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Q Fever in Intensively Managed Dairy Goats: Transmission Dynamics, Production Losses and Control Interventions

A thesis by
JOSÉ CANEVARI
Submitted for the Degree of Doctor of Philosophy

Q Fever in Intensively Managed Dairy Goats: Transmission Dynamics, Production Losses and Control Interventions

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Abstract

Q fever is a zoonotic disease with a significant impact on public health worldwide. It is caused by the highly infectious bacterium *Coxiella burnetii*. In humans, Q fever typically manifests as a self-limiting febrile syndrome. *C. burnetii* infection can also lead to chronic disease presentations and be life-threatening for individuals with underlying risk factors. Australia ranks among the countries with the highest Q fever report rates. Livestock species are the main source of infection for humans and some of the largest Q fever outbreaks recorded in recent times have been linked to dairy goats.

In Victoria, Australia, a Q fever outbreak linked to a large dairy goat enterprise occurred in 2012 – 2014. This was the largest farm-associated Q fever outbreak recorded in the country. In the context of a steadily growing dairy goat sector, an improved understating of Q fever dynamics and the efficacy of available control strategies in dairy goats is required. This thesis documents a series of studies designed to better understand factors contributing to the spread of Q fever in this herd and the potential efficacy of different control measures.

As a first step, we developed a software prototype for reporting herd performance in intensively managed dairy goat herds. This allowed productivity, reproductive performance, health and mortality to be documented, and enabled the performance of Q fever positive and Q fever negative does to be compared. With this system in place, a panel study was conducted to document the prevalence of *C. burnetii* shedding at the time of kidding. A heterogeneous *C. burnetii* shedding pattern was found, characterised by the presence of small numbers of 'super shedder' does. Further, does identified as 'super shedders' had reduced total lactation milk yields compared with *C. burnetii* negative does.

Demographic data gathered using the herd health software prototype and data on Q fever prevalence from our panel study were used to develop a within-herd transmission model of Q fever. The model was used to assess the expected time to eradication by means of vaccination. In addition, the efficacy of segregation of pregnant does and culling of *C. burnetii* shedders at the time of parturition or abortion was assessed. Vaccination consistently led to disease eradication, although it required this intervention to be sustained for at least 6.5 (95% CrI: 4.1 to 11.3) years. A combination of vaccination with segregation of pregnant does or culling of *C. burnetii* shedders at parturition or abortion shortened the median time to eradication. The model was adapted to account for heterogenous shedding patterns and used to test the potential efficacy of a test and remove intervention. We found the removal of super shedders does alone would lead to Q fever eradication. However, tests with a high sensitivity for early detection of super shedders are required for this strategy to be effective.

Declaration by author

This is to certify that:

(i) This thesis comprises only my original work towards the degree of Doctor of Philosophy, that to my knowledge has not been published previously;

(ii) The text in this thesis was prepared and submitted by the author;

(iii) Relevant information from published or unpublished work from other individuals included in this thesis have been acknowledged in the text, with a list of references also given.

José T. Canevari
June 10, 2020

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- Canevari JT, Firestone SM, Vincent G, Campbell A, Tan T, Muleme M, Cameron AWN, Stevenson MA. The prevalence of *Coxiella burnetii* shedding in dairy goats at the time of parturition in an endemically infected enterprise and associated milk yield losses. BMC Veterinary Research 14 (2018) 353 DOI: 10.1186/s12917-018-1667-x

- Canevari JT, Firestone SM, Vincent G, Campbell A, Cameron AWN, Tan T, Muleme M, Stevenson MA, 2018. The association between *Coxiella burnetii* shedding at the time of parturition in dairy goats and daily milk yields produced during the subsequent lactation. In: Proceedings of the 15^{th} Symposium of the International Society for Veterinary Epidemiology and Economics, 12 - 16 November 2018, Chiang Mai, Thailand, p. 77.

- Stevenson MA, Tan T, Muleme M, Canevari JT, Firestone SM, Vincent G, Campbell A, Cameron AWN, 2018. What we know and what we need to know about the distribution of Q fever risk in humans and livestock. In: ASAV, SCGV and AVBIG Conference 2018: The Abdomen and Beyond, 12 - 16 August 2018, Melbourne Convention Centre, Melbourne, Australia, pp. 300–304.

- Canevari JT, Firestone SM, Vincent G, Campbell A, Tan T, Muleme M, Cameron AWN, Stevenson MA. Q fever associated milk yield losses in dairy goats. In: ASAV, SCGV and AVBIG Conference 2018: The Abdomen and Beyond, 12 - 16 August 2018, Melbourne Convention Centre, Melbourne, Australia, pp. 200–203.

- Canevari JT, Firestone SM, Vincent G, Campbell A, Cameron AWN, Tan T, Muleme M, Stevenson MA. The prevalence of dairy goats shedding *Coxiella burnetii* at the time of parturition in an endemically infected herd and associated milk yield losses. Meeting of the Epidemiology Chapter of the Australian and New Zealand College of Veterinary Scientists, 5 - 7 July 2018, Gold Coast, Queensland, Australia.

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- Firestone SM, Bond K, Muleme M, Vincent G, Graves S, Franklin L, Sutton B, Cameron AWN, Cowan R, Lim K, Athan E, Harris O, Macfarlane-Berry L, Segal Y, Devlin J, Campbell A, Tan T, Canevari JT, Islam A, Wilks C, Hufschmid J, Browning G, Stevenson MA, Stenos J. Controlling Q fever through a series of coordinated investigations and interventions focused on humans, goats and the farming environment in Victoria, Australia. Proceedings of the 4^{th} International One Health Congress and the 6^{th} Biennal Conference of the International Association for Ecology and Health, 4 - 7 December 2016, Melbourne Convention Centre, Melbourne, Australia.

I acknowledge the intellectual contributions of all authors included in each of the publications listed above.

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List of abbreviations and symbols

μL	Microlitres
APSW	Abortion, premature delivery, stillbirths and weak offspring complex
ABM	Agent-based model
AIC	Akaike Information Criterion
ACIVM	American College of Internal Veterinary Medicine
AI	Aritificial insemination
ARRL	Australian Rickettsial Reference Laboratory
BTM	Bulk tank milk
CDC	Centers for Disease Control and Prevention (USA)
CFT	Compement fixation test
CI	Confidence interval
com1	Coxiella outer membrane protein 1
CrI	Credible interval
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
EID	Electronic individual identification
ELISA	Enzyme-linked immunosorbent assay
EFSA	European Food and Safety Authority
AR1	First order autoregressive covariance structure
FMD	Foot and mouth disease
GE	Genome equivalent
IFA	Indirect immuno-fluorescence assay
IS	Insertion sequence
INTA	Instituto Nacional de Tecnologia Agropecuaria
IFN-γ	Interferon gamma
km	kilometre
LCV	Large cell variant
LHS	Latin hypercube sampling
LPS	Lipopolysaccharide
MAP	*Mycobacterium avium* subspecies *paratuberculosis*
MCMC	Monte Carlo Markov Chain

ODE	Ordinal differential equations
PRCC	Partial rank correlation coefficient
PSK	Planned start of kidding
PSM	Planned start of mating
PCR	Polymerase chain reaction
$\mathbf{Q}_1$	25% percentile
$\mathbf{Q}_3$	75% percentile
QFS	post Q-fever fatigue syndrome
PABAK	Prevalence adjusted kappa
qPCR	Quantitative polymerase chain reaction
RML	Rocky Mountain laboratory
SMC	Sequential Monte Carlo
SCV	Small cell variant
SCC	Somatic cell count
SE	Standard error
UV	Ultra-violet
UK	United Kingdom
USA	United States of America
OIE	World Organisation for Animal Health

Chapter 1

Literature review

1.1 The history of Q fever

Q fever is a zoonotic disease caused by *Coxiella burnetii*, a small obligate intracellular gram-negative bacterium. The disease was first described as a clinical entity in 1937 by Edward Derrick, a physician investigating an outbreak of febrile illness among abattoir workers in Queensland, Australia. Derrick succeeded in reproducing the disease in guinea pigs by injecting them with blood from his patients. However, his attempts to isolate the causal agent were unfruitful, which led him to name the disease Q fever, the 'Q' standing for 'query' (Derrick, 1937). Frank Macfarlane Burnet and Mavis Freeman (1937) published a series of studies in which monkeys and mice were inoculated with a liver emulsion from infected guinea pigs sourced from Derrick. The authors described the presence of rickettsial organisms in 'large intracitoplasmatic colonies' in stained sections and smears of mouse liver. They remained cautious at the time of attributing the disease to a rickettsia and left open the alternative explanation of a viral agent. Later, Burnet was able to culture *C. burnetii* in chicken embryos, which supported the hypothesis of a rickettsial causal agent for Q fever (Burnet, 1938). A number of accidental human laboratory infections are reported in Burnet's publication. Back when Burnet's study was carried out, it was not known *C. burnetii* was highly transmissible via aerosols. Indeed, a mite (*Ornithonyssus bacoti*) was believed to the vector responsible for laboratory infections.

This seminal research on Q fever in Australia overlapped in time with those carried out at the Rocky Mountain Laboratory (RML) in Montana, USA. In fact, articles published in the late 1930s mention the exchange of biological material between Australian and American research groups working on rickettsial diseases (Dyer, 1938). Researchers from the RML described a rickettsia-like filter-passing agent isolated from ticks collected at Nine Mile Creek, Montana, in a series of papers published between 1938 and 1939 (Davis and Cox, 1938; Parker and Davis, 1938a,b; Dyer, 1938; Cox, 1939). Laboratory animals inoculated with this bacterium developed a febrile disease. A laboratory accident whereby a staff member at the laboratory in Montana contracted Q fever and developed clinical symptoms like those described by Derrick, Burnet and Freeman in Australia, led to the hypothesis that the two newly discovered infectious agents in these two distant continents were in fact the same (Dyer, 1939).

1.2 Bacteriology

C. burnetii is a small pleomorphic rod and its membrane resembles that of a Gram-negative bacterium (Maurin and Raoult, 1999). It belongs to the gamma group of Protobacteria, within the order Legionellales (Raoult et al., 2005). The capacity of *C. burnetii* to survive in the environment coupled with its highly infectious nature has led to its classification as a potential bioterrorism agent by the United States Centers for Disease Control (CDC) (2017). The bacterium is highly adapted to the harsh environment of intracellular vacuoles where it replicates with an estimated doubling time of 20–45 hours (Zamboni et al., 2002).

C. burnetii exists in two morphological variants, the large cell variant (LCV), which is metabolically active and replicates within infected cells, and a spore-like small cell variant (SCV), which is metabolically inactive and has an increased viability in the face of temperature changes and desiccation (McCaul and Williams, 1981). Scott et al. (1990) tested *C. burnetii* resistance to chemical disinfectants and found the bacteria can remain viable after 24h of exposure to some commonly used disinfectants, like 0.5% hypochlorite and 5% formalin. The high stability of the SCV of *C. burnetii* led to an increase in pasteurisation temperatures in the 1950s (Enright et al., 2010). Two antigenic variants of *C. burnetii* occurs, phase I and II, based on the composition of its immunogenic lipo-polysaccharide (LPS) region. The phase I variant is virulent and contains LPS with a complete O antigen. The phase I variant transitions to the avirulent phase II variant, which lacks O antigen sugars, after multiple passages in a non-immunologically competent host (Stoker and Fiset, 1956).

The full genome of *C. burnetii* was sequenced for the first time in 2003 (Seshadri et al., 2003). The strain was the RSA493 Nine Mile phase I, isolated from ticks in the 1930s. A number of other full and incomplete genomes have been published in GenBank since then (D'Amato et al., 2014; Sidi-Boumedine et al., 2014; Freylikhman et al., 2018). Genetic analysis has allowed the characterisation of particularly pathogenic strains, as is the case of the Cb175 strain from Cayenne, French Guiana, associated with high incidence of respiratory disease in humans in this country (Epelboin et al., 2012). The high pathogenicity of this strain has been hypothesised to be linked with a deleted region in the genome (D'Amato et al., 2015). The 6105 bp fragment present in the Nine Mile strain but missing in Cb175 encodes for proteins that are part of a Type 1 secretion system. Interestingly, other epidemic bacterial strains have been found to have fewer secretion proteins as compared with non-epidemic strains (Georgiades and Raoult, 2011).

1.3 Diagnostic tools

Presence of *C. burnetii* can be demonstrated in several ways. Isolation of the agent can be undertaken in embryonated chicken eggs, cell cultures and also in axenic ('cell free') media (Raoult et al., 1993; Omsland et al., 2009; Samuel and Hendrix, 2009). These techniques are time consuming and require level three biosafety laboratories. If abortion due to *C. burnetii* is suspected in livestock, smears of placental tissue (particularly cotyledons) or vaginal discharge, as well as of liver, lung or abomasal content from the aborted foetus can be stained using techniques like Stamp, Gimenez or Macchiavello. Stained smears can be observed under the microscope using oil-immersion objective lens ($\times$ 500 or above) (World Organisation for Animal Health, 2018). Also, detection of *C. burnetii* in paraffin-embedded tissues or acetone fixed smears can be attained by immunohistochemistry (Raoult et al., 1994). A detailed description of methods for performing immunohistochemistry for detection of *C. burnetii* can be found in Muleme et al. (2017).

PCR techniques, particularly real time quantitative PCR (qPCR), are now widely used to detect *C. burnetii* DNA. The two most commonly used PCR assays are those targeting the insertion sequence IS*1111* and the *com1* gene (Jones et al., 2011). Whereas the IS*1111* assay is highly sensitive, an expected feature given the insertion sequence occurs up to 110 times in each *C. burnetii* genome, the *com1* assay is preferred for the quantification of genome equivalents (GE) given the gene is present as a single copy in each *C. burnetii* genome (Klee et al., 2006; Fenollar and Raoult, 2007).

Regarding detection of antibodies against *C. burnetii*, among the serological tests available the most often used are the immunofluorescence assay (IFA), the complement fixation test (CFT) and the enzyme-linked immunosorbent assay (ELISA) (Rousset et al., 2007; World Organisation for Animal Health, 2018; Muleme et al., 2016). The relatively low sensitivity of CFT has led to a decline in its use in favour of the other techniques (Natale et al., 2012). The ELISA and the IFA have similar accuracy with some evidence suggesting IFA is marginally more sensitive in goats (Muleme et al., 2016). However, this seems not to be the case in cattle (Wood et al., 2019). The ELISA presents the advantage over IFA of a high throughput, which may explain its extended use. Whereas, the IFA can be easily adapted to test for antibodies targeting phase specific antigens, providing further indication of the recency of exposure.

1.4 Q fever in humans

1.4.1 Clinical symptoms

Infection with *C. burnetii* is often asymptomatic but can also lead to a variety of clinical presentations (Raoult et al., 2005). After an incubation period with a median of 18 days (95% CI: 7 to 32), symptomatic patients typically develop a flu-like, self-limiting illness along with myalgia and severe headache (Todkill et al., 2018). Occasionally, pneumonia or hepatitis are also observed (Maurin and Raoult, 1999) with a frequency that seems to vary across different locations. Pneumonia was reported in 86% of the 183 patients admitted in a hospital located in Noord-Brabant province, the area that was most affected by the 2007–2010 Q fever outbreak in The Netherlands (Wielders et al., 2014). On the other hand, hepatitis is a more common symptom than pneumonia in hospitalised patients in Spain and France (Espejo et al., 2014; Edouard et al., 2014). A relatively low proportion of individuals contracting Q fever (i.e. 2 to 5%) can develop persistent focalised infections, a form of disease that can be life threatening and requires a prolonged treatment with antibiotics. This presentation is more common in patients with pre-existing heart valve disease, aneurysms or valvular prostheses (Wegdam-Blans et al., 2012; Eldin et al., 2017).

C. burnetii infection can also lead to a presentation known as Q fever fatigue syndrome (QFS) in 20% to 30% of patients (Ledina et al., 2007; Morroy et al., 2016). The definition of QFS is not standardised at an international level, but the use of the term is generally accepted to refer to a deteriorated health status and social functioning characterised by severe fatigue following acute Q fever (Morroy et al., 2016). QFS may last up to 10 years and accounted for the largest part of economic costs associated with the Dutch outbreak in 2007–2010 (Tempelmann et al., 2011). Indeed, van Asseldonk et al. (2013) estimated above 60% of the total estimated public health costs resulting from this outbreak were attributed to QFS (van Asseldonk et al., 2013). In Australia, the courts have recognised QFS as a sequela of *C. burnetii* infection (Tozer, 2015). Persistence of living *C. burnetii* as the cause of QFS has been suggested but as yet has not been demonstrated (Melenotte et al., 2019).

An alternative causal hypothesis for QFS is the persistence of non-viable *C. burnetii* cell components which can induce alterations in cytokine production (Melenotte et al., 2019). Failure to distinguish between QFS and latent infections can have serious consequences since latent infections can lead to *C. burnetii* dissemination and/or endocarditis with potentially fatal results (Harris et al., 2000; Sukocheva et al., 2016).

1.4.2 Sources of infection

Inhalation of *C. burnetii* contaminated dust particles is considered the main route of infection in humans (Babudieri, 1959). The infectivity of *C. burnetii* has been demonstrated to be high enough to pose a serious risk to people even when present in very small numbers in the environment (Brooke et al., 2013; Tiggert et al., 1961). Human infections are often associated with contact with livestock (Norlander, 2000; McQuiston and Childs, 2002). Certainly, people working in the livestock industry like farmers, veterinarians or abattoir workers are at an increased risk of contracting Q fever (Marrie and Raoult, 1997). However, Q fever cases have also been attributed to contact with wildlife (Sivabalan et al., 2017) or infected pets like dogs or cats (Pinsky et al., 1991; Buhariwalla et al., 1996). Because *C. burnetii* can survive for a prolonged time in the environment and travel long distances carried by wind (Tissot-Dupont et al., 2004), the general community dwelling in areas in the proximity of livestock farms or saleyards can be exposed and acquire Q fever (Dupuis et al., 1987; O'Connor et al., 2014; Clark and Soares Magalhães, 2018). Q fever outbreaks have also occurred in urban areas with no apparent connection with farming environments. For example, Amitai et al. (2010) described a large Q fever outbreak in a school in Tel Aviv, Israel, where 322 cases of febrile illness occurred. Amitai and collaborators hypothesised that infection could have been dispersed via the air conditioning systems and that the source could have been infected feral cats. Person to person transmission has been reported but it is considered to be very uncommon (Marmion and Stoker, 1950; Milazzo et al., 2001). Contaminated clothes can serve as an inanimate vector of infection although this is also considered rare (Serbezov et al., 1999; Bond et al., 2016). *C. burnetii* can be found in milk from infected animals and remain viable if milk is not pasteurised (Loftis et al., 2010). Further, viable *C. burnetii* has been found in cheeses made with unpasteurised milk and ripened up to 8 months (Barandika et al., 2019). However, reports of infection through ingestion of raw milk are scarce and, overall, the oral route is considered to be of negligible epidemiological importance (Fishbein and Raoult, 1992; European Food Safety Authority, 2010). It has been suggested that age and gender are significant determinants of the symptomatology associated with *C. burnetii* infection, with adults and male individuals being at a higher risk of developing clinical symptoms compared to children and women, respectively (Maltezou and Raoult, 2002; Eldin et al., 2017). However, confounding by level of exposure cannot be ruled out in these studies.

1.4.3 Q fever in Australia

Australia has one of the highest reported rates of human Q fever in the world (Gidding et al., 2009). Local costs related to Q fever have been estimated to be around A$ 1 million and more than 1,700 weeks of work time annually (Garner et al., 1997). Notification of human Q fever cases is mandatory in all states and territories since 1977 (Garner et al., 1997). According to the National Notifiable Diseases Surveillance System, the national average of human cases per year throughout the last ten years ranged between 1.4 and 2.5 cases per 100,000 population (Anonymous, 2018). Official case reports have been used for both descriptive and analytic epidemiological studies. A study into Q fever incidence rates between 1991 and 2014 found two states in

Australia, Queensland and New South Wales, accounted for around 87% of the total human cases annually reported in the country (Sloan-Gardner et al., 2017). The notification rate for males was four times higher compared to females and the highest notification rate was among the 40-59 years age group. Occupations that involved contact with livestock were the most frequently listed among cases (i.e. 16%). Interestingly, 9% of the cases had a no known risk occupation. The reasons behind the relatively high Q fever report rates in Australia, and in particular in Queensland and New South Wales, are unclear. At the national level, a relatively large population working in the livestock sector and perhaps a level of awareness and development of diagnostic capabilities above that of other countries for historical reasons could partially explain this. The presence of wildlife known to be *C. burnetii* potential hosts (Tozer et al., 2014) could be an additional explanation for this phenomenon. The fact that a considerable proportion of human Q fever reports were not associated with risk occupations underscores the need for further research into Q fever in wildlife and companion animals.

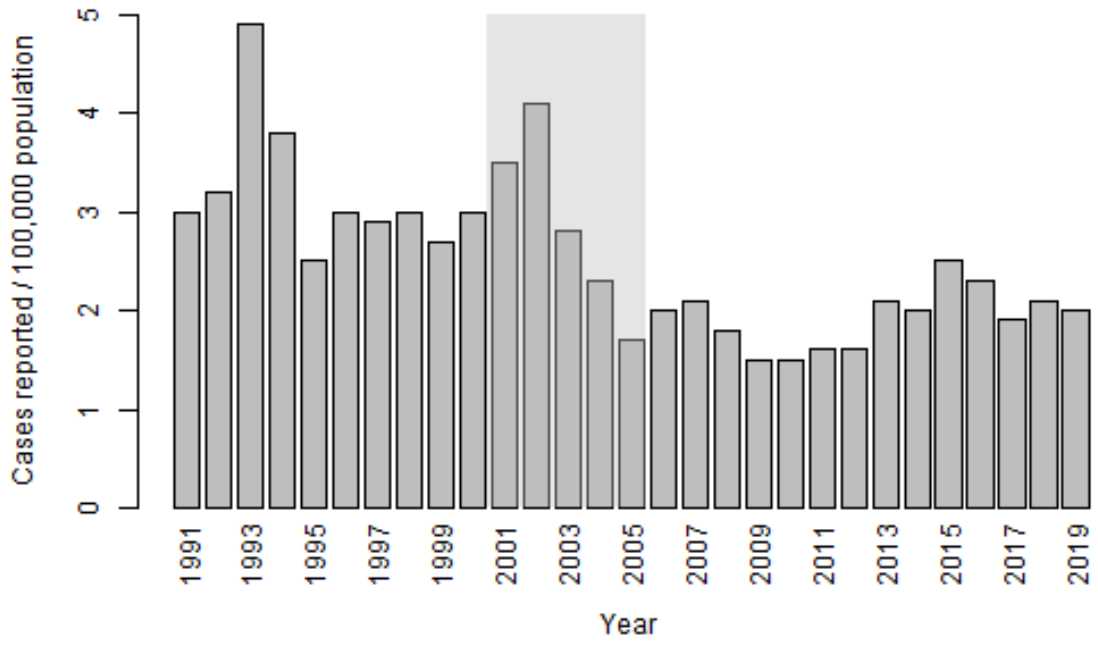

Figure 1.1: Bar chart showing the incidence rate of Q fever (expressed as the number of reports per 100,000 head of population per year) in Australia from 1991 to 2019. The greyed area shows the time window during which a national level program supported vaccination of at-risk populations. Data sourced from the National Notifiable Diseases Surveillance System, Australian Government Department of Health (Anonymous, 2018).

Australia is the only country in the world with a vaccine licensed for use in humans. The vaccine, a whole-cell preparation of phase 1 Henzerling strain inactivated with formalin, was released in 1989 and it has proven to be very effective in preventing infection in at-risk populations (Gidding et al., 2009). From 2001 to 2006, a national level control program including vaccination of at-risk populations markedly reduced disease incidence (Sloan-Gardner et al., 2017). Starting in 2011, a slow rise of incidence rates has been observed (Figure 1.1), although incidence rates have so far remained below levels observed before the vaccination program. Vaccination is only recommended in people above 15 years of age and with no evidence of previous exposure, which is assessed by means of antibody testing as well as a skin test (Ormsbee and Marmion, 1990). A series of barriers for vaccination uptake have been identified including costs of pre-screening and vaccination, lack of

perceived risk, difficulties to access health services and being under the recommended age limit for vaccination (Gidding et al., 2009; Eastwood et al., 2018).

1.4.4 Historical outbreaks

Although Q fever in humans usually occurs as sporadic cases, large scale outbreaks are also observed (Sawyer et al., 1987; Lyytikäinen et al., 1998; Bellini et al., 2014). Some of the largest Q fever human outbreaks had dairy goat farms implicated as the source of infection for people (Serbezov et al., 1999; Panaiotov et al., 2009; Roest et al., 2011a). It is not the aim of this brief section to provide a comprehensive listing of all major human outbreaks reported in the literature. Rather, it will focus on what were the two largest Q fever outbreaks recorded in the literature. Also, it aims at introducing the reader to an outbreak in Australia that is central to the work presented in this thesis.

A Q fever outbreak of unprecedented scale occurred in The Netherlands in 2007–2010 (Enserink, 2010). In 2007, 168 human cases were reported, mainly in the southern province of Noord-Barbant. The number of cases reported in 2008 escalated to 1000 and continued to rise in 2009 when 2354 people were diagnosed with Q fever. In 2010 the epidemic started receding as evidenced by a drop in the number of reported cases which totalled 504 (Schneeberger et al., 2014). The source of the outbreak was traced back to infected dairy goat herds with elevated abortion rates caused by *C. burnetii* infection. A total of 130 dairy goat herds and 5 dairy sheep herds were found to be infected throughout the course of the outbreak (Roest, 2013). *C. burnetii* isolates from infected farms since 2007 were genetically almost identical (Roest et al., 2011b), which suggest a single point introduction and subsequent between-herd dispersion. Also, *C. burnetii* isolates from human patients were found to be genetically similar to isolates obtained from infected animals (Roest et al., 2011a; Tilburg et al., 2012).

The rapid increase in the number of goats in the region, mainly kept in medium to large farms under intensive housing conditions, and the proximity of these dairy farms to human populations may be key to explain the Q fever outbreak in The Netherlands. Within the 20 years that preceded the outbreak the size of the national goat herd had approximately quadrupled. This rise of goat numbers was in part due to the conversion of pig farmers to dairy goat producers as a result of a large classical swine fever outbreak with a devastating impact on the pig industry in the late 1990s (Stegeman et al., 2000). Also, the introduction of the European milk quotation system for dairy cattle in 1984 resulted in an increase number of dairy goat farms (Enserink, 2010). The size of the average dairy goat farm in The Netherlands was relatively large compared with that of, for example, France, with approximately 600 and 200 animals per farm respectively (Roest et al., 2009).

It has also been hypothesised that the unprecedented scale of the outbreak could be in part attributable to a more virulent strain of *C. burnetii* (Roest et al., 2011a). Indeed, *C. burnetii* was already prevalent among livestock in The Netherlands long before the outbreak without it representing a major health issue either for animals or humans (Wolf and Kouwenaar, 1954). However, the epidemiology of Q fever in livestock appears to have changed in the years that preceded the outbreak. In 2006, *C. burnetii* had been identified as the main cause of abortion in the dairy goat population in The Netherlands (Wouda and Dercksen, 2007), a finding that interpreted retrospectively highlights the importance animal health and human health services interaction.

A series of control measures were progressively put in place during the Dutch outbreak. In June 2008, Q fever became a notifiable disease in The Netherlands. Also, surveillance on the occurrence of abortions was enhanced. Notification was made mandatory for abortion rates above 5% in herds with more than 100 breeding animals, or 3 or more abortions within a 30-day period for smaller size farms. From 2009 onward, monitoring of

bulk tank milk (BTM) using PCR for the detection of *C. burnetii* DNA was carried out in all small ruminant dairy farms. A voluntary vaccination campaign in goats started in autumn 2008. At the start of 2009 vaccination was made mandatory for goats and sheep in the regions most affected by the outbreak and by 2010 vaccination was imposed in the whole country for small ruminant dairies and recreational farms. The vaccine used was a phase I whole-cell inactivated vaccine (Coxevac®, CEVA Santé Animale).

In February 2009, the Dutch authorities regulated the disposal of manure from the kidding/lambing stables imposing a 30-day waiting time after the end of parturition period for its removal. Also, manure had to be covered when stored or transported and, if spread in the fields, it had to be immediately ploughed to avoid dispersal by the wind. Products of abortions had to be rendered and farmers were encouraged to send foetuses and/or placentas for pathological examination. Recommendations were also made to limit the access of visitors to small ruminant dairy farms. In December 2009, the Dutch government decided to cull all pregnant goats and sheep in Q fever infected farms, and imposed life-long breeding bans for the remaining non-pregnant goats and sheep in these same farms. An estimated total of 58,150 animals were culled during this phase of the control campaign (van Asseldonk et al., 2013; Vellema and van den Brom, 2014).

In Bulgaria, in 1993, a Q fever outbreak of a scale comparable to that of the Dutch epidemic occurred. More than 2000 people were likely infected in the first 6 months of the outbreak. Reports of Q fever occurrence suggest *C. burnetii* had been already circulating in livestock populations from as far back as 1949. Following the collapse of large state-owned farming premises and cooperative farms in the 1990s, there was a rapid decline in the number of cows and sheep, and a rise in the number of goats, seen by farmers as an accessible source of food. This prompted a sudden increase in the goat population in Bulgaria which almost tripled from 1990 to 1997. The fact that goats were kept near households and in close contact with family members could have played a role in facilitating Q fever spread (Serbezov et al., 1999). Publications in English describing this outbreak are scarce.

In Victoria, Australia, in 2012, an outbreak of Q fever linked to a goat and sheep dairy enterprise resulted in seventeen employees and one family member confirmed with Q fever, as well as six additional suspected cases (Bond et al., 2016). The dairy is comprised of three farms located within less than 3 km one from the other. There is a cheese factory operating within the same premises as the dairy, and a total of 100 employees work for the company. At the time of the outbreak, the dairy kept close to 5,000 goats and 800 sheep. This is an intensively managed production system, with animals kept in sheds and fed total mixed rations. The entry date of Q fever into this enterprise could not be determined. Farm staff members reported an increase frequency of abortions since 2004, which could be indicative of disease entry around that year.

Applied control measures included Q fever vaccination of farm staff and banning of unvaccinated farm visitors. Also, recommendations on the use of personal protective equipment and hygiene practices were made. As for management of manure and aborted foetuses, the same practices put in place during the outbreak in The Netherlands were recommended. Perhaps mainly attributed to vaccination of the farm's personnel and the absence of urban areas in the near proximity, no further human cases were reported. However, the disease remained endemic among goats (Muleme et al., 2017b). Importation of an existing animal vaccine from Europe was not allowed due to biosecurity concerns, and works towards the development of an autogenous animal vaccine were initiated.

1.5 Q fever in livestock

1.5.1 Disease occurrence and risk factors

The use of the term coxiellosis — as opposed to Q fever — for referring to *C. burnetii* infection in animals was suggested by Lang (1990). However, both terms are interchangeably used in the literature and so in this thesis the disease in animals is termed as either Q fever or coxiellosis.

Q fever is prevalent in cattle, sheep and goats in all 5 continents as shown in a review carried out by Guatteo et al. (2011). New Zealand is commonly mentioned as a rare exception where *C. burnetii* is considered exotic. This claim is supported by evidence gathered in a survey carried out in 1991–1992 in which 2,181 serum samples from aborted cattle and 12,556 serum samples from sheepdogs tested negative for presence of antibodies to *C. burnetii* (Hilbink et al., 1993). The overall mean for animal level prevalence in the studies included in the review carried out by Guatteo et al. ranged between 15% and 30% for all of the three ruminant species. Most published studies reporting disease frequency in livestock are based on serology and relatively few report shedding prevalence. Further, those that do report shedding prevalence often report shedding in milk, an excretion route that is considered of relatively low importance in the epidemiology of the disease compared to shedding in products of conception (Angelakis and Raoult, 2010). A need for further studies looking at shedding prevalence, particularly in small ruminants, was highlighted by Guatteo and collaborators.

Like humans, livestock acquire infection mainly through inhalation of contaminated aerosols (Lang, 1990). Infection by the oral route is possible but appears not to be as effective as the respiratory route (Roest et al., 2012). *C. burnetii* was isolated from numerous tick species and several of them were shown to be competent vectors under experimental conditions (Duron et al., 2015). Further, trans-stadial transmission as well as transovarian transmission can occur in ticks, which provides weight to the hypothesis of ticks being involved in disease transmission (Eldin et al., 2017). In that line, van Engelen et al. (2014b), found dairy cattle herds infested with ticks had two times the odds of *C. burnetii* DNA being detected in bulk tank milk (BTM) relative to herds that were free from ticks. A subject of recent research has been the fact that some ticks can harbor *Coxiella*-like endosymbionts, microorganisms likely to be misclassified as *C. burnetii* using PCR techniques. This finding may mean some of the previous studies that identified *C. burnetii* in ticks by means of PCR should be reconsidered and possibly repeated (Duron et al., 2015).

Numerous wildlife species have been found to be infected with *C. burnetii* and could be a potential source of infection for humans and livestock (Gonzalez-Barrio and Ruiz-Fons, 2019). In a study carried out by Enright et al. (1971) in Mendocino County, California, antibodies against *C. burnetii* were detected in 17 different species of mammals using CFT. Coyotes (*Canis latrans*), foxes (*Urocyon cinereoargenteus*), brush rabbits (*Sylvilagus bachmani*) and deer (*Odocoileus hemionus columbianus*) were among the species with the highest prevalence of exposure to *C. burnetii*. Furthermore, the authors were able to isolate *C. burnetii* from 9 different wild mammal species. In a more recent study in The Netherlands, *C. burnetii* DNA as well as antibodies were detected both in black rats (*Ratus ratus*) and brown rats (*Ratus novergicus*), which suggests these rodents could play a role as Q fever reservoirs (Reusken et al., 2011).

In Australia, Cooper et al. (2013) found *C. burnetii* DNA in blood collected from a variety of native marsupial species as well as in different tick species infesting them. Some of these marsupials (e.g. bandicoots and possums) are relatively abundant both in rural areas and in urban and peri-urban areas to which they are highly adaptable. Serological studies have also been carried out in native and introduced Australian fauna with

results that add evidence to the existence of a wild cycle of Q fever in this country (Cooper et al., 2011, 2012). Whereas Cooper's studies were carried out mainly in the northern state of Queensland, Banazis et al. (2010) and Potter et al. (2011) studied the prevalence of exposure to *C. burnetii* in grey kangaroos (*Macropus fuliginosus*) in Western Australia. Presence of antibodies against *C. burnetii* ranged from 24.1% to 33.5% of grey kangaroos sampled, depending on the study. Also, in both studies *C. burnetii* DNA was found in kangaroo faeces. The existence of wildlife reservoirs for *C. burnetii* should be factored in at the time of developing control measures given the risk of spill over to livestock and human populations.

Viable *C. burnetii* were found in semen of naturally infected bulls (Kruszewska and Tylewska-Wierzbanowska, 1997) and sexual transmission has been observed in mice (Tylewska-Wierzbanowska and Kruszewska, 1990). Sexual transmission has also been observed in humans, although only as rare isolated cases, representing negligible epidemiological importance (Milazzo et al., 2001). *C. burnetii* DNA has been found in the reproductive tract of naturally infected non-pregnant goats, which means there is a potential risk for disease transmission during embryonic transfer from the donor to the recipient in this species (Alsaleh et al., 2011). There is not clear evidence of disease transmission occurring via artificial insemination or natural service either in cattle or other livestock species.

The stability of the SCV form of *C. burnetii* can potentially result in viable organism persisting in the environment long periods of time after being shed by infected animals (Minnick and Raghavan, 2012). Welsh et al. (1958) carried out an experimental study to assess the onset and persistence of *C. burnetii* in aerosols in relation to the time of lambing. Six sheep were challenged with *C. burnetii* intravenously and kept in isolated pens from which air samples were obtained throughout a 3-week period starting one week before the expected lambing date. All pens had positive air samples following the lambing of the sheep kept in them. Further, positive air samples were detected up to the last day of sampling (i.e. day 14 post lambing) in one of the pens (Figure 1.2).

Results from an observational study by Welsh et al. (1959) have been misinterpreted by some authors as evidence of the capacity of *C. burnetii* to survive in the environment for 150 days. Welsh and collaborators recovered viable *C. burnetii* from soil samples obtained within the window of time that defined the lambing season at a sheep ranch in California (i.e. for 150 days), but sustained re-infection of the environment from lambing sheep was not ruled out. Further, the authors did not recover viable *Coxiella* beyond the end of the lambing season. Similar results were found by Astobiza et al. (2011a) who detected *C. burnetii* DNA in air samples taken from premises where a Q fever positive sheep flock was lambing within a time window of two months during which the majority of the ewes lambed. However, *C. burnetii* was not detected towards the end of the lambing season, when only a reduced number of ewes were lambing. On the other hand, in a longitudinal study carried out in a dairy goat farm in Spain where a high abortion rate due to *C. burnetii* infection was observed, viable *C. burnetii* was found in dust collected around the parturition period and up to 2 months from the last parturition (Álvarez-Alonso et al., 2018). Evstigneev et al. (2007) seeded *C. burnetii* into different types of soils and detected viable bacteria until day 20 from seeding, although no follow up beyond that time was carried out. The viability of *C. burnetii* in the environment and how different environmental conditions such as temperature, humidity and exposure to UV light impact on its survival is still poorly understood and warrants further research.

The role of wind in between-farm transmission of Q fever has been the subject of numerous studies. Nusinovici et al. (2017) found a significant association between the *C. burnetii* environmental burden in farms located upwind from a given farm and the risk of this farm becoming Q fever positive. Animal movements

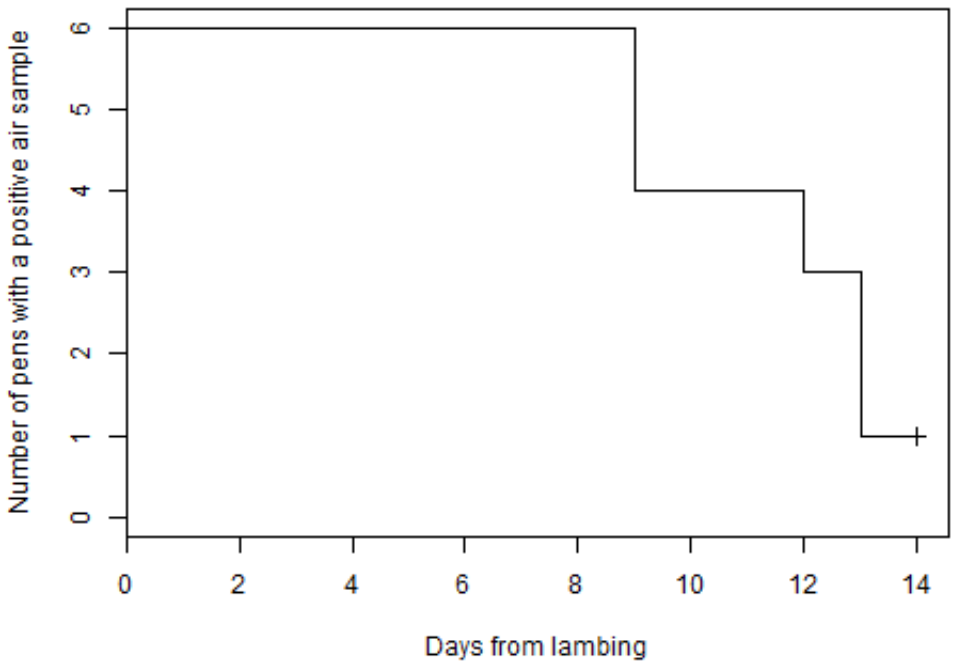

Figure 1.2: Kaplan-Meier curve showing the time to the last detection of viable *C. burnetii* in air samples taken from six individual pens where experimentally infected sheep had lambed. The follow-up period ended 14 days after lambing. Data adapted from Welsh et al. (1958).

throughout the study period were not recorded and could be a potential source of bias in the analysis. This is because farmers are likely to buy cows from farms located in the proximity of their own farms and thus transmission from one herd to another could be explained by movement of cows from infected to non-infected herds. Other studies found an increased risk of Q fever in livestock in high livestock density areas compared to low livestock density areas, independent from the effect of animal movements (Nusinovici et al., 2014; Pandit et al., 2016). These studies attributed the estimated increase in the risk of infection in high livestock density areas to the effect of windborne environmental contamination. The fact that wind can play a role in the spread of *C. burnetii* infection among livestock herds makes disease control challenging in the event of a large-scale outbreak in areas of high livestock density.

