	0.36 (0.07, 1.14)	0.3 (0.0, 1.0)

CI95%: 95% confidence interval
CrI95%: 95% credible interval

human infection may be associated with high prevalence in livestock [32]. Hence, investigation of *C. burnetii* seroprevalence in sheep from New South Wales and Queensland is warranted.

There are several aspects of this study that limit the generalisability of the seropositivity to *C. burnetii* observed in these flocks to the general sheep population in southern Australia. Firstly, serological testing targeted primiparous ewes with evidence of abortion and perinatal lamb mortality. Bias towards ewes that failed to rear lambs could be expected to overestimate prevalence in the general sheep population if *C. burnetii* was an important contributor to abortion and perinatal deaths. Very low seropositivity to *C. burnetii* in this sampled population suggests that coxiellosis was not an important contributor to abortion and perinatal lamb mortality in these flocks. Very low seropositivity to *C. burnetii* in the sampled population of primiparous ewes was consistent with that observed for randomly selected mature ewes on these farms. Secondly, blood samples for primiparous ewe samples were collected close to the time of lambing or abortion, and this may increase probability of detection of *C. burnetii* seropositivity [33]. Lastly, whilst the inclusion criteria for farms included sheep genotype and management that were generally representative of standard commercial sheep farms in the region, inclusion criteria involved ability to monitor ewes over study period and some sheep studs were included in the study. Further investigation is required to confirm if very low seroprevalence is consistently observed across the general population of breeding ewes on commercial farms in southern Australia.

Sampling younger ewes likely contributed to the low seropositivity to *C. burnetii* reported in this study. Age is recognised as an important risk factor for *C. burnetii* seropositivity, with older animals more likely to be seropositive [34–36]. Notwithstanding this, no apparent difference in seropositivity was observed between primiparous ewe lambs (approximately 13 months old at lambing), yearlings (2 years old at lambing) and mature ewes (3 years or older).

There is no reference test for serological diagnosis of coxiellosis, and sensitivity and specificity for *C. burnetii* serological tests are not well described [37]. The commercial indirect ELISA for *C. burnetii* that was used in this study has been used in other seroprevalence studies in sheep [25,37–41] and the World Organisation for Animal Health (OIE) recommends ELISA as the preferred method for *C. burnetii* seroprevalence studies [42]. In our study, the three samples categorised as seropositive using indirect ELISA were negative by CFT. It was not possible to determine if these were false positives. Complement fixation tests are reported to have lower sensitivity than ELISA, but high specificity for elevated levels of anti-*C. burnetii* antibodies in flocks with *C. burnetii*-associated abortions [42]. Discordant results can be observed using different ELISA kits [43], therefore testing samples with more than one kit is an alternative option for validating animal status [42]. Validation for commercial ELISA in Australian sheep under field conditions could better inform estimation of true prevalence. However, coxiellosis is not frequently diagnosed in Australian sheep which presents challenges for evaluating assay sensitivity and specificity under field conditions.

Seroprevalence surveys may underestimate *C. burnetii* shedding in livestock. Banazis, et al. [18] detected *C. burnetii* in Australian sheep faecal samples in the absence of *C. burnetii* seropositivity. Other studies have also demonstrated poor correlation between seropositivity and antigen detection [10,12,37,44]. Joulié, et al. [37] reported good correlation between high *C. burnetii* burden on vaginal swabs and seropositivity one-month post-abortion or post-lambing using the same commercial indirect ELISA kit as used in our study. However, it is possible that some ewes in our study were shedding *C. burnetii* without evidence of seropositivity, and thus represent a reservoir of *C. burnetii* infection for other sheep or humans. Nevertheless, the combination of testing methodology used and timing of blood sample collection (within 6 weeks of parturition) in conjunction with the absence of detection of *C. burnetii* using molecular techniques on tissues from aborted or stillborn lambs suggests that coxiellosis was not a major contributor to abortion and lamb mortality observed on these farms.