Risk factors associated with herd-level presence of antibodies against *C. burnetii* in small ruminants have been assessed by Meadows et al. (2015a; 2015b). In cross-sectional studies carried out in Canada, the size of the herd was positively associated with the odds of exposure to *C. burnetii* at the herd level both in sheep and goats. This is in agreement with the results of other studies looking at risk factors associated with Q fever in sheep and goats (Schimmer et al., 2011; Lambton et al., 2016). The studies carried out by Meadows and collaborators also found that farms where animals were moved to lamb/kid in a separate airspace had an increased risk of being seropositive. The authors suggested this could be due to restricted ventilation in the lambing/kidding area where farmers were keeping their pregnant stock. In the same line, Capuano et al. (2001) found that herds under management practices that involved housing of cattle had an increased risk of exposure to *C. burnetii* compared with herds in which cattle where grazed in paddocks. Also, Schimmer et al. (2011) found the use of windbreak curtains increased the animal level risk of *C. burnetii* exposure in dairy goat farms. Regarding hygiene practices

around the time of parturition, farms where disinfection of the kidding/lambing pen was carried out had lower odds of herd level *C. burnetii* exposure compared to those where hygiene practices were limited to adding bedding material and removal of birthing products (Meadows et al., 2015a,b). Also, the frequency with which cleaning of the litter was performed was found to be negatively associated with the risk of *C. burnetii* exposure in ruminants (Cantas et al., 2011). As for type of production system, dairy farms were at a higher risk of being seropositive compared to meat farms. Likely reasons mentioned by the authors include the lower population turnover in dairy farms compared with meat farms, which can result in exposed animals being kept in the herd for longer. In agreement with these results, Van den Brom and collaborators (2013) reported a significantly higher risk of farm-level seropositivity in dairy farms as compared to non-dairy farms for both goats and sheep. Furthermore, within farm prevalence of *C. burnetii* antibodies was also significantly higher in dairy systems.

1.5.2 Pathophysiology and excretion routes

The first Q fever challenge studies in livestock date from the late 1940s. Bell et al. (1949) inoculated four heifers with a suspension of an infected yolk-sac culture of *C. burnetii* via the teat canal and were able to isolate the bacteria from blood up to day 5 post inoculation. Infectiousness of milk and other tissues obtained up to 68 days post challenge was demonstrated by inoculation in guinea pigs. The dose of *C. burnetii* with which cows were challenged was not reported in this study, however, it was likely very high given the severity of the lesions in udder tissues reported by the authors. Therefore, results should be considered cautiously as such a scenario is remote from field experience.

Challenge studies in sheep were carried out by Martinov et al. (1989) and Brooks et al. (1986). Martinov and collaborators inoculated three sheep intravenously and a fourth sheep using the intraperitoneal route with a suspension of the *C. burnetii* Tchilnov strain. All four sheep were pregnant at the time of inoculation although gestational ages were not uniform. Acute clinical signs including fever, depression, rhinitis and tachypnea were observed five to seven days post inoculation. Challenged ewes delivered either stillborns or weak non-viable lambs. In the challenge study carried out by Brooks et al., six sheep were challenged subcutaneously around day 100 of gestation. Presence of viable *C. burnetii* in placental tissues was demonstrated by inoculation into mice. No acute signs were reported but weak lambs and perinatal mortality were observed. The main histopathological finding of both studies was placentitis.

Arricau Bouvery et al. (2003) carried out a study to investigate Q fever clinical signs and shedding routes after experimental infection. One-year-old pregnant goats ($n = 19$) at 90 days of gestation were inoculated subcutaneously with three different doses of *C. burnetii* (i.e. 10^4, 10^6 and 10^8 infective mouse doses). Fever was observed in some animals around days 5–7 p.i. and was more marked within the group of goats that received the most concentrated inoculum. Seroconversion started around day 21 p.i. although antibody levels were below the threshold of positivity of the serological assay used (iCHEKIT-Q-Fever enzyme immuno-assay kit; Bommeli diagnostics, Switzerland) until approximately week 6–7 p.i. All challenged goats aborted, irrespective of the infective dose used. The first abortion happened at day 12 post infection (p.i.) and the remaining were clustered around day 29 and 43 p.i. Presence of *C. burnetii* was detected by PCR in cotyledons of all aborted goats and in a variety of tissues including liver and lungs. Excretion of *C. burnetii* was detected in faeces, vaginal discharges and milk of all infected goats. *C. burnetii* DNA was detected as far as 14 days after parturition in vaginal discharges and 52 days after parturition in milk. Faeces collected from some challenged goats were PCR positive before each of them had aborted (although after the first abortion had occurred) and the mean

duration of faecal excretion was 20 days. Shedding of *C. burnetii* in milk and faeces was found to be intermittent. *Coxiella* excretion from each excretion route and at the different sampling time points was assessed as either detectable or not-detectable, but shedding quantities were not determined.

In another experimental study, Sanchez et al. (2006) inoculated subcutaneously 12 goats that were 90 days pregnant. Two goats were euthanised on day 26 p.i. and 2 were killed on day 40 p.i. The remaining 8 exposed goats were sacrificed after abortion; 2 on day 8 post abortion (p.a.) and 6 on day 120 p.a. *C. burnetii* was detected by PCR in the choriallantonic membranes and placentomes as early as 26 p.i. All exposed goats aborted around day 42 ± 4 p.i. Histopathological lesions of the placenta increased in severity with time from infection. As for foetal organs, the liver was the only organ were lesions were observed, although *C. burnetii* was detected by PCR in spleens, liver and lung. Trophoblast cells were found to be the main site of multiplication of *C. burnetii*. Major immuno-histopathological changes were observed in the mammary glands and *C. burnetii* was detected in milk in all goats that aborted, from the abortion date up to in average 17 days. Although *C. burnetii* was detected in liver and lung, no obvious lesions were found in these or other maternal organs apart from the mammary glands.

Albeit the above-mentioned studies provide valuable insight into the pathophysiology of coxiellosis in goats, they have in common that the route of infection used (i.e. subcutaneous injection) is not representative of field conditions, where goats acquire Q fever mainly through inhalation of *C. burnetii* contaminated particles. Roest et al. (2012) were able to reproduce Q fever in goats using the intranasal route. In their experiment, they challenged 10 goats in their 76^{th} day of gestation. Both abortions and delivery of healthy kids were observed in challenged goats and shedding of *C. burnetii* in vaginal discharges was detected irrespective of the gestation outcome. *C. burnetii* was detected in the upper respiratory tract at 14 p.i. and in the placenta between day 14 and 28 p.i. In agreement with the findings of Sanchez et al., the trophoblasts of the chorioallantois were found to be the primary target cells of *C. burnetii*. Shedding of *C. burnetii* by exposed goats was not detected until abortion or parturition. After delivery, *C. burnetii* DNA was found in milk for 38 days, and in vaginal discharges and faeces until the end of the experiment (i.e. 141 days p.i.). Lochia discharge following parturition could explain the sustained detection of *C. burnetii* in vaginal swabs. Several foetal tissues (i.e. spleen, liver, kidney, lung and heart) returned positive results to PCR, indicating in-utero infection can occur.

Altogether, results from experimental infections in pregnant goats indicate *C. burnetii* has a strong tropism for trophoblasts where it replicates to high numbers. However, this does not rule out the probability of persistent infection in non-pregnant goats. Moreover, as already mentioned, *C. burnetii* DNA was found in oviducts and uterine flushing media and in genital tract tissues of non-pregnant goats (Alsaleh et al., 2011). Also, Arricau-Bouvary et al. found *C. burnetii* DNA in the liver of a non-pregnant goat challenged subcutaneously (Arricau Bouvery et al., 2003). Furthermore, Muleme et al. (2017a) followed up young goats that became infected early in life and found 36% of them to be shedders *C. burnetii* at parturition. Muleme's results could be interpreted as evidence of persistent infection occurring in non-pregnant goats, although the alternative scenario of re-infection from the environment cannot be ruled out.

Experimental infections show abortions in goats are a consequence of placentitis and result in large quantities of *C. burnetii* being excreted. Excretion of *C. burnetii* can also occur in goats that deliver healthy kids. *C. burnetii* can also be found in faeces, urine, saliva and milk of infected animals though in smaller quantities than in birth products. Presence of *C. burnetii* DNA in foetal tissues mean intrauterine infection is possible. In the challenge study carried out by Roest and collaborators *C. burnetii* DNA was detected in blood in some of the challenged goats towards the end of their gestation. Although the sensitivity of detection in this tissue

seems to be low, the finding opens the question on the potential use of qPCR techniques for early detection of infected does. Further, the sensitivity of qPCR in blood has recently been found to improve using lyophilisation techniques (Edouard and Raoult, 2016). Comparison of clinical signs (e.g. pregnancy outcome) observed for cattle, sheep and goats challenged with *C. burnetii* should be considered with caution given the differences in *Coxiella* strains, inoculation routes, concentrations of inoculum, and gestational age at the time of challenge in the different experiments. Although the use of the intranasal route for infection used in the study carried out by Roest et al., as opposed to the subcutaneous or intraperitoneal routes used in other studies, may result in a course of disease closer to that expected under field conditions, other factors such as a wide range of exposure doses and times of exposure relative to variables like age or gestational status are difficult to capture in experimental studies. Observational studies carried out in *C. burnetii* naturally infected herds could help better understand shedding dynamics in the context of farming environments.

A number of observational studies have contributed to the understanding of *C. burnetii* shedding patterns in livestock. In the early 1950s, the presence of viable *C. burnetii* in placental tissues of naturally infected sheep was studied by Welsh et al. (1951). The infectiousness of placental tissues was demonstrated by inoculation in laboratory animals. Further, sheep from which placental tissues were recovered were also tested for antibodies using CFT, and it was found close to 30% (6 out of 21) of the sheep with positive placentas had no antibodies against *C. burnetii*. The titration in laboratory animals of placental tissues showed placentas obtained from CFT positive sheep tended to have higher concentrations of *C. burnetii* than those from CFT negative sheep. This pattern in which some seronegative animals are infectious (i.e. *C. burnetii* shedders but have not detectable antibodies) was repeatedly found in subsequent studies (Berri et al., 2005a; Rousset et al., 2009b; Natale et al., 2012).

The advent of PCR and qPCR techniques significantly facilitated the laboratory task of testing specimens for presence of *C. burnetii*, the latter also allowing quantification of genome copies. This led to an increase in the number studies looking at *C. burnetii* shedding patterns in naturally occurring Q fever in livestock (Berri et al., 2001; Guatteo et al., 2006; Rousset et al., 2009b). de Cremoux et al. (2012) studied the serological responses and shedding patterns of three dairy goat herds where abortions attributed to *C. burnetii* had happened. Vaginal shedding was found to be very high (i.e. above 1×10^6 GE per sample) in more than 90% of goats that aborted. Interestingly, 30% of shedding goats that did not abort showed shedding levels of similar magnitude. Out of a total of 654 goats classified as shedders, 396 (60%) were positive when tested by ELISA. Further studies looking at the distribution of *C. burnetii* shedding quantities by infected animals could contribute to the development of control measures that target specific subgroups of animals likely to shed large quantities of *C. burnetii* in the environment. Longitudinal studies carried out in sheep (Berri et al., 2002) and goats (Berri et al., 2007) provided evidence of the occurrence of shedding in at least two consecutive parturitions by some infected animals. However, the frequency with which this occurs is not clear and further information could be of value for the development of control interventions.

1.5.3 Impacts on production

Q fever in cattle, sheep and goats can lead to the entire spectrum of the abortion, premature delivery, stillbirth and weak offspring (APSW) complex (Lang, 1990; Agerholm, 2013). Abortion late in pregnancy was observed in Holstein-Friesian dairy cows challenged with *C. burnetii* (Behymer et al., 1976). Also, placentitis and abortion in cows has been associated with naturally occurring *C. burnetii* infection (Bildfell et al., 2000). However,

C. burnetii is a relatively infrequent cause of abortion in cattle under field conditions (Anderson et al., 1990; Kirkbride, 1993a), and abortion outbreaks associated with Q fever in this species have not been reported. In sheep, on the other hand, abortion outbreaks attributed to *C. burnetii* infection have been reported (Zeman et al., 1989; Berri et al., 2002). However, in common with cattle, results from some diagnostic surveys suggest Q fever may not be a common cause of abortion in this species (Buxton and Henderson, 1999). For example, out of 1,784 abortions submitted for diagnosis to the South Dakota Animal Disease Research and Diagnostic Laboratory in USA throughout a 10-year period, only 0.1% were attributed to *C. burnetii* infection (Kirkbride, 1993b).

Goats appear to be at higher risk of having a *C. burnetii* associated abortion than other ruminants (Lang, 1990), and *C. burnetii* is one of the main causes of abortion in goats in the USA and Europe (Moeller, 2001; Chanton-Greutmann et al., 2002). The proportion of goats in a herd that abort following introduction of *C. burnetii* vary significantly in published reports, ranging from 3% to 90% (Arricau Bouvery et al., 2003; Arricau-Bouvery et al., 2005; Berri et al., 2007; Reichel et al., 2012). During the Dutch outbreak of 2007–2011, out of 94 infected goat herds close to 30% presented abortion risks above 5%, and the maximum abortion risk observed was 80% (Hogerwerf et al., 2013). The underlying level of immunity due to previous exposure, management practices (particularly those related to reproductive management, housing and stock density), and differences in the virulence of the *C. burnetii* strain involved could explain the variability in abortion risks observed.

Although there is agreement in that *C. burnetii* infection is linked to weak offspring in livestock (Agerholm, 2013), the ongoing impacts to these animals are unknown. Increased perinatal mortality and/or increased incidence of diseases in young animals would be expected in herds were *C. burnetii* infection causes low birth weights (Wu et al., 2006). Also, low birth weights could lead to an increase in the age of first service, shortening the productive life of replacements (Pardo et al., 2019). Epidemiological studies in which variables like birth weight, weight gain, age of first service, removal rates, removal causes and disease incidence in young animal categories are compared between Q fever positive and negative herds — or between animals born from Q fever positive and Q fever negative mothers — are required to assess the impact of Q fever on the efficiency of the rearing cycle.

On top of production losses due to reproductive disease and weak offspring, *C. burnetii* infection in cattle has been associated with subclinical mastitis (Barlow et al., 2008). Further, Freick et al. (2017) found milk fat yields were lower in primiparous cows that were shedding *C. burnetii* after calving as well as in cows that seroconverted during their first 42 days in milk compared with their negative mates. Also, viable *Coxiella burnetii* has been isolated from sheep with clinical mastitis (Martinov, 2007). The relationship between *C. burnetii* infection and milk production in goats was studied by Muleme et al. (2017b). The authors found seroconversion to phase 1 antibodies was associated with an extra 0.28 L of milk per day (95% CI: 0.01 to 0.54), and hypothesised the observed difference was attributed to a protective effect associated with humoral immunity targeting phase I antigens. To assess whether active infection with *C. burnetii* is associated with milk yield losses, milk volumes could be assessed against individual *C. burnetii* shedding status.

Clinical signs associated with *C. burnetii* infection in livestock are sometimes unapparent, as evidenced by the fact that often diagnosis is only made following the occurrence of human cases (Berri et al., 2005a; Bond et al., 2016). Because impacts on production are often subtle, the use of consistent methods for recording and reporting herd performance is of key importance. This would enable early detection of changes in herd health, which could be indicative of coxiellosis. Farmers are nowadays making use of highly efficient data recording technologies, which increased the availability of production data and facilitated the assessment of

herd performance trends in livestock production (Ait-Saidi et al., 2014; Alejandro, 2016). However, farmers still make limited use of health management data to know where they gain or lose income from their herds (Kaler and Green, 2013; Lima et al., 2018). In emerging livestock industries, like the dairy goat industry in Australia, the lack of published benchmarks precludes the comparison of herd performance with a set of local targets. Standardised methods for measuring herd performance are needed in order to assess the impact of Q fever and other health disorders in dairy goats.

1.5.4 Control strategies

Vaccination with vaccines prepared from phase I *C. burnetii* are an effective means for reducing environmental contamination and consequently disease transmission (Arricau-Bouvery et al., 2005). Arricau Bovary et al. (2005) carried out an experimental study in goats to compare the efficacy of two commercially available vaccines in preventing abortion and shedding of *C. burnetii* to the environment. The two vaccines used in the study were a *Coxiella* phase I vaccine (Coxevac®, CEVA, Santé Animale, Libourne, France) and a *Coxiella* phase II vaccine (Chlamyvax-FQ, Mérial, France). Although neither of the vaccines prevented infection, the phase I vaccine effectively prevented abortions and shedding in milk. The phase I vaccine was also effective in reducing the amount of *C. burnetii* shed in placental tissues, vaginal mucus and faeces. In contrast, the phase II vaccine achieved similar results to the control group (non-vaccinated). Also, the antibody response induced by vaccination was higher in animals vaccinated with the phase I vaccine than in those vaccinated with a phase II vaccine.

A phase I vaccine was also tested in clinically affected goat herds by Rousset et al. (2009b) and by de Cremoux et al. (2012). Both studies found the vaccine had an overall effect in reducing the amount of *C. burnetii* shedding. This effect was more pronounced in renewal (nulliparous) animals. Assuming the prevalence of exposure was lower in renewal goats compared to adult goats, the differences observed could be attributed to the pre-vaccination Q fever status, as suggested by the findings of a study carried out in cattle by Guatteo et al. (2008). In Guatteo's study, a five fold reduction in the probability of becoming a shedder was observed in cows that were non-pregnant and susceptible (i.e. non-shedders and serologically negative) at the time of vaccination. In contrast, in cows that were either exposed/infected or pregnant at the time of vaccination no significant reduction in the probability of becoming a shedder was observed. An improved efficacy of vaccination in young small ruminants was also found in an observational study carried out in The Netherlands by Hogerwerf et al. (2011). This case-control study found an overall reduction in both the odds of shedding and in the quantities of *C. burnetii* being shed in uterine fluid, vaginal mucus and in milk in vaccinated animals compared to non-vaccinated animals. Vaccination with a phase I vaccine became mandatory in The Netherlands in 2010 as part of the outbreak response. Notably, by 2014 no abortions due to *C. burnetii* had been reported in The Netherlands since the start of vaccination (van Engelen et al., 2014a). It is worth mentioning that *C. burnetii* had been found to be the most frequent cause of abortion in goats between 2009 and 2012 (van den Brom et al., 2012).

Treating pregnant animals with antibiotics (tetracycline mainly) with the objective of reducing *C. burnetii* shedding at parturition is a relatively common practice of veterinary practitioners in parts of Europe (Berri et al., 2005a; Taurel et al., 2012; de Cremoux et al., 2012). In a cohort study carried out by Taurel et al. (2012), cows treated with long acting oxytetracycline at a dose of 20 mg/kg at drying off had close to half the odds of shedding at calving compared to untreated cows (OR: 0.4; 95% CI: 0.21 to 0.75). No additional protective effect was found in animals that received a second dose of oxytetracycline 15 days after drying off. No statistically

significant differences were obsserved in bacterial loads shed by cows that had received antibiotic treatment and those in the control group. Astobiza et al. (Astobiza et al., 2013), assessed the efficacy of oxytetracycline treatment in ewes in reducing *C. burnetii* sheddiing at lambing. A group of ewes was administered two doses of 20 mg/kg of oxytetracycline on gestation days 100 and 120, and their shedding status at lambing was compared with a group of untreated ewes. Vaginal swabs, faeces and milk samples from both treated and non-treated ewes within 30 days of lambing were obtained and qPCR was used to test for presence of *C. burnetii* DNA. No significant differences were found in *C. burnetii* shedding quantities between ewes that had been treated and those that had not. The relative scarcity of evidence of antibiotic treatment efficacy for controlling coxiellosis in livestock has led the European Food and Safety Authority (EFSA) to discourage their use (European Food Safety Authority, 2010). Moreover, the emergence of antibiotic resistance as a major global public health concern makes mass treatment of animals in infected farms highly questionable in the absence of sound evidence of its benefit.

There are no reports in the literature of the use of test and remove interventions at the individual level for the control of Q fever in livestock in field conditions. In a simulation study, Bontje et al. (2016) assessed the efficacy of removal of does with a positive test result in milk — assuming a 50% probability of detection — and found this intervention would not lead to disease eradication from a herd. During the 2007–2010 Netherlands outbreak, farms were monitored by bulk tank milk (BTM) PCR testing, and breeding of dairy goats and dairy sheep was banned in *Coxiella* positive farms. Culling of all pregnant animals in herds with two consecutive positive results was performed by the end of 2009 (Roest et al., 2011a). An explanation on why test and cull interventions at the individual level have not been attempted is that the agreement between serological status and shedding status at the individual level is often poor. Shedding animals and even animals that had aborted due to coxiellosis are sometimes found to be seronegative (Arricau-Bouvery et al., 2005; Guatteo et al., 2008; Reichel et al., 2012). The agreement between serology and shedding status seems to improve among the sub-group of animals shedding large quantities of *C. burnetii*, as shown by Natale et al. (2012). However, it is unknown if the removal of high shedders only would result in eradication of Q fever from an infected herd.

Hygiene measures recommended for infected farms include composting of manure, rendering of aborted foetuses and placentas, vermin control, and disinfection of premises (Vellema and van den Brom, 2014; Bond et al., 2016). Under-ploughing of composted manure after spreading across fields has also been recommended to prevent dispersion by wind (Bond et al., 2016). There is anecdotal evidence of a reduced presence of *C. burnetii* contaminated aerosols in farms using slatted floors and periodic manure removal systems as opposed to straw bedding (Astobiza et al., 2011a). Elimination of manure and disinfection seem to have sufficed to control disease transmission and even eradicate disease from livestock farms after an outbreak occurred in Hungary in 2015 (Gyuranecz et al., 2015). Berri et al. (2005b) reported a case where no shedding of *C. burnetii* was detected in a goat herd in the kidding season that followed an outbreak of abortions due to Q fever. A detail description of the outbreak in their paper makes no mention of specific control measures applied. These examples contrasts with reports of other outbreaks in goats where coxiellosis seem to have reached endemic levels despite the introduction of a series of hygiene measures (Bond et al., 2016; Muleme et al., 2017a).

The use of a segregated kidding/lambing environment has been proposed as a strategy to reduce within-farm disease transmission (Arricau-Bouvery et al., 2001; Reichel et al., 2012; Plummer et al., 2018). Though intuitively isolating kidders would effectively reduce the pool of susceptible individuals exposed to environmental contamination, there is no published evidence of the efficacy of this practice in reducing disease transmission.

1.6 Mathematical models of Q fever

1.6.1 Introduction to mathematical models

Models are simplified abstract representations of real-world complex systems and have multiple applications in human and animal disease prevention and control. One of the aims of disease modelling is to predict the spreading of diseases in outbreak situations so that preventive measures and allocation of resources is carried out efficaciously. Perhaps the best known example of the use of models to this end was during the response to the 2001 FMD outbreak in the UK (Keeling et al., 2001). Some authors have criticised the overuse of mathematical models for decision making during this response (Campbell and Lee, 2003; Mansley et al., 2011). Much of this criticism was centered on the use of models to guide the decision of pre-emptively culling all FMD-susceptible stock in farms that shared a boundary with an infected premise — without a case by case on the ground assessment of the risk of infection for these animals — which may have unnecessarily inflated the number of livestock culled in an effor to control disease spread (Kitching et al., 2006).

Mathematical models are also useful tools to predict the impact of control interventions for endemically or seasonally occurring diseases (French et al., 1999; Wall et al., 2000) or to assist in the design of targeted surveillance strategies for exotic or rarely occurring diseases (England et al., 2004; Cowled et al., 2012; VanderWaal et al., 2017). Moreover, the development process of a mathematical model often reveals information gaps and prompts relevant questions about key drivers of disease dynamics (de Jong, 1995). Modeling can be particularly helpful in instances where experimental or field trials are impractical or impossible to carry out due to logistic restrictions or resource limitations.

Mathematical models of disease transmission can be compartmental or agent-based. Compartmental mathematical models use algebraic formulae to describe the flow of individuals through different compartments that represent different health statuses. One of the most commonly used compartmental models are SIR models. In SIR models, subjects are classified as either susceptible (S), infectious (I) or recovered (R). The flow of subjects from one compartment to another in a simple SIR model can be described by a series of differential equations (Figure 1.3). In these equations, the parameter β, known as the transmission rate, is the product of the contact rate between infectious and susceptible individuals and the transmission probability given a contact. The recovery rate, often represented using the Greek letter γ, equals the inverse of the infectious period (Keeling and Danon, 2009). From these two rates, a key epidemiological parameter, known as the basic reproductive ratio or R_0, can be derived as,

$$R_0 = \beta/\gamma \tag{1.1}$$

R_0 represents the average number of secondary cases expected to occur in a fully susceptible large population after the introduction of an infected individual (Vynnycky and White, 2010). The value of R_0 determines the likelihood of occurrence of an outbreak. Values of $R_0 < 1$ mean an outbreak is not likely to spread, whereas values of $R_0 > 1$ mean the chain of transmission can be maintained and hence an outbreak can spread effectively (Keeling and Danon, 2009). If a newly introduced infection induces immunity, as the epidemic progresses some of the contacts that could have led to infection will fall on non-susceptible individuals, leading to a rate of secondary infections per infected individual below R_0. The *effective* or *net* reproduction number (R_n) refers to the number of secondary cases resulting from introduction of one infectious individual into a population in

which some individuals may already be immune because of natural exposure or vaccination (Vynnycky and White, 2010). Mathematically, (R_n) is defined as follows,

$$R_n = R_0 \times S, \tag{1.2}$$

where S represents the proportion of susceptible individuals in the population at time t. From this equations, it follows that when the proportion of susceptible individuals is below $1/R_0$ there will be less than one secondary case per infected individual and hence the incidence of disease should decrease. This phenomenon is known as the *herd immunity threshold* and it has direct implications on the design of control policies. For example, it derives from it that for a vaccination program to be effective a proportion of the susceptible population equal or higher to $1 - 1/R_0$ should be immunised.

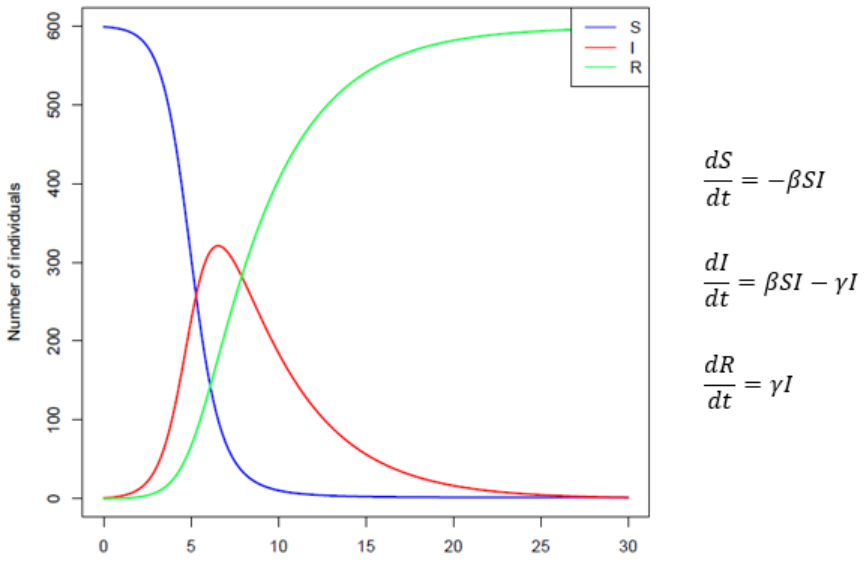

Figure 1.3: Ordinary differential equations and model predictions for an SIR model. The total population N was set to 600 individuals, a transmission rate β of 1.5 and a recovery rate *gamma* of 0.25 were used. The initial conditions were set to one infected individual and the remaining of the population being susceptible.

Additional compartments can be added to SIR models. For instance, SEIR models incorporate the 'exposed' compartment, in which individuals are infected but still not infectious to others. Since the environment can be a reservoir for infection in certain diseases, some models incorporate one or more additional compartments to represent the burden of a microorganism in the environment (Andrews and Basu, 2011). As more complexity is added to models, that is more compartments and relationships among them are included, they may (or may not) render more accurate results, vitally depending on the additional assumptions required along with additional complexity (Saltelli, 2019). Whilst models should be as complex as required to fulfill the purpose for which they were developed, complexity should be kept at a level in which the role of its components and the interactions among them can still be understood and supported with existing knowledge. Also, models should be flexible enough to be able to be relatively easily adapted to new situations (Keeling and Rohani, 2008).

As opposed to compartmental models where the system keeps track of the count of individuals in each disease state over time, in agent based models (ABM) each subject and its disease state is individually tracked. Hence, population disease dynamics emerge from aggregating the individual disease states over time (Rahmandad and Sterman, 2008). Detailed individual-level information can be included into a disease transmission model

using this approach. For example, ABMs can keep track of individual spatial location and adjust contact rates accordingly (Eubank et al., 2004). One feature that limits the use of agent based models is that they become computationally intensive when the size of the population being modeled is relatively large. Also, communicating their internal structure can be challenging when their complexity is high. Nevertheless, agent-based modelling is becoming more popular alongside the generalised use devices that record individual data and the increasing access to computering systems that can cope with the computational demand of these models, such as high-performance cluster and/or cloud computing systems that facilitate parallel computing (Macal, 2016; Lafayette et al., 2016).

Models can also be classified according to whether or not they incorporate stochasticity. Determinisitic models, like the example in Figure 1.3, render unique results for a given set of parameters and initial conditions. On the other hand, stochastic models account for the effect of chance in model outcomes and will therefore generate results that cannot be entirely predicted from the parameters used alone. This means stochastic models need to be run multiple times before output trajectories for a given set of parameters can be characterised. While deterministic models of disease transmission are accurate for large systems, for smaller populations, or at the start or end of an epidemic, stochastic processes can have a considerable impact on disease dynamics. Therefore, stochasticity becomes important when studying the efficiency of control strategies for disease eradication (Mancy et al., 2017).

Often, stochasticity alone cannot explain fluctuating temporal patterns of disease occurrence in populations. This is in part because host-agent systems can be influenced by multiple seasonal processes. Disease transmission can be modified for example by changes in contact rates, fluctuations in the relative abundance of competent vectors, or changes in environmental conditions affecting the survival of a given pathogen (Altizer et al., 2006). The increased incidence of influenza infections and other respiratory diseases in human populations in winter provides a clear example of seasonal disease patterns (Dietz, 1976). On top of seasonal variations affecting disease transmission rates, changes in the host demographic rates like pulses of births can impact on the relative abundance of susceptible individuals and lead to variations in disease transmission rates (White et al., 1996). Livestock systems are often consistent with this scenario because management practices commonly result in births being aggregated over a restricted temporal window. Seasonal variations in the degree of susceptibility to infection can also occur (Waller et al., 2004). Elucidating the link between seasonal environmental changes and disease patterns can aid in forecasting long-term disease risks and in developing control strategies to mitigate disease impact.

Most mathematical models take as inputs parameters and initial conditions for independent and dependent model variables. The degree of certainty around these inputs will be affected, for example, by their inherent natural variation, measurement errors or the availability of published studies that quantify their values. Uncertainty analysis can be used to assess the extent to which model outputs vary as a result of the uncertainty in model inputs. Uncertainty analysis are often coupled with sensitivity analysis, by which the source of variability in model outputs can be apportioned to specific model inputs. Hence, the result of a sensitivity analysis is a qualitative and quantitative characterisation of how, and to what extent, each model input source impacts on model results (Sanchez and Blower, 1997). Therefore, sensitivity analysis allows to prioritise efforts for experimental or observational studies aiming to quantify specific components of disease transmission mechanisms. Also, sensitivity analysis can shed light on inconsistencies in the model structure when changes in model inputs do not result in changes in the output consistent with the biology of the system (Owen et al., 2011).

When there is relatively low uncertainty around model inputs, a local sensitivity analysis can be used. In

local sensitivity analysis, parameter values in relative proximity to a nominal value are selected to assess the resulting variation in model outputs. Often, uncertainty around parameter values is relatively high and global sensitivity analysis using sampling-based methods are favoured instead (Marino et al., 2008). Monte Carlo (MC) algorithms are sampling-based approaches commonly used to perform global sensitivity analysis (Marino et al., 2008). Essentially, parameter vectors are randomly sampled from probability distributions selected to represent the existing knowledge for each parameter and passed as arguments for model simulations. A large number of samples may be required to recreate input parameter distributions and ensure the parameter space is covered. Latin hypercube sampling (LHS) techniques can be used to increase implementation efficiency and obtain an un-biased estimate of the average model output (McKay et al., 1979; Blower and Dowlatabadi, 1994). In LHS, the proposed distributions for model parameters are divided into N intervals of equal area under the curve (i.e. total probability) and a value from within each of these intervals is randomly sampled. The result of LHS is a matrix with N rows and a number of columns equal to the number of parameters varied. For each parameter, the choice of probability density functions and their boundaries is based in the degree of knowledge on biological processes that each parameter attempts to capture. Uniform distributions with a conservatively large range of possible values comprised between the minimum and maximum boundaries should be used when knowledge about the biological process is scarce. On the other hand, more informative probability distributions (e.g. beta pert, normal or lognormal distributions) can be used when evidence suggests areas of higher probability across the range of plausible values (Owen et al., 2011). Once parameter candidates are selected, model simulations can be produced and the correlation between each parameter and the model output(s) of choice can be assessed.

The correlation between input sources and model outputs can be visually assessed using scatter plots. Scatter plots are also useful to detect nonlinearities and non-monotonicities. A monotonic relationship is one in which the response variable either increases or decreases as the input variable increases, independent on whether the rate of that change is constant or not. Measures of correlation for monotonic relationships between input and output can be obtained using partial rank correlation coefficients (PRCC) (Blower and Dowlatabadi, 1994). With PRCC a measure of monotonicity for each varied parameter can be obtained while accounting for the linear effects of the remaining variables. In stochastic models, the variability in model outputs reflects both aleatory and epistemic uncertainty. The aleatory uncertainty is that resulting from the inherent stochastic components of the model, whereas the epistemic uncertainty is that resulting from the experimentally introduced variation in model parameters. One method that has been proposed to reduce the relative weight of aleatory uncertainty in sensitivity analysis is to average the output of multiple model realisation with each set of sampled parameters and use that average to estimate PRCCs (Marino et al., 2008).

Uncertainty and sensitivity analysis are diagnostic tools that can be used to identify key model parameters and measure the uncertainty in model predictions associated with each parameter in the model. However, these techniques are not intended to provide information about the distribution of model parameters in real systems. Model parameters can be obtained from the literature or elicited from experts (Garner and Hamilton, 2011). However, information about plausible parameter values is not always available and the lack of agreement among experts can lead to considerable levels of uncertainty. Further, selection of parameters based on experiments carried out under disparate experimental conditions could result in unrealistic assumptions (Conlan et al., 2012). When empirical data about the system being model is available, statistical methods can be used to infer model parameters (van der Vaart et al., 2015). Bayesian methods have increasingly been used to this end. Among the advantages of Bayesian approaches are their ability to accommodate unobserved data and the fact that previous knowledge can be incorporated using prior probability distributions. Also, interpretation of the resulting

posterior probability distributions can be more intuitive than the output of classical statistical methods (Lopes and Beaumont, 2010). Some of the most commonly used Bayesian methods for inference of model parameters are those within the class of Approximate Bayesian Computation (ABC) (Sunnåker et al., 2013).

Originally developed as a tool for population genetic analysis (Pritchard et al., 1999), ABC methods are nowadays increasingly used for parameter inference in epidemiological models (Lopes and Beaumont, 2010). An advatage of ABC is that they do not require an explicit estimation of likelihood functions, which can be intractable or too costly to evaluate in complex models. Instead, ABC uses Monte Carlo simulations and rejection methods to evaluate posterior distributions (Toni et al., 2009). The cost of ABC flexibility is that it is a relatively inefficient process and therefore demands high computing capacity. However, the significance of this drawback is reduced by the increasing availability of super-computers and the fact that parallelisation of ABC is straight forward (Kosmala et al., 2016). The efficiency of the basic sampling-rejection ABC algorithm have also been improved by embedding it into Monte Carlo Markov Chain algorithms, like the Metropolitan-Hastings type algorithm (Marjoram et al., 2015). Also, Sequential Monte Carlo algorithms can be coupled with ABC, which results in an iterative refinement process where parameter values are initially sampled from the prior and finally sampled from the approximate posterior (Sisson et al., 2009; Brooks-Pollock et al., 2014).

1.6.2 Published within-herd Q fever transmission models

The first published model of Q fever transmission in livestock was authored by Courcoul et al. (2010) who modelled the disease in cows. This model was coded as an ABM in discrete time and a Markov Chain Monte Carlo method was used to infer parameters governing the transition rates between the compartments. The model includes four different disease states: susceptible, infectious with antibodies, infectious without antibodies and recovered. Infectious individuals contribute to environmental contamination and new infections are acquired from the environment. Susceptible cows can become infectious without antibodies and either transition back to being susceptible or become infectious with antibodies. Cows that are infectious with antibodies can recover and become temporarily immune. Recovered individuals can move back to be infectious with antibodies. The underlying structure of Courcoul et al. model entails the assumption that shedding of *C. burnetii* invariably precedes the presence of detectable antibodies. Given the scarcity of experimental infection studies in cows, it is hard to assess whether this is the most common case in this species. However, in experimental studies carried in goats a rise of antibodies to *C. burnetii* was detected as soon as 3 weeks after exposure, 4 weeks before shedding started (Roest et al., 2013b). Although the underlying structure of this model can potentially capture the inter-relation of antibody presence and shedding status in cattle, it may not be an appropriate approach for modelling Q fever in goats.

The model developed by Courcoul and collaborators was later expanded to incorporate herd demography and individual variability in *C. burnetii* shedding patterns in terms of routes, intensity, and duration of shedding (Courcoul et al., 2011b). Additional complexity was added by including more disease states into the model described in their previous publication (Courcoul et al., 2010). New parameters were incorporated to account for the additional complexity, which were obtained from the literature or calibrated so that model simulations were in agreement with expert knowledge on disease dynamics. Key parameters were explored using a sensitivity analysis. The parameters governing shedding levels, proportion of *C. burnetii* excreted that reached the environment, and the decay rate of *C. burnetii* in the environment were found to have the highest influence on model predictions. The authors reported model predictions showed complex disease dynamics; this phenomenon

could potentially be associated with the heterogeneous shedding patterns accounted for in the model (Grassly and Fraser, 2008).

A version of the model developed by Courcoul et al. (2011b) was used to test the effectiveness of reactive vaccination under three scenarios with varying vaccination strategies and vaccine efficacy assumptions (Courcoul et al., 2011a). In the first scenario, heifers and cows were vaccinated for a 10-year period. In the second scenario, vaccination was carried out in the same animal categories but vaccination was only sustained for 3 years. In the third scenario, heifers only were vaccinated for a 10-year period. The vaccine was assumed to be effective when applied to non-pregnant uninfected individuals as supported by Guatteo et al. (2008). When vaccinated under these conditions, cows that became infected were not allowed to abort and were assumed to shed lower quantities of *C. burnetii* (i.e. 30 to 3000 times lower) than non-effectively vaccinated infectious cows. In addition, for scenario 1, three different transition rates from the susceptible vaccinated state to the infectious state were assessed in which either 5%, 11% or 90% of infected cows that had been effectively vaccinated became shedders, respectively. The proportion of simulations that resulted in disease extinction within a 10-year period was relatively high (i.e. 0.84), including when 90% of vaccinated infected cows were allowed to become shedders. This suggests the reduction in shedding quantities induced by vaccination had a larger impact reducing disease transmission than the reduction in the prevalence of shedders. The median time to disease extinction for this scenario ranged from 5.2 to 7 years. For scenario 2 (i.e. reactive vaccination for 3 years only) a relatively prportion of simulations resulted in disease extinction (i.e. 0.08 to 0.43 depending on whether vaccine-induced immunity was assumed to wane over time or not, respectively). As for the third scenario (i.e. vaccination of heifers only), 95% of simulations resulted in disease extinction, and the median time to disease extinction was 6.7 years. Overall, results from this study suggest that eradication of coxiellosis from an endemic dairy cow herd by means of vaccination should be considered as a relatively long-term strategy. Time to disease extinction under the different scenarios assessed was reported using central tendency statistics (i.e. mean or median). However, no measure of variability was reported, which would have aided the interpretation of results given the underlying stochasticity of the model.

The model developed by Courcoul et al. (2011b) was used to assess whether the abortion storms observed in approximately 30% of the infected dairy goat herds during the Dutch outbreak of 2007–2011, but typically not observed in dairy cows, could be explained by differences in demographic factors alone (Hogerwerf et al., 2013). In this simulation study, disease related parameters were kept fixed for both cows and goats, while herd size and demographics were modified to reproduce average conditions of dairy goat farms in The Netherlands. Resulting disease dynamics for dairy cow herds and dairy goat herds were then compared. Seasonal fluctuations in the prevalence of shedders in simulated goat herds were observed. On the other hand, the prevalence of shedders in dairy cow herds reached a relatively constant steady state. This is an expected pattern resulting from the temporally restricted occurrence of parturitions in dairy goat farms as opposed to all year round calvings assumed for dairy cow herds. Higher median bacterial loads in the environment as well as a higher frequency of abortions were predicted for cow herds compared with goat herds of the same size. However, from observational studies it is known that abortions are more likely to occur in goats than in cows (Agerholm, 2013). The authors concluded that observed differences in the frequency of abortions in cattle and goats could not be explained by differences in the herd size and demographics alone, and that there was a need for changes in the model structure as well as for information on species-specific parameters.

Bontje et al. (2016) developed a within-farm Q fever transmission model for dairy goats and used it to study Q fever dynamics and the impact of different control interventions. A deterministic version of the model

was first developed using ordinary differential equations (ODEs). A stochastic version of the model was then developed using an agent-based approach. The underlying structure of the model is that of an SIR model, although further complexity was included by accounting for gestation status (i.e. pregnant or non-pregnant) and the addition of compartments for goats infected late in pregnancy, goats persistently infected and for the environment. Parameters were obtained from the literature or calibrated to obtain model outputs resembling the observed dynamics in dairy goat farms that were infected during the Dutch outbreak and had abortion storms as a consequence of Q fever. A local elasticity analysis was performed on the deterministic version of the model. The parameters governing the transmission rate, fraction of infected does that become persistently infected and the fraction of infected does that abort were found to have a medium to high elasticity, that is small changes in their value had a relatively large impact on model outputs.These parameters were also considered to have a medium to large uncertainty given the limited availability of published studies where their values could be inferred from.

The stochastic version of the model was used to study the efficacy of different control interventions by comparing time to disease eradication, mean number of infected placentas that reached the environment and mean number of abortions per year. The effect of vaccination was assessed for vaccination starting before introduction of disease into the herd (i.e. preventive vaccination) and as a reactive control measure (i.e. after occurrence of an abortion rate above 5% or after detection of *C. burnetii* in BTM). The vaccine was assumed to be 90% effective in susceptible non-pregnant goats and was modelled by changing the status of effectively vaccinated goats to recovered (immune) non-pregnant. Bontje et al. also assessed the effect of imposing a permanent breeding ban on all goats in the herd at the time of detection of *C. burnetii* in BTM. Finally, two control measures including culling interventions were assessed. Namely, test and cull based on detection of shedding in individual milk samples and culling of all pregnant goats if the abortion rate reached a 5% threshold. In The Netherlands, the use of prolonged lactation is increasing. Hence, each of the mentioned control measures were studied under three different reproductive managements. These were: (1) joining of goats every year; (2) joining goats every two years; and (3) joining goats the first two years only. Preventive vaccination followed by reactive vaccination after an abortion storm or detection of *C. burnetii* in BTM were found to be the most effective control strategies to reduce disease prevalence and environmental contamination. For reactive vaccination, the average time from start of vaccination to disease eradication ranged from 4.1 to 6.2 years, with differences depending mainly on the type of reproductive management being simulated. Reproductive strategies that reduced the number of pregnant goats per year led to shorter times to disease eradication. The control strategies based on culling of either does that were positive in milk or culling of all pregnant does after a BTM positive result did not lead to disease eradication. The use of a lifelong breeding ban on animals present at the time disease detection (restated in case of disease re-occurrence) was also not effective in eradicating coxiellosis from the herd.

So far, no models developed to simulate within-farm Q fever transmission in dairy goats have been parameterised using a formal statistical method and observed data on Q fever occurrence. Moreover, high uncertainty and a relatively high impact on model predictions was observed for some parameters needed to model Q fever within farm transmission. Vaccination has been shown to be an effective control measure both in models developed for dairy cows and for dairy goats. Other control measures, like culling of goats shedding in milk, were predicted to be insufficient for eradicating disease within a reasonable time-frame. It remains unknown if the combination of some of these strategies with vaccination could reduce the time required to eradicate disease. Segregation of pregnant goats has been suggested as a potential control measures by EFSA (European Food

Safety Authority, 2010), the American College of Internal Veterinary Medicine (ACIVM) (Plummer et al., 2018) and other authors (Arricau-Bouvery et al., 2001; Rodolakis et al., 2009). However, this strategy has not been assessed either in field studies or by means of mathematical models.

1.6.3 Conclusions

Q fever can lead to serious health consequences in infected people and large scale outbreaks with significant public health impact have been reported. Livestock are often identified as the source of infection for humans. Goats in particular have been linked to some of the largest Q fever human outbreaks recorded. Trends towards more intensified management systems and a relative proximity of livestock farms to urbanised areas can lead to an increased risk of Q fever occurrence and disease spill over from animals to humans. The sequence of events in The Netherlands' outbreak illustrate how an increased circulation of Q fever among small ruminants can precede a major human outbreak. Therefore, it is of utmost importance to develop strategies for early detection and control of Q fever at the animal level to reduce the risk of occurrence of human Q fever.

Clinical signs of *C. burnetii* infection are more prevalent in goats than in other susceptible livestock. Reproductive disease outcomes associated with infection can lead to abortion waves or can be less conspicuous. Improved methods for recording and reporting herd performance and analysis of data collected for extended periods are required for the development of a more detailed characterisation of production losses caused by Q fever in intensively managed dairy goats. There is some evidence suggesting Q fever in dairy goats could have a negative impact on milk production.

Challenge studies in goats have largely contributed to the understanding of the pathophisiology and shedding routes of *C. burnetii*. However, relatively few studies have been published assessing shedding dynamics in naturally infected herds. Additional research in this area would contribute to the understanding of how *C. burnetii* infection spreads and to identify key determinants of its persistence in endemic herds.

Mathematical models of disease transmission can be used to test hypothesis that would be too costly or logistically unfeasible to test in experimental or observational studies. For example, mathematical models can be used to test the long term efficacy of control interventions like vaccination or changes in management practices. When empirical data is available model parameters can be inferred using statistical methods, which contributes to the robustness of model predictions.

1.7 Research aims and objectives

Based on the background provided by this literature review, the aims and objectives of the studies presented in this thesis are to :

- Develop a means for reporting various aspects of herd performance in intensively managed dairy goat herds.
- Assess the prevalence of *C. burnetii* shedding at the time of kidding and compare total lactation milk yields in *C. burnetii* positive and *C. burnetii* negative does.
- Develop a mathematical model of Q fever using empirically-derived generated data and use this model to test the efficacy of candidate Q fever control and eradication strategies.

Chapter 2

Information management in intensively managed dairy goat herds

Abstract – The dairy goat industry in Australia has grown steadily since 2000, largely a result of increased demand by the Australian consumer for specialist European style cheeses. The median herd size of dairy goat enterprises in Australia has increased, making the task of managing herds effectively more difficult. The widespread use of state-of-the-art data recording technologies in intensive dairy goat systems has resulted in a considerable increment in the amount and quality of production data available. However, improved herd performance reporting tools are required to capitalise on the investment in these technologies. Standardised measures of herd performance allow performance shortfalls to be detected and allow responses to subsequent management interventions to be monitored. There is a limited availability of information management systems capable of generating a suit of action list, tactical and strategic herd level reports for intensively managed dairy goat herds. In this chapter, an approach for data recording and quantifying milk production and milk quality, reproductive performance, removal rates and disease frequency in dairy goat herds is outlined. We describe a relational database schema that can be used to ensure that individual doe data are managed in such a way to make herd-level analyses of performance straight forward. We exemplify these methods using a web based application.

2.1 Introduction

There has been a sustained growth of the dairy goat industries in Australia in the last two decades, with a transition from small-scale cottage type enterprises to increasingly organised and market-oriented businesses (Stubbs and Abud, 2009). In 2017, the annual farm-gate value of dairy goat milk produced in Australia was estimated to be AU$ 20.2–27.0 million. As of 2016 there were 68 licensed dairy goat farms in Australia. The state of Victoria in the southeast of the country concentrated approximately one third of these farms, with 36 farms in total. New South Wales and Queensland counted 12 and 10 farms each, respectively (Zalcman and Cowled, 2017). The mean herd size of dairy goat enterprises in Australia increased 35% from 2006–2007 to 2011–2012, moving from 185 to 250 goats per farm. Moreover, there has been a shift towards more intensive management styles with use of total mixed ration feeding systems (Abud and Stubbs, 2009; Foster, 2014). A similar trend towards intensification of management has been observed in some European countries (Castel et al., 2011; Pulina et al., 2018) . In common with intensively managed dairy cow herds, intensively managed dairy

goat herds now routinely make use of technologies such as individual animal electronic identification (EID), automatic sorting gates and milking machines that measure and record individual milk volumes (Alejandro, 2016).

Legislation making it mandatory for small ruminants (in addition to cattle and deer) to be uniquely identified has been introduced in countries that produce animals and animal products for export. Examples include the cattle passport system in the European Union (2003) and the National Livestock Identification System in Australia (2017). While the primary purpose of mandatory identification is to expedite backward and forward traceability in the event of incursions of exotic diseases such as foot-and-mouth disease (Mathews, 2011), a beneficial side effect is that EIDs, when used in combination with EID-compatible data collection devices (e.g. milking machines and walkover scales), enhance both the frequency and accuracy of lifetime event recording at the individual animal level. When a comprehensive range of individual animal lifetime event data is collected (e.g. kidding dates, service dates, dates and details of pregnancy testing and dry-off dates) standardised measures of herd performance can be generated, allowing herd performance to be compared with industry benchmarks and responses to interventions to be objectively monitored (Dohoo, 1993). This process provides an evidence-based approach for herd decision making, consistent with contemporary management styles that favour provision of tangible proof of the efficacy of interventions applied at either the individual animal or herd level (Sargison and Scott, 2010; Anneberg et al., 2016).

Reports of performance in ruminant dairy herds can be grouped into three main categories: (1) action reports, (2) tactical reports and (3) strategic management reports. Action reports provide lists of animals who, based on their documented lifetime event history, are due for key events (e.g. due to kid) or due for management events (e.g. due to mate, due to be pregnancy tested, due to be dried off) on a given day. Tactical reports provide information that allow current shortfalls in performance to be rectified (e.g. reports of median individual doe somatic cell counts stratified by age group). Strategic management reports allow a herd manager to assess how current herd performance aligns with long-term production goals. Compilation of strategic management reports from groups of herds allow industry benchmarks to be quantified (Cook et al., 2016).

Dairy information management systems range from bureau-based approaches where a herd advisor or representatives of a dairy herd improvement authority take responsibility for entering data into the system and generating herd reports on behalf of a herd manager (Stevenson, 2000; Espetvedt et al., 2013) to on-farm systems where farm staff manage the process of data collection, data entry and generation of reports using dedicated herd health software (Wenz and K Giebel, 2012). The reporting capabilities of dairy information management systems is variable, with those developed and marketed by companies whose primary focus is on-farm machinery (e.g. milking machines, walkover scales) generally limited to basic action list reports compared with a more comprehensive range of reports (covering the three categories listed above) provided by companies whose sole focus is dairy herd health software development; for example DairyCOMP 305 (Valley Agricultural Software, Tulare, California, USA) or DairyWIN (Massey University, Palmerston North, New Zealand). To the best of our knowledge herd health software options for dairy goat enterprises are limited, with most producers using software developed and marketed by on-farm machinery companies.

While numerous studies have been published about herd health software and the productivity gains obtained from their appropriate usage in dairy cattle (Tomaszewski et al., 2000; Noordhuizen and Wentink, 2001), relatively little has been published in the area of dairy goat production. A likely reason for this information gap is a lack of demand for herd health management tools by dairy goat producers because the scale of production in individual herds has historically been relatively small. The current trend towards larger herd sizes and a

change to more intensive management systems combined with more widespread use of modern data recording technologies has increased the need for better information management systems, particularly in medium to large herds (Olivier et al., 2005; Barkema et al., 2015). In this paper we describe an approach for quantifying herd performance in intensively managed dairy goat herds. Our objectives were three-fold. Firstly, to specify the minimum data required to produce measures of herd performance sufficient for routine monitoring. Secondly, to define the calculation methods and assumptions behind a series of reports to quantify milk production and milk quality, herd reproductive performance, disease frequency and replacement and culling rates. Thirdly, to develop a software prototype for quantifying and reporting herd performance in dairy goats using freely available software tools.

2.2 Materials and methods

2.2.1 Data and data analysis tools

Data from the existing information management system of a large dairy goat enterprise comprised of around 5,000 milking does in Victoria, Australia was retrieved and used to prototype the herd performance reports described in this paper. The data were transferred from a series of spreadsheet files (Microsoft Excel, Microsoft Corporation, Redmond, USA) into a relational database (PostgreSQL, The PostgreSQL Global Development Group, USA). The contributed R package RODBC (Ripley and Lapsley, 2018) was used to extract the data from the relational database and import it into the statistical environment R (R Core Team, 2017) for report development. A graphical user interface for each report was developed using the contributed R package Shiny (Chang et al., 2018).

2.3 Dairy goat demographics

An average dairy goat herd is comprised of approximately 20% replacement kids, 17% does aged between 7 to 12 months, 59% adult does and 4% bucks (Solis-Ramirez et al., 2011). Figure 2.1 shows key demographic and management events in the production cycle. Female kids kept in the herd as replacements are commonly weaned at 2 months of age. The first ovulation occurs at 5 to 7 months of age (Jainudeen et al., 2016) and does are first joined when they are 7 months of age. In herds where rams are run in the paddock with the does, the length of the breeding period is approximately 35 to 45 days (Haibel, 1990). The gestation length of goats is 150 days, with only relatively small variations across different goat breeds (Jainudeen et al., 2016). Age at first kidding is expected to be approximately 12 months. Pregnancy testing can be carried out 35 days from the joining date using ultrasound (Haibel, 1990). The average lactation length in Saanen goats is 270 days (Belanche et al., 2019). Does are dried-off 60 days prior their expected kidding date. Does producing below a milk volume threshold determined by the herd manager can have their drying-off date brought forward (Zamuner et al., 2020). Mean longevity varies from herd to herd. A study carried out in 164 dairy goat herds New Zealand reported mean longevity values ranging from 4.5 to 5.7 years (Scholtens et al., 2018). The peak of milk production is commonly observed in third lactation does, with a decline in cumulative milk production in fourth and above parity does (Zamuner et al., 2020).

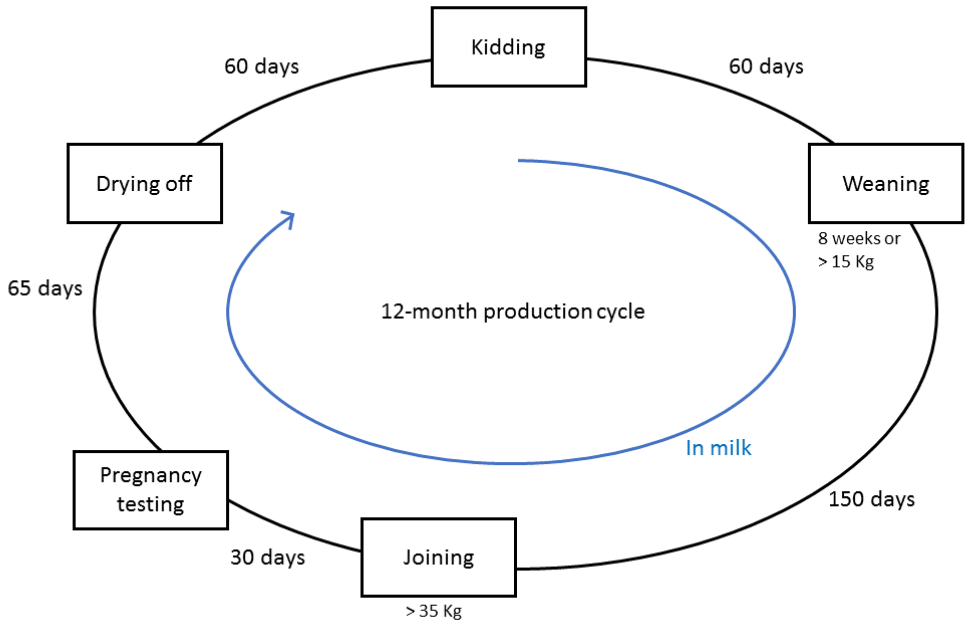

Figure 2.1: Schematic representation of the production cycle of dairy goats.

2.4 Data recording

A description of the types of data that need to be recorded in intensive dairy goat farms is shown in Table 2.1. The types of data recorded for a given herd will depend on the available technological infrastructure, available human resources and their level of training and herd management practices in place. In herds where either artificial insemination (AI), hand mating or mating of individual bucks with in-season groups of does occurs in all, or a subgroup of the herd, reproductive data including PSM dates, the dates and details of oestrus and service events, and details of kids born from each doe are likely to be part of the recording routine. On the other hand, when billies are run with the herd in the paddock, start and end of joining period as well as the identity of the billies used are recorded. In the latter case, the exact date of conception (and therefore the expected date of parturition) cannot be estimated for individual does. Typically, large groups of does that are due to kid are housed together which makes it difficult to associate kids with their respective dams once they are born. The increasing affordability of DNA parentage testing will likely help overcome this challenge in the future (Bolormaa et al., 2008). The information management system proposed in this paper has been designed to accommodate both AI and natural service matings.

Figure 2.2 shows a relational database schema that can be used for data recording for dairy goat enterprises. The general approach is as follows. Details of animals that comprise the herd are registered into the system using the Animals table, with a 1 to n integer identifier used as a primary key. The animals table records static details, that is, details about the animal that do not change during the animal's lifetime: date of birth, the animal's unique identifier, sire, dam and breed. While for the most part individual animals stay in their herd of origin for the duration of their productive life, a one-to-many relationship exists with the Animals Enter and Animals Exit table allowing individuals to enter and leave a herd for any reason on any number of occasions. A one-to-many relationship exists between the Animals table and each of the lifetime event tables (e.g. kiddings, oestrus events, service events, reproductive examinations, production details, weight and condition score estimations, disease and treatment events, and dry off events).

Of need for particular mention is the Planned Start of Mating (PSM) table which lists, for each doe, the date on which she will be mated following kidding if observed in oestrus. In other words, the PSM date is the calendar date timed so that if mating occurs the doe will kid at the desired date in the following kidding season. In a seasonal mating system, the objective of reproductive management is to promptly detect does in oestrus following PSM date and submit them for service. Submission rate and in-kid assessments, therefore, provide a measure of how quickly does are submitted for their first service following PSM or how quickly does are confirmed in-kid following PSM, respectively. An advantage of this approach is that it easily handles the relatively common situation where, if a doe fails to become pregnant within a defined time frame following PSM, she can be 'carried over' to be mated with does that kid in the next kidding season. In this situation the doe would have a second PSM date applied to her lifetime event record, appropriate for the next kidding season. Thus, while in most situations there will be a one-to-one relationship between kidding events and PSM dates, there will be small numbers of animals with two (or more) PSM dates attached to a given kidding event.

Automated records can be used to check the completeness and the quality of manually recorded data. In Appendix A.2, we describe an approach that can be used to infer kidding dates from daily milk yield records. A similar approach could be used to infer drying-off dates.

Table 2.1: Details of individual animal event information to be recorded in dairy goat herds.

Event type	Details
Static animal details	Date of birth, permanent lifetime identifier, visual (ear tag) identifier, electronic identifier, sire, dam, breed.
Kidding	Date of kidding, number of kids born, identity of kids born.
Planned Start of Mating	Planned Start of Mating date.
Oestrus events	Date heat observed.
Services	Date of service event, service type, service sire.
Joinings	Date billy entered the herd, date billy exited the herd, identity of billy run with the herd.
Abortions	Date of abortion event, cause of abortion event (if known).
Vet reproductive examinations	Date of reproductive examination, reason for reproductive examination, diagnosis.
Re-numbering	Date of animal re-tagged, old ear tag number, new ear tag number.
Production	Date of herd test, herd test milk fat percentage, herd test milk protein percentage, herd test milk volume.
Enter herd	Date animal entered herd, how animal entered herd (born, purchased, transferred), stock class on enter, fertility status on enter, lactation status on enter, estimated due to kid date, estimated due sire.
Exit herd	Date animal exited herd, fate of animal (culled, died, sold or lost), reason for removal.
Diseases	Date of disease event, category of disease event, diagnosis.
Treatments	Date of treatment event, category of treatment event, type of treatment event.
Samples	Date of sample event, type of sample taken, name of test performed, result of test.
Dry offs	Date of dry-off event.
Weight, height, body condition score	Date of weight, height or body condition score event, type of measure recorded (weight, height, body condition score), value recorded.

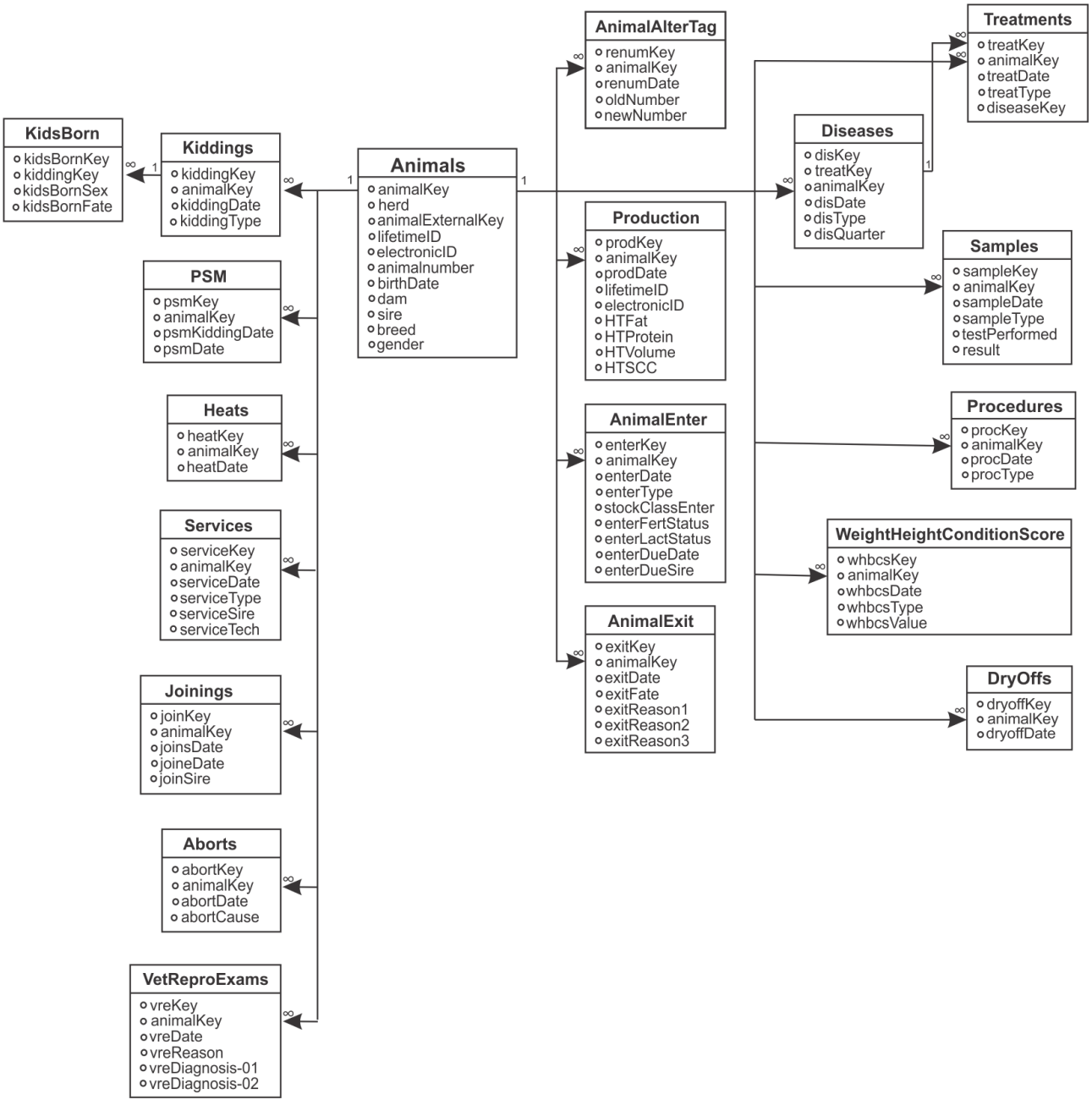

Figure 2.2: Relational database schema for recording individual dairy goat (doe and billy) life time event data. A list of definitions for abbreviated variables in this figure is provided in Appendix Table A.1.

2.5 Key performance indicators

A dairy goat herd is comprised by distinct stock classes. For the purpose of reporting animals can be divided into kids (from birth to weaning), maidens (from weaning to first parity) and adults (from first parity onward).

2.5.1 Reproduction

For assessment and reporting herd reproductive performance we covered the following areas: (1) kidding rates, (2) submission rates, (3) return interval analyses, (4) conception rates, (5) in-kid rates, and (6) abortion rates. Breaking reproductive performance down in this way provides a structured approach to analysis and herd problem solving. Structured in the sense that the time taken for does to become pregnant is influenced by submission rates and a combination of heat detection intensity and accuracy and conception rates. Finally, once does are confirmed in-kid reproductive performance may be adversely affected by abortion events, quantified by abortion rate analyses. We draw the reader's attention to the fact that most of the reproductive outcomes described in this paper are, in fact, incidence risk measures where the numerator equals the number of individuals experiencing the event of interest over a defined time frame and the denominator equals the number of individuals initially at risk. The term 'rate' is used in this paper to allow our nomenclature to be consistent with the equivalent measures used in dairy cattle.

Kidding rates

Herd kidding performance can be quantified in two ways. Firstly, simply as the number of kidding events per week relative to the Planned Start of Kidding date (PSK, the calendar date on which the herd manager desires kidding to start). The second method involves reporting kidding rate, defined as the number of does that have kidded by defined time frames after PSK divided by the total number of does eligible to kid at PSK. The second method is reliant on all does being pregnancy tested following the end of the mating period. Where no pregnancy testing is carried out, kidding events are reported using the first method and kidding rates can only then be calculated at the end of the respective kidding season. In this situation herd managers should be aware of the potential for bias in reported kidding rates if there are substantial numbers of pregnancies lost between the end of the mating period the subsequent expected kidding date.

Submission rates

Submission rate is defined as the proportion of does eligible for mating that have received their first service event at defined intervals following a Planned Start of Mating (PSM) date. Since goats are seasonal breeders a single PSM date is typically used for an entire group of animals to be mated (regardless of the date on which they kidded). The proportions of does submitted for their first service at defined intervals from PSM are calculated using the Kaplan-Meier technique (Kaplan and Meier, 1958; Morton, 2004). Here, the start of the follow-up period is the PSM date and the outcome event of interest is the day on which the doe received her first service, relative to her PSM date. Does that leave the herd during the submission rate analysis period (defined as PSM plus 6 weeks) are right censored on the day they leave the herd; does that do not have a service event recorded during the submission rate analysis period are right censored at PSM date plus 6 weeks. Submission rate reports quote the proportion of does submitted for their first service at weekly intervals following PSM.

Return interval analysis

Return intervals are defined as the number of days between consecutive oestrus events, oestrus and service events, service and oestrus events and consecutive service events. Where oestrus detection intensity is less than optimal animals returning to oestrus 18 to 24 days after a service event will not be observed and will be often detected in oestrus when they return at the next cycle (39 to 45 days after the original service event). Therefore, the ratio of 18 to 24-day services to 39 to 45-day services can be used to assess to what extent heats are being missed. Where heat detection accuracy is poor submission rates will often be satisfactory, but conception rates will be below accepted targets. An example return interval analysis report for a dairy goat herd is shown in Figure 2.4.

Conception rates

Conception rate is defined as the proportion of all services given during a defined date range that result in a pregnancy. Those services with an unknown outcome (i.e. those where the doe has been sold or the doe has died prior to pregnancy testing) are excluded from the conception rate analysis. We propose that conception efficiency is reported for all services and for 18 to 24-day services. For the 18 to 24-day conception rate analysis the services included in the denominator for the report is reduced to include: (1) those where an oestrus event occurred 18 to 24 days later, and (2) services that resulted in pregnancy. The idea of the 18 to 24-day conception rate analysis is to filter the group of eligible services to include only those does that were truly in oestrus at the time of mating. This removes the confounding effect of poor oestrus detection accuracy on reported conception rate. When no pregnancy test data are available non-return to service can be used to estimate conception rates. The caveat using this approach is that non-return to service can be highly influenced by heat detection intensity (the proportion of does that are in oestrus that are detected).

In-kid rates

In-kid rate is defined as the proportion of does eligible for mating that have conceived at defined intervals following a PSM date. Similar to the submission rate analyses, proportions of does pregnant at defined intervals from PSM are calculated using the Kaplan-Meier technique. For in-kid rate analyses, the start of the follow-up period is the PSM date and the outcome event of interest for each doe is the day on which conception occurred, as identified from pregnancy testing. Does that leave the herd during the mating period (defined as PSM plus 10 weeks) are right censored on the day they leave the herd. Does that do not have a pregnancy outcome defined are right censored at the end of the mating period. In-kid rate reports quote the proportion of does confirmed pregnant at weekly intervals following PSM. For herds where service details are recorded, in-kid rate reports can be produced when does are pregnancy tested and the pregnancy test assessments link pregnancy to a given service event. For herds where service details are not recorded, in-kid reports can only be produced when does kid again in the subsequent kidding season. In this situation, dates of conception are assumed to be 150 days prior to the date of kidding for each doe. As for the kidding rate report (described above) herd managers should be aware of the potential for bias in reported in-kid rates if there are substantial numbers of does removed from the herd between the end of mating and the subsequent kidding season or there are pregnancies lost between the end of the mating period and the subsequent kidding season.

Figure 2.3 shows an example of an in-kid analysis report produce with our reporting system. Since the data used for this report came from a herd were natural service is used the date of conception was back calculated

using the kidding date minus the average gestation length (i.e. 150 days).

Abortion rates

Abortion rates are defined as the proportion of does confirmed pregnant by pregnancy testing that have an abortion event recorded. If abortion events are not actively or are intermittently recorded failure to kid rates (similar to failure to farrow rates, as used in intensively managed pig herds) can be used as a proxy measure. Failure to kid is defined as the proportion of does confirmed pregnant that do not kid at any time throughout the subsequent kidding period.

2.5.2 Removal rates

Removal rates for each age class can be used to identify stock and/or age classes where involuntary loss is greater than that expected. Recording of removal fate (i.e. death, culled or sold) provides an important piece of information at the time of estimating mortality rates and culling rates. This prevents misinterpretations when the number of exits in dairies that sell adult does for milk production or breeding are compared with the number of does exiting the herd in dairies that do not market adults.

Stillbirth rate is defined as the proportion of kids delivered during a defined kidding period that are dead (i.e. stillborn) at the time of delivery. Pre-weaning mortality rate is the proportion of kids that are born alive that die between birth and the time of weaning (usually 6 to 8 weeks of age). Post-weaning mortality rate equals the number of deaths that occur between weaning and first kidding as a proportion of the total number of kids that are weaned.

For herds where the number of live births that occur per day are not routinely recorded an inferred pre-weaning mortality rate can be calculated as follows. The number of does kidding on a given day is defined as the number of does that enter the milking herd on the same day. The total number of births (i.e. the number of male and female kids delivered) is estimated by multiplying the number of does that kid by average litter size. An inferred pre-weaning survival rate equals the number of kids that receive an ear tag at weaning divided by half the estimated total number of births. Inferred pre-weaning mortality rate then equals one minus the pre-weaning survival rate. Using this approach, pre-weaning mortality rate includes those kids that were stillborn. In herds where kids are tagged before 48 hours of age a similar approach can be used to estimate periparturient mortality rate (i.e. the number of stillbirths or deaths occurring in the first 48 hours of life as a proportion of the total number of kids born).

The culling rate for adult members of the herd (i.e. does that have kidded at least once) is defined as the total number of animals culled over a defined period (e.g. 12 months) divided by the average number of animals at risk of culling throughout the follow up period. To account for open herds, in which adult does can enter as either replacements or purchases and does may leave the herd as sales, the average number of animals at risk equals the total number present at the start of the follow-up period plus half the number of animals that enter the herd (as purchases) minus half the number that are removed for reasons unrelated to culling. Mortality rate for adult members of the herd is calculated in a similar fashion.

2.5.3 Disease frequency

Diseases of dairy goats can be categorised into disorders of kidding, infectious and/or zoonotic disorders, lameness disorders, metabolic disorders, miscellaneous disorders, poisonings and toxicoses, reproductive disorders, skin conditions, and udder disorders (Gautam, 2012). The frequencies of disease events are reported as either incidence risk or incidence rate estimates, depending on the nature of the disease of interest. For kidding disorders disease frequencies are reported as incidence risks, where the numerator is the number of identified disease events and the denominator is the number of animals that kidded during the analysis period. For the remaining categories, disease frequencies are reported as incidence rates, where the numerator is the number of identified disease events and the denominator equals the total amount of animal time at risk during the analysis period. Incidence rates are useful because they allow to account for individual animals experiencing multiple disease events and provide a means for removing individuals from the population at risk during the recovery period (Stevenson, 2000).

Often, it is useful to report the time to disease occurrence relative to the time elapsed from an event of interest (e.g. relative to management events like weaning or drying-off). Survival analysis can be used when the variable of interest represents time to event. An additional advantage of survival analysis is that changes in the number of individuals at risk over time can be readily incorporated into the analysis by censoring. The Kaplan-Meier method can be used to quantify incidence rates for each of the disease event categories defined above. A start and end date for the analysis period is defined and the outcome of interest is the date on which a given disease event occurs. Does that leave the herd during the analysis period are right censored on the day they leave the herd. Does that do not have a disease event recorded during the analysis period are right censored at the end of the analysis period. The incidence rate of disease is presented as an instantaneous hazard plot where time is shown on the horizontal axis and the number of disease events per head of population per unit time is shown on the vertical axis (Kleinbaum and Klein, 2012). Disease frequencies can be reported either as a function of calendar time or animal referent time, for example the number of days since kidding. This allows the identification of seasonal patterns of disease or times of the production cycle when the risk of a given disease increases.

2.5.4 Milk production and milk quality

Milk production at the individual level can be reported as milk volume as a function of days in milk (DIM). Lactation length is the time from kidding to drying-off. Total milk yields can be standardised to a given lactation length (e.g. 290 days) to facilitate comparisons across individual does or across lactations for a single doe. Linear interpolation for imputation of milk yields between two herd tests is the simplest way to deal with missing herd tests and was set as the official method for calculating lactation records in the US in 1969 (Vanraden, 1997). Other methods require fitting lactation curve models to milk yield data (Gipson and Grossman, 1989). An advantage of using lactation curve models is that this approach allows the projection of milk production beyond the date of the last herd test.

Milk somatic cell counts (SCCs) form the basis of milk quality monitoring. Because milk secretion in goats is mainly apocrine, SCCs in goats are generally higher than SCCs in cows or sheep (Paape and Capuco, 1997). Also, the physiology of milk secretion in goats results in the presence of large anucleated cytoplasmatic particles in the milk. Therefore, cell counting methods specific for DNA should be used to estimate SCCs in goats (Dulin et al., 1982). Average SCC scores range from 270 to 2000 $\times$ 10^3 and 659 to 4213 $\times$ 10^3 cells/mL for healthy

and infected goat mammary glands, respectively (Sanchez et al., 1998). In the USA and France, the legal limit for SCCs in goat milk is 1000×10^3 cells/mL, although industry targets are 400×10^3 cells/mL (Foster, 2014).