This was an observational study with sheep managed extensively, reflecting standard sheep management in these regions of Australia. Although foetal and lamb mortality between scanning and lamb marking were high for some flocks, average lamb mortality in the primiparous flocks was consistent with ranges previously reported in Australian studies [45]. It is unclear from the current study whether reproductive performance of maiden ewes would be impacted in flocks where *C. burnetii* seroprevalence was greater.

This study focussed on *C. burnetii*, but foetal and lamb mortality are often multifactorial [45]. Endemic diseases other than coxiellosis were contributing to abortions and perinatal lamb mortality in flocks in this study. *Chlamydia pecorum* was detected in aborted foetuses, stillborn lambs and lambs with evidence of polyarthritis post-weaning in a subset of flocks [27,46]. Campylobacteriosis (*Campylobacter foetus* foetus) was identified in one flock [47]. There was no evidence that infection with *Toxoplasma gondii* [48] or *Neospora caninum* [49] were important contributors to foetal and lamb mortality observed in these flocks. Further investigations using data from this study will include multivariable analysis to evaluate the relative importance of different pathogens on reproductive performance.

Despite low seropositivity to *C. burnetii* detected in this study, contact with sheep should still be considered a risk factor for Q-fever in humans and precautions should be taken to reduce the risk of zoonotic *C. burnetii* transmission. Sheep have been associated with cases of Q-fever in humans in Australia and overseas [10,15,32,50–54]. *Coxiella burnetii* shedding can occur from both symptomatic and asymptomatic sheep, and in the absence of detectable seropositivity [55,56]. Control strategies include use of appropriate personal protective clothing when handling birth material or lambing ewes, good hygiene practices, controlling dust and vaccination of people with an occupational risk including farm, abattoir and veterinary staff.

5. Conclusion

There was no evidence to implicate *C. burnetii* as an important contributor to abortions or perinatal lamb mortality observed for primiparous ewes on the farms in this study. Furthermore, exposure to *C. burnetii* was not widespread in sheep from farms in southern Australia included in the study. Whilst ewes on these farms were not an important

reservoir for *C. burnetii,* the occupational risk associated with transmission of *C. burnetii* from Australian sheep has public health implications and people at risk should maintain appropriate measures to avoid zoonotic transmission.

CRediT authorship contribution statement

Tom Clune: Conceptualisation, Methodology, Data curation, Formal analysis, Investigation, Writing – original draft and Writing – review & editing. **Caroline Jacobson:** Conceptualisation, Methodology, Data curation, Formal analysis, investigation, Writing – original draft, Funding acquisition, Resources, Supervision and Writing – review & editing. **Amy Lockwood:** Conceptualisation Data curation Formal analysis, Investigation, Writing – original draft and Writing – review & editing. **Serina Hancock:** Conceptualisation, Formal analysis, Investigation, Writing – review & editing, Funding acquisition, Resources and Supervision. **Andrew Thompson:** Conceptualisation, Writing – review & editing, Funding acquisition, Resources and Supervision. **Sue Beetson:** Conceptualisation, Methodology, and Writing – review & editing. **Ryan O'Handley:** Resources, Methodology and Writing – review & editing. **Mieghan Bruce:** Formal analysis, Investigation, Writing – review & editing, and Supervision. **Angus Campbell:** Formal analysis, Investigation and Writing – review & editing. **Elsa Glanville:** Formal analysis, Investigation and Writing – review & editing. **Daniel Brooks:** Formal analysis, Investigation and Writing – review &editing. **Colin Trengove:** Formal analysis, Investigation and Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the participating farmers who provided access to their animals and facilities, conducted lambing rounds, and collected and stored lambs for necropsy. We thank Celia Smuts, Janine Simmonds and the staff at VetPath for their assistance with the serological testing. We thank Shane Besier, Sam Hair, Cameron Loomes, Richmond Loh and Anna Erickson at DPIRD for their assistance with the laboratory diagnostic testing through the DPIRD Abortion Surveillance Scheme. We thank Tom La and Nyree Philip (Murdoch University), Louis Lignereux and Rob Paterson (University of Adelaide), Andrew Whale, Mary McQuillan and Patrick Hannemann (Livestock Logic, Hamilton, Victoria), Sean McGrath (Millicent Veterinary Hospital), Simon Edwards and Michelle Smart (Willunga Veterinary Hospital), and Lauryn Stewart and Deb Lehmann (Kangaroo Island Veterinary Hospital) for assistance with sample collection and feedback. We thank Johann Schroder for helpful feedback on the manuscript.