Does with a SCC in the order of 1000×10^3 cells/mL should be screened for udder infection (Foster, 2014). Setting a SCC threshold allows one to report the proportion of the herd that is above that threshold over time. This can be analysed and reported using different metrics to try to identify times of higher risk of mammary infections in the lactation cycle. The lactation new infection rate is the proportion of does that moved from below to above a defined SCC threshold between two consecutive milk tests within a lactation. The proportion of the herd infected is defined as the proportion of does above the SCC threshold at given point in time. The proportion of does chronically infected is defined as the proportion of does that were above the SCC threshold in two of the last three milk tests. Dry period infection rates can be inferred using milk test data from the first 30 days of lactation. The proportion of does above the SCC threshold within the first 30 days of a lactation is a useful indicator of does kidding with an intramammary infection. Combining this with the last SCC measurement from the previous lactation can help differentiate new infections acquired during the dry-off period from existing infections that failed to cure. The net transmission rate (NTR) is the ratio of the number of does moving from below to above the SCC threshold to the number of does moving from above to below the SCC threshold between two milk tests. The NTR is an easy-to-interpret indicator of transmission dynamics both in the lactation and dry period. An NTR above one indicates that more infections are occurring than cures, suggesting ongoing transmission. For interpretation of SCCs it is important to consider factors other than intramammary infection that can influence SCCs, like lactation number, yield and stage of lactation (Bradley et al., 2012).

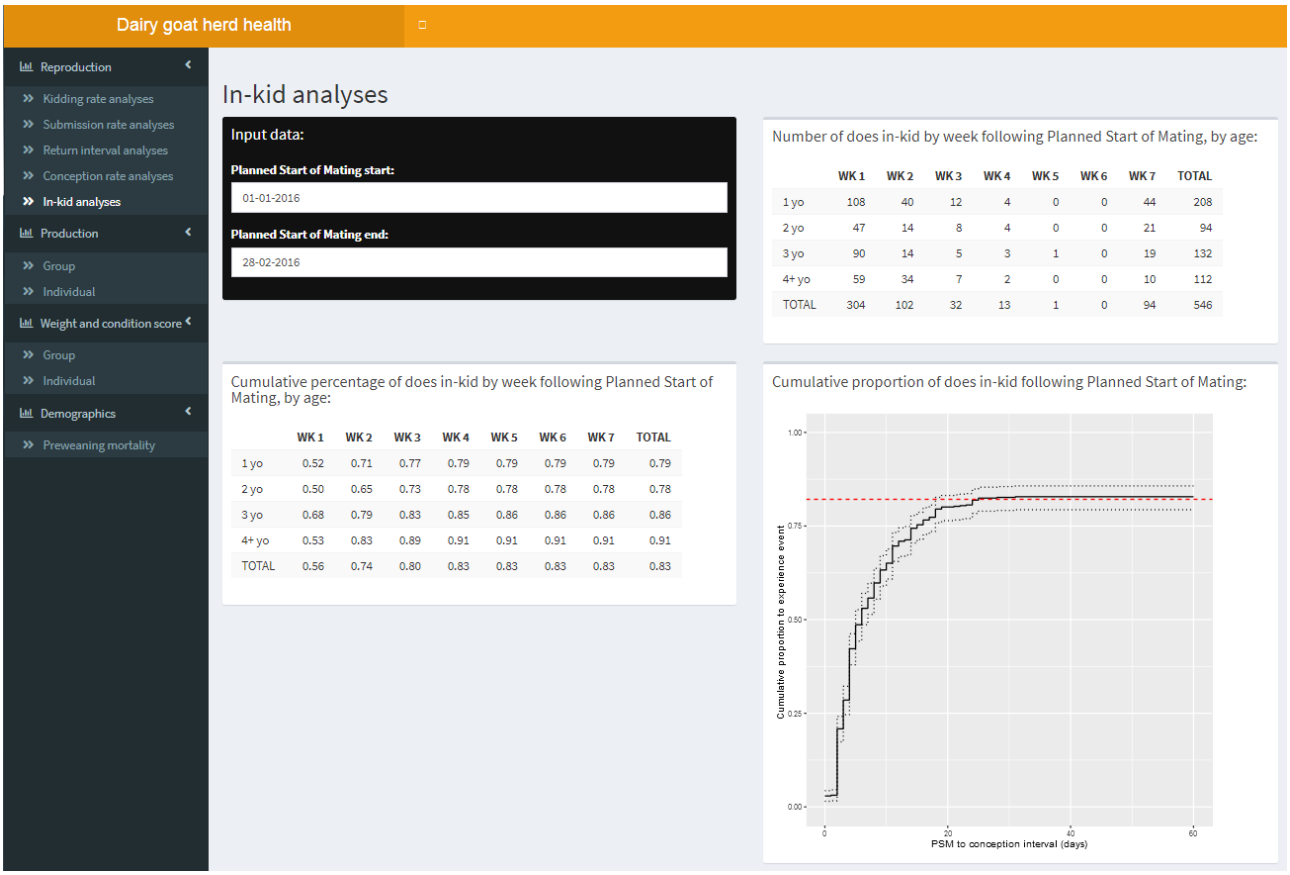

Figure 2.3: Graphic user interface of a dairy goat herd health reporting system showing the results of in-kid analyses. The red dashed line in the bottom right plot marks the 8 week in-kid rate target.

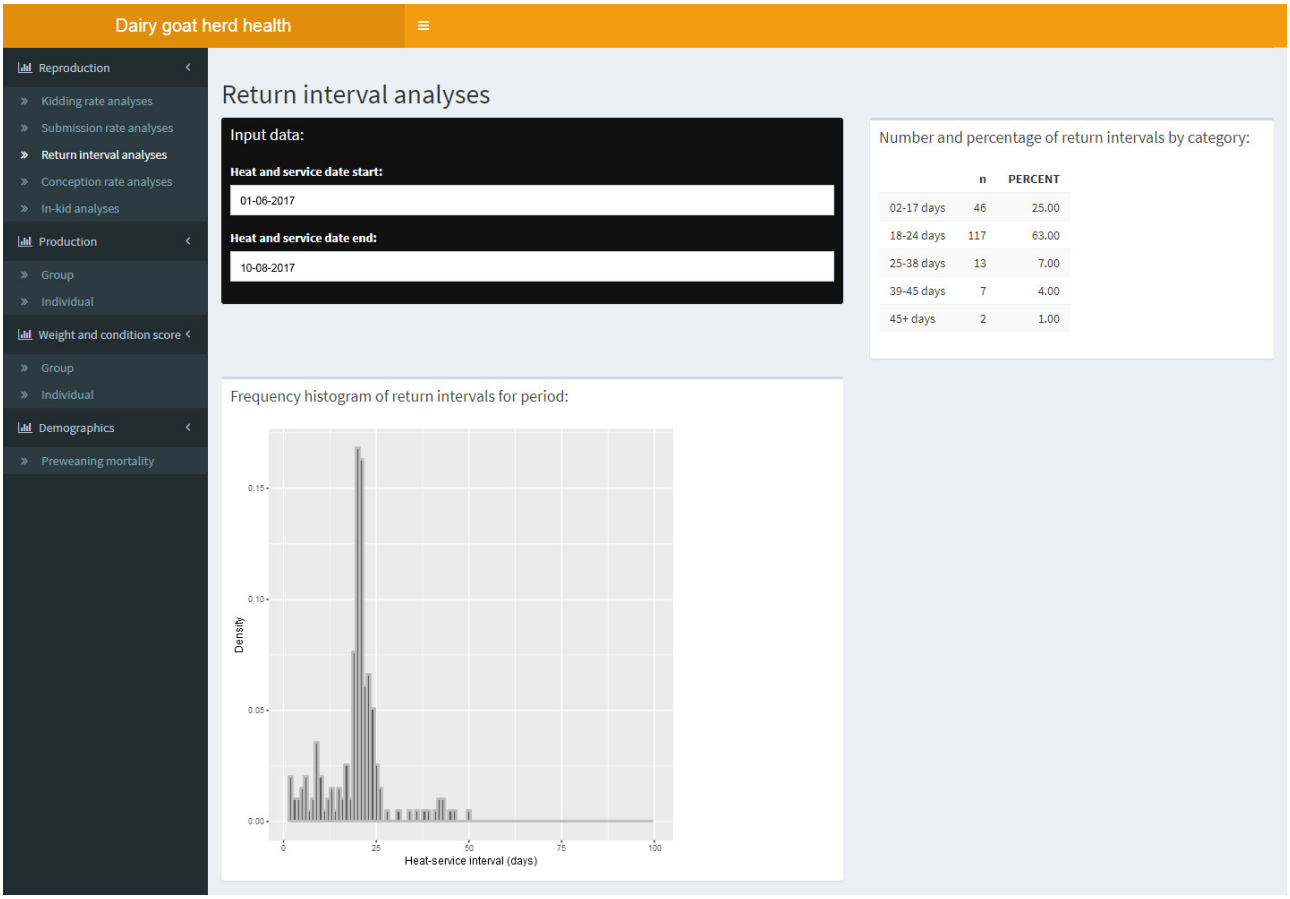

Figure 2.4: Graphic user interface of a dairy goat herd health reporting system showing the results of return interval analyses. Top right: Counts and percentages of return intervals by category (2 to 17 days, 18 to 24 days, 25 to 38 days, 39 to 45 days and greater than 45 days). Middle left: Frequency histogram of return intervals for the specified heat and service interval date range.

2.6 Estimating reproductive performance benchmarks

Given the absence of publications reporting targets for herd reproductive performance in intensively managed dairy goats, we used a simulation method to estimate key reproductive targets. Dates of oestrus events following a PSM for a large population of dairy goats ($n = 1000$) were randomly sampled from a normal distribution with a mean of 21 days and a standard deviation of 2 days (Medan et al., 2005). Time to subsequent oestrus events for each of the goats where then randomly sampled from the same distribution. For each oestrus event an 80% probability of heat detection was assumed. When detected in oestrus, the probability of conception following service was set to 60% (Motlomelo et al., 2002; Leboeuf et al., 2008). Each doe was given a maximum of three services. Reproductive outcomes (including the date of oestrus, service and conception events) were recorded and the data were then analysed using each of the reproductive analyses described in this paper. Report results were used to provide point estimates of reproductive performance targets (e.g. 4 week and 8 week in-kid rates). Herd reproductive targets based on the simulation approach described above are summarised in Table 2.2. Simulations were carried out using R (R Core Team, 2017). The R script used to produce the simulations is included in Appendix A.1. Following widespread uptake of the methods described in this paper by the dairy goat industry it is our expectation that strategic management reports can be compiled to allow empirical industry benchmarks to be developed.

Table 2.2: Suggested targets for kidding rates, submission rates, return interval analyses, conception rates and in-kid rates in intensively managed dairy goat herds.

Outcome	**Target**
Kidding:	
4 week kidding rate	≥66%
8 week kidding rate	≥95%
Submission:	
21 day submission rate	≥78%
28 day submission rate	≥85%
Return intervals:	
11 to 17 days	≤5%
18 to 19 days	≤14%
20 to 22 days	≥46%
23 to 24 days	≤17%
25 to 38 days	≤2%
39 to 46 days	≤3%
46 to 59 days	≤1%
Ratio 18-24 day cycles to 39-45 day cycles	≥9:1
Conception rates:	
First service	≥60%
Second service	≥60%
Third service	≥60%
Total services	≥60%
Number of services per conception	≤1.7%
In kid rates:	
4 week in-kid rate	≥57%
8 week in-kid rate	≥82%
Percentage of group no in-kid by PSM date plus 70 days	≤14%

PSM: Planned start of mating.

2.7 Discussion

The application of new technologies in livestock production systems such as EIDs have made it easier for individual animal event details to be consistently and accurately recorded over time. National regulatory frameworks making it mandatory for producers to identify their animals electronically makes it likely that these technologies will be used by greater numbers of producers in the years to come. There is therefore a need for herd information management systems to be re-engineered to capitalise on the benefits obtained from appropriate use of these technologies (VanderWaal et al., 2017). The relational database structure proposed in this paper provides a necessary first step to facilitate this process.

The economic efficiency of the rearing cycle is tied to achieving low mortality rates in young stock. Dates of mortality events and mortality reasons are often poorly recorded in dairy goat herds, in particular the fate of kids that are not to be kept as replacements (Malher et al., 2001). A factor contributing to the accuracy of quantification of mortality rates in young stock is the age at which young stock are permanently identified, with mortality events typically poorly recorded for those kids that have not been permanently identified. To overcome this issue, we propose a way of estimating periparturient and pre-weaning mortality rates in the absence of detailed information on the number and identity of kids born. Although imperfect, if applied consistently, this system allows comparisons of periparturient and pre-weaning mortality among sub groups of the herd and over time. Similar approaches, where losses of animals from a herd are assumed to be mortalities, have been used successfully in various other goat and sheep farming systems (Campbell et al., 2009; Hanks et al., 2018).

In dairy systems increasing longevity improves profitability since the cost of rearing replacements is amortised over a longer period of productive life (Delaney, 2018). Understanding the factors that contribute to longevity is therefore of strategic importance (Hadley et al., 2006; Gautam et al., 2017). For recording culling events, a predefined set of removal reasons should be established to facilitate automation of data analysis. Examples for categories for culling reasons in dairy goats can be found in Mahler et al. (2001). It has been shown that adding a second and third removal reason can substantially aid interpretation of records and reporting (Fetrow et al., 2006). Removal fates (i.e. does sold, culled or died) should also be recorded.

For disease recording, delineating a series of robust case definitions helps to eliminate the likelihood of over- or under-reporting of disease events by farm staff. In turn, this allows the frequency of disease to be monitored over time. Compared with reproduction where events are recorded for most, if not all members of the herd, disease events in dairy herds are relatively infrequent which means that a large amount of data must be recorded for (often) a long period before meaningful analyses can be carried out. Further, human resources in the intensive dairy sector are characterised by high levels of staff turnover and work patterns that are usually organised in shifts (Durst et al., 2018), from which it is implicit that disease recording will be a task shared by multiple individuals. Also, farm staff typically have variable levels of training and skill in animal husbandry. In this context, the absence of a unified nomenclature will likely lead to difficulties at the time of aggregating disease event data. Nonetheless, syndromic-based approaches to disease observation and recording that can be used by people with different clinical expertise have been used successfully in other livestock systems (Hanks et al., 2018; Pfeiffer et al., 2018). Additionally, smart phone applications have been successfully used for the recording of disease events in dairy cows, improving accuracy and completeness of disease related data (Tremblay et al., 2016; Beyene et al., 2018). Similar technologies offer a promising means for facilitating and standardising the

recording of disease events in dairy goat herds.

On top of production benefits derived from herd health management, the current context of growing public awareness about animal welfare constitutes an additional reason for improving herd health information systems (Olivier et al., 2005; von Keyserlingk and Weary, 2017). Moreover, herd health programs for dairy cows are already mandatory in some European countries. For example, in Denmark dairy herds with more than 100 cows or 200 head of young stock are required to have a herd health program in place (Anneberg et al., 2016). Livestock businesses that can provide documentary evidence of a high standard of animal welfare using a herd health information management system will be better equipped to address animal welfare concerns raised by members of the general public.

In years to come, there is likely to be an increase in the use of tools to provide reports of herd performance in real time and to provide predictions of the effect of interventions that might be applied to address identified performance shortfalls. In this regard, mathematical and/or simulation models that assess the impact of management interventions on herd performance of dairy goats have been published by Guimarães et al. (2009) and Puillet et al. (2010). More recently, a web-based application that incorporates observations relating to pasture growth, the weather, parasite abundance prediction models and stocking rates has been developed to provide managers of sheep flocks in Australia with the ability to predict pasture growth, animal performance and animal health and climate risks (Kahn et al., 2017). The validity of model predictions relies heavily on the presence of accurate details of the herd or flock population at risk. The collection and assimilation of data for this purpose is likely to be greatly enhanced using the approaches described in this paper.

Chapter 3

The prevalence of *Coxiella burnetii* shedding in dairy goats at the time of parturition

Abstract – This was a panel study of the prevalence of *C. burnetii* infection in does in an endemic dairy goat enterprise in Victoria, Australia. Our first objective was to determine the prevalence of does shedding *C. burnetii* at the time of parturition and to quantify the concentration of genome equivalents (GE) present in each *C. burnetii* positive sample. Our second objective was to determine the proportion of positive does that were persistent shedders. Our final objective was to quantify the association between *C. burnetii* qPCR status at the time of kidding and daily milk volumes produced during the subsequent lactation.

Vaginal swabs (n = 490) were collected from does at the time of kidding and analysed using a quantitative polymerase chain reaction (qPCR) assay. Shedding of *C. burnetii* was detected in 15% (95% CI 12% to 18%) of the sampled does. Does were classified as qPCR-negative, qPCR-positive low and qPCR-positive high based on the estimated concentration of GE from the qPCR. Persistent shedding at relatively low concentrations was detected in 20% (95% CI: 10% to 35%) of shedding does sampled again at their subsequent parturition. After controlling for possible confounders and adjusting for variation in daily milk yields at the individual doe level, daily milk yields for qPCR-positive high does were reduced by 17% (95% CI: 3% to 32%) compared to qPCR-negative does (p = 0.02).

Shedding concentrations of *C. burnetii* were highly skewed, with a relatively small group of does shedding relatively high quantities of *C. burnetii*. Further, high shedding does had reduced milk yields compared to qPCR-negative does. Early detection and culling of high shedding does would result in increased farm profitability and reduce the risk of Q fever transmission.

Canevari JT, Firestone SM, Vincent G, Campbell A, Tan T, Muleme M, Cameron AWN, Stevenson MA. The prevalence of *Coxiella burnetii* shedding in dairy goats at the time of parturition in an endemically infected enterprise and associated milk yield losses. *BMC Veterinary Research* 14 (2018) 353 DOI: 10.1186/s12917-018-1667-x

3.1 Introduction

Q fever is a zoonotic disease caused by the rickettsia-like bacterium *Coxiella burnetii*. The microorganism occurs in two antigenic variations, phase I and phase II, which differ in the composition of the cell wall lipopolysaccharides (Maurin and Raoult, 1999). Q fever affects a wide range of mammalian species among which domestic livestock constitute the main reservoir of infection for humans (Guatteo et al., 2011). Once in the environment *C. burnetii* can survive for prolonged periods of time due to its capacity to differentiate

into a spore-like form known as the small cell variant. In the acute phase, clinically infected humans may develop symptoms including high fever, headaches and joint and muscle pain (Maurin and Raoult, 1999). Life-threatening persistent focalised *C. burnetii* infection(s), mainly endocarditis, can also occur in a relatively small percentage of cases (Million and Raoult, 2017). Goats are frequently identified as a source of Q fever outbreaks in humans (Lennette et al., 1949; Hatchette et al., 2001), as was the case in the outbreak of Q fever that occurred in The Netherlands in 2007–2010 where more than 4500 people were infected (Dijkstra et al., 2012). Goats are also the livestock species most likely to show clinical signs of disease, which include increased rates of abortion and an increase in the number of stillbirths and weak offspring (Agerholm, 2013). An association between subclinical mastitis and *C. burnetii* infection has been reported in dairy cattle (Barlow et al., 2008). Also, puerperal *C. burnetii* vaginal shedder primiparous cows were found to yield less milk fat compared to negative cows (Freick et al., 2017). While histopathological udder lesions have been found in goats artificially infected with *C. burnetii* (Sánchez et al., 2006), and an association has been detected between recent *C. burnetii* exposure and milk yield (Muleme et al., 2017b), we are aware of no studies that have been published investigating *C. burnetii* shedding at the time of kidding and its association with either subclinical or clinical mastitis or milk yield.

Infected goats can shed large quantities of *C. burnetii* into the environment at the time of parturition. Other documented shedding routes include the milk, feces, urine and saliva, though the amounts of bacteria shed via these routes have been shown to be relatively low (Arricau Bouvery et al., 2003; Rodolakis et al., 2007; Sting et al., 2013). *C. burnetii* DNA can be detected in vaginal swabs using quantitative polymerase chain reaction (qPCR) techniques, making it possible to identify does that are shedding the infective agent at the time of kidding (Berri et al., 2000; Roest et al., 2012). Also, qPCR can be used to monitor the presence of *C. burnetii* DNA in bulk tank milk (Kim et al., 2005; van den Brom et al., 2012; Boarbi et al., 2014). Out of an array of available qPCR assays those targeting the *com1* gene and the insertion sequence IS*1111* are the most frequently used (Jones et al., 2011). qPCR assays targeting the *com1* gene have the advantage that the target gene occurs only once within the *C. burnetii* genome, making it possible to quantify the number of genome equivalents in a sample. Compared with the *com1* qPCR the IS*1111* qPCR is more sensitive; an expected feature considering the insertion sequence it targets can occur up to 110 times within the *C. burnetii* genome (Klee et al., 2006; Fenollar and Raoult, 2007; Jones et al., 2011). Antibodies against *C. burnetii* can be assessed using a range of serological techniques of which the immunofluorescence assay (IFA) has the highest diagnostic sensitivity and specificity when used in goats (Muleme et al., 2016)[1].

Australia has one of the highest reported incidence rates of Q fever in humans with between 1.5 and 4.9 notified cases per 100,000 head of population at risk per year (Gidding et al., 2009). The frequency of Q fever in humans in Victoria has historically been low compared with other states, with incidence rates ranging from 0.3 to 2.0 notified cases per 100,000 head of population at risk per year since 1991 (Anonymous, 2018; Bond et al., 2018). In 2012–2014 an outbreak of Q fever was associated with a large dairy goat enterprise in Victoria. A total of 24 people were infected; all of them farm staff with the exception of one individual who was a staff family member suspected as having acquired infection via contaminated fomites (Bond et al., 2016). Vaccination of all farm staff and elective vaccination of staff contacts with Q-VAX® (the vaccine used to prevent Q fever in humans in Australia) has, so far, prevented further human cases from occurring. Nevertheless, subsequent investigations carried out on this enterprise showed that coxiellosis was still prevalent among does and that *C.*

[1]The diagnostic sensitivity and diagnostic specificity of IFA in goats is 84.5% (95% CI: 54.4% to 98.7%) and 94.4% (95% CI: 79.8% to 99.5%), respectively (Muleme et al., 2016).

burnetii DNA as well as specific antibodies to *C. burnetii* were present in bulk tank milk (Muleme et al., 2017b).

Long-term studies carried out in dairy cattle, sheep and goat farms where Q fever outbreaks have occurred have shown that infection can persist in herds-flocks for at least two years, with some animals shedding *C. burnetii* in two consecutive parturitions (Hatchette et al., 2003; Berri et al., 2007; Astobiza et al., 2011a; Piñero et al., 2014). There have been few published reports describing the dynamics of infection in endemically infected herds-flocks. A better understanding of the stages of the production cycle when infection transmission risk is greatest and which age groups or stock classes are responsible for infection transmission provides essential information for the design of herd-flock level Q fever control and eradication strategies.

This was a panel study of the prevalence of *C. burnetii* in an intensively managed dairy goat enterprise related to a human outbreak (Bond et al., 2016) of Q fever in Victoria, Australia. There were three main objectives. Firstly, to retrieve vaginal swabs from does within 24 hours of kidding and to determine both the prevalence of does shedding *C. burnetii* and the concentration of genome equivalents (GE) present in each *C. burnetii*-positive sample. Our second objective was to select a subset of does identified as *C. burnetii*-positive at the first round of testing and to re-sample them at their subsequent kidding event to determine the proportion that were persistent *C. burnetii* shedders. Our final objective was to quantify the association between *C. burnetii* qPCR status at the time of kidding and both milk yields and somatic cell counts (SCCs) during the subsequent lactation. Results from qPCR testing of vaginal swab samples from aborted does submitted by farm staff were also included in this paper.

3.2 Materials and methods

3.2.1 Study design and study population

The source population for this study were does on three of the four farm units that comprise a large dairy goat enterprise in south western Victoria, Australia. The eligible population were does that kidded during the period 1 March to 31 December 2016. At the start of the study there were up to 1,500 does milking in each of the three study herds. The majority of does in each herd were Saanen with smaller numbers of Toggenburgs and Alpines. Does were housed in sheds using a deep litter system and fed a mixture of concentrates and roughages *ad libitum* throughout the lactation. In each of the herds mating was timed so that does kidded in four batches throughout the year (March to April, June to July, September to October and November to December). Each herd was visited once weekly during each of the four kidding seasons and vaginal swab and whole blood samples were taken from each doe that had kidded during the previous 24 hours, leading to a total of 490 samples from full-term kidding does[2]. A sub-sample of does (n = 40) identifed as *C. burnetii*-positive from the 2016 sampling had vaginal swabs taken again at their subsequent kidding event in 2017. Throughout 2016 and 2017, an additional 26 vaginal swabs from aborted does were obtained by farm staff and submitted for qPCR *com1* testing. The volume of milk produced by each doe for each day of lactation was measured and recorded using the milking machine used on each farm. Individual doe milk samples were collected on four occasions throughout the year and submitted for individual doe somatic cell count estimation. These details were collated with each doe's biographical data, with most of the does included in the study having at least one SCC estimate. Biographical

[2]Figure B.1 is a frequency histogram of all kidding events recorded in 2016 for each of the three herds and four kidding seasons and the count of does sampled in this study.

data for each doe, the dates of sample collection and the results of vaginal swab and blood tests were stored in a relational database.

DNA from each of the vaginal swab samples was extracted using a HiYield Genomic DNA Mini Kit (Real Biotech Corporation) according to the manufacturer's instructions. Real time PCR analyses targeting the *com1* gene were carried out using the methodology described by Lockhart et al. (2011). The number of *C. burnetii* GE per μL of sample was estimated using a standard curve described by Muleme et al. (2017a). Vaginal swabs from the 40 does identified as positive to the *com1* qPCR in 2016 and resampled in 2017 were tested using both the *com1* and IS*1111* qPCR. The IS*1111* qPCR assay was carried out following the methodology described in (Banazis et al., 2010). Only samples presenting the typical amplification curve and with a Ct (cycle threshold) value below 40 were considered positive. High-pure water served as the negative control for the qPCR assays. The *com1* amplicon cloned into a plasmid and DNA extracted from Vero cell cultures of *C. burnetii* Nine Mile RSA439 (Phase II, Clone 4) were used as positive controls for the *com1* and IS*1111* assays, respectively. Serological testing was carried out using the methods described by (Muleme et al., 2016) using a 1/160 dilution cut-off. For IFA negative controls serum from New Zealand goats (a country declared free of *C. burnetii* by the World Organisation for Animal Health) was used. Serum samples from two goats from a known *C. burnetii*-positive farm that tested positive to the complement fixation test (CFT) were used as IFA positive controls.

3.2.2 Statistical analyses

Impact of qPCR status on daily milk yields

Individual daily milk volume records of up to 305 days in milk were available for 462 of the 490 does sampled in 2016 (n = 110,024 records in total). Daily milk volumes greater than 15 litres (n = 311) were deemed implausible and were discarded from the analyses. Does were grouped into three categories based on the estimated concentrations of *C. burnetii* present in their vaginal swab samples. The number of *C. burnetii* GE per μL present in each qPCR-positive vaginal swab sample was plotted as a frequency histogram. Does shedding *C. burnetii* concentrations greater than the third quartile plus 1.5 times the interquartile range[3] (i.e. outliers) comprised the first group (n = 14). Does shedding less than this threshold comprised the second group (n = 56), and those that were *C. burnetii*-negative comprised the third group (n = 392). In the remainder of this paper we use the terms qPCR-positive high, qPCR-positive low, and qPCR-negative (respectively) to refer to does in each of these categories. The mean Ct values for groups qPCR-positive low and qPCR-positive high were 34.9 (95% CI: 34.1 to 35.6) and 23.9 (95% CI: 19.7 to 28.2), respectively. To compare daily milk yields in the first 305 days of lactation among the three *C. burnetii* qPCR *com1* status groups a generalised additive mixed-effects model was developed. Parity, kidding season, farm and days in milk were included as fixed effect terms in the model to adjust for their potentially confounding effect on daily milk yield. A penalised spline term was used to account for the non-linear association between days in milk and daily milk yield. Lack of independence in the data arising from repeated measurements of milk yield for each doe were accounted-for by including a random effect term at the individual doe level. Several options for covariance structure of the random effect term were tested and compared using the Akaike Information Criterion (AIC), and the best fitting of these, a first order autoregressive (AR1) covariance structure was used to account for autocorrelation of error terms (Dohoo et al., 2009). The final model was of the form:

[3]The cut-off value was 1140 GE per μL.

$$MY_{ij} = \beta_0 + \beta_1 Status_i + \beta_2 Parity_i + \beta_3 Season_i + \beta_4 Farm_i + \beta_5 s(dim_i j) + D_i + \varepsilon_{ij}$$

$$D_i \sim N(0, \sigma^2_{doe}), \qquad \varepsilon_{ij} \sim N(0, \sigma^2)$$

The outcome variable MY_{ij} is the milk yield (in litres) of the i^{th} doe on the j^{th} day of lactation. Daily volume of milk produced was a function of an intercept term β_0 and regression coefficients $\beta_1 ... \beta_5$ representing the effect of the 3-level categorical variable *C. burnetii* qPCR *com1* status, (described above), parity of each doe at the time of kidding (first, second, third, and fourth parity or greater), kidding season in which the doe kidded (March to April, June to July, September to October and November to December), farm ('A', 'B' and 'C') representing which of the three farms a doe was located for the duration of her lactation and days in milk (an integer ranging from 1 to up to 305), respectively. The normally-distributed random effect for each doe is represented by the term D_i and ε_{ij} is the residual error (assumed to be independent and following a Normal distribution).

Impact of qPCR status on somatic cell counts

Individual SCC records were available for 401 of the goats sampled (one test per goat). Following the same methodology described in Section 3.2.2, does were grouped as qPCR-positive high (n = 11) , qPCR-positive low (n = 53), and qPCR-negative (n = 337). To compare SCCs among the three *C. burnetii* qPCR *com1* status groups a multiple linear regression model was developed. SCCs were transformed to a log (natural) scale to meet normality assumptions. The variables parity, kidding season, milk yield on the day of the test (available in the database aforementioned), days in milk and farm were included as fixed effect terms to account for their potentially confounding effect on SCCs. The final model was of the form:

$$LogSCCi = \beta_0 + \beta_1 Status_i + \beta_2 Parity_i + \beta_3 Season_i + \beta_4 Yield_i + \beta_5 Farm_i + \beta_5 dim_i + \varepsilon_i$$

$$\varepsilon_i \sim N(0, \sigma^2)$$

The outcome variable $LogSCC_i$ is the natural log SCC for the i^{th} doe. The log transformed SCCs were a function of an intercept term β_0 and regression coefficients $\beta_1 ... \beta_5$ representing the effect of the 3-level categorical variable *C. burnetii* qPCR *com1* status, (described above), parity of each doe at the time of kidding (first, second, third, and fourth parity or greater), the season in which the doe kidded (March to April, June to July, September to October and November to December), farm ('A', 'B' and 'C'), milk volume produced on the day of the SCC test and days in milk at the time of SCC test. Finally, ε_{ij} represents the residual error term (assumed to be independent and to follow a Normal distribution).

For both regression models, all explanatory variables were forced into the model irrespective of their statistical significance on the basis that they were considered *a priori* to be potential confounders of the association of interest. Frequency histograms of the residuals and plots of the residuals versus predicted values were developed to check that the assumptions of normality and homogeneity of variance had been met. The observed data was superimposed over a line plot of model predictions to visually assess model fit. Significance level was set at α = 0.05. Statistical analyses were carried out using R version 3.4.3 (R Core Team, 2017) and the contributed packages gamm4 (Wood and Scheipl, 2017), lme4 (Bates et al., 2015) and epiR (Stevenson et al., 2018).

3.3 Results

Does that had vaginal swabs positive for *C. burnetii* were present on all three sampled farms units in all kidding seasons with the exemption of Farm B in the March to April kidding season. Across the four kidding seasons and the three study farms there were 15 (95% CI: 12 to 18) *com1* qPCR-positive does per 100 does at risk. The prevalence of shedding increased from the first to second parities and then decreased for does of parity three and above. Exceptions were the March to April kidding season when shedding was not detected in second parity does and the November to December kidding season when first parity does had a slightly higher prevalence than those in their second parity. When does were grouped by farm and parity the prevalence of shedding was stable over the four kidding seasons ranging from 13% in the September to October season to 17% in the November to December season. For IFA serology the prevalence followed a similar pattern to that of qPCR though the decrease in seroprevalence for second to third parity does was less marked as the decrease in prevalence using the *C. burnetii* qPCR *com1*[4]. When does were grouped by farm and parity the IFA seroprevalence ranged from 24% to 44% in the March to April and June to July kidding seasons, respectively (Table 3.1).

The distribution of *C. burnetii* GE concentrations in vaginal swabs was highly skewed. While the majority of does that were *C. burnetii* qPCR *com1* positive shed relatively low quantities of bacteria, the concentration of a *C. burnetii* GE in a small number of does (n = 14) was above $>10^3$ and as high as 10^9 GE per μL L of sample. There was a fair agreement between serology and shedding status after adjusting for bias (PABAK 0.37; 95% CI: 0.28 to 0.45). Nevertheless, 39 out of 71 does that were *C. burnetii* qPCR *com1* positive returned a negative result when tested with the IFA. The risk of a doe being IFA positive if she was shedding more than 10^3 GE per μL L as measured by the qPCR *com1* assay was 2.6 (95% CI 1.8 to 3.8) times the risk of a doe that was negative to the qPCR *com1* assay (Table 3.2). Log10 transformed *C. burnetii* GE concentrations for does of parities 1, 2, 3 and 4+ identified as positive were not statistically significantly different (Kruskall-Wallis test statistic 5.45; p = 0.14).

Of the 40 does that were *C. burnetii* qPCR *com1* positive in 2016 and re-sampled when they kidded again in 2017, none were positive when tested with the *com1* assay (0%; 95% CI: 0% to 9%) whereas 8 (20%; 95% CI: 10% to 35%) were positive when tested with the IS*1111* assay. Out of the 26 vaginal swabs from aborted does submitted for *com1* qPCR testing, 14 (54%; 95% CI: 35% to 71%) returned a positive result.

Mean daily milk volume yield for all does during the follow-up period (i.e. lactations starting with the kidding event at which does were sampled and up to 305 days in milk) was 3.03 (95% CI: 3.02 to 3.04) litres. After adjusting for the effect of farm, season, parity and days in milk and using a random effect term at the individual doe level, does in the qPCR-positive high group produced 0.53 (95% CI 0.08 to 0.98) litres less milk per day than does in the qPCR-negative group (Table 3.3). Daily milk yields for does in the qPCR-positive low group were similar to daily milk yields in the qPCR-negative group. No statistically significant association at the $p \leq 0.05$ level was found for qPCR status and SCC.

[4]Figure B.2 shows the prevalence of *C. burnetii* shedding and prevalence of exposure by parity number.

Table 3.1: Seroprevalence (IFA) and *C. burnetii* shedding prevalence assessed by qPCR in vaginal swabs (*com1*) in recently kidded does on a large dairy goat enterprise in south western Victoria, Australia in 2016.

Kidding season	**Assay**	**Does positive / total sampled**				**Prevalence as no. of positive does per 100 does at risk (95% CI)**			
		Farm A	Farm B	Farm C	**Total**	Farm A	Farm B	Farm C	**Total**
March to April	*com1*	0/34	11/33	1/22	12/89	0 (0, 10)	33 (20, 50)	4 (1, 22)	13.5 (7.9, 22.1)
June to July	*com1*	16/41	4/55	2/51	22/147	39 (26, 54)	7 (3, 17)	4 (1, 13)	15 (10.1, 21.6)
September to October	*com1*	15/52	2/40	1/42	18/134	29 (18, 42)	5 (1, 16)	2 (0, 12)	13.4 (8.7, 20.2)
November to December	*com1*	17/41	2/40	2/39	21/120	41 (28, 57)	5 (1, 16)	5 (1. 17)	17.5 (11.7, 25.3)
March to April	IFA	13/34	15/33	11/22	39/89	38 (24, 55)	45 (30, 62)	50 (31, 69)	28.3 (21.4, 36.3)
June to July	IFA	9/40	9/55	17/51	35/146	22 (12, 37)	16 (9, 28)	33 (22, 47)	24 (17.8, 31.5)
September to October	IFA	9/51	13/39	9/41	31/131	18 (10, 30)	33 (21, 49)	22 (12, 37)	23.7 (17.2, 31.6)
November to December	IFA	18/41	13/40	9/39	40/120	18 (10, 30)	32 (20, 48)	23 (13, 38)	33.3 (25.5, 42.2)
Total	*com1*	48/168	19/168	6/154	73/490	29 (22, 36)	11 (7, 17)	4 (2, 8)	14.9 (12, 18.33)
	IFA	49/166	49/166	46/153	145/486	29 (23, 37)	30 (23, 37)	30 (23, 38)	29.8 (25.9,34)

Table 3.2: Levels of shedding (GE/μL) determined by qPCR *com1* assay and serological status (IFA) in an endemically infected dairy goat enterprise in Victoria, Australia.

Shedding level	**Term kidders**				**Aborted does***
(GE per μL L)	**IFA+**	**IFA-**	**IFA missing**	**Total**	**Total**
Negative	113	302	2	417	12
1-10^3	22	35	2	59	4
$>10^3$	10	4	-	14	10
Total	145	341	4	490	26

* No serology was available for aborted goats.
GE = genome equivalents

Table 3.3: Multiple linear regression model outputs of factors influencing daily milk yields in does on a large dairy goat enterprise in south western Victoria, Australia.

Variable	**Coefficient (SE)**	*t*	**p value**	**95% CI**
Intercept	2.38 (0.11)	21.26	<0.001	2.15 to 2.59
qPCR status:				
Negative	Reference	-	-	-
Positive low	- 0.08 (0.12)	- 0.68	0.495	- 0.33 to 0.16
Positive high	- 0.53 (0.23)	- 2.33	0.02	- 0.98 to - 0.08 [a]
Parity:				
1	Reference	-	-	-
2	0.34 (0.10)	7.29	<0.001	0.54 to 0.94
3	1.22 (0.11)	10.7	<0.001	0.99 to 1.44
4+	0.71 (0.13)	5.5	<0.001	0.46 to 0.96
Kidding season:				
Mar to Apr	Reference	-	-	-
Jun to Jul	0.06 (0.11)	0.49	0.62	- 0.17 to 0.28
Sep to Oct	0.53 (0.12)	4.52	<0.001	0.3 to 0.77
Nov to Dec	0.54 (0.12)	4.57	<0.001	0.3 to 0.77
Herd:				
A	Reference	-	-	-
B	0.24 (0.10)	2.34	0.02	0.04 to 0.44
C	- 0.71 (0.11)	- 6.72	<0.001	- 0.92 to - 0.5
Random effect	SD			
Goat	0.82			
Within goat temporal correlation with first order autoregressive structure (AR1): 0.23				

AIC: 343081.1
[a] Interpretation: After adjusting for the effect of parity, kidding season, days in milk and farm, daily milk yields from does that were qPCR-positive high were 0.53 (95% CI 0.08 to 0.98) litres less than does that were qPCR negative. The random effect term at the individual doe level accounts for repeated measures and individual animal variability.
SE: standard error
CI: confidence interval

3.4 Discussion

The results of the present study show that under intensive management conditions coxiellosis in dairy goats can become endemic, with a relatively high prevalence of *C. burnetii* shedding does at parturition after several years from introduction of disease in the herd. Vaccination with a phase I vaccine is regarded as an effective control measure for *C. burnetii* infection in dairy goats (European Food Safety Authority, 2010). However, no Q fever vaccines are licensed for use in animals in Australia. Efforts are currently underway to develop an autogenous vaccine to immunise animals in the enterprise where the present study was carried out. A study by Astobiza et al (2010) showed treatment of pregnant sheep with oxytetracycline did not reduce the risk of *C. burnetii* shedding at lambing. The impact of changes in management practices (like the use of prolonged lactations to reduce the number of kidding events per year) on disease transmission have been modeled by Bontje et al. (2016). Predictions from this model suggest that while these interventions can lead to a reduction in the amount of environmental contamination, they alone are not sufficient to allow the disease to be eradicated. While the study by Bontje and collaborators provides useful insights into the effectiveness of coxiellosis control methods in a European herd management context, its ability to be applied to Australian dairy goat management conditions is limited mainly due to the complex seasonal kidding patterns that are feature of intensively managed dairy goat herds in Australia. There is a clear need for herd-flock managers to have access to tools and/or management strategies to allow coxiellosis to be controlled and/or eradicated in livestock farms in Australia. Ideally, the relative efficacy of recommended tools should be based on empirical evidence. The results presented in this paper partially address this need.

Our results show that while most qPCR-positive does shed relatively low concentrations of *C. burnetii* in their vaginal fluids at the time of parturition, a small proportion shed very high concentrations. Interestingly, the small proportion of does that were shedding high concentrations of *C. burnetii* (which we term 'super shedders') shed similar concentrations to does that had aborted. The phenomenon of super shedders has previously been described for coxiellosis as well as for other infectious diseases of livestock. In Germany, a single *C. burnetii* super-shedding ewe that lambed in a farmers' market was the source of a Q fever outbreak that resulted in 299 infected people (Porten et al., 2006). Capparelli et al. (2009), studied *Brucella abortus* shedding patterns in the milk of water buffaloes and found that while the majority of *Brucella abortus*-positive buffalo were shedding low concentrations of bacterium a small proportion shed very high concentrations. Similar findings were reported by Omisakin et al. (2003) in a study of *Escherichia coli* O157 in cattle in the United Kingdom. In their cross-sectional study Omisakin and collaborators estimated that approximately 97% of *Escherichia coli* O157 recovered from the environment was attributable to 9% of animals that were shedding.

In a cross-sectional study following an outbreak of coxiellosis in goats in France de Cremoux et al (2012) quantified the number of *C. burnetii* GE recovered in vaginal swabs in recently kidded does using the IS*1111* assay. de Cremoux et al (2012) showed that between 6% and 33% of does across three flocks were high shedders (greater than 10^6 bacteria per swab). While the findings of de Cremoux and collaborators are not directly comparable with ours because GEs were estimated using a different assay and results were reported as absolute GE counts (as opposed to concentrations), it is evident that in both studies the distribution of GE estimates is skewed, meaning that while most qPCR-positive does shed small amounts of *C. burnetii*, a substantially smaller fraction shed large amounts. If does contributing to the majority of environmental contamination can be

identified and culled before either aborting or kidding this could have a major impact on reducing the level of environmental contamination and the probability of disease transmission.

While super shedders are likely to play an important role in the transmission of disease, persistent shedders can perpetuate coxiellosis transmission. Of the 40 does that tested positive to *com1* in 2016 and were tested again in 2017, all were negative to the *com1* assay and 20% ($n = 8$) were positive to the IS*1111* assay. Due to the superior sensitivity of the IS*1111* assay compared with the *com1* assay our inference is that the level of shedding in the persistently shedding group of does was low. Berri et al. (2005b) conducted a longitudinal study in a goat herd in France that had experienced an abortion outbreak due to coxiellosis and found no PCR positives in vaginal swabs when does were sampled at the subsequent kidding season. This finding led the authors to hypothesise that *C. burnetii* shedding by infected does was limited to one parturition. We note however, that vaginal swabs in that study were obtained two weeks after parturition, which could have markedly reduced the likelihood of detecting *C. burnetii* shedding does. Later, in an investigation of an outbreak of abortions in another dairy goat herd in France, Berri et al. (2007) found that 26% of the does that were *C. burnetii* shedders at the time of kidding had also been shedders at their previous kidding. *C. burnetii* GE concentrations for the repeat-shedding does were not reported. In our study we show that infected does can shed *C. burnetii* over at least two consecutive parturitions. A weakness of previously published research in this area has been that the change in *C. burnetii* infection status of individual does from one parturition to the next has not been unambiguously reported. Instead, authors have simply documented the group-level prevalence of *C. burnetii* for groups of consecutively kidding does. This approach is likely to misrepresent the true proportion of does that are persistent shedders due to (among other factors) individual herd replacement and culling rates. We conclude that if one was attempting to eradicate Q fever from a dairy goat herd, priority should be placed on identifying and isolating high shedders as opposed to identifying and removing persistently shedding does. Our reasoning for this is two-fold. Firstly, the proportion of persistently shedding does is relatively small and secondly the quantities of *C. burnetii* shed by persistently shedding does is relatively small. Our results support that for monitoring the IS*1111* assay is preferred over the *com1* assay due to its superior sensitivity for detecting relatively low levels of *C. burnetii* DNA.

Does presented for sampling were those that had kidded during the previous 24 hours. Due to logistic limitations we were unable to determine the exact time of kidding for each doe. For this reason it is possible that detected differences in *C. burnetii* shedding quantities were influenced by the interval from kidding to the time of sampling, with higher concentrations of *C. burnetii* likely to be shed closer to the time of delivery, leading to missclassification of individual doe qPCR category assignment. An argument against this explanation is the negative association between daily milk yields for *C. burnetii* qPCR-positive high does as discussed below. Abortion rates reported by the herd managers in this enterprise ranged from 2% to 8% across the three farms and four kidding seasons. It cannot be ruled out that abortion causes other than coxiellosis also played a role in fetal loss in these herds. Throughout 2016–2017 14 vaginal swab samples from the 26 does that aborted returned a qPCR *com1* positive result. The concentration of *C. burnetii* GE found in positive samples from aborted does was high (Table 3.2) and similar to those found in a prospective study of sheep and goat abortion cases in Ontario, Canada (Hazlett et al., 2013). An area of interest for future work on this farm would be to investigate abortion causes and the extent to which coxiellosis alone, or in combination with, other abortion-causing agents is responsible for reproductive losses.

The agreement between serology and qPCR was not substantial, which is consistent with findings from other studies (Rousset et al., 2009a; de Cremoux et al., 2012; Guatteo et al., 2012; Bauer et al., 2016). However,

the agreement improved in does that were shedding high concentrations of *C. burnetii* (Table 3.2). Out of the 14 goats shedding above 10^3 GE/μL, 10 had a positive result to IFA. The reasonably strong agreement between qPCR and serology for high-shedder does indicates that there may be some use for serology as a means for detecting high-shedder does in endemically infected herds. We note that in the present study vaginal swabs and blood samples were collected at the same time (within 24 hours after kidding), hence, it cannot be assumed that high shedders could actually be detected using serology before kidding. A useful area of future research would be to collect blood samples for IFA serology from does entering their last third of gestation and to compare the IFA results to qPCR *com1* results from vaginal swabs taken at the time of kidding. Detection of *C. burnetii* in whole blood samples by qPCR is a possible alternative to serology for this purpose, which could have the additional benefit of being a method for detecting super shedders in vaccinated herds.

With regards to the impact of *C. burnetii* infection on milk yield we identified that daily milk volume yields were lower in does that comprised the qPCR-positive high group compared with qPCR-negative does. Given average daily milk yields in these herds were in the order of 3 litres, our inference is that *C. burnetii* infection in the relatively small proportion of does classed as high shedders results in a substantial (i.e. 3% to 32%) reduction in daily milk yields. In a study that assessed seroconversion dynamics at the time of kidding Muleme et al. (2017b) found that does that had seroconverted to phase I IgG produced 0.2 L of milk per day more than does with no protective antibody response in the first 9 weeks of lactation. We hypothesise these results represent two sides of the same coin, the drop in milk yields associated with *C. burnetii* infection found in the present study relates to the gain in daily milk yields observed by Muleme et al. in does with a protective immune response. Infection with *C. burnetii* in goats can lead to placentitis, endometritis and mild lesions in the mammary gland (Sánchez et al., 2006), and these conditions provide plausible biological explanations for the milk losses identified in this study. In cows, Barlow et al. (2008) found an association between the presence of *C. burnetii* in individual milk samples and increased SCCs. In the present study, no statistically significant association between individual SCCs and *C. burnetii* qPCR results in vaginal swabs was found. We note, however, that only one individual SCC estimate was available per doe per lactation. Testing for presence of *C. burnetii* in individual milk samples, as opposed to vaginal swabs, and controlling for presence of most common microorganisms that cause mastitis in goats would be a more appropriate way of addressing the question on whether *C. burnetii* can cause subclinical mastitis in goats. This is the first time a negative association between *C. burnetii* infection status and milk yield in dairy goats has been reported.

3.5 Conclusions

Shedding concentrations of *C. burnetii* were highly skewed, with a relatively small group of does shedding relatively high quantities of *C. burnetii*. Further, high shedding does had reduced milk yields compared to qPCR-negative does. Agreement between serology and shedding status was improved in does shedding high concentrations of *C. burnetii*. Future studies should aim at developing improved methods for early detection of high shedding does. Eradication of *C. burnetii* from intensively managed dairy goat herds should have a positive impact on herd profitability (in terms of increasing daily milk yields) as well as reducing the likelihood of dairy goats being a source of *C. burnetii* infection for humans.

Chapter 4

Modelling Q fever transmission and control interventions in an intensively managed dairy goat herd

Abstract – Q fever is a zoonotic disease caused by the bacterium *Coxiella burnetii*. Large Q fever outbreaks have been traced back to dairy goat herds. In this study, we developed an agent-based model of within-herd Q fever transmission using demographic and epidemiological data obtained from a Q fever endemic dairy goat enterprise in Victoria, Australia. Epidemiological parameters were inferred using Approximate Bayesian Computation. The model was used to assess the efficacy of vaccination, segregation of pregnant does, culling of does shedding *C. burnetii* at the time of delivery and combinations of thereof. Model predictions suggest eradication is achievable using vaccination, although this should be regarded as a relatively long-term strategy. Whereas segregation of pregnant does or culling of *C. burnetii* shedders at the time of delivery did not have a marked impact on disease transmission when applied alone, they reduced the time to disease eradication when applied in combination with vaccination. Out of all the control strategies assessed, the shortest time to disease eradication was achieved when vaccination was applied in combination with culling of does identified as *C. burnetii* shedders at kidding or abortion.

4.1 Introduction

Q fever is a zoonotic disease that commonly occurs as sporadic human cases among populations that work in close contact with livestock (Maurin and Raoult, 1999). Large Q fever outbreaks affecting populations in relative proximity to farms can also occur (Clark and Soares Magalhães, 2018). In 2007–2010 the largest Q fever outbreak ever recorded occurred in The Netherlands where more than 4000 people contracted Q fever. Dairy goat farms were traced back as the source of the outbreak (Roest et al., 2011a). A series of control measures were taken in an effort to control disease spread, including hygiene measures, movement restrictions, breeding bans, vaccination and stamping out (van der Hoek et al., 2010). The overlapped implementation of multiple control strategies made it difficult to estimate the relative contribution of each control measure applied (Hogerwerf et al., 2011). Mathematical models of disease transmission can be used to simulate disease transmission and assess the direct and combined efficacy of several different control interventions (Tildesley et al., 2006).

Vaccination is regarded as an effective control measure for Q fever in goats (European Food Safety Authority, 2010). Overall, studies that assessed the efficacy of vaccination in livestock support the hypothesis that vaccination reduces *Coxiella* excretion by infected individuals and that vaccine efficacy is conditioned by gestational status as well as disease status at the time of vaccination (O'Neill et al., 2014). Segregation of kidding does has been proposed as a potential control strategy by the European Food Safety Authority (EFSA) (2010) and the American College of Veterinary Internal Medicine (ACVIM) (Plummer et al., 2018). However, no published studies have assessed the impact of segregation of kidding does either applied alone or in combination with other control measures. There is limited information on the efficacy of control interventions based on identification and removal of *C. burnetii* shedders.

Q fever transmission in goats has been modelled by other authors (Hogerwerf et al., 2013; Bontje et al., 2016). Parameters for these models were obtained from the literature or quantitatively calibrated to make model outputs match observed disease dynamics. Statistical inference methods can be used to formally assess the ability of a model to explain data (Conlan et al., 2012). Furthermore, the use of rigorous statistical methods for estimation of key model parameters using existing data is an important step towards improving the validity of model predictions (Grassly and Fraser, 2008). Approximate Bayesian Computation (ABC) is a class of methods that can be used to estimate parameters of dynamic models when evaluation of the likelihood is impracticable (Toni et al., 2009).

In Victoria, Australia, a Q fever outbreak associated with a large dairy goat enterprise resulted in 24 human cases (18 laboratory confirmed) between 2012 and 2014 (Bond et al., 2016). Vaccination of all farm staff with Q-Vax, a vaccine licensed for use in humans in Australia, effectively prevented further human cases. However, infection remained endemic among goats as shown by studies carried out subsequently (Muleme et al., 2017a; Canevari et al., 2018). To this day, there are no Q fever vaccines licensed for use in animals in Australia. Efforts are underway to develop an autogenous vaccine to immunise goats at the farm identified as the source of this outbreak. The relatively small scale at which the vaccine will be produced is expected to result in a non-negligible price per dose. Therefore, the expected time from start of vaccination to disease eradication is key to determine the economic feasibility of eradication by means of vaccination.

The objective of this study was to estimate the expected time from start of vaccination to Q fever eradication in a Q fever endemic dairy goat herd in Victoria, Australia. In addition, we assessed if the effect of either segregation of pregnant does or culling of does shedding *Coxiella* at parturition or abortion, both applied alone or in combination with vaccination, would have a positive impact on disease control. An ABM was used to address these questions. Demographic parameters were estimated from farm records, and epidemiological parameters were inferred using ABC and data on Q fever incidence obtained in the field study presented in Chapter 3.

4.2 Materials and methods

4.2.1 Study population

The model was developed using data from a large dairy goat enterprise endemically infected with *C. burnetii* located in Victoria, Australia. This enterprise is comprised of three farms independently managed, three known to be highly endemic for coxiellosis and located less than 3 kilometres apart (See Muleme et al. 2017a for further detail). In turn, does in each of these three farms are allocated into one of four mobs depending on the time of the year in which they are expected to kid (i.e. autumn, winter, spring or summer kidders, respectively).

Replacements are weaned at 2 months of age and joined 5 months later when they are 7 months of age. Therefore, maidens are expected to kid at 12 months of age in the same kidding season they were born in. From then on, does are managed to kid once per year. Thus, each of these four mobs is independently managed, with mixing of does from different mobs within each farm kept to a minimum. For the purpose of this study, we modelled the spring kidding mob from one of the three farms. Given that the intervals between key demographic events (i.e. conceptions, births, weanings) are the same for any of the four mobs, in any of the three farms, selection of which mob to model was arbitrary.

The time of entry of *C. burnetii* into this enterprise could not be established with certainty. Early outbreak investigations led to two main hypotheses in this regard. The first suggested disease was introduced into the herd with a group of goats imported from the state of Queensland in 2011. Queensland is located in the north-east of Australia and has the highest Q fever report rates in humans in the country (Eastwood et al., 2018). However, when the herd where the imported goats and their contacts had been kept was tested, no evidence of *C. burnetii* exposure was found (Muleme et al., 2017b), which led to rejection of this hypothesis. A second hypothesis was that the time of entry was as early as 2004, time since which an increase in abortion rates had been perceived by farm staff (Bond et al., 2016).

4.2.2 Model description

In goats, *C. burnetii* infection can lead to placentitis and abortions, which typically occur in advanced stages of pregnancy (Lang, 1990; Álvarez-Alonso et al., 2018). With abortions, large quantities of *C. burnetii* are released to the environment. Furthermore, infected does that deliver on-term healthy kids can shed *C. burnetii* in quantities comparable to those that abort (Roest et al., 2012). *C. burnetii* can differentiate into a spore-like form known as small cell variant which allows it to remain viable in the environment for a prolonged time (McCaul and Williams, 1981). New infections occur via aerosolisation of contaminated particles that are inhaled by susceptible individuals (Woldehiwet, 2004).

Goats can acquire infection early in life and, while a proportion of young goats seem to be able to clear the infection, others remain persistently infected and can shed *C. burnetii* at their first parturition (Muleme et al., 2017a). Also, shedding can occur in at least two consecutive parturitions, suggesting *C. burnetii* has the capacity to evade the immune system and resume replication when conditions are optimal (Berri et al., 2007). *C. burnetii* DNA has been detected in oviducts and uterine flushing media and in genital tract tissues of non-pregnant goats (Alsaleh et al., 2011), and reactivation of *C. burnetii* infection with pregnancy has been demonstrated in guinea pigs and mice (Sidewell and Gebhardt, 1966).

We adapted a model originally developed by Bontje et al. (2016) to account for demographics and transmission dynamics observed in a Q fever endemic dairy goat farm in Victoria, Australia. One of the main differences with Bontje's model is that, in our version, non-pregnant infected goats can become persistently infected. Also, instead of having two compartments for the environment (i.e. manure and dust), we used a single compartment to represent the environment. This decision was made based on the absence of data to inform the fraction of *C. burnetii* shed by infected goats expected to go to one or the other compartment, as well as for the absence of substrate specific *C. burnetii* decay rates. The transmission rate was also modified following the method described by Courcoul et al. (2010). We adopted this density-dependent approach so that changes in the population size resulting from one of the control interventions here assessed (i.e. segregation) did not result in spurious changes in the transmission rate. Disease was seeded into the model by introducing one infected

pregnant doe on calendar day 103 (peak of the joining season).

A schematic representation of the model is shown in Figure 4.1. The underlying framework of the model is an adaptation of the standard SIR model (Keeling and Rohani, 2008). Goats can be susceptible (S), infected (I) or recovered (R) and be in turn pregnant (P) or non-pregnant (NP) (i.e. $S_{NP}, S_P, I_{NP}, I_P, R_{NP}, R_P$). Susceptible goats are infected from the environment (E). Environmental contamination is measured on an arbitrary scale as in (Bontje et al., 2016). The environmental bacterial load is dynamic; it increases when infected does shed *Coxiella* into the environment, and exponentially decreases over time at a rate μ. Infection can lead to recovery and immunity, or to a persistently infected state (J_{NP}). If goats are infected beyond their kidding date minus the incubation period, they move to the late-infected category (I_{P2}). Late-infected goats will not abort or shed in their subsequent kidding event, but can become persistently infected and their following pregnancy lead to abortion or shedding of *C. burnetii* at kidding. Abortions are restricted to happen within the last third of gestation. Arricau Bouvery et al. found shedding in faeces can occur before parturition or abortion for an average time extent of 3 weeks (Arricau Bouvery et al., 2003). However, other authors found shedding in faeces only occurred after parturition or abortion and that shedding via this route extended for several months (Roest et al., 2012). In this model, the worst case scenario was assumed and all does in infected categories (i.e. $I_{NP}, I_P, I_{P2}, J_{NP}$) were assumed to shed relatively small quantities of *C. burnetii* in faeces.

The model was coded as an agent-based model (ABM) in *C++* programming language and compiled and run in R (R Core Team, 2017) using the contributed package *Rcpp* (Eddelbuettel and François, 2011). A combination of Markov and non-Markov events was used following the methodology described by Conlan et al. (2015) for modelling bovine tuberculosis. Epidemiological events were modelled as Markov events, with stochasticity incorporated using a Gillespie algorithm (Gillespie, 1976). Demographic processes were modelled as non-Markov events and, their time of occurrence was randomly sampled from appropriate distributions. A description of both epidemiological and demographic events included in the model is shown in Table 4.1.

4.2.3 Model parameters

Demographic model parameters were informed using empirical data collated from the study herd. Dates of birth and removal dates of individual goats ($n = 1653$) where collated from the farm's data base and used to compute individual doe length of productive life. The contributed *R* library *fitdistrplus* (Delignette-Muller and Dutang, 2015) was used to fit a Weibull distribution to the observed density distribution of lifespan (Figure C.1-A). Kidding dates from the spring kidding season of 2016 ($n = 594$) were plotted as a density histogram and a normal distribution was fit to these data (Figure C.1-B). Conception dates were modelled by subtracting 150 days (gestation length) from kidding dates. The in-kid rate estimated for the farm in 2016 was 83% (95 CI: 79% to 86%). In our model, the probability of a doe conceiving when joined was sampled for a Beta distribution centered on 0.83, with 95% of the distribution density above 75% (shape 1 = 69.42, shape 2 = 15.1). The entry of replacements into the herd was assumed to start 2 months after the average kidding date — when newborns are weaned. Individual entry times were randomly sampled from an exponential distribution so that 95% of the entries occurred within a two-week period ($\lambda = 0.214$). The maximum total population size was set to 900 individuals. This led to approximately 430 kidding events per year, which is consistent with the number of kidding events registered in the farm under study (range: 275 to 648 in years 2012–2016). Only female goats — from weaning to removal — were considered in the model, as billies likely play a negligible role in within-herd Q fever transmission.

Epidemiological model parameters were inferred using Approximate Bayesian Computation (ABC) rejection-sampler (Toni et al., 2009) (Table 4.3). Five million candidate parameter vectors were sampled from prior distributions and used to simulate disease dynamics. Each simulation was run for 12 years, time at which the age-stratified prevalence of shedding at kidding was calculated. Similarly, the average abortion rate in the 12^{th} year from disease introduction was computed. These metrics were compared with estimations of prevalence of *C. burnetii* shedding at kidding and failure to kid risk obtained in a panel study carried out in 2016 in the farm described above (Canevari et al., 2018). When simulated summary metrics were within a tolerance interval defined as the estimated 95% CI for the point estimates of prevalence of *C. burnetii* shedding at kidding and failure to kid risk (see Table 4.4), the candidate parameter vectors were retained as samples from the approximate posterior distributions, otherwise they were discarded. The decision to compare observed data with metrics calculated at year 12 of simulations responds to the fact that available evidence suggested disease could have entered the farm around 2004, and information on age-stratified prevalence and failure to kid risk (i.e. the proportion of does with a positive pregnancy test that have no kidding event recorded in the subsequent kidding season) were available for 2016. Further, by year 12 simulations showed an endemic pattern of disease (See Figure 4.5A). To cope with the computational demand of the ABC, the process was run in parallel using a NeCTAR cloud of 16 processors and the contributed *R* package *doParallel* (Weston, 2019). A description of epidemiological parameters and prior distributions used for the ABC are shown in Table 4.3. Flat priors were used for all parameters, providing support to only biologically plausible values. For reporting approximate posteriors, credible intervals were estimated using the highest posterior density method (McElreath, 2016).

Selection of priors for the ABC rejection-sampler

The parameter α dictates the proportion of infected does that become persistently infected. In a panel study carried out in a dairy goat farm in Australia, 40 goats shedding *C. burnetii* at parturition were sampled again in their subsequent kidding and 8 (20%, 95% CI: 11% to 35%) were found to be persistent shedders (Canevari et al., 2018). In another study carried out in the same endemic farm, out of 41 primiparous goats that had seroconverted before week 28 of age, shedding at parturition was detected in 15 (37%, 95% CI: 24% to 52%) (Muleme et al., 2017a). However, other authors have suggested the proportion of goats that become chronically infected could be 70% (Bontje et al., 2016). Acknowledging the uncertainty around this parameter value, we took a conservative approach and used a uniform wide prior distribution for α, with a minimum of 1% and a maximum of 75%.

In the model, the decay of *C. burnetii* in the environment is governed by the parameter μ. A longitudinal study carried out in 4 sheep ranches located in an enzootic area for Q fever in California, Welsh et al. (1959) found viable *C. burnetii* in soil samples during the lambing season but not beyond the end of the lambing season. On the other hand, Álvarez-Alonso et al. (2018) recovered viable *C. burnetii* from dust up to 60 days beyond the end of the kidding season in a dairy goat farm were abortions had occurred. We used a uniform prior distribution for μ, with a minimum and maximum of 0.025 and 0.428 units day^{-1}, respectively (equivalent to 95% decay ranging from 7 to 120 days).

Regarding the incubation period, σ, after a point source exposure at a rural fair, abortions in different sheep flocks occurred within the subsequent 21 to 118 days (Sanford et al., 1994). In a challenge study carried out in pregnant goats by Arricau-Bouvary et al. (2003), the time from *C. burnetii* inoculation to abortion ranged from 12 to 48 days, with all but one abortion occurring between day 25 and 48 post inoculation. Based on results of

another experimental study in goats, Roest et al. (2012) found it takes 14 to 28 days for *C. burnetii* to colonise the trophoblast cells up to detectable levels using immunohistochemistry and PCR. Sanchez et al. inoculated pregnant goats on day 90 of gestation and observed abortions 42 ± 4 days later (Sánchez et al., 2006). Based on the published data, we chose a uniform prior distribution for σ with a minimum of 14 and a maximum of 67 days.

As for the proportion of infected does that abort, (θ), abortions reported in experimental infections range from 80% to 100% of challenged does (Arricau Bouvery et al., 2003; Sánchez et al., 2006; Roest et al., 2012). However, results from experimental studies likely overestimate this parameter given the relatively high concentrations of *C. burnetii* to which goats were exposed. Also, in published studies goats were infected in the last third of their pregnancy when the risk of placental colonisation and abortion is likely to be higher. Therefore, we used a uniform prior distribution for θ ranging from 5% to 75%.

Shedding quantities of *C. burnetii* at abortion or kidding of infected does is governed by ε_p. Given that an arbitrary scale was used to model environmental contamination no data was available to inform a prior for this parameter. A uniform prior distribution ranging from 0.01 and 0.2 *C. burnetii* scaled units was arbitrarily chosen for ε_p. Shedding quantities of *C. burnetii* in faeces have been estimated to be approximately 1 million times lower than quantities shed in products of conception (Bontje et al., 2016). Thus, for ε_f we chose a uniform distribution ranging from 1e-8 to 2e-07 *C. burnetii* scaled units.

4.2.4 Sensitivity analysis

A global sensitivity analysis was carried out to assess the relative importance of each epidemiological parameter in the model output following the method described by (Blower and Dowlatabadi, 1994). A Latin Hypercube Sampling (LHS) scheme was used to sample 1000 sets of parameter values from the distributions described in Figure 4.3. Infection dynamics were simulated for 12 years after introduction of one infected pregnant doe into a fully susceptible herd using each of the 1000 sets of parameters sampled. We run 200 model iterations for each parameter set. The total number of infected placentas (i.e. abortions and kidding events of infected does) was recorded as output. The mean cumulative number of infected placentas across the 200 iterations run for each parameter set was used to calculate the partial rank correlation coefficient (PRCC) (Marino et al., 2008). The PRCC indicates whether there is a positive or a negative relationship between the input setting (parameter values) and the output (cumulative number of infected placentas). The relative contribution of each of the parameters to the output can be assessed by comparing the magnitude of the PRCC.

4.2.5 Control interventions

To compare the efficacy of different control scenarios, 200 model iterations for each of the 6 following scenarios were run: (1) no intervention; (2) culling of *C. burnetii* shedders at parturition or abortion; (3) segregation of pregnant does; (4) vaccination; (5) vaccination and culling of *C. burnetii* shedders at parturition or abortion; and (6) vaccination and segregation of pregnant goats. All control interventions were modelled to start in year 12 after introduction of an infected pregnant doe into a fully susceptible herd. Following the start of control interventions the model was run for 14 years. Disease eradication was defined as absence of infected does and an environmental contamination below 10^{-6} theoretical units. To allow comparisons between interventions, the starting point for calculation of time to eradication for all control interventions was standardised to the average

day of conception (i.e. calendar day 103) in year 12 since introduction of an infected animal into the herd. Time to eradication for the different control strategies was compared using Kaplan-Meier curves. Summary statistics of the total number of infected goats over time were plotted for each scenario. The simulations were generated using parameters sampled from the approximate posterior distribution.

Culling of *C. burnetii* shedders at parturition or abortion

We modelled testing of vaginal swab samples obtained at the time of parturition/abortion using qPCR. We assumed all does were presented for sampling at the time of delivery. The qPCR assay was assumed to have both perfect sensitivity and specificity. Goats returning a positive result were modelled to leave the herd one week after they had kidded/aborted. Culled goats were replaced with newborns at weaning as with the rest of the modelled exits.

Segregation of pregnant does

Segregation of kidding does was modelled by adding a separate environment to which pregnant does were moved 30 days after the end of the joining season (when pregnancy testing is routinely carried out). Does were moved back to the main environment immediately after kidding or abortion. The environmental contamination in the segregated kidding environment was assumed to exert a pressure of infection only on does that were in this environment, and not on does in the main environment. The equation describing the probability of infection for a susceptible pregnant doe in the segregated kidding environment is shown in Table 4.1. We represent the bacterial load in the segregated kidding environment with the letter K. Just as with the main environment E, the bacterial load in the segregated kidding environment is measured in *C. burnetii* scaled units and its value is dynamic; it increases with shedding from infected does and decreases over time at a rate μ. To differentiate does in the segregated kidding environment from does in the main environment we use the suffix κ to refer to does in the segregated environment. For example, $S_{P(\kappa)}$ represents the number of susceptible pregnant goats in the segregated kidding environment.

Vaccination

In a challenge study carried out by Arricau Bouvary et al. (2005) vaccination effectively prevented abortions, reduced the risk of shedding at parturition and reduced the quantity of *C. burnetii* shed by vaccinated positive does. During the 2007–2010 Q fever outbreak in The Netherlands, Hogerwerf et al. (2011) tested does from both vaccinated and unvaccinated farms and found a reduction in the risk of *C. burnetii* detection by qPCR in uterine fluid, vaginal swabs and milk in individuals from vaccinated herds. Also, bacterial loads in qPCR positive samples from vaccinated farms were reduced compared with those from unvaccinated farms. In a randomised controlled field trial carried out by de Cremoux et al. (2012) vaccinated goats where found to excrete significantly reduced quantities of *C. burnetii*. The reduction of *Coxiella* excretion in vaccinated individuals was more marked in individuals that had no evidence of infection/exposure at the time of vaccination. Disease status at the time of vaccination was also found to have an impact on vaccine efficacy in cows. Guatteo et al. (2008) found no effect of vaccination on either the probability of shedding or bacterial loads found in shedding cows when vaccination was carried out in individuals that either had been classified as infected or were pregnant at the time of vaccination. On the other hand, when vaccinated as 'susceptible' and 'non-pregnant', a five-fold reduction in the probability of a cow becoming a shedder was observed. Pregnancy status was also found to

have an impact on vaccination efficacy by Tutusaus et al. (2014), who found vaccination lacked efficacy when applied to pregnant cows.

In the model, only susceptible non-pregnant (S_{NP}) individuals were assumed to benefit from vaccination. The same rate of disease progression was assumed for vaccinated and unvaccinated individuals (i.e. vaccination did not prevent infection). Only 10% of does infected as vaccinated shed *Coxiella* with products of conception. The quantity of *C. burnetii* shed by vaccinated does that became shedders was reduced by 95% compared to unvaccinated infected does (O'Neill et al., 2014). Also, it was assumed vaccinated does did not abort (Arricau Bouvery et al., 2003). For the vaccination control strategy, replacements were modelled to enter the herd as vaccinated. Also, 3 weeks prior to the start of the joining period a round of vaccination was simulated.

Combined control strategies

Segregation of kidding does and culling of shedders at parturition or abortion were also modelled in combination with vaccination to assess whether there was a synergistic effect of the combined interventions.

4.3 Results

4.3.1 Parameter estimation

Marginal approximate posterior distributions are shown in Figure 4.2. The parameters α, θ and σ were identified by the ABC rejection-sampler. For the parameter ε_p, lower values were better supported by the data. On the other hand, the parameters μ and γ and ε_f could not be identified. The marginal approximate posterior distribution for the parameter α, which dictates the fraction of infected goats that become persistently infected, had a median of 0.29 (95% CrI: 0.20 to 0.39). The median of the approximated posterior for the parameter θ, the fraction of infected pregnant does that abort, was 0.28 (95% CrI: 0.16 to 0.4). The posterior distribution for the incubation period σ, defined as the minimum time in days required from *C. burnetii* exposure to either abortion or shedding at kidding, had a median of 49 (95% CrI 29 to 67) days. Figure C.2 shows the bivariate relationships between the model parameters.

Epidemiological parameters with the highest influence on the cumulative number of infected placentas by year 12 from introduction of disease into the herd coincided with those identified by the ABC rejection-sampler (see Figure 4.3). Parameters α, σ and θ were found to have the highest correlation with the cumulative number of infected placentas by year 12 from introduction of infection into the herd. On the other hand, the decay rate of *C. burnetii* in the environment and the recovery rate for infected non pregnant does γ were not significantly correlated with the cumulative number of infected placentas by year 12.

Model simulations in the absence of control interventions were generated using a random sample of 200 sets of model parameters accepted by the ABC. Model predictions for prevalence of shedding at kidding stratified by age and abortion rates 12 years from the introduction of an infected doe into the herd are shown in Figure 4.4. Targets for these metrics are included in the plot to allow a visual assessment of model fit. Overall, the model predictions appeared consistent with the observed data. Introduction of one infected pregnant doe mid-way through the mating period led to an outbreak followed by endemicity (defined as disease still present at year 12 from introduction) in 94% of the simulations; in the remaining 6% of simulations, an outbreak did not occur.

4.3.2 Control strategies

Predictions for the total number of infected goats from 200 iterations of the model under six different scenarios are shown in Figure 4.5. In the absence of control interventions, model predictions showed a peak incidence of infected individuals around year 2 following the introduction of an infected pregnant doe. The median prevalence of shedding at kidding for that year was 18.7% (95% CrI: 6.9% to 40.8%). In turn, the median maximum abortion rate was 7.9% (95% CrI: 1.5% to 14.9%). Culling of does shedding *C. burnetii* at kidding or abortion had no marked impact on disease incidence when applied alone. Further, the increased pool of susceptible individuals resulting from a higher number of replacements introduced each year led to slightly larger peaks of disease incidence after the implementation of culling. Segregation of pregnant does led to only a marginal reduction in the median total number of infected does over time. This intervention prevented early infection of replacements by precluding exposure to environmental contamination from the kidding season ending two months before they entered the herd. However, most of these susceptible individuals were later exposed to infection in the subsequent kidding season. Vaccination and the combination of vaccination with either culling or segregation of pregnant does were the most effective control measures for reducing disease incidence.

For comparison of time to eradication only simulations that led to an endemic state were used. Figure 4.6 are a series of Kaplan-Meier survival curves showing time to eradication for the different control scenarios assessed. Neither culling of shedders at kidding/abortion nor segregation of pregnant does alone were enough to achieve eradication in any of the simulations. Control strategies including vaccination resulted in disease eradication within 14 years in 98% of the simulations. There were differences in the median time to eradication for each of the assessed vaccination scenarios (Figure 4.7). When vaccination alone was modelled, the median time to disease eradication was 6.5 (95% CrI: 4.1 to 11.3) years. Compared with vaccination alone, vaccination and segregation reduced the median time and the uncertainty around the estimated time to eradication to 5.7 (95% CrI: 4.5 to 8.7) years. Moreover, vaccination combined with culling of shedding does had a median predicted time to eradication of 4.7 (95% CrI: 3.2 to 9.1) years, the lowest of all assessed scenarios.

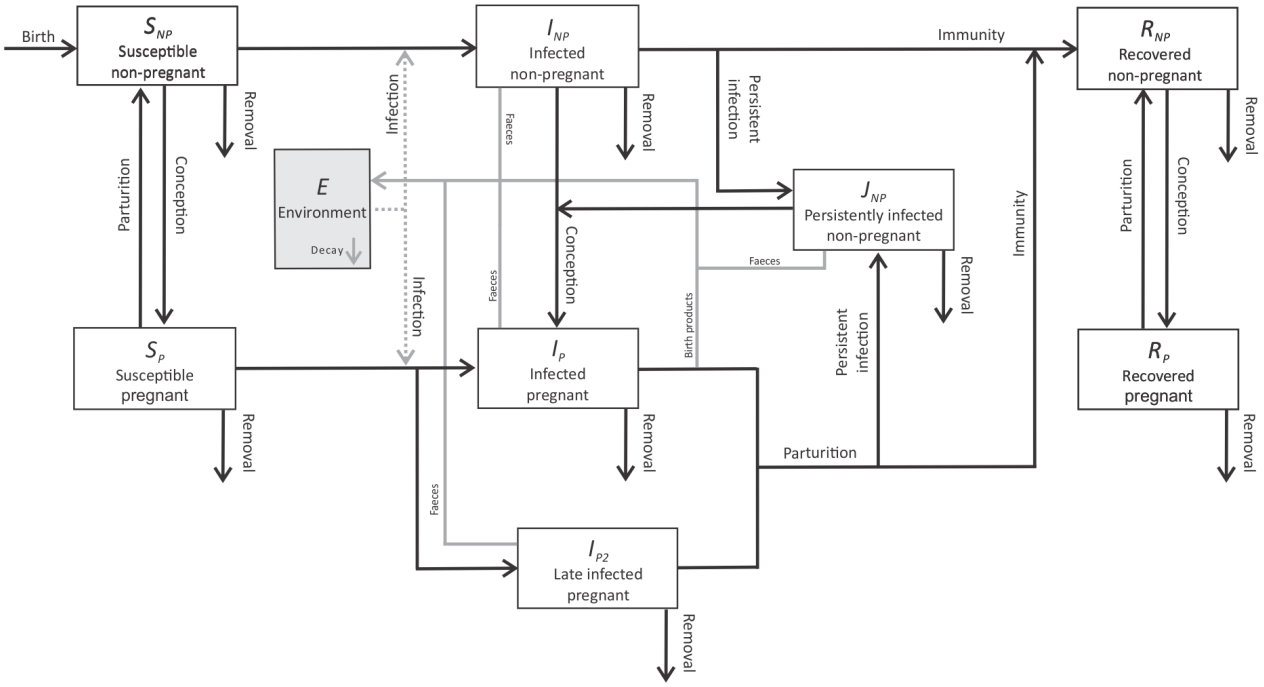

Figure 4.1: Schematic representation of the model structure. Susceptible goats (S_{NP} and S_P) can acquire infection from the environment (E), which is contaminated with birth products from infected pregnant does (I_P) that either abort or deliver a kid on-term. Also, all infected categories are assumed to shed a relatively small quantity of *C. burnetii* to the environment in faeces. The environmental bacterial load decays over time at a rate μ. When the time between infection and the expected time of delivery is less than the incubation period, does become late-infected (I_{P2}) and will not abort or shed *Coxiella* at kidding. After kidding or abortion, infected does can become persistently infected (J_{NP}) or recovered (R_{NP}). Infected non-pregnant does (I_{NP}) can gain immunity after a recovery period or become persistently infected. Replacements enter the herd at weaning, and exit the herd at a time sampled from a distribution fitted to the observed life-span frequency distribution in a large dairy goat enterprise in Victoria, Australia. The model structure is an adaptation of that published by Bontje et al. (2016).