Funding

This study was funded by Meat and Livestock Australia (B. AHE.0318). The manuscript was approved for publication by the funding body (Meat and Livestock Australia), but Meat and Livestock Australia was not involved in the collection, analysis or interpretation of data, or in the writing of the manuscript. Molecular diagnostic testing for aborted and stillborn lambs was performed under the Western Australian Ewe Abortion and Newborn Lamb Death Surveillance Programme (Department of Primary Industries and Regional development, Western Australia). Tom Clune received post-graduate scholarships from Meat and Livestock Australia and Sheep Industry Business Innovation (Department of Primary Industries and Regional Development, Western Australia). Equipment used for this project was funded by the Murdoch University Veterinary Trust.

Declaration of interest

None of the authors of this paper have a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.cimid.2021.101727.

References

[1] P.R. Shorten, S.J. Edwards, J.L. Juengel, The role of reproductive loss on flock performance: a comparison of nine industry flocks, Transl. Anim. Sci. 5 (1) (2021) txab013-txab013.
[2] M.B. Allworth, H.A. Wrigley, A. Cowling, Fetal and lamb losses from pregnancy scanning to lamb marking in commercial sheep flocks in southern New South Wales, Anim. Prod. Sci. 57 (10) (2017) 2060–2065.
[3] D.O. Kleemann, S.K. Walker, Fertility in South Australian commercial Merino flocks: sources of reproductive wastage, Theriogenology 63 (8) (2005) 2075–2088.
[4] R. Kilgour, Lambing potential and mortality in Merino sheep as ascertained by ultrasonography, Aust. J. Exp. Agric. 32 (3) (1992) 311–313.
[5] G. Refshauge, T. Atkinson, S.M. Robertson, M. Hernandez-Jover, B. Allworth, M. Friend, Reducing Kid Loss – Select and Project. Phase 1 Final report, Meat and Livestock Australia, North Sydney, 2020.
[6] T. Clune, S. Beetson, S. Besier, G. Knowles, R. Paskin, G. Rawlin, R. Suter, C. Jacobson, Ovine abortion and stillbirth investigations in Australia, Aust. Vet. J. 99 (3) (2021) 72–78.
[7] T. Tan, A pilot study of the seroprevalence of Q fever in cattle, sheep and goats in Victoria (Masters by Research thesis), University of Melbourne, 2018.
[8] N. Arricau-Bouvery, A. Rodolakis, Is Q fever an emerging or re-emerging zoonosis? Vet. Res. 36 (3) (2005) 327–349.
[9] S.P. Martinov, P. Neikov, G.V. Popov, Experimental Q fever in sheep, Eur. J. Epidemiol. 5 (4) (1989) 428–431.
[10] M. Berri, A. Souriau, M. Crosby, D. Crochet, P. Lechopier, A. Rodolakis, Relationships between the shedding of *Coxiella burnetii,* clinical signs and serological responses of 34 sheep, Vet. Rec. 148 (16) (2001) 502–505.