Table 4.1: Description of epidemiological and demographic events included in the model.

Epidemiological events				
Event	Status	Effect	Rate	Notes
Infection	S_{NP}	$S_{NP} \rightarrow I_{NP}$	$S_{NP} \times (1 - e^{-E})$	
	S_P	$S_P \rightarrow I_P$	$S_P \times (1 - e^{-E})$	In main environment
	$S_{P(\kappa)}$	$S_{P(\kappa)} \rightarrow I_{P(\kappa)}$	$S_{P(\kappa)} \times (1 - e^{-K})$	In segregated environment
Recovery	I_{NP}	$I_{NP} \rightarrow R_{NP}$	$(1-\alpha) \times \gamma \times I_{NP}$	
Persistent infection	I_{NP}	$I_{NP} \rightarrow J_{NP}$	$\alpha \times \gamma \times I_{NP}$	
Demographic events				
Event	Status	Effect	Distribution	Notes
Entry	Y	$Y \rightarrow SNP$	$\text{WeaningDate}_{year} + Exp(0.214)$	
Exit	$S_{NP}, S_P, I_{NP}, I_P, I_{P2}, J_{NP}, R_{NP}, R_P$	$(...) \rightarrow Y$	$t + Weib(944, 1.9)$	
Conception date	S_{NP}	$S_{NP} \rightarrow S_P$	$Norm(\text{JoiningDate}_{year}, 8.8 \text{ days})$	
	I_{NP}	$I_{NP} \rightarrow I_P$	$Norm(\text{JoiningDate}_{year}, 8.8 \text{ days})$	$t + \sigma \leq \text{Kidding\_date}_{goat}$
		$I_{NP} \rightarrow I_{P2}$	$Norm(\text{JoiningDate}_{year}, 8.8 \text{ days})$	$t + \sigma > \text{Kidding\_date}_{goat}$
	R_{NP}	$R_{NP} \rightarrow R_P$	$Norm(\text{JoiningDate}_{year}, 8.8 \text{ days})$	
	J_{NP}	$J_{NP} \rightarrow I_P$	$Norm(\text{JoiningDate}_{year}, 8.8 \text{ days})$	
Kidding date	S_P	$S_P \rightarrow S_{NP}$	$\text{Joining\_date}_{goat} + 150$ days	
	I_P	$I_P \rightarrow R_{NP}$	$\text{Joining\_date}_{goat} + 150$ days	$(1-\alpha)$
		$I_P \rightarrow J_{NP}$	$\text{Joining\_date}_{goat} + 150$ days	α
	I_{P2}	$I_{P2} \rightarrow R_{NP}$	$\text{Joining\_date}_{goat} + 150$ days	α
		$I_{P2} \rightarrow J_{NP}$	$\text{Joining\_date}_{goat} + 150$ days	$(1-\alpha)$
Conception rate			$Beta(69.42, 15.1)$	

Exp stands for exponential distribution; in brackets the distribution mean. *Weib* stands for Weibull distribution; in brackets distribution parameters shape and scale. *Norm* stands for Normal distribution; in brackets distribution parameters mean and standard deviation. *Beta* stands for Beta distribution, in brackets distribution parameter shape 1 and shape 2.

Table 4.2: Individual demographic and epidemiological variables.

Data field	Data type	Details
Epi_status	Categorical (S_{NP}, S_P, I_{NP}, I_P, I_{P2}, J_{NP}, R_{NP}, R_P, Y)	Epidemiological status
Entry_date	Numeric (days)	Used to track age of goats
Exit_date	Numeric (days)	Entry date plus random sample from population lifespan distribution
Joining_date	Numeric (days)	Date joined
Kidding_date	Numeric (days)	Date joined plus gestation length
Preg_status	Boolean (true or false)	Pregnancy status
Ab_status	Boolean (true or false)	Whether current pregnancy will result in abortion or not. Determined when *INP* or *JNP* become pregnant or *SP* become infected
Vac_status	Boolean (true or false)	Vaccination status

Table 4.3: Description of epidemiological parameters and prior distributions used in the ABC rejection-sampler.

Parameter	Description	Units	Prior distribution
α	Fraction of infected does that become persistently infected	NA	Unif. (min = 0.01, max = 0.75)
μ	*Cb** environmental decay	*Cb* scaled units day^{-1}	Unif. (min = 0.025, max = 0.428)
σ	Incubation period (i.e. average time from infection to abortion)	Days	Unif. (min = 14, max = 67)
θ	Fraction of infected does that abort	NA.	Unif. (min = 0.05, max = 0.75)
γ	Recovery rate	Day^{-1}	Unif. (min = 1/42, max = 1/14)
ε_f	*Cb* quantity shed in faeces	*Cb* scaled units day^{-1}	Unif. (min = 1e-8, max = 2e-7)
ε_p	*Cb* quantity shed in products of conception	*Cb* scaled units	Unif. (min = 0.01, max = 0.2)

Cb: *Coxiella burnetii*.

Table 4.4: Metrics and range of acceptance used in the ABC rejection-sampler.

Metric	Level	Range of acceptance
Prevalence of shedding at kidding[a]	Parity 1	0.12 to 0.22
	Parity 2	0.14 to 0.28
	Parity 3+	0.06 to 0.15
Failure to kid risk [b]		0.04 to 0.08

[a] 95% CI for shedding prevalence stratified by age were obtained from (Canevari et al., 2018).
[b] 95% CI for failure to kid risk (i.e. the number of does with a positive pregnancy diagnosis during the dry period with no kidding event recorded up to 180 days later) was used as a proxy for abortion risk.

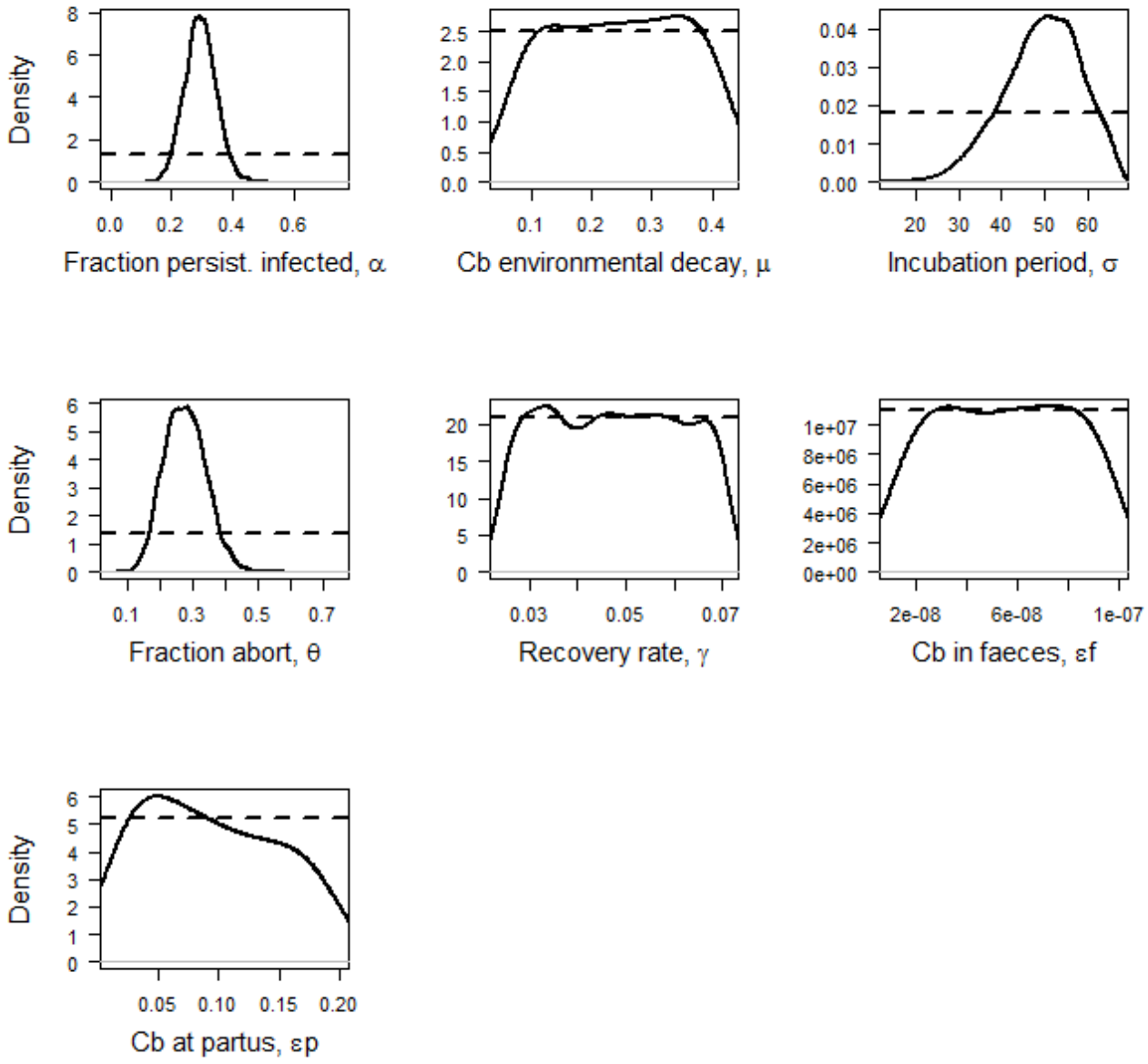

Figure 4.2: Kernel density estimates for the marginals of the approximate posterior distributions of the seven model parameters included in the ABC. Dashed lines show prior distributions from which parameter candidates where sampled. Uniform distributions were used as priors. Parameters θ, α and σ show a peaked distribution that substantially differs from their priors, indicating these parameters were informed by the data. Lower values of ε_p were better supported by the data.

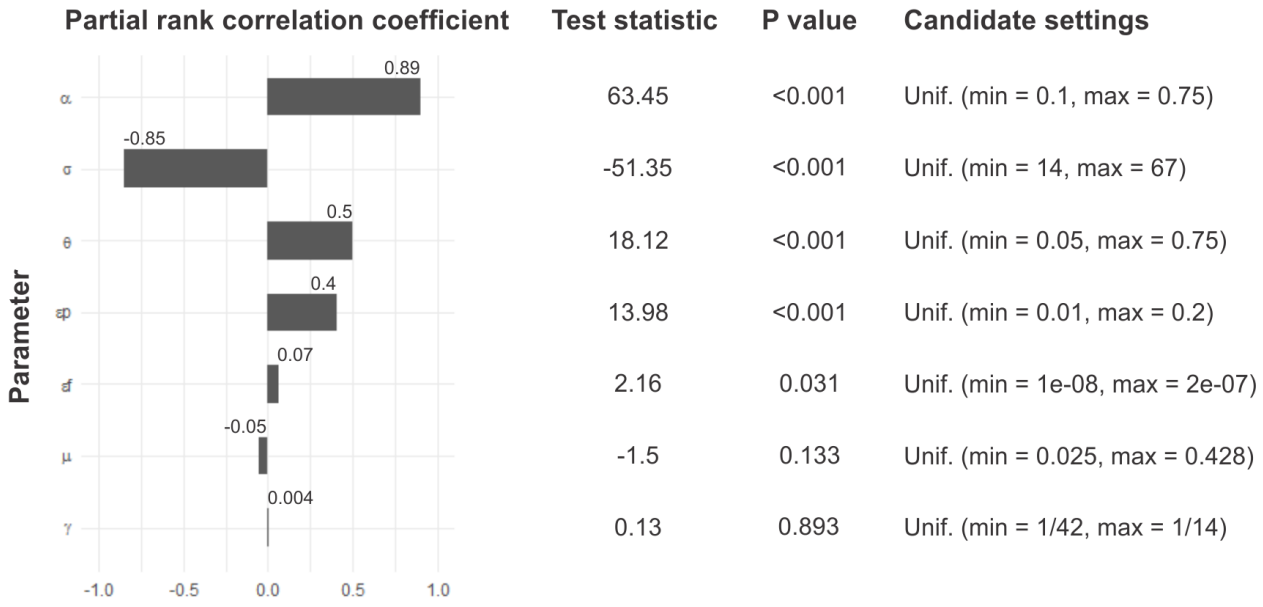

Figure 4.3: Tornado plot showing estimated partial rank correlation coefficients (PRCC) for parameters included in the sensitivity analysis. The statistical significance of a non-zero PRCC value was tested using a t test statistic with $N-2$ degrees of freedom, where N equals the number of model runs.

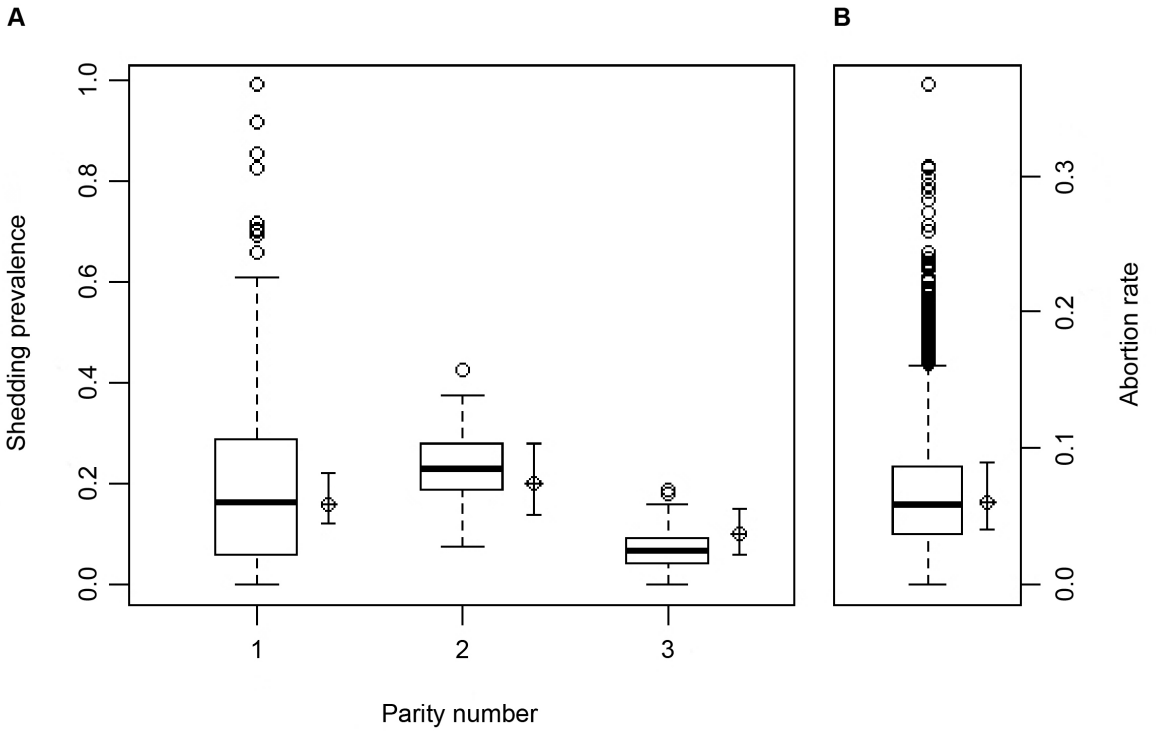

Figure 4.4: Comparison between posterior predictive distributions (box and whisker plots) and observed data (error bar plots) for: **A.** Prevalence of *C. burnetii* shedders at parturition for parities 1, 2 and 3 and above; and **B.** Abortion risk.

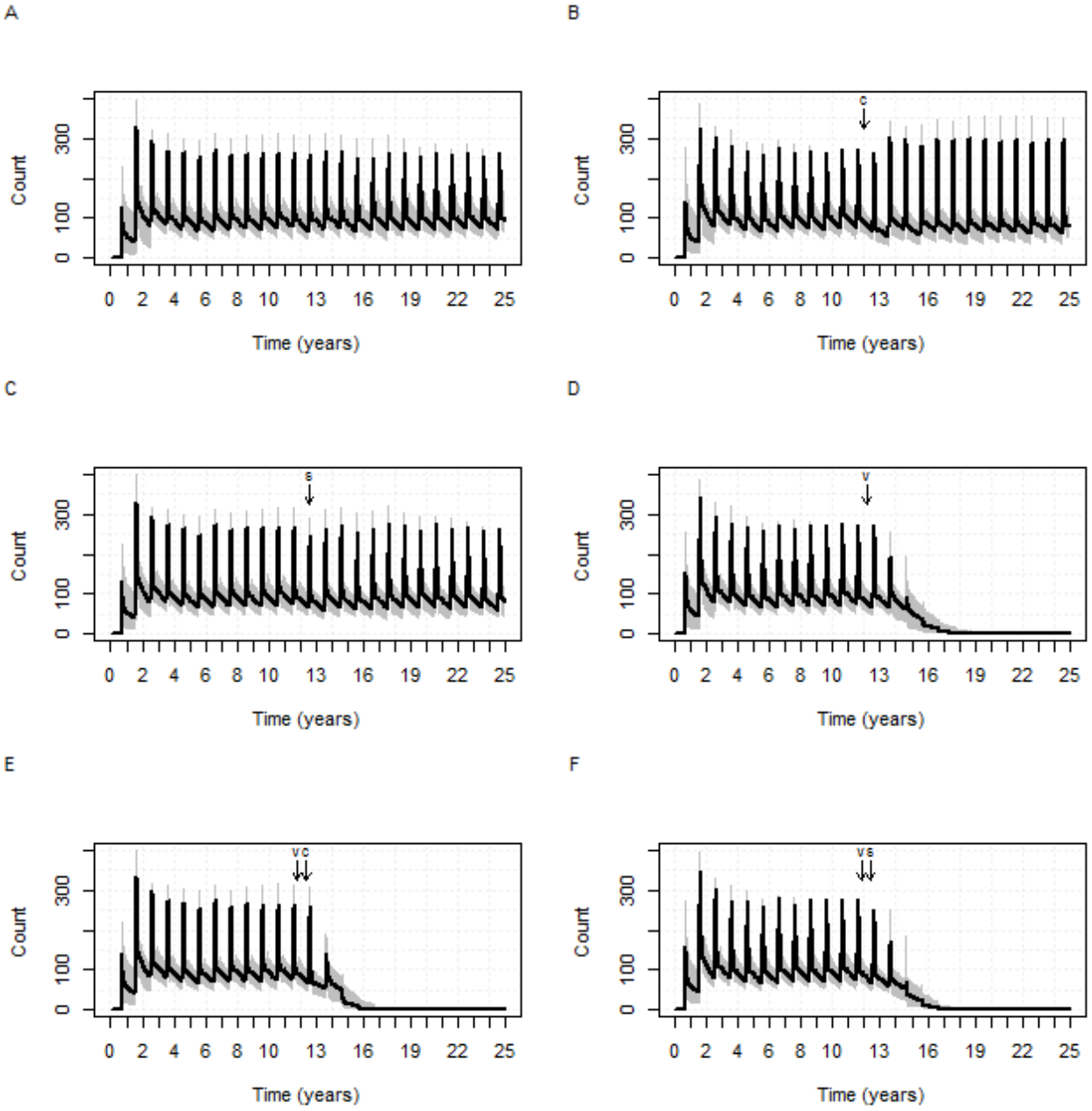

Figure 4.5: Line plots showing the median across 200 simulations of the total number of infected does as a function of the number of years since simulation start under six different control scenarios. Infection was seeded at the start of each simulation by including one infected pregnant doe into the herd at the peak of the joining season. The greyed areas show the 5% and 95% percentiles. Vertical arrows show starting point of control interventions: **A.** No intervention; **B.** Culling of *C. burnetii* shedders after parturition or abortion; **C.** Segregation of pregnant does after pregnancy diagnosis; **D.** Vaccination; **E.** Vaccination and culling of *C. burnetii* shedders after parturition or abortion; and **F.** Vaccination and segregation of pregnant does after pregnancy diagnosis.

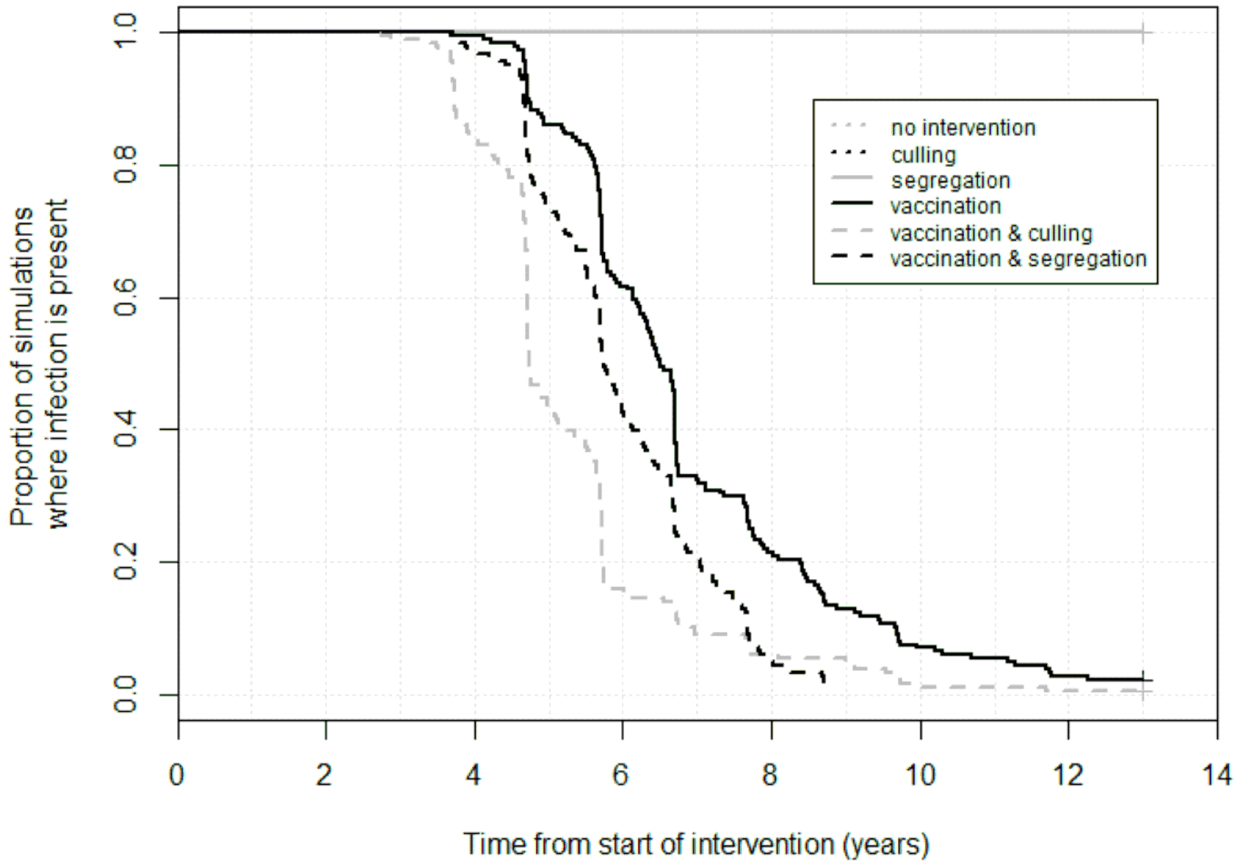

Figure 4.6: Kaplan-Meier survival curves showing the number of years from the start of intervention to disease eradication for no intervention and five alternative control and eradication strategies. Note that curves for the first three intervention listed in the legend are overlapped as no simulations under these interventions led to disease eradication.

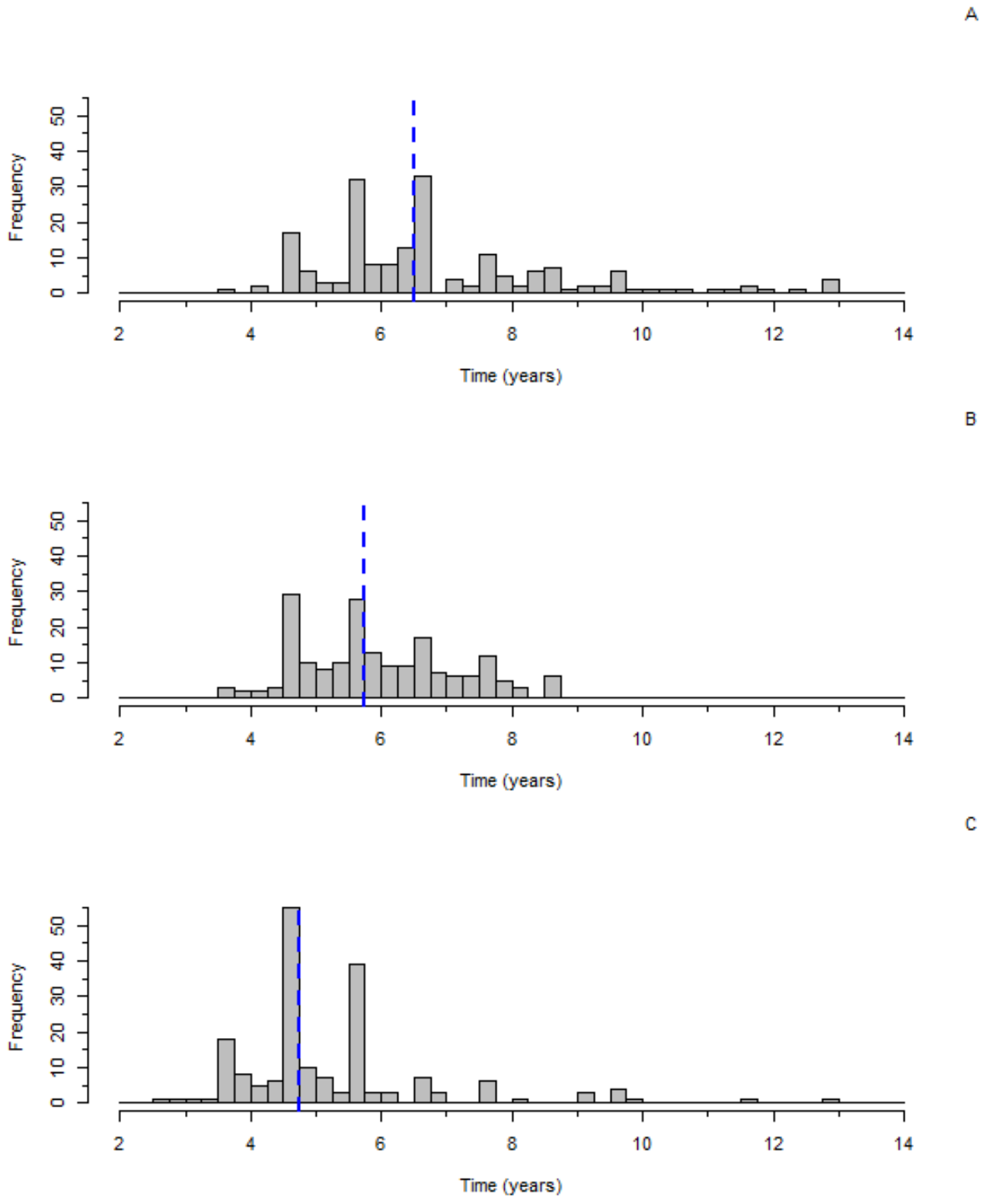

Figure 4.7: Distribution of time to eradication (in years) for three control and eradication strategies: **A.** Vaccination; **B.** Vaccination and segregation of pregnant does after pregnancy diagnosis; and **C.** Vaccination and culling of *C. burnetii* shedders after parturition or abortion. The vertical dashed lines on each plot indicate the median number of years to eradication.

4.4 Discussion

We present a model of within-herd Q fever transmission in intensively managed dairy goat herds. The model was used to assess the efficacy of a series control interventions. We parameterised the model using an ABC rejection-sampler algorithm and empirical data from an endemically infected herd. Our results show that vaccination would lead to eradication of Q fever in approximately six and half years in an endemically infected intensively managed dairy goat herd. Neither segregation of pregnant goats nor culling of shedders had a major impact on the incidence of disease when applied alone. However, when applied in combination with vaccination, both strategies reduced the predicted median time to eradication, with culling providing the greatest reduction. To the best of our knowledge this is the first study that has modelled Q fever in dairy goats using empirical data for validation. Also, this is the first study that has assessed the efficacy of segregation of pregnant does and culling of *C. burnetii* shedders combined with vaccination for Q fever control and eradication.

In a field study where vaccination was tested in a sheep flock with endemic Q fever, a significant decrease in abortion rates and incidence of shedding was observed already in the first two years following the start of vaccination. However, after four years of vaccination *C. burnetii* was still detected in environmental samples (Astobiza et al., 2011b). This is in agreement with our model predictions that show a rapid decline in the incidence of disease after vaccination followed by a long tail of very low incidence. The average extinction time predicted by a model developed by Bontje et al. (2016) ranged from 5 to 6 years when vaccination was applied either after detection of infection in bulk tank milk or after an abortion storm. In Bontje's model, persistently infected goats could only shed *Coxiella* in two consecutive parturitions. In our model, persistent shedding could occur in more than two consecutive parturitions. This could partially explain the differences obtained in model predictions, although differences in the model structure and in parameter values make direct comparisons difficult.

Segregation of pregnant does when used alone as a disease control strategy did not lead to disease eradication. On the other hand, combining vaccination with segregation of pregnant does was effective at eradicating Q fever from the herd. Further, the median time to eradication was reduced by 10 months compared with vaccination alone. An explanation for this difference is that the segregation of pregnant does prevents young replacements from getting infected as a result of exposure to the tail of peak environmental contamination occurring at each kidding season. When replacements become infected before their first pregnancy they will, on most occasions, become clear of infection over a relatively short period of time. However, a small proportion will become persistently infected, thus extending the time to eradication.

Separating does that are due to kid from the rest of the herd is a common practice in intensively managed dairy goat herds (Gunther et al., 2019). However, to the best of our knowledge, herd managers do not routinely apply strict biosecurity measures alongside segregation in a way that would effectively restrict dispersal of *C. burnetii* to surrounding areas (e.g. changing clothes and footwear before entering and leaving the kidding area, regular cleaning and disinfection, etc). Hence, real life scenarios may lay between total segregation and sharing of the same environment by kidders and the rest of the herd. In this sense, a study by Muleme et al. (2017b) found seroconversion for *Coxiella* to be higher around the kidding period in does that were not kidding but were kept near the pen where a group of does were kidding. Although we have shown isolation of kidders in the absence of vaccination does not have a marked impact on Q fever incidence, this measure should still be considered if its implementation can minimise exposure to contaminated environments by farm staff or facilitate disinfection of kidding areas.

The potential use of test and cull strategies for the control of Q fever in livestock has not been explored in depth. This is because serological status is not a good predictor of the presence of *C. burnetii* shedding at kidding (de Cremoux et al., 2012). The correlation between serological status and shedding has been shown to improve when measured in goats shedding comparatively higher concentrations of *C. burnetii* (Natale et al., 2012; Canevari et al., 2018). However, these observations were based on samples obtained at the time of delivery. Thus, there is still uncertainty about the accuracy of serology in predicting shedding status if the presence of antibodies were to be assessed with enough anticipation to allow culling of infected does before parturition. Testing vaginal swabs by qPCR at the time of delivery can be highly sensitive and specific, the caveat being shedding of *C. burnetii* can only be detected at exactly the time when environmental contamination occurs (Roest et al., 2012). However, considering that a fraction of shedding goats will become persistently infected and shed in at least two consecutive kidding events, we assessed whether elimination of *C. burnetii* shedders could still reduce environmental contamination and, consequently, disease transmission.

Culling goats that were shedders at parturition or abortion did not have a marked effect on the median number of infected goats over time. Nevertheless, when combined with vaccination it resulted in a reduction of approximately 21 months compared to vaccination only in the median time from start of vaccination to eradication. This was the most effective of the control interventions assessed. However, applying this strategy would imply an additional cost over vaccination alone associated with: i) the cost of sampling and qPCR testing all does at kidding; and ii) the requirement for an increased number of replacements to maintain herd size. A logistically reasonable sampling strategy would be to sample does in their first day of a new lactation. All does that abort should be culled given the likelihood of them being infected is relatively high. Although the cost of qPCR has decreased considerably, costs associated with testing many animals may still represent a limitation. With regards to the costs associated with replacing an increased number of removed animals, these would be concentrated in the first years the intervention is applied as in subsequent years the prevalence of shedding decays. The number of goats removed for other reasons is expected to decrease over time because the median age of the herd will be reduced following the higher culling rates that occur during the first years of the program. A profitable area of future research would be to expand the model presented here to quantify the economic impacts of different control interventions.

It has been shown that the quantity of *C. burnetii* shed at parturition by infected does follows a skewed distribution, with a relatively low number of does likely contributing a large proportion of environmental contamination (de Cremoux et al., 2012; Canevari et al., 2018). Differences in the quantity of *C. burnetii* shed were also observed in persistently shedding does, suggesting a reduction of shedding quantities in chronically infected individuals (Canevari et al., 2018). A limitation of the present study is that our model did not account for heterogeneous shedding patterns. When the incidence of disease is relatively high, individual doe-level effects (some does shedding high quantities of *C. burnetii* and others shedding low quantities) will tend to cancel the heterogeneity out. However, when the incidence of infection is low, heterogeneous shedding patterns may lead to complex model behaviours, including resurgent outbreaks (Grassly and Fraser, 2008). Thus, real life scenarios may result in a higher level of uncertainty than that captured by our model due to heterogeneous shedding dynamics.

In our model, demographic events were modelled as random samples from distributions fitted to empirical data of kidding dates and the length of productive life of does collated from a large dairy goat enterprise. Epidemiological parameters were estimated using an ABC rejection-sampler. However, not all epidemiological parameters could be identified by the ABC rejection-sampler. The rate of decay of *C. burnetii* in the environment

μ, the recovery rate of I_{NP} does γ, and the quantity of *C. burnetii* shed in faeces by infected goats ε_f, were not informed by the data. Nevertheless, epidemiological parameters that were found to be key drivers of disease dynamics in our sensitivity analysis were identified by the ABC rejection-sampler.

The quantity of *C. burnetii* shed by infected in goats in faeces is much lower than that shed in products of conception (Bontje et al., 2016). Further, our sensitivity analysis showed shedding in faeces had a relatively low impact on disease dynamics. It was therefore expected that the recovery rate of I_{NP} goats had no major impact on the behavior of the model, as the contribution to environmental contamination of goats within this category was via shedding in faeces. In our model, the endemic state was characterised by a relatively large prevalence of *C. burnetii* shedding goats at the time of parturition. This explains why new infections occurred as marked spikes during the kidding season. A relatively low number of susceptible does were left in the population after peaks of environmental contamination and until the herd was replenished with new susceptible does. With disease dynamics following this behaviour, differences in the persistence of *C. burnetii* in the environment within the ranges assessed had no major impact on disease transmission when no control interventions were applied.

In conclusion, out of the control strategies assessed in this study, only those including vaccination resulted in disease eradication. Consistent with previous studies, our model shows that vaccination should be considered as a relatively long term intervention. The combination of vaccination with segregation of pregnant does or culling of does shedding *C. burnetii* at the time of delivery resulted in a reduction of time to eradication. The shortest time to eradication was observed with the combined strategy vaccination and culling of *C. burnetii* shedders at delivery.

Acknowledgements

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Chapter 5

Modelling test and cull strategies for the control of Q fever in an intensively managed dairy goat herd

Abstract – Q fever is caused by *Coxiella burnetii*, a highly infectious bacterium capable of infecting many animal species, including humans. Following infection, goats can shed large quantities of *C. burnetii* with products of conception. This is the main source of environmental contamination in infected herds and therefore the main driver of infection. The quantities of *C. burnetii* shed by infected goats at the time of delivery is highly skewed, with a small number of goats likely being responsible for most of the environmental contamination. In this simulation study we assessed whether control strategies aiming at detection and removal of high-shedding does before parturition or abortion could lead to disease eradication from an endemically infected intensively managed dairy goat herd. In the absence of scientific literature providing evidence on the accuracy of available tests for the detection of *C. burnetii* shedding does before parturition or abortion, we assessed several scenarios with varying test accuracy assumptions. We found that test and remove strategies using a test with perfect specificity and 90% sensitivity would lead to disease eradication in approximately 7 years. Our results provide support for the development of field studies aiming at further clarifying the potential role of test and remove interventions for the control of Q fever in dairy goats.

5.1 Introduction

Goats have been identified as the source of numerous human Q fever outbreaks (Panaiotov et al., 2009; Roest et al., 2011a; Bond et al., 2016). When does are infected with *C. burnetii* they can develop placentitis and shed large quantities of *Coxiella* into the environment at either the time of abortion or the time of kidding (Roest et al., 2012; Sánchez et al., 2006). Inhalation of particles contaminated with *Coxiella* is the main route of infection for both humans and animals (Lang, 1990). Quantities of *Coxiella* eliminated by naturally infected does at the time of parturition have been found to be highly skewed, with a relatively low proportion of *Coxiella*-positive does likely to be contributing to the majority of environmental contamination (Sting et al., 2013; Canevari et al., 2018). This being the case, if high-shedder does can be identified and removed before parturition or the time of abortion a major reduction in *C. burnetii* environmental contamination would be expected. This strategy could potentially lead to eradication of coxiellosis from endemically infected herds.

Test and removal strategies using serological tests for the detection of infected animals have played an important role in disease control and eradication campaigns such as bovine tuberculosis and bovine brucellosis in Australia (Chamberlin, 1985; More et al., 2015) and other countries (Ragan, 2002). The question of whether a test and removal strategy could be used for Q fever control and eradication in domestic ruminant populations has been raised by other authors (Angelakis and Raoult, 2010; Hogerwerf et al., 2014). Several studies have shown poor agreement between serology and shedding of *C. burnetii* at kidding (Natale et al., 2012; Canevari et al., 2018). In contrast, agreement between the presence of antibodies as detected by immuno-fluorescence assay (IFA) and shedding status determined by quantitative Polymerase Chain Reaction (qPCR) in vaginal swabs improved within subgroups of high-shedding does (Canevari et al., 2018). This suggests that does with a more severe intrauterine infection are more likely to have detectable antibody levels. In the study of Canevari and colleagues (2018) 71% of does identified as high-shedders at parturition were positive to IFA. On the other hand, the percentage of low-shedders that were positive to IFA was 39% and, of the does that were negative to qPCR on vaginal swabs, 27% returned a positive result to IFA.