[11] D.L. Brooks, R.W. Ermel, C.E. Franti, R. Ruppanner, D.E. Behymer, J.C. Williams, E. H. Stephenson, J.C. Stephenson, Q fever vaccination of sheep: challenge of immunity in ewes, Am. J. Vet. Res. 47 (6) (1986) 1235–1238.
[12] M. Berri, D. Crochet, S. Santiago, A. Rodolakis, Spread of *Coxiella burnetii* infection in a flock of sheep after an episode of Q fever, Vet. Rec. 157 (23) (2005) 737–740.
[13] J.S. Agerholm, *Coxiella burnetii* associated reproductive disorders in domestic animals–a critical review, Acta Vet. Scand. 55 (1) (2013) 13, 13-13.
[14] M. Berri, E. Rousset, J.L. Champion, P. Russo, A. Rodolakis, Goats may experience reproductive failures and shed *Coxiella burnetii* at two successive parturitions after a Q fever infection, Res. Vet. Sci. 83 (1) (2007) 47–52.
[15] E.H. Derrick, J.H. Pope, D.J. Smith, Outbreak of Q fever in Queensland associated with sheep, Med. J. Aust. 46 (18) (1959) 585–588.
[16] I.D. Smith, Reproductive wastage in a merino flock in central western Queensland, Aust. Vet. J. 38 (10) (1962) 500–507.
[17] L.C. Rowan, J.C. Keast, Q Fever antibodies in New South Wales sheep, Aust. Vet. J. 41 (11) (1965) 356–359.
[18] M.J. Banazis, A.S. Bestall, S.A. Reid, S.G. Fenwick, A survey of Western Australian sheep, cattle and kangaroos to determine the prevalence of *Coxiella burnetii*, Vet. Microbiol. 143 (2) (2010) 337–345.
[19] H.F. Gidding, C. Wallace, G.L. Lawrence, P.B. McIntyre, Australia's national Q fever vaccination program, Vaccine 27 (14) (2009) 2037–2041.
[20] K. Eastwood, S. Graves, P. Massey, K. Bosward, D. van den Berg, P. Hutchinson, Q. Fever, Aust. J. Gen. Pract. 47 (2018), 5555-5555.
[21] P.E. Fournier, T.J. Marrie, D. Raoult, Diagnosis of Q fever, J. Clin. Microbiol. 36 (7) (1998) 1823–1834.
[22] E. Angelakis, D. Raoult, Q fever, Vet. Microbiol. 140 (3) (2010) 297–309.
[23] T.S. Sloan-Gardner, P.D. Massey, P. Hutchinson, K. Knope, E. Fearnley, Trends and risk factors for human Q fever in Australia, 1991–2014, Epidemiol. Infect. 145 (4) (2016) 1–9.
[24] K.A. Bond, G. Vincent, C.R. Wilks, L. Franklin, B. Sutton, J. Stenos, R. Cowan, K. Lim, E. Athan, O. Harris, L. Macfarlane-Berry, Y. Segal, S.M. Firestone, One Health approach to controlling a Q fever outbreak on an Australian goat farm, Epidemiol. Infect. 144 (6) (2016) 1129–1141.
[25] T. Lurier, E. Rousset, P. Gasqui, C. Sala, C. Claustre, D. Abrial, P. Dufour, R. de Crémoux, K. Gache, M.L. Delignette-Muller, F. Ayral, E. Jourdain, Evaluation using latent class models of the diagnostic performances of three ELISA tests commercialized for the serological diagnosis of *Coxiella burnetii* infection in domestic ruminants, Vet. Res. 52 (1) (2021) 56.
[26] T.M. Ellis, M.D. Barton, Australia and New Zealand Standard Diagnostic Procedures: Q fever, Australian Government, The Department of Agriculture and Water Resources, 2003, pp. 1–7.

[27] T. Clune, S. Besier, S. Hair, S. Hancock, A. Lockwood, A. Thompson, M. Jelocnik, C. Jacobson, *Chlamydia pecorum* detection in aborted and stillborn lambs from Western Australia, Vet. Res. 52 (1) (2021) 84.
[28] L.D. Brown, T.T. Cat, A. DasGupta, Interval estimation for a proportion, Stat. Sci. 16 (101–133) (2001).
[29] E.S.G. Sergeant, Epitools epidemiological calculators, 2021. ⟨http://epitools.ausvet.com.au⟩. (Accessed 20 February 2021).
[30] N. Speybroeck, B. Devleesschauwer, L. Joseph, D. Berkvens, Misclassification errors in prevalence estimation: Bayesian handling with care, Int. J. Public Health 58 (5) (2013) 791–795.
[31] T. Clune, A. Lockwood, S. Hancock, A. Thompson, S. Beetson, A. Campbell, E. Glanville, D. Brookes, C. Trengove, R. O'Handley, G. Kearney, C. Jacobson, Abortion and lamb mortality between pregnancy scanning and lamb marking for maiden ewes in southern Australia, Animals (2021).
[32] K.A. Bond, L. Franklin, B. Sutton, M.A. Stevenson, S.M. Firestone, Review of 20 years of human acute Q fever notifications in Victoria, 1994–2013, Aust. Vet. J. 96 (6) (2018) 223–230.
[33] M. Muleme, J. Stenos, G. Vincent, C.R. Wilks, J.M. Devlin, A. Campbell, A. Cameron, M.A. Stevenson, S. Graves, S.M. Firestone, Peripartum dynamics of *Coxiella burnetii* infections in intensively managed dairy goats associated with a Q fever outbreak in Australia, Prev. Vet. Med. 139 (2017) 58–66.
[34] M. Klaassen, H.-J. Roest, vdW. Hoek, B. Goossens, A. Secka, A. Stegeman, *Coxiella burnetii* seroprevalence in small ruminants in The Gambia, PLoS One 9 (1) (2014) e85424-e85424.
[35] J. Muema, S.M. Thumbi, M. Obonyo, S. Wanyoike, M. Nanyingi, E. Osoro, A. Bitek, S. Karanja, Seroprevalence and factors associated with *Coxiella burnetii* infection in small ruminants in Baringo County, Kenya, Zoonoses Public Health 64 (7) (2017) e31–e43.
[36] A.L. García-Pérez, I. Astobiza, J.F. Barandika, R. Atxaerandio, A. Hurtado, R. A. Juste, Short communication: Investigation of *Coxiella burnetii* occurrence in dairy sheep flocks by bulk-tank milk analysis and antibody level determination, J. Dairy Sci. 92 (4) (2009) 1581–1584.
[37] A. Joulié, E. Rousset, P. Gasqui, E. Lepetitcolin, A. Leblond, K. Sidi-Boumedine, E. Jourdain, *Coxiella burnetii* circulation in a naturally infected flock of sheep: individual follow-up of antibodies in serum and milk, Appl. Environ. Microbiol. 83 (13) (2017) e00222-17.
[38] R. Cruz, F. Esteves, C. Vasconcelos-Nóbrega, C. Santos, A.S. Ferreira, C. Mega, A. C. Coelho, H. Vala, J.R. Mesquita, A Nationwide Seroepidemiologic Study on Q Fever Antibodies in Sheep of Portugal, Vector Borne Zoonotic Dis. 18 (11) (2018) 601–604.
[39] S. Villari, P. Galluzzo, M. Arnone, M. Alfano, F. Geraci, G. Chiarenza, Seroprevalence of *Coxiella burnetii* infection (Q fever) in sheep farms located in Sicily (Southern Italy) and related risk factors, Small Rumin. Res. 164 (2018) 82–86.
[40] A. Conan, A.A.M.J. Becker, V. Alava, A. Chapwanya, J. Carter, K. Roman, H. Avsaroglu, C.A. Gallagher, Detection of *Coxiella burnetii* antibodies in sheep and cattle on a veterinary campus in St. Kitts: implications for one health in the Caribbean region, One Health 10 (2020), 100163.
[41] M.-È. Turcotte, S. Buczinski, A. Leboeuf, J. Harel, D. Bélanger, D. Tremblay, C. A. Gagnon, J. Arsenault, Epidemiological study of coxiella burnetii in dairy cattle and small ruminants in Québec, Canada, Prev. Vet. Med. 191 (2021), 105365.