The probability of early detection of shedding animals by means of serology could be affected by the lag between infection and the development of circulating antibodies at detectable levels. In an experimental study where goats were challenged with *C. burnetii* seroconversion started around the third week post inoculation (Arricau Bouvery et al., 2003). Similarly, Roest et al. (2013b) identified a rise in antibody levels 2 to 3 weeks post infection. In both of these challenge studies all inoculated goats developed antibodies. Once antibodies have developed, the time circulating antibodies persist at detectable levels could impact on the accuracy of serological tests to detect *C. burnetii* shedders. There is limited knowledge on the duration of detectable antibody levels following *C. burnetii* infection in goats. In a longitudinal study carried out in an endemic dairy goat herd, Muleme et al. (2017a) found a high degree of variability in the duration of antibody presence in kids followed from birth until the date of their first parturition. Some of the kids in the Muleme study had antibodies against *C. burnetii* detectable up to 12 months of age.

Early detection of 'super shedders' by means of diagnostic techniques other than serology could also be attempted. *C. burnetii* DNA can be detected in serum during the acute phase of Q fever infection in humans (Vincent et al., 2015) and this has been proposed as a method for early detection of infection (Turra et al., 2006; Schneeberger et al., 2010). Detection of *C. burnetii* in blood has also been attempted in livestock. Das et al. (2014) isolated *C. burnetii* in blood samples from cows and buffaloes with a history of reproductive disorders. Further, Roest et al. (2012) detected *C. burnetii* DNA in the blood of does experimentally challenged on day 76 of gestation (Roest et al., 2012). The analytic sensitivity of *com1* and IS*1111* qPCR assays has been found to be remarkably high when used in DNA extractions from human blood, with a limit of detection found to be approximately one copy of the target gene. Also, no false positive amplification signal was observed in specimens spiked with a number of intracellular bacteria (Lockhart et al., 2011). An additional advantage of the use of qPCR in blood is that the risk of cross-contamination at the time of sampling is negligible. For comparison, when a large number of goats are sampled using vaginal swabs there may be an increased risk of accidental sample contamination from either lapses in sampling technique or from environmental contamination. Because of the lower risk of cross contamination, it would be reasonable to expect a low number of false positive results to qPCR in blood. A drawback of serology is that if it is implemented alongside vaccination there is a requirement that one should be able to distinguish antibody responses from vaccination with antibody responses from natural infection (in effect, a DIVA strategy[1]). Contrarily, test and remove interventions based on the use

[1]DIVA: Differentiation of infected from vaccinated animals.

of PCR would not be affected by vaccination.

The objective of this study was to determine if detection and subsequent removal of high-shedding does alone would lead to *C. burnetii* eradication from intensively managed endemically infected dairy goat herds. To this end, we modified the mathematical model of within-farm *C. burnetii* transmission presented in Chapter 4 to account for heterogeneous *C. burnetii* shedding patterns. We assessed the potential efficacy of test and cull interventions based on the use of IFA or qPCR testing of blood samples obtained before the expected kidding date. Given the uncertainty around the accuracy of these tests for early detection of shedders, multiple scenarios with a range of diagnostic test accuracy assumptions were studied. By doing so, we aimed to determine how accurate a diagnostic test would have to be for a test and cull strategy to achieve eradication of coxiellosis from a herd.

5.2 Materials and methods

5.2.1 Model description

The mathematical model presented in Chapter 4 was modified to incorporate heterogeneous *C. burnetii* shedding patterns. We assumed that there were three classes of *C. burnetii* infected does at the time of delivery: (1) those that delivered a kid at full term and were low *Coxiella* shedders; (2) those that delivered a kid at full term and were high *Coxiella* shedders; and (3) does that aborted due to infection with *C. burnetii*. We assumed that all of the aborted does went on to become high shedders. The probability of a doe being a high-shedder was determined by the parameter λ, which follows a *Beta* distribution centered on 0.2 with 95% of the distribution's density less than 35%. These assumptions were based on our analyses of the *C. burnetii* genome equivalent (GE) data presented in Chapter 3. A schematic representation of the model showing the inclusion of shedding categories is shown in Figure 5.1. As in the first model version presented in Chapter 4, abortions can only occur in the last third of gestation. If no control interventions are in place, goats that abort remain in the herd until their removal date.

An additional modification to the model presented in Chapter 4 was the inclusion of a contact parameter F in the transmission terms (Equation 5.1). The contact parameter F modulates the level of contact with environmental contamination. This approach for modelling infection from the environment is similar to that described by Kirkeby et al. (2016) who modelled paratuberculosis transmission in cattle accounting for heterogeneous shedding patterns. The transmission terms were then as follows:

$$\begin{aligned} &S_P \times (1 - (1/exp(E \times F))), \\ &S_{NP} \times (1 - (1/exp(E \times F))). \end{aligned} \tag{5.1}$$

In Equation 5.1 E corresponds to the environmental bacterial load at time t expressed in *C. burnetii* infectious doses. S_P and S_{NP} are the number of susceptible pregnant and susceptible non-pregnant does, respectively. The value of F was calibrated so that in the endemic state the age-stratified prevalence of *C. burnetii* shedding at kidding was in agreement with the overall age-stratified prevalence of shedding at kidding reported in Chapter 3.

5.2.2 Model parameters

The epidemiological parameters used for this study are shown in Table 5.1. Demographic parameters were the same as presented in Chapter 4. For parameters that were identified by the ABC rejection-sampler in Chapter 4 (i.e. α, σ and θ), distributions were fit to approximate marginal posteriors using the contributed *R* package *fitdistrplus* (Delignette-Muller and Dutang, 2015). The parameter μ, governing the mortality rate of *C. burnetii* in the environment, was set to 0.05 scaled *C. burnetii* units day^{-1} (equivalent to 95% decay over 2 months) based on (Álvarez-Alonso et al., 2018). The parameter γ, defining the recovery rate of infected non-pregnant does was set to 0.035 day^{-1}. The average duration of infection in non-pregnant does was then 4 weeks (Bontje et al., 2016). The recovery of infected pregnant does was modelled as a non-Markov event since it was assumed clearing of infection could only occur after delivery. The time from infection to development of detectable antibodies was set to 3 weeks as supported by *C. burnetii* challenge studies in goats (Arricau Bouvery et al., 2003; Roest et al., 2013b). Waning of antibodies was not included in the model as long-term duration of antibodies to *C. burnetii* has been described in goats (i.e. up to one year from onset of detection) (Muleme et al., 2017a). Moreover, re-exposure from the environment in an endemic scenario would be expected to favour the persistence of circulating antibodies over time.

The quantity of *C. burnetii* shed by infected does at the time of delivery has been shown to be highly variable. The analysis of qPCR results obtained in the study presented in Chapter 3 showed the median concentration of *C. burnetii* GE in vaginal swabs from the combined group of aborted does and full term kidders classified as high shedders was 10,000 times greater than that found in full term kidders classified as low shedders. A similar order of magnitude in the range of variation in *C. burnetii* shedding concentrations were found in vaginal mucus from infected cattle (Guatteo et al., 2007). In a within-herd transmission model of Q fever in cattle published by Courcoul et al. (2011b), high shedder cows were assumed to shed 3000 times more than low shedder cows. In our model, based on the empirical data presented in Chapter 3 high *Coxiella* shedder does were assumed to shed 10,000 times more than low *Coxiella* shedder does. The quantity of *C. burnetii* shed in faeces by infected does was set to a small fraction of that shed in products of conception based on (Bontje et al., 2016) (i.e. in the order of 10^6 times less).

5.2.3 Simulation conditions

Model simulations were started with 780 does on calendar day 103 (peak of the joining season). The herd was replenished with weaned does up to a maximum of 900 individuals two months after the end of each kidding season. At the time of entry, each doe had its removal date sampled from a distribution fitted to the observed length of productive life in the study herd (see Figure C.1-A). Infection was seeded into the herd by introducing one infected pregnant doe at the start of each simulation. To increase the chance of one infected doe leading to an outbreak, the introduced infected doe was assumed to abort. The model was coded in continuous time. To assess control interventions, a burn-in period of 12 years was used to ensure an endemic disease state had been reached. We ran 200 model iterations for the assessment of disease dynamics in the absence of interventions, and 200 model iterations for each of the control strategies assessed.

5.2.4 Control interventions

Eradication was defined as the absence of infected does and an environmental contamination below 10^{-6} *C. burnetii* scaled units. Each model simulation was stopped when eradication was achieved or at 30 years from the beginning of the simulation, whatever occurred first. Results from both IFA and qPCR testing were assumed to be available one week after the date of sampling. When a goat returned a positive result, she was immediately removed from the herd and replaced with a weaned kid two months after the end of each kidding season as with exits that occur for other reasons. The proportion of simulations that resulted in eradication, time to disease eradication (in years), number of does tested and the number of does culled were recorded. Details of each of the scenarios assessed are presented below.

Test and cull based on detection of antibodies using IFA

To assess the efficacy of test and remove interventions based on IFA testing, three scenarios with varying probabilities of detection of high *Coxiella* shedders, low *Coxiella* shedders and non-shedders by IFA were used (see Table 5.2, scenarios A, B and C). These scenarios were based on the estimated probability of detection for each shedding category found in Chapter 3. Also, two different testing times were assessed, namely 4 and 6 weeks prior to the start of each kidding season, which in the model occurs approximately on day 235 each year. Thus, a total of 6 different test and remove strategies using IFA were assessed (i.e. 3 test accuracy scenarios for each of the 2 testing times). A baseline (i.e. no intervention) scenario was included as reference.

Test and cull based on detection of *C. burentii* DNA in blood using qPCR

Only does with relatively severe intrauterine infection — here assumed to be high *Coxiella* shedders or does that abort — were modelled to have circulating *C. burnetii* DNA in blood at detectable levels when approaching parturition. Low *Coxiella* shedders were assumed to be not detected by the test. A minimum of three weeks from the time of infection to the presence of detectable *Coxiella* DNA in blood was assumed. The specificity of the test was set to 100%. A range of sensitivity values starting from 0.5 and up to 0.99 were assessed. As with test and cull based on IFA, two different testing times were assessed (i.e. 4 and 6 weeks prior the start of the kidding season). A total of 18 combinations of testing time and test accuracy were assessed. A reference scenario with no interventions was also included in the analysis.

5.3 Results

The predicted age-stratified prevalence of *C. burnetii* shedding at the time of kidding in the absence of interventions is shown in Figure 5.2. A peak in prevalence of shedding at kidding was observed in the second year after the introduction of an infected, aborting doe. The overall median prevalence of *C. burnetii* shedding at kidding across all parities stabilised from year three onward with a median close to 15%. In the endemic state, second parity does showed the highest median prevalence of *C. burnetii* shedding at kidding compared with first parity does and third and above parity does. From year three onward, a marked decrease in the prevalence of *Coxiella* shedding at kidding was observed in third and above parity does, which reflects acquisition of immunity from previous exposure in this age category, consistent with the qPCR results presented in Figure B.2 in Chapter 3.

Results from the test and remove simulations using IFA are shown in Table 5.2. When no intervention was applied, all simulations led to an endemic state. As expected, the efficacy of test and cull shortened the interval from incursion to eradication for scenarios where the values for test accuracy were higher. The proportion of simulations that led to disease eradication within 5 and 10 years increased when testing occurred 6 weeks prior to the start of the kidding season compared with simulations where testing occurred 4 weeks prior to the start of the kidding season. However, the efficacy of IFA testing was relatively low in all scenarios assessed, with at the most 65% of simulations achieving eradication within 10 years.

Results from test and remove simulations using qPCR are shown in Table 5.3. When test and remove interventions were modelled to occur four weeks prior to the start of the kidding season only those scenarios with almost perfect sensitivity (i.e. 99%) led to eradication in more than half of the simulations, with up to 71% of the simulation leading to eradication by year 10 from start of testing. When the testing date was brought forward to 6 weeks prior to the start of kidding the efficacy of the qPCR test and removal was improved and scenarios with sensitivities of 90% or greater led to eradication in above 70% of the simulations and up to 100% by year 10 from the start of testing.

Figure 5.3 shows how as testing time is moved closer to the start of the kidding season abortions can occur, resulting in environmental contamination and new infections. For scenarios where eradication was achieved, the time to disease eradication ranged from 3.2 to 14.7 years. Using an almost perfect test for detecting high *Coxiella* shedders (i.e. 99% sensitivity and 100% specificity) 6 weeks prior to the start of the kidding season the median time to disease eradication was 3.2 years (Q_1 2.7, Q_3 3.7). The median number of animals culled was higher for interventions based on IFA than those based on qPCR, which is an expected result given the relatively low specificity of IFA.

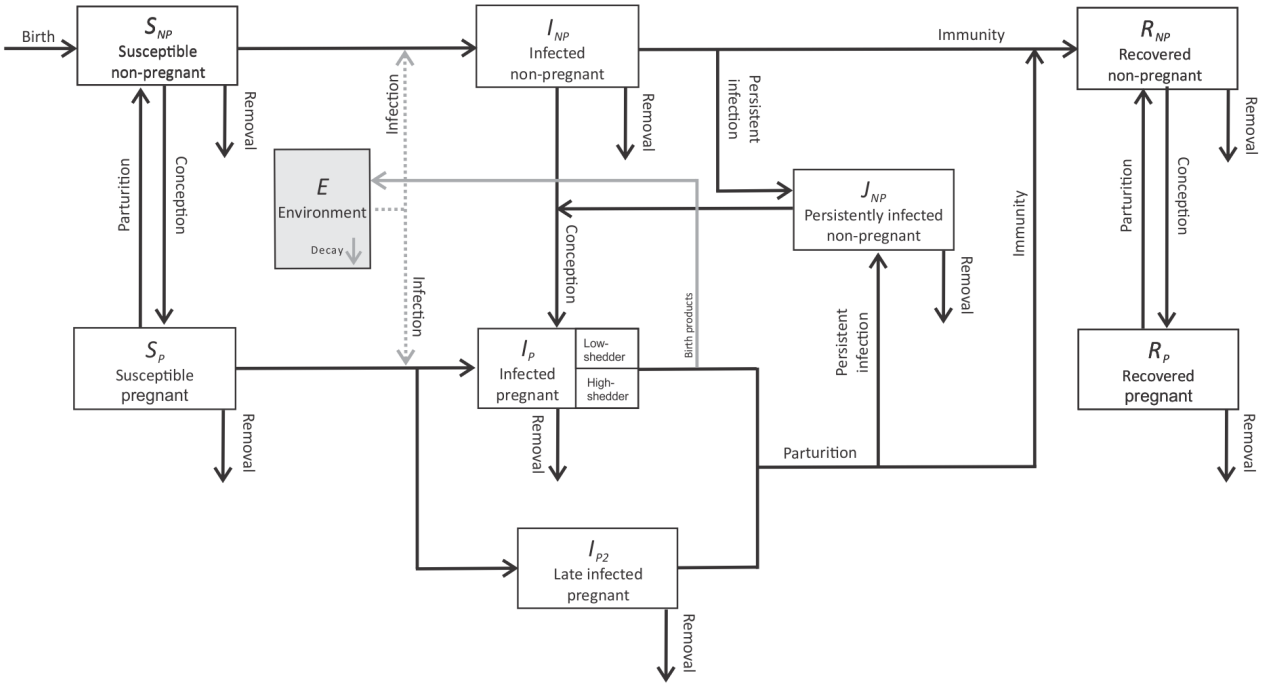

Figure 5.1: Schematic representation of model structure accounting for heterogeneous shedding. The underlying structure of the model is the same as that presented in Chapter 4 except that I_P does can either behave as high-shedders or low-shedders at the time of delivery. Arrows indicating shedding in faeces were removed to facilitate interpretation. All infected does (i.e. I_{NP}, I_P, I_{P2} and J_{NP}) were assumed to shed a relatively small quantity of *C. burnetii* to the environment in faeces.

Table 5.1: Description of epidemiological parameters

Paramater	Description	Units	Value
α	Fraction of infected does that become persistently infected	n.a.	Beta (shape1 = 18.8, shape2 = 43.8)
μ	*Cb** mortality rate in the environment	*Cb* scaled units day^{-1}	0.05
σ	Incubation period (i.e. average time from infection to abortion)	Days	Gamma (shape = 15.3, scale = 2.9)
θ	Fraction of infected does that abort	NA	Beta (shape1 = 18.8, shape2 = 43.8)
γ	Recovery rate	day^{-1}	0.035
$\varepsilon_p H$	*Cb* quantity shed in products of conception by high-shedders	*Cb* scaled units	0.3
$\varepsilon_p L$	*Cb* quantity shed in products of conception by low-shedders	*Cb* scaled units	3e-05
ε_f	*Cb* quantity shed in faeces	*Cb* scaled units	9.5e-08
λ	Fraction of infected that become high *Cb* shedders	NA	Beta (shape1 = 5.1, shape2 = 17.6)
F	Contact parameter	NA	0.04

Cb stands for *Coxiella burnetii*.

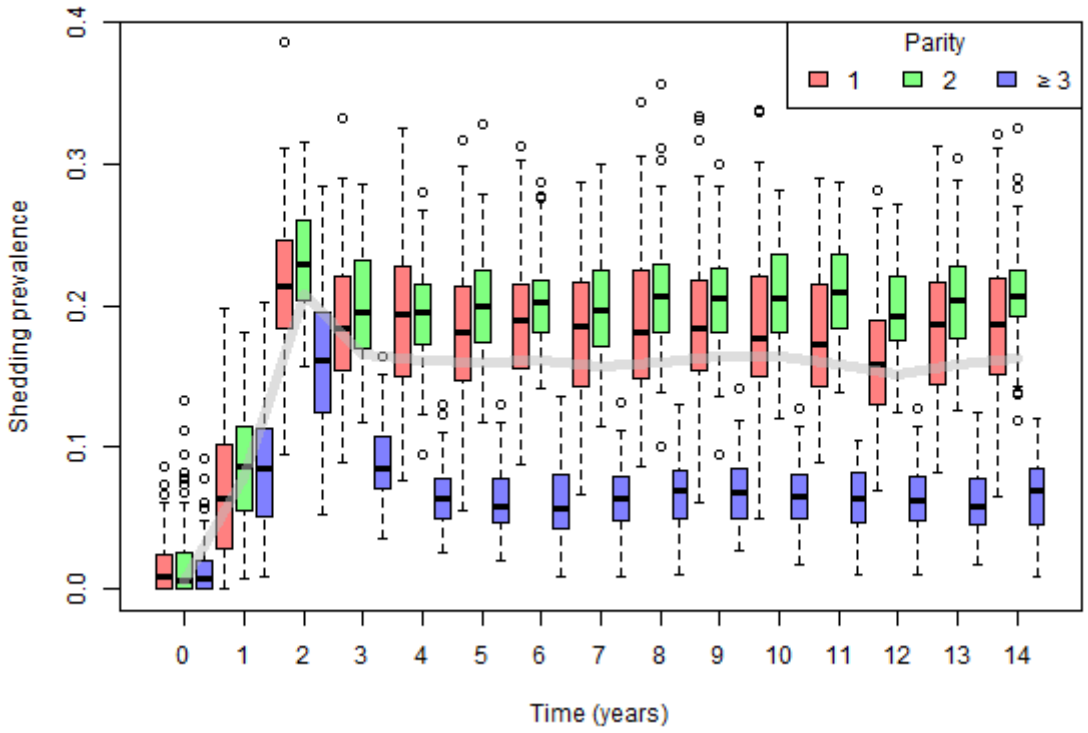

Figure 5.2: Model predictions for the prevalence of *C. burnetii* shedding at kidding (expressed as the number of does shedding at the time of kidding divided by the total number of kidding does) stratified by parity number for the baseline run with no interventions. The light gray line shows the overall median prevalence of shedding at kidding across all parities. Results from 200 model iterations.

Table 5.2: Values of probability of returning a positive IFA result for high-shedders (HS), low-shedders (LS) and non-shedding (NS) does for the three different scenarios assessed and predicted efficacy of test and remove interventions. Results from 200 model simulations.

Scenario	Probability of detection HS	Probability of detection LS	Probability of detection NS
A	0.45	0.27	0.32
B	0.71	0.39	0.27
C	0.88	0.52	0.21

Simulations							
Intervention	Scenario	Testing time (weeks prior kidding)	% 5-year extinction (95% CI)	% 10-year extinction (95% CI)	Median extinction time (Q1, Q3)*	Median number tested (Q1, Q3)	Median number culled (Q1, Q3)
Baseline	-	-	0 (0, 1.9)	0 (0, 1.9)	-	-	-
Test & remove	A	4	0 (0, 1.9)	0 (0; 0, 1.9)	-	10725 (10681, 10763)	3417 (3384, 3454)
	B	4	0 (0, 1.9)	0.5 (0.1, 2.8)	7.4 (7.4, 7.4)	10689 (10653, 10720)	3202 (3156, 3239)
	C	4	5.5 (3.1, 9.6)	12.5 (8.6, 17.8)	10.7 (6.7, 14.9)	10632 (9900, 10674)	2944 (2686, 2987)
	A	6	0 (0, 1.9)	0 (0, 1.9)	-	10895 (10866, 10936)	3503 (3470, 3540)
	B	6	1.5 (0.5, 4.3)	3 (1.4, 6.4)	5.5 (3.6, 7.7)	10864 (10824, 10913)	3256 (3221, 3292)
	C	6	35.5 (29.2, 42.3)	65 (58.2, 71.3)	6 (3.8, 9.7)	4226 (2812, 8528)	1163 (706, 2251)

* Estimation (in years) based on simulations where eradication was achieved.

Table 5.3: Predicted efficacy of culling pregnant goats returning a positive result to qPCR in blood 4 and 6 weeks prior the start of each kidding season under different assay sensitivity assumptions. Results from 200 model simulations.

Intervention	Sensitivity of the test	Testing time (weeks prior kidding)	% 5-year extinction (95% CI)	% 10-year extinction (95% CI)	Median extinction time (Q1, Q3)*	Median number tested (Q1, Q3)	Median number culled (Q1, Q3)
Baseline	-	-	0 (0, 1.9)	0 (0; 0, 1.9)	-	-	-
Test Cull	0.5	4	0 (0, 1.9)	0 (0, 1.9)	-	10171 (10137, 10201)	283 (272, 293)
	0.65	4	0 (0, 1.9)	0 (0, 1.9)	-	10189 (10157, 10233)	357 (346, 370)
	0.7	4	0 (0, 1.9)	0 (0, 1.9)	-	10191 (10156, 10220)	379 (366, 391)
	0.75	4	0 (0, 1.9)	0 (0, 1.9)	-	10198 (10162, 10231)	401 (386, 417)
	0.8	4	1 (0.3, 3.6)	1.5 (0.5, 4.3)	5.6 (4.7, 13.7)	10197 (10157, 10230)	419 (402, 438)
	0.85	4	0.5 (0.1, 2.8)	2.5 (1.1, 5.7)	14.7 (9.7, 15.6)	10200 (10158, 10235)	434 (409, 454)
	0.9	4	5.5 (3.1, 9.6)	16.5 (12, 22.3)	9.7 (6.7, 14.4)	10188 (9120, 10237)	438 (350, 465)
	0.95	4	17.5 (12.9, 23.4)	41.5 (34.9, 48.4)	7.5 (4.6, 10.1)	9092 (3434, 10206)	352 (123, 458)
	0.99	4	30 (24.1, 36.7)	71 (64.4, 76.8)	5.9 (4.3, 9)	3942 (2799, 7395)	130 (70, 259)
	0.5	6	0 (0, 1.9)	0 (0, 1.9)	-	10359 (10321, 10402)	286 (273, 300)
	0.65	6	0 (0, 1.9)	0 (0, 1.9)	-	10384 (10353, 10417)	362 (349, 374)
	0.7	6	0 (0, 1.9)	1 (0.3, 3.6)	7.8 (7.3, 8.2)	10380 (10352, 10412)	375 (361, 386)
	0.75	6	0.5 (0.1, 2.8)	2.5 (1.1, 5.7)	6.7 (6.3, 8.5)	10384 (10345, 10425)	391 (372, 406)
	0.8	6	1.5 (0.5, 4.3)	8.5 (5.4, 13.2)	9 (5.8, 15.7)	10378 (10343, 10418)	394 (359, 416)
	0.85	6	9.5 (6.2, 14.4)	29.5 (23.6, 36.2)	7.7 (5.5, 11.8)	10336 (5150, 10398)	339 (144, 396)
	0.9	6	22 (16.8, 28.2)	70.5 (63.8, 76.4)	6.7 (5, 8.7)	4078 (3416, 6512)	118 (70, 198)
	0.95	6	65.5 (58.7, 71.7)	94.5 (90.4, 96.9)	3.9 (3.5, 5.8)	2334 (2261, 3452)	61 (45, 91)
	0.99	6	94.5 (90.4, 96.9)	100 (98.1, 100)	3.2 (2.7, 3.7)	1753 (1724, 2294)	43 (37, 51)

* Estimation based on simulations where eradication was achieved.

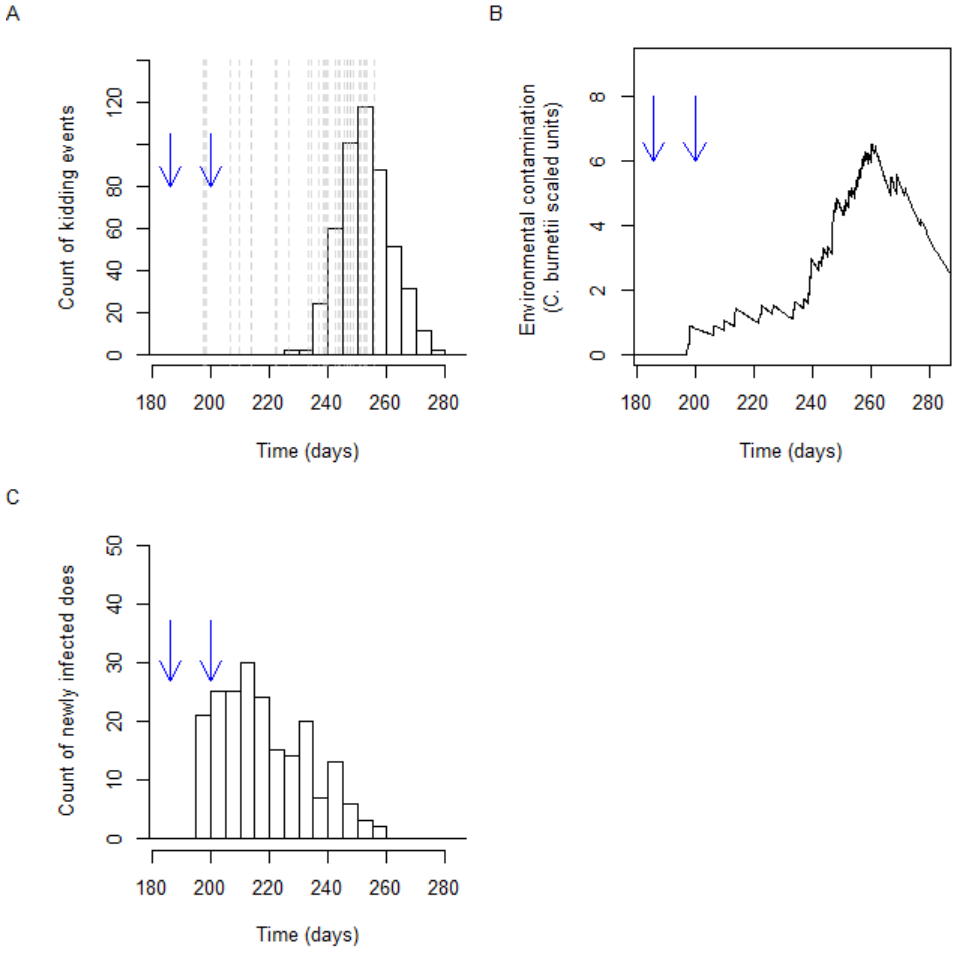

Figure 5.3: Results from a single model iteration with no interventions showing the relevance of time of testing for the success of test and remove interventions. Time in the x axis represents calendar time (i.e. days from 1st of January) on year 12 from the start of the simulation. **A**. Frequency histogram of kidding events. Vertical dashed lines show the time of abortions. **B**. Line plot showing environmental contamination over time. **C**. Frequency histogram of new infections. Vertical arrows show the two testing times assessed in this study (i.e. 4 and 6 weeks prior the start of each kidding season). When testing was carried out 4 weeks prior the start of the kidding season (arrow on the right in each plot) abortions had already started, leading to environmental contamination and new infections.

5.4 Discussion

Our study shows that test and cull interventions based on identification of high *Coxiella* shedding does before parturition or abortion can successfully eradicate coxiellosis from an endemic dairy goat herd. To our knowledge, this is the first study to quantify the efficacy of test and cull control interventions for Q fever in dairy goats based on early detection of shedders. Tests with relatively high sensitivity (i.e. 90% or above) were required to achieve eradication within ten years in more than two-thirds of the simulations. The median time to eradication with a 90% sensitive test was comparable with that estimated for vaccination in Chapter 4.

The predicted overall prevalence of *C. burnetii* shedding at kidding in the endemic state from our model was consistent with the mean prevalence of shedding found in the panel study presented in Chapter 3. Moreover, the predicted age stratified prevalence of *C. burnetii* shedding at kidding in the endemic state was highest in second parity does and then decreased for does in their third or greater parity, which is again consistent with our field observations. The predicted decrease in prevalence of shedding at kidding based on the model was greater than that observed in our panel study. An underestimation of prevalence of shedding at kidding in does in their third or greater parity was also observed in the original model presented in Chapter 4 (Figure 4.4). An explanation for this difference may be that immunity acquired after natural infection is not 100% effective as assumed in the model. Recognising this issue, a modification of the model to account for does transitioning back to a susceptible state after recovery could be included. However, quantifying this probability may be challenging as re-infections and chronic infections would be difficult to distinguish under field conditions.

Empirical data collected in the study presented in Chapter 3 was used to estimate the probability of detection of shedders by IFA. The 95% CI estimates for the probability of obtaining a positive IFA result stratified by shedding category were used to develop three scenarios. Only the best case scenario (scenario C) led to more than half of the simulations resulting in disease eradication in less than 10 years when testing was carried out 6 weeks prior to the start of the kidding season. The efficacy of test and cull using IFA improved when testing was timed to occur 6 weeks prior to the start of each kidding season compared with testing at 4 weeks. Test and remove interventions in Q fever endemic dairy goat herds should then be timed to occur before abortion high-risk periods. This highlights the importance of recording incident abortion events. This would allow high abortion-risk periods to be identified, which in turn would identify the most appropriate times for testing.

In the absence of empirical data reporting the accuracy of PCR in blood as a means for detecting *C. burnetii* shedders, the efficacy of test and remove interventions based on PCR can only be assessed at a theoretical level. Under the model assumptions, if high *Coxiella* shedders could be detected with a sensitivity of 90% or above it would be feasible to eradicate Q fever from an endemic herd by applying a test and remove intervention. Tests other than PCR in blood could also be considered for test and remove interventions. For example, in humans, increased white blood cell counts and high levels of C-reactive protein in blood were found to be good predictors of an inflammation of the foetal membranes (amnion and chorion) due to a bacterial infection (Popowski et al., 2011). It is not known if similar associations occur in does. Identification and removal of high *Coxiella* shedding does alone prior to parturition or abortion would lead to disease eradication. A key determinant of the success of test and remove strategies is the ability to detect high-shedding does.

Other authors have considered the potential use of test and cull interventions for the control of Q fever in goats. The accuracy of qPCR in vaginal mucus and both qPCR and ELISA in milk as a means of detection of intrauterine infection in pregnant does was assessed by Hogerwerf et al. (2014). Hogerwerf and collaborators collected uterine fluid, vaginal mucus and milk samples from 203 does that belonged to three herds culled during

the Q fever outbreak response in The Netherlands in 2010. All samples were obtained at the time of culling. The qPCR results in uterine fluid were used as a reference to estimate the accuracy of tests used in vaginal mucus and in milk for detection of intrauterine infection. Almost all vaginal mucus samples were positive to qPCR (i.e. 200 out of 203). Thus, the estimated sensitivity of qPCR in vaginal mucus used as a test to detect intrauterine infection was virtually 100%. However, the specificity was remarkably low (i.e. 3%). These results are puzzling, as it would be expected that the presence of *C. burnetii* DNA in vaginal mucus is the result of intrauterine infection. Similarly, the proportion of goats for which *C. burnetii* DNA was detected in individual milk samples was high (i.e. 80% overall, with a subgroup of does from one herd being 95% positive). The resulting estimated sensitivity and specificity for PCR in milk as means for detecting intrauterine infection was 80% and 21%, respectively. As for the ELISA in milk, the estimated sensitivity and specificity relative to the qPCR result in uterine fluid was 58% and 52%, respectively. Given samples in this study were collected on-farm at the time culling, the possibility of cross contamination as a reason for the poor diagnostic specificities reported in this study cannot be ruled out. If the estimates of diagnostic sensitivity and specificity reported by Hogerwerf et al. are externally valid, our simulation analysis show that none of these diagnostic methods are useful for test and remove interventions.

Based on results from our panel study presented in Chapter 3, we assumed environmental contamination and therefore disease transmission to be mainly driven by high shedding events. The relative contribution of high shedders to the force of infection in diseases where transmission is mediated by environmental contamination remains poorly understood. Some authors have suggested that high shedding individuals are not necessarily high spreaders (Slater et al., 2016). For example, in the case of paratuberculosis in cattle, shedding quantities of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) by infected individuals can be 10,000 times higher in a small proportion of high MAP shedders compared with average MAP shedders (Whitlock et al., 2005). Results from a simulation study where longitudinal data were used for parameterisation of a within-herd transmission model of paratuberculosis show the relative contribution to the force of infection attributed to high MAP shedders would only be 10 times higher than that of average shedders (Slater et al., 2016). Mathews et al. (2006) studied the relative contribution of high shedding cows in the within-herd transmission of *Escherichia coli* O157 using field data to inform a mathematical model. The authors concluded that approximately 80% of transmission was attributable to the top 20% most infectious individuals. A future area of research could be the use of longitudinal field studies to elucidate the relative contribution of low *Coxiella* shedders and high *Coxiella* shedders to Q fever transmission in intensively managed dairy goat herds.

In the present study, the time at which testing was carried out had a marked impact on the efficacy of the interventions assessed. Contrary to the case of paratuberculosis — where high shedding individuals persistently contribute to environmental contamination over an extended period of time (Mitchell et al., 2015) — the bulk of shedding in coxiellosis occurs at the time of delivery, when heavily contaminated products of conception are released to the environment (van den Brom et al., 2015). Elimination of high *Coxiella* shedders after kidding or abortion would only result in a marginal reduction of environmental contamination associated with subsequent shedding of *C. burnetii* in faeces, milk or lochia (Arricau Bouvery et al., 2003).

The time at which testing is carried out could also have an impact on the accuracy of diagnostic tests used to detect shedding does. Indeed, antibody levels in does challenged with *C. burnetii* have been shown to rise as parturition approaches (Roest et al., 2013b). In herds were does are artificially inseminated, testing could be timed taking into consideration the expected day of parturition for each doe. In turn, in herds where natural service is used, the estimation of gestational age by means of ultrasound scanning could be used to approximate

expected kidding dates (Suguna et al., 2008). Timing the testing of does in this way could reduce the chance of abortions occurring before disease screening and would likely improve the average accuracy of diagnostic tests.

It could be argued that in the case of coxiellosis early detection and removal of high *Coxiella* shedders should have a comparable effect on reducing disease transmission to that of vaccination. Experimental studies have shown vaccination does not prevent *C. burnetii* infection but it prevents abortions and reduces *C. burnetii* shedding quantities (Arricau-Bouvery et al., 2005). In essence, this is homologous to the scenario where no high shedding events occur. In our simulation study the predicted efficacy of test and cull interventions was only sufficient for disease eradication in more than 70% of the simulations when test sensitivity for detection of high *Coxiella* shedders was assumed to be 90% or above and when testing was carried out 6 weeks prior to the start of the kidding season. Field studies are needed to assess the accuracy of different methods for early detection of high *Coxiella* shedding does before kidding or abortion. Further research is also needed to validate the relative contribution of low *Coxiella* shedders and high *Coxiella* shedders to disease transmission in dairy goats.

Chapter 6

General discussion

6.1 Major outcomes of this study

6.1.1 Reporting herd performance in intensively managed dairy goats

One of the challenges with assessing the epidemiology and economic impacts of disease in dairy goat herds is the lack of robust and consistent metrics for recording animal health, welfare, and performance. Chapter 2 in this thesis provides guidelines for monitoring and reporting herd performance in intensively managed dairy goat herds. The adoption of our reporting system by intensive dairy goat farms in Australia would allow monitoring the impacts of Q fever on dairy goat production and the development systems for early detection of Q fever in dairy goat herds. Moreover, generalised adoption of our system would enable better understand the impact of other diseases in the dairy goat industry.

Q fever in dairy goats is known to cause abortions and perinatal mortality (Agerholm, 2013). The information management system developed in this thesis can be used to consistently quantify these losses. Also, this tool can be used to assess the potential impacts of Q fever on other areas of herd performance (e.g. milk production, the proportion of involuntary culling events or the efficiency with which replacements are reared). This information can be used for the cost-benefit evaluation of interventions directed towards prevention, control and eradication of Q fever in intensively managed dairy goat herds. Moreover, consistent and thorough recording of the occurrence of abortions can be a valuable input for the design of Q fever control strategies, as it would help identify high-risk periods for disease transmission. When control interventions are applied in endemic herds, monitoring of herd performance can contribute to the assessment of the efficacy of these interventions over time. Our information management system can also be used for the development of Q fever surveillance programs based on the detection of deviations from historical or regional reproductive performance benchmarks.

A recent cross-sectional study carried out in Victoria found a herd level prevalence of *C. burnetii* exposure in farmed goats of 1.6% (95% CrI: 0 to 2.8). In this same study, the animal level prevalence in herds identified as exposed was 19.1% (95% CrI: 3.7 to 43.3) (Tan, 2018). These figures suggest that the risk of Q fever occurrence in dairy goat herds is relatively low in the state of Victoria. However, when *C. burnetii* incursion occurs, within-herd spread of disease can result in a large proportion of goats being infected, which represents a clear public health risk. Based on the available data, coxiellosis in dairy goats in Victoria could be characterised as a low-probability but high-consequence event (Miller and Parent, 2012). In common with low-probability but high-consequence events, early detection and preventative measures are expected to be the most cost-effective

control measures (Belay and Monroe, 2014). The importance of developing sensitive surveillance strategies for the detection of *C. burnetii* incursion into livestock herds is highlighted by the fact that clinical signs of Q fever in livestock often go unnoticed and disease is only detected after human cases are diagnosed (Lyytikäinen et al., 1998; Tissot-Dupont et al., 1999; Cutler et al., 2007).