[42] World Organisation for Animal Health, Q fever (chapter 3.1.16), Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019, World Organisation for Animal Health (OIE), Available: ⟨https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/⟩ (accessed June 2021), 2019.
[43] M.W. Horigan, M.M. Bell, T.R. Pollard, A.R. Sayers, G.C. Pritchard, Q fever diagnosis in domestic ruminants: comparison between complement fixation and commercial enzyme-linked immunosorbent assays, J. Vet. Diagn. Invest. 23 (5) (2011) 924–931.
[44] N. Arricau Bouvery, A. Souriau, P. Lechopier, A. Rodolakis, Experimental *Coxiella burnetii* infection in pregnant goats: excretion routes, Vet. Res. 34 (4) (2003) 423–433.
[45] G.N. Hinch, F. Brien, Lamb survival in Australian flocks: a review, Anim. Prod. Sci. 54 (6) (2014) 656.
[46] N. Ostfeld, M.M. Islam, M. Jelocnik, M. Hilbe, T. Sydler, S. Hartnack, C. Jacobson, T. Clune, I. Marsh, N. Sales, A. Polkinghorne, N. Borel, *Chlamydia pecorum*-induced arthritis in experimentally-induced and naturally infected sheep, Vet. Pathol. 58 (2) (2020) 346–360.
[47] T. Clune, A. Lockwood, S. Hancock, A.N. Thompson, S. Beetson, M. Bruce, A.J. Campbell, E. Glanville, D. Brookes, C. Trengove, R. O'handley, C. Jacobson, Seropositivity to Campylobacter and association with abortion and lamb mortality in maiden ewes from southern Australia, Available at: (2021).
[48] T. Clune, A. Lockwood, S. Hancock, A.N. Thompson, S. Beetson, M. Bruce, A.J. Campbell, E. Glanville, D. Brookes, C. Trengove, R. O'Handley, C. Jacobson, *Toxoplasma gondii* is not an important contributor to poor reproductive performance of primiparous ewes from southern Australia: a prospective cohort study, Available at: DOI: 10.21203/rs.3.rs-955972/v1 (2021).
[49] T. Clune, A. Lockwood, S. Hancock, M. Bruce, A.N. Thompson, S. Beetson, A. J. Campbell, E. Glanville, D. Brookes, C. Trengove, R. O'Handley, C. Jacobson, *Neospora caninum* is not an important contributor to poor reproductive performance of primiparous ewes from southern Australia: evidence from a cross-sectional study, Parasitol. Res. 120 (3875–3882) (2021) 3875–3882.
[50] S.R. Graves, A. Islam, Endemic Q fever in new south wales, Australia: a case series (2005-2013), Am. J. Trop. Med. Hyg. 95 (1) (2016) 55–59.
[51] P.J. Boland, N.R. Parker, Q fever in south west Queensland, Med. J. Aust. 171 (8) (1999) 446, 446-446.
[52] B.A. O'Connor, I.G. Tribe, R. Givney, A windy day in a sheep saleyard: an outbreak of Q fever in rural South Australia, Epidemiol. Infect. 143 (2) (2015) 391–398.
[53] H. Tissot-Dupont, M.-A. Amadei, M. Nezri, D. Raoult, Wind in November, Q fever in december, Emerg. Infect. Dis. 10 (7) (2004) 1264–1269.
[54] D. Webster, D. Haase, T.J. Marrie, N. Campbell, J. Pettipas, R. Davidson, T. F. Hatchette, Ovine-associated Q fever, Epidemiol. Infect. 137 (5) (2009) 744–751.
[55] R.M. Jones, D.F. Twomey, S. Hannon, J. Errington, G.C. Pritchard, J. Sawyer, Detection of *Coxiella burnetii* in placenta and abortion samples from British ruminants using real-time PCR, Vet. Rec. 167 (25) (2010) 965–967.
[56] A. Rodolakis, M. Berri, C. Héchard, C. Caudron, A. Souriau, C.C. Bodier, B. Blanchard, P. Camuset, P. Devillechaise, J.C. Natorp, J.P. Vadet, N. Arricau-Bouvery, Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine, and ovine herds, J. Dairy Sci. 90 (12) (2007) 5352–5360.
[57] Australian Government Bureau of Meteorology, Australian Climate Averages - Rainfall, 2021. ⟨http://www.bom.gov.au/jsp/ncc/climate_averages/rainfall/index.jsp⟩. (Accessed June 2021 2021).