Other countries have developed surveillance systems for Q fever and other zoonotic diseases based on mandatory reporting of abortions in livestock (Bellini et al., 2014; de Cremoux et al., 2018). For example, during the 2007–2010 Q fever outbreak response in The Netherlands mandatory reporting of abortion rates above 5% for herds of 100 or more animals or 3 abortions within a 30-day time window was implemented (Roest et al., 2011a). In France, occurrence of abortions in cloven hoofed animals is mandatory (Anonymous, 2008). Since 2005, France has been officially recognised as being brucellosis-free, therefore early detection of potential re-incursions is of much interest. A caveat of surveillance systems based on reporting of abortions is the potential for under-reporting. The percentage of abortions that are reported by French farmers has been estimated to be 20% and 39% for beef cattle and dairy cattle, respectively (Bronner et al., 2013). The relatively low perceived risk of brucellosis by farmers as well as the perceived lack of benefit from reporting abortion events have been identified as factors that negatively impact on the willingness to report abortion events (Bronner et al., 2014). These same factors could hamper the effectiveness of programs aimed at monitoring Q fever occurrence based on reporting of reproductive disease. Bronner and collaborators suggested that the French brucellosis monitoring scheme should move towards one in which each farmer records occurrence of abortions using their own information management systems and authorities are notified only if abortions exceed a pre-defined threshold. These authors also suggested to develop methods for identifying abortions using additional data sources, for example, insemination dates or calving intervals. A similar approach could be used for Q fever passive surveillance in dairy goat herds in Australia and the information management system presented here can contribute to that end.

The information management system developed as part of this thesis could also be used to address herd health issues other than Q fever. Studies carried out in dairy cows and dairy goats have shown that the adoption of computerised information management systems can lead to increased milk yields and profitability of dairy farms (Jofre-Giraudo et al., 1990; Tomaszewski et al., 2000; Belanche et al., 2019). Results from a 3-year follow-up study carried out in Andalusia, Spain, showed dairy goat farms that adopted an information management tool were able to shorten goats' unproductive periods by reducing the age of first kidding and the dry period length. Moreover, higher milk yields and an increment in the off-season milk production were also observed compared to farms that had not adopted an information management tool (Belanche et al., 2019). Improvements in herd performance observed in farms that used an information management tool during the follow-up period were attributed to the enhanced ability to identify the best does to keep as replacements, select elite does in which to use artificial insemination (AI), identify low-performing does for culling and the ability to continuously monitor the productivity and physiological stage of each doe. A profitable area of future research could be an economic evaluation of the implementation of the information management system developed in this thesis in intensively managed dairy goat herds in Australia.

We expect the generalised use of the proposed system will allow the estimation of local industry benchmarks against which Australian dairy goat producers can assess the performance of their animals and their herds. One key advantage of the implementation of standardised measures of individual production merit is that it would allow a national progeny testing scheme of bucks, which could foster the more wide spread use of AI within Australia and lead to the genetic improvement of the national herd (Olivier et al., 2005; Stubbs and Abud, 2009).

The main components of the system presented here could also be used to develop information management systems for other relatively small-scale but incipient dairy industries in Australia, like the dairy camel and the dairy buffalo industries.

The software prototype presented here can be expanded to include predictive models to assist on-farm decision making. For example, reporting estimations of the retention pay-off of individual goats could aid culling decisions (Groenendaal et al., 2004). The development of a module for early detection of sub-clinical disease based on automatically recorded animal level data (e.g. daily milk yields) could also be the subject of further research (Hogeveen et al., 1991; Edwards and Tozer, 2004). In this regard, an exploratory analysis of individual daily milk yields and culling records showed some does for which 'sudden death' had been recorded as a removal reason had marked declines in milk yields preceding the date of their death (Figure 6.1). As further data are recorded into the system the possibility of using daily milk yields to detect sick does could be assessed. Additional routinely recorded data, for example the position in which does enter the milking parlour, could be used to increase detection accuracy.

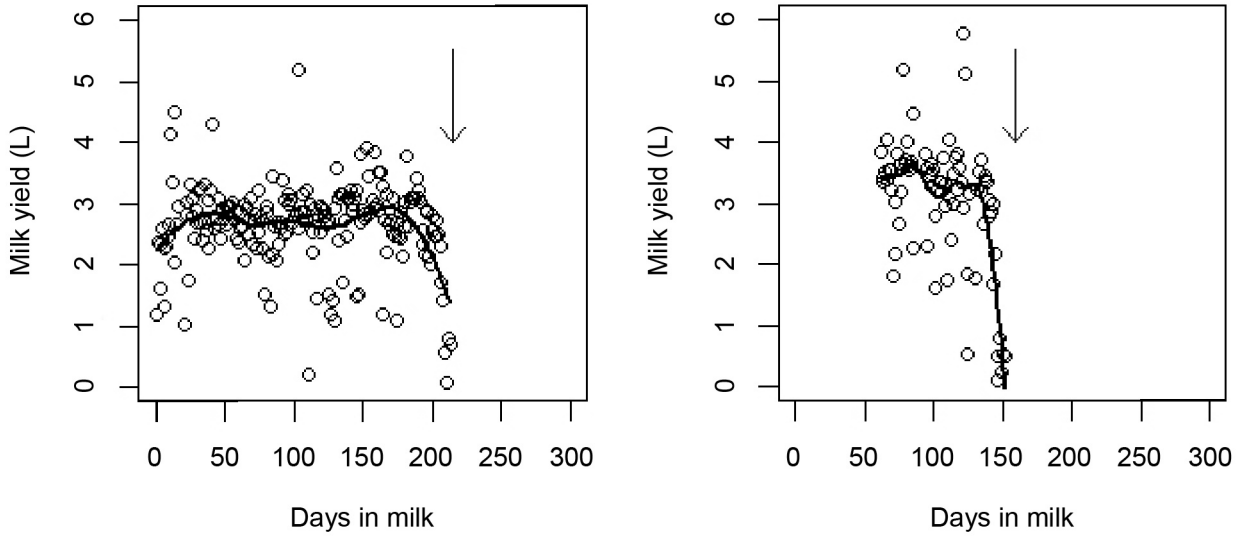

Figure 6.1: Daily milk yields of two individual does for which sudden death was reported as a removal reason. The vertical arrow shows the reported day of death. The plots show an abrupt reduction in daily milk yields preceding death date. This drop in milk production could potentially allow sick does to be detected and treated promptly.

Individual and herd level performance reports can only be as good as the quality of the data used as input. Whereas reports that rely on data that are automatically recorded (e.g. individual milk production or animal weights) can be straight forward to develop, those that depend on manually entered information (e.g. disease occurrence) may require more time and veterinarians working in close contact with herd managers before data of sufficient quality is gathered (Stevenson, 2000). To optimise efficiency, farm staff should ideally only collect data that will be used to produce useful information for management (Etherington et al., 1995). Based on this principle, the minimum data required to produce sufficient reports on individual and herd level performance was specified in Chapter 2.

Automatically recorded data can be used to validate manually recorded data or even replace manual records altogether for certain variables. For example, individual daily milk yields can be used to estimate kidding dates, as a milk test record for a given doe following a period of no records indicates the start of new lactation.

Similarly, drying-off dates can be estimated by detecting interruptions in milk tests for a given doe. Also, missing recordings for removal events can be flagged when a predefined time lapses without any event recorded for an individual goat. This way, data quality can be improved when the use of highly efficient recording technologies is combined with software that can implement algorithms for the detection of certain key events. In the appendix A.2, we included the description of an algorithm that can be used to infer kidding events from daily milk test data with high accuracy. Provided pregnancy test data are entered into the system, the automated recording of kidding events would allow failure to kid rates to be readily estimated and this used to trigger a veterinary investigation including Q fever as a differential diagnosis every time a predetermined failure to kid rate threshold is exceeded. This would be an efficient way to address the potential shortfalls associated with mandatory abortion reporting systems of the type used in some European countries (Bronner et al., 2013).

6.1.2 Q fever disease dynamics and impacts on milk production in dairy goats

Results from our panel study show that, in the absence of vaccination or stamping, out Q fever in intensively managed dairy goat herds can become endemic with the occurrence of abortions and a relatively high prevalence of *C. burnetii* shedding at the time of delivery. The ability of *C. burnetii* to persist within intensively managed dairy goat herds highlights the importance of taking decisive action for disease control to reduce the risk of Q fever spillover to humans. In this study, out of all does found to be shedding *C. burnetii* at parturition close to 20% were shedding very high quantities of *C. burnetii*, with the remaining 80% shedding relatively low levels. This finding led us to classify shedding does into low-shedders and high-shedders. High-shedding does were more likely to have detectable antibodies by IFA than does that were low-shedders. Also, high-shedders produced significantly lower daily milk volumes. After accounting for potential confounders, the average milk yield of high-shedding does was 17.5% (95% CI: 3 to 32) lower than that of non-shedding does. This is the first report of a negative association between active shedding of *C. burnetii* and milk production in goats. Persistent shedding, defined as shedding of *C. burnetii* over two consecutive kidding events, was observed in approximately one fifth of the does identified as shedders and re-sampled at their subsequent kidding. Although persistent shedding has been described in previous studies (Berri et al., 2002, 2007), our study has been the first to quantify the frequency of its occurrence.

We believe disease persistence within this enterprise could be attributed to herd size and management practices. Certainly, a large herd size (close to 5000 does in milk) and a relatively high level of population turnover (the average doe lifespan in this enterprise is close to 3 years) results in a sustained influx of a large number of susceptible individuals over time. Also, high stocking densities resulting from confinement of does in sheds — as opposed to extensive or semi-extensive pastoral systems — are likely to lead to increased opportunities for exposure to *C. burnetii*. Apparent disease extinction after an abortion storm and in the absence of vaccination has been reported in a large dairy goat farm in the UK where does were kept indoors (Reichel et al., 2012). An additional factor that was present in the enterprise where our study was carried out was that reproduction was managed so that does kidded in four distinct seasons per year. Shedding of *C. burnetii* at kidding was detected during each of the four kidding seasons in 2016. Does expected to kid at each of the four kidding seasons are managed independently in distinct management groups. Therefore, it would be reasonable to assume that *C. burnetii* infection within a mob is mainly explained by intra-mob transmission. However, it is also possible that wind and fomites contribute to new infections within a mob. Field studies are required to quantify the relative importance of these infection transmission mechanisms.

When the prevalence of shedding at the time of kidding was stratified by age, a peak of shedding prevalence was observed in second parity does. The prevalence of antibodies against *C. burnetii* showed a similar pattern. The decrease in shedding prevalence that was observed in does that were in their third or greater parities could be attributed to naturally acquired immunity. Experimental studies have shown that goats challenged with *C. burnetii* develop both a humoral immune response and a cell-mediated immune response (Arricau Bouvery et al., 2003; Roest et al., 2013b). The protective effect of antibodies against *C. burnetii* was demonstrated in mice challenged 24 hours after administration of immune serum (Humphres and Hinrichs, 1981). Mice that had been administered immune serum showed an increased clearance rate of infection. Whereas antibodies play an important role protecting hosts from *C. burnetii* challenge at an early stage, T cell-mediated immune response has been shown to contribute to the elimination of *C. burnetii* at later stages of infection (Zhang et al., 2007).

Regarding the distribution of *C. burnetii* GE concentrations detected in qPCR positive vaginal swab samples obtained at the time of kidding, a highly skewed pattern was observed. While *C. burnetii* GE concentrations were relatively low in the majority of positive samples, a reduced group of positive samples had very large concentrations of *C. burnetii* (Figure 6.2). Tropism of *C. burnetii* for the trophoblast cells in the placenta has been shown in experimental studies (Sánchez et al., 2006; Roest et al., 2012). An explanation for the very high concentration of *C. burnetii* GE in vaginal swabs found in a subgroup of apparently full-term kidders is that they had developed placentitis but managed to maintain their foetus until full-term, which is consistent with findings from experimental studies (Roest et al., 2012). On the other hand, samples where GE concentrations were relatively low could be attributed to: i) infections occurring at an advanced stage of pregnancy; ii) infections in hosts that had some level of immunity because of earlier *C. burnetii* exposure; or iii) exposure to a relatively low *C. burnetii* dose. Shedding concentrations for does classified as high-shedders were similar to that of aborted qPCR positive does. This is in agreement with de Cremoux et al. (2012) who found aborted does had very high concentrations of *C. burnetii* in vaginal swabs and that about 31% of qPCR positive full-term kidders had similar concentrations of *C. burnetii* DNA in vaginal swabs compared with does that had aborted (de Cremoux et al., 2012).

Does that were found to be high-shedders at parturition had significantly lower daily milk yields in their subsequent lactation. Although high-shedding does were found to represent a relatively low proportion of the herd and so the economic impact of these losses may be limited, their reduced milk production represents an additional incentive for identifying and removing high-shedding does. The biological explanation behind the identified milk yield loss is not yet clear. Occurrence of retained placenta or metritis was not recorded in our study but they represent potential explanations for the observed milk yield losses (Laven and Peters, 1996). In an experimental study carried out by Sanchez et al. (2006), major histopathological changes were observed in the mammary glands of does challenged with *C. burnetii*. Further, clinical mastitis was observed in one of the challenged does. Martinov et al. (2007) isolated *C. burnetii* from sheep with mastitis from six different dairy sheep farms in Bulgaria. However, no control animals were included in this study and therefore the putative association between the presence of viable *C. burnetii* in individual milk samples and the occurrence of mastitis could not be assessed. It is noteworthy that staff members from the farm where our panel study was carried out reported recurrent cases of mastitis with no response to antibiotic treatment (Cameron, personal communication). Routine recording of mastitis events, treatment of clinical mastitis and assessment of responses to treatment using the information management system presented in Chapter 2 would provide a more quantitative basis for assessing these putative associations. A logical approach would then be to use these data for the design of a

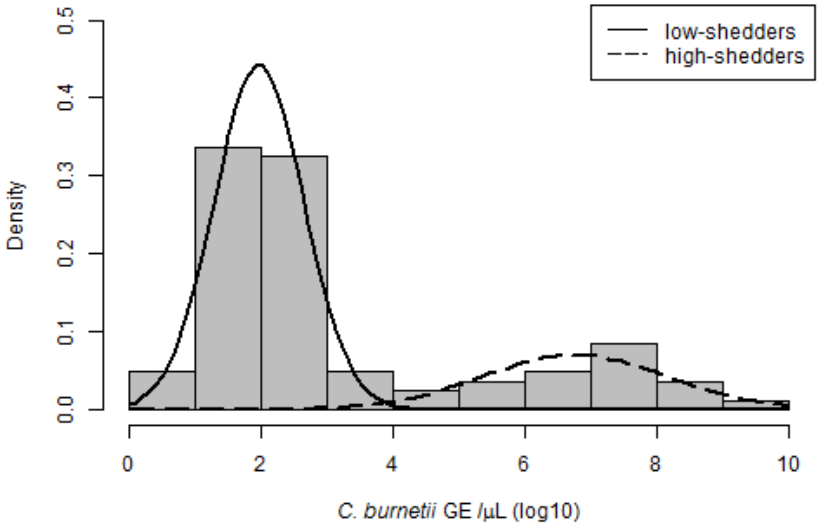

Figure 6.2: Histogram showing *C. burnetii* concentrations in qPCR positive vaginal swabs obtained from aborted (n = 14) and full-term kidders (n = 72) (expressed as GE per μL on a log 10 scale). Superimposed on this plot is a probability density function comprised of a mixture normal distribution. The probability density function was computed using the contributed *R* package *mixtools* (Benaglia et al., 2009) (mean1 = 1.87, sd1 = 0.68, mean2 = 6.75, sd2 = 1.41, lambda = 0.25).

case-control study to assess whether natural infection with *C. burnetii* is associated with or a cause of clinical mastitis in dairy goats.

Throughout the study period, farm staff members obtained vaginal swabs ($n = 26$) from does that had aborted and submitted them for qPCR testing. We detected *C. burnetii* DNA in 54% (95% CI: 35% to 71%) of these swabs. Abortion causes other than Q fever may be responsible for some of the abortions observed in this farm. We note it would have been helpful to report more descriptive statistics about abortions, such as stage in gestation, doe characteristics, and overall abortion rates for each of the herds. However, this information was not available at the time it was requested. This circumstance was, in part, what drove us to pursue the development of standardised methods for recording and reporting herd performance in intensively managed dairy goat systems. We expect future studies farm will benefit from these tools and will be able to provide valuable insight into abortion patterns in Q fever endemic dairy goat herds.

We found the agreement between serology and qPCR in vaginal swabs at the time of kidding was not substantial. This is in line with previous studies carried out in dairy goats (Rousset et al., 2009a; de Cremoux et al., 2012). It could be argued that, since we sampled does within 24 hours post-kidding, there might have not been enough time for an immune response to develop, and that this could have negatively impacted the agreement between the tests. However, in challenge studies, a rise in antibody levels was observed in goats as soon as 2 weeks from the time of exposure to *C. burnetii* (Roest et al., 2013a). On the other hand, colonisation of the trophoblast cells by *C. burnetii* takes approximately 2 to 4 weeks from exposure (Roest et al., 2012). It derives from this that, when *C. burnetii* was detected by PCR in vaginal swabs at the time of parturition, enough time from exposure had elapsed for an antibody response to mount in the most individuals. An alternative explanation for the lack of agreement could be the attributed to test accuracy imperfections. Muleme et al. (2016) estimated the sensitivity of IFA was 94.8% (95% CI: 80.3% to 99.6%) and 88.8% (95% CI: 58.2% to 99.5%) for

the detection of IgG and IgM antibodies, respectively. In turn, the estimated diagnostic specificity was 92.5% (95% CI: 77.1% to 99.3%) and 92.4% (95% CI: 83.0% to 99.2%) for IgG and IgM antibodies, respectively. To our knowledge, no studies assessing the diagnostic accuracy of qPCR in vaginal swabs have been published. Future studies addressing this knowledge gap are needed.

Simulation based studies have shown the proportion of goats that become persistent shedders is a key determinant of disease persistence within a herd and the levels of environmental contamination over time (Bontje et al., 2016). In agreement with other published studies, we found a fraction of the sampled does were shedding *C. burnetii* in two consecutive parturitions. It remains unanswered whether goats can shed *C. burnetii* over more than two parturitions. Also, it cannot be ruled out that some of the goats that were classified as persistently infected were in fact individuals that were repeatedly exposed and re-infected in-between sampling intervals. Our results suggested that persistent shedders where shedding relatively low concentrations of *C. burnetii* during their second sampling event. This was initially interpreted as the result of individuals having developed immunity and consequently being able to contain infection, a hypothesis that was supported by other authors' findings (Berri et al., 2005b). However, since results from other studies showed reproductive failure attributed to *C. burnetii* can occur in two consecutive parturitions (Berri et al., 2007) we concluded that this hypothesis could not be generalised.

6.1.3 Modelling Q fever control interventions in dairy goats

The information gathered in the first sections of this thesis was used to develop an agent-based mathematical model of within-herd Q fever transmission. A novelty of this model is that a formal statistical method was used to estimate model parameters using empirical data on *C. burnetii* shedding prevalence at kidding and failure to kid risk from a dairy goat herd in which *C. burnetii* was endemic. The model was used to test the efficacy of a series of control interventions. Vaccination was found to be an effective means for eradicating Q fever from the herd. However, simulations from our model showed vaccination must be sustained for a relatively long time to achieve eradication, with the predicted median time to eradication being 6.5 (95% CrI: 4.1 to 11.3) years. This highlights the importance of identifying control interventions that can be combined with vaccination to reduce the time to eradication. The segregation of pregnant does in a separate environment has been proposed by other authors as a strategy to reduce Q fever transmission (European Food Safety Authority, 2010; Plummer et al., 2018). In our model, this intervention did not have a marked effect on disease incidence when applied alone. Nevertheless, the combination of vaccination and segregation of pregnant does led to a reduction in the time to eradication. Also, culling of does shedding *C. burnetii* at the time of kidding or abortion reduced the time to eradication. To best of our knowledge, this has been the first study to assess the efficacy of these interventions either applied alone or in combination.

Model parameters were estimated using an ABC rejection-sampler algorithm. ABC methods encompass a suit of Bayesian computational methods that can be used to sample parameter values from the approximate posterior in contexts where calculation of the likelihood function would be computationally too costly to evaluate or otherwise unavailable (Sisson et al., 2019). One of the parameters that was identified by the ABC rejection-sampler was α, which governs the fraction of infected does that became persistently infected. In absence of published information about the value of this parameter the authors of previous mathematical models of Q fever transmission in dairy goats have assumed an average value of 70% (Bontje et al., 2016). From our ABC rejection-sampler the median of the approximate posterior distribution for α was 29% (95% CrI: 20 to 39).

This is close to the 20% (95% CI: 10 to 35) we obtained in our panel study where a group of does shedding at the time of kidding were re-tested in their subsequent kidding. The relative proximity of both estimations is noteworthy given they were obtained using two very different estimation procedures. The parameters governing the fraction of infected does that abort and the average incubation period were also identified by the ABC rejection-sampler. The efficiency of the ABC rejection-sampler algorithm was considerably improved when simulations were run in a version of the model coded in the C++ programming language compared with a version coded in R (R Core Team, 2017). Further efficiency was gained by parallelising the algorithm using 16 computer cores. Relatively more complex but more efficient versions of ABC, for example the ABC Sequential Monte Carlo (SMC) algorithm (Beaumont et al., 2009), could be used to reduce the computational cost of the model fitting process. This would in turn facilitate the assessment of the ability of different model versions to capture observed disease patterns.

Segregation of does that are close to their kidding date is a widespread practice among dairy goat producers in Australia (Gunther et al., 2019). However, segregation of kidders is likely to be carried out mainly for management purposes (e.g. to allow parturient does to be readily accessible in the event of dystocia and/or to keep newborns in an environment protected from harsh weather) and not to address a perceived biosecurity risk. This is supported by the fact that the perceived risk of Q fever is relatively low among dairy goat farmers in Australia (Gunther et al., 2019). Simulations from our model showed no marked impact of this intervention on the incidence of disease over time. Similarly, culling of shedders after parturition or abortion did not achieve a significant reduction in disease incidence. However, we found a reduction in the median time to disease eradication of approximately 10 and 21 months when vaccination was combined with segregation of pregnant does or culling of shedders, respectively. This said, an improved knowledge about the risk of re-introduction of disease from wildlife is of utmost importance to develop recommendations around whether vaccination should be sustained indefinitely in large intensively managed dairy goat enterprises in Australia.

To date, no vaccines licensed for use in livestock are available in Australia. A whole-cell formalin-inactivated vaccine is currently being developed by the Australian Rickettsial Reference Laboratory in Geelong, Victoria. In an experimental study where kids where inoculated with this vaccine a satisfactory antibody response was elicited and no secondary effects apart from mild swelling at the inoculation site were observed (Muleme, 2017). The vaccine was developed from an isolate obtained from the dairy goat enterprise where the panel study presented in this thesis was carried out. Field studies to assess the protective effect of this vaccine are likely to commence in the near future in the same enterprise. Results from these studies can be used to inform the mathematical model presented in Chapter 4, further improving the predictive ability of the model.

It has been shown that vaccination against Q fever in livestock is more effective in susceptible non-pregnant individuals (Guatteo et al., 2008; Tutusaus et al., 2014). Muleme et al. (2017a) showed that in an endemic dairy goat herd *C. burnetii* infection of newborn kids can occur as early as 9 weeks of age. For this reason, for effective control in endemic dairy goat herds, vaccination of replacements should be carried out at 8 weeks of age in all replacements. Given the high population turnover that characterises intensively managed dairy goat herds, a full vaccination coverage could be achieved in a relatively short time by vaccinating replacements only. Yearly booster vaccination of does before joining is recommended by the manufacturers of Coxevac®, the Q fever vaccine used in Europe. A key piece of information for predicting the efficacy of vaccination of replacements only is the duration of the protective effect induced by vaccination. In humans, a single dose of the Q-Vax® vaccine provides long-lasting immunity (Marmion et al., 1990; Kersh et al., 2013). Experimental studies to assess the level of residual protection at 1, 2 and 3 years post vaccination are required to inform

recommendations regarding the need for booster vaccinations.

Removal of infected animals has been used for the control of diseases such as bovine brucellosis and bovine tuberculosis (Chamberlin, 1985; More et al., 2015). The accuracy with which available tests can detect infected individuals is a key determinant of the feasibility of test and remove interventions (Kudahl et al., 2007). A lack of sensitivity of diagnostic tests would lead to a large proportion of infected animals not being detected, whereas a low specificity would increase the cost of test and remove interventions. The combination of two or more diagnostic tests can be used to improve the accuracy with which infected animals are detected. For example, test and remove interventions for the control of brucellosis in cattle are based on the use of a screening test (typically Rose Bengal) and a confirmatory test (e.g. a complement fixation test) (Samartino, 2002). The prevalence of disease is another factor that needs to be taken into account when assessing the feasibility of test and remove interventions. If the prevalence of disease is high the associated cost of test and remove interventions could be a major limitation. Conversely, when the prevalence of disease is low, there are marked reductions in the positive predictive value of diagnostic tests with imperfect sensitivity and specificity (Thrusfield, 2005; Rothman et al., 2008). This means that herd managers need to be well warned that in the final stages of an eradication program there will be an increase in the proportion of test-positive animals that are actually false positives (Schiller et al., 2011). At the time of writing, implementation of a national compensation scheme for Q fever in dairy goats is unlikely. However, for other infectious diseases the existence of compensation schemes can markedly increase the probability of success of test and remove interventions (Blasco and Molina-Flores, 2011).

In this thesis, a simulation-based study was carried out to assess the efficacy of control interventions based on the detection and removal of infected does before parturition or abortion. Since results from our panel study showed a subgroup a high-shedding does was likely to be responsible for the majority of environmental contamination, the mathematical model previously developed was modified to account for heterogeneous *C. burnetii* shedding patterns. A series of scenarios were assessed with varying diagnostic test accuracy assumptions and testing times relative to the start of each kidding season. Under model assumptions, removal of high-shedders alone before parturition or abortion would lead to disease eradication from an endemic dairy goat herd. Diagnostic tests with a relatively high sensitivity for the detection of high *Coxiella* shedding does are needed for test and remove interventions to be effective. For diseases that have a chronic course and a relatively low R_0 (e.g. bovine tuberculosis) test and remove interventions can reduce the prevalence of disease and achieve eradication from a herd even when highly accurate diagnostic tests are not available (Álvarez et al., 2012). As shown by our model, this may not be the case for Q fever in small ruminants. This is because the occurrence of one abortion by an infected animal can result in a marked peak in disease transmission (Sanford et al., 1994). For this same reason, the time at which testing was carried out relative to the start of the kidding season had a marked effect on the efficacy of test and removal interventions. Results from this study should be complemented with field investigations. A longitudinal study to investigate different diagnostic methods for the detection of does with placentitis caused by *C. burnetii* in naturally infected herds before high-risk periods for abortions is currently underway. This study will also investigate *C. burnetii* shedding dynamics following parturition or abortion. We believe this new study will generate valuable data to inform further modelling research.

6.2 Conclusions

- We developed a software prototype for monitoring and reporting herd performance in intensively managed dairy goat herds. This tool can contribute to early detection of Q fever in intensively managed dairy goat herds and the economic evaluation of preventive and control interventions.
- We found a highly skewed pattern of *C. burnetii* shedding in naturally infected does, with a small proportion of does shedding relatively quantities of *C. burnetii*. Daily milk yields for 'super shedder' does were 17% (95% CI 3% to 32%) less than their *C. burnetii* negative herd mates.
- We developed a mathematical model of within-herd Q fever transmission. The model was used to assess the efficacy of potential control interventions. Vaccination was an effective means for disease eradication in endemically infected herds with a median time to eradication of 6.5 years (95% CrI: 4.1 to 11.3).
- Time to disease eradication by means of vaccination can be reduced by segregating pregnant does or culling does shedding *C. burnetii* at parturition or abortion. If the sub-group of super shedder does can be identified and removed before parturition or abortion eradication of coxiellosis from an endemically infected herds would be feasible.

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Appendix A

Chapter 2 supplementary material

A.1 Suplemmentary tables and figures

Table A.1: List of abbreviations in Figure 2.1

Table	Variable	Description
Animals	animalKey	Unique animal identifier
	herd	Herd unique idetifier
	animalExternalKey	External unique identifier
	lifetimeID	National unique identifier
	electronicID	Electronic individual identifier
	animalnumber	Visual eartag
	birthDate	Date animal born
	dam	Unique ID of mother
	sire	Unique ID of female progentior
	breed	E.g. Saanen, Alpine
	gender	Sex
KidsBorn	kidsBornKey	Unique identifier of kid born
	kiddingKey	Kidding event unique identifier
	kidsBornSex	Sex of born kid
	kidsBornFate	Kept as replacement, slaughtered
Kiddings	kiddingKey	Kidding event unique identifier
	animalKey	Unique animal identifier
	kiddingDate	Date of kiddding event
	kiddingType	Single, twins, triplets
PSM	psmKey	Unique identifier for
	animalKey	Unique animal identifier
	psmKiddingDate	Kidding date when PSM defined
	psmDate	Assigned PSM
Heats	heatKey	Unique identifier for oestrus record
	animalKey	Unique animal identifier
	heatDate	Date oestrus detected
Services	serviceKey	Unique identifier for service
	animalKey	Unique animal identifier
	serviceDate	Date of service
	serviceType	e.g. fresh semen, frozen semen
	serviceSire	Unique identifier of sire used for service
	serviceTech	Technician ID/name

Continued on next page

Table A.1 – continued from previous page

Table	Variable	Description
Joinings	joinKey	Joining unique identifier
	animalKey	Unique animal identifier
	joinsDate	Joining start date
	joineDate	Joning end date
	joinSire	Sire(s) used
Aborts	abortKey	Unique identifier for abortion event
	animalKey	Unique animal identifier
	abortDate	Date of abortion
	abortCause	Cause of abortion
VetReproExams	vreKey	Unique identifier of veterinary reproductive examination
	animalKey	Unique animal identifier
	vreDate	Date of reproductive examination
	vreReason	Reason for reproductive examination
	vreDiagnosis-01	Diagnosis
	vreDiagnosis-02	Diagnosis
AnimalAlterTag	renumKey	Unique identifier of tag alteration
	animalKey	Unique animal identifier
	renumDate	Date eartag changed
	oldNumber	Previos eartag number
	newNumber	New eartag number
Production	prodKey	Unique identifier of production record
	animalKey	Unique animal identifier
	prodDate	Date of herd test
	lifetimeID	National unique identifier
	electronicID	Electronic individual identifier
	HTFat	Fat content in milk
	HTProtein	Protein content in milk
	HTVolume	Milk volume
	HTSCC	Somatic cells count
AnimalEnter	enterKey	Unique identifier of animal entry event
	animalKey	Unique animal identifier
	enterDate	Date of entry
	enterType	e.g. transferred, bought
	stockClassEnter	Stock class at the time of entry
	enterFertStatus	Pregnant/empty at time of entry
	enterLactStatus	In-milk/dry at time of entry
	enterDueDate	Expected date of entry
	enterDueSire	Unique sire identifier
AnimalExit	exitKey	Unique identifier of animal exit event
	animalKey	Unique animal identifier
	exitDate	Date of exit
	exitFate	Sold, death, culled
	exitReason1	First exit reason
	exitReason2	Second exit reason
	exitReason3	Third exit reason
Diseases	disKey	Unique identifier of disease event
	treatKey	Unique identifier of treatment event
	animalKey	Unique animal identifier
	disDate	Date of disease event
	disType	e.g. udder disorder, metabolic disorder
	disQuarter	Quarter affected

Continued on next page

Table A.1 – continued from previous page

Table	Variable	Description
Treatments	treatKey	Unique treatment Identifier
	animalKey	Unique animal identifier
	treatDate	Date of treatment
	treatType	Type of treatment (e.g. antibiotic)
	diseaseKey	Unique identifier of disease event
Samples	sampleKey	Unique identifier of sampling event
	animalKey	Unique animal identifier
	sampleDate	Date sample obtained
	sampleType	Type of sample (e.g. milk, blood, faeces)
	testPerformed	Test performed (e.g. ELISA, FEC)
	result	Outcome of the test
Procedures	procKey	Unique identifier for procedure event
	animalKey	Unique animal identifier
	procDate	Date procedure was performed
	procType	e.g. marking, dehorning
WeightHeightCoditionScore	whbcsKey	Unique identifier of weight/height/body condition score event
	animalKey	Unique animal identifier
	whbcsDate	Date of event
	whbcsType	Weight, height or body condition score
	whbcsValue	Measurement
DryOffs	dryoffKey	Unique identifier for drying-off event
	animalKey	Unique animal identifier
	dryoffDate	Date of drying-off event

A.2 Inferring kidding dates from daily milk tests

The increasing use of data recording technologies that register individual level variables on a daily basis allows to infer key demographic and management events using relatively simple algorithms. We developed and tested the accuracy of an algorithm that uses daily milk test data to estimate kidding dates. We used kidding event records and individual level daily milk test records from the dairy goat farm described in Section 2.2.1. A kidding event was assumed when: i) a milk test corresponds to a doe for which there has been no previous milk test record; or ii) the time distance with the nearest milk yield record for a given doe is 21 days or more. The use of a unique animal key that can be linked to multiple ear tags via the *AnimalAlterTag* table (see Figure 2.2), precludes the occurrence of a false kidding event recording when an ear tag is replaced.

Figure A.1 shows a frequency histogram of manually recorded kidding events (upper row) and those inferred from daily milk tests (lower row). Out of all kidding events recorded by farm staff members over the first two kidding seasons of 2017 (n = 889), 98.5% were detected by our algorithm. Further, 18 additional kidding events detected by the algorithm had not been manually recorded. This approach can be used to flag potentially missing or inconsistent data.

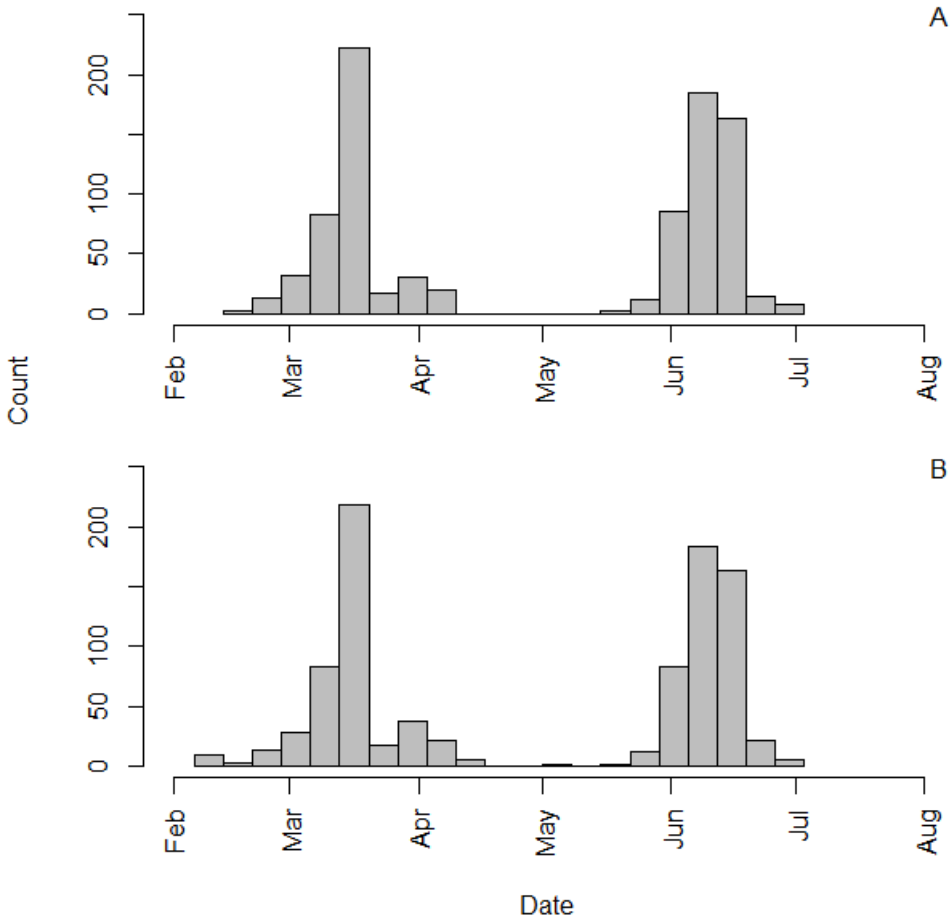

Figure A.1: Frequency histogram of kidding events from a large dairy goat farm in Victoria, Australia (2017). (A) Manually entered by farm staff. (B) Inferred from individual daily milk test data.

A.3 R code for simulation of reproductive events

```
library(dplyr)

# Define parameters. Mean length of oestrus cycle 20.8 days, sem 0.4 days
# Reference Castro et al. (1999):
mx <- 21
sdx <- 2

# Sensitivity and specificity of oestrus detection:
Se <- 0.900
Sp <- 0.995

# Conception rate (Leboeuf et al. 2000):
pRate <- 0.6

# Number of goats to simulate:
ngoats <- 1000

# Number of cycles to follow:
ncycles <- 3

# Create a data frame:
id <- rep(1:ngoats, each = ncycles)
oestNumber <- rep(1:ncycles,ngoats)
day <- NA
df <- data.frame(cbind(id, oestNumber,day))

df$day[df$oestNumber == 1] <- sample(1:mx, ngoats, replace = TRUE)
# df$day[df$oestNumber == 1] <- rexp(ngoats, 1/mx) # Cycle day for each goat at PSM
df$day[df$oestNumber == 2] <- df$day[df$oestNumber == 1] +
   rnorm(ngoats, mean = mx, sd = sdx)
df$day[df$oestNumber == 3] <- df$day[df$oestNumber == 2] +
   rnorm(ngoats, mean = mx, sd = sdx)

df <- df %>%
  group_by(id) %>%
  mutate(diff = day - lag(day, default = first(day)))
```

```
# Test if length of cycle is correct:
mean(df$diff[!df$oestNumber==1])

# Days don't start at 0 but in 1 and are integers:
df$day <- floor(df$day + 1)
df <- df[,-4]

# Second data frame with all days:
ndays <- max(df$day)
id <- rep(1:ngoats, each = ndays)
days <- rep(1:ndays,ngoats)
dat <- data.frame(cbind(id, days))
dat <- merge(dat, df[1:3],
   by.x = c("id", "days"),
   by.y = c("id", "day"),
   all.x = TRUE, all.y = TRUE)
dat$oestNumber[is.na(dat$oestNumber)] <- 0
dat$detection <- NA
heatDetF <- function(x){ifelse(x[3] == 0,sample(c(0,1),1,
   prob = c(Sp,1-Sp)),sample(c(0,1),1, prob = c(1-Se,Se)))}

dat$detection <- apply(dat,1,heatDetF)

# What's observed?
obs <- subset(dat, detection==1) # Keep only detected oestrus

# Simulate conceptions:
obs$pregnant <- NA

obs$pregnant[obs$oestNumber == 1] <- sample(c(0,1),
   length(obs$pregnant[obs$oestNumber == 1]),

prob <- c(1-pRate,pRate), replace = TRUE)
obs$pregnant[obs$oestNumber == 2] <- sample(c(0,1),
   length(obs$pregnant[obs$oestNumber == 2]),

prob <- c(1-pRate,pRate), replace = TRUE)
obs$pregnant[obs$oestNumber == 3] <- sample(c(0,1),
   length(obs$pregnant[obs$oestNumber==3]),

prob = c(1 - pRate, pRate), replace = TRUE)
obs$pregnant[obs$oestNumber == 0] <- 0
```

```
# Remove rows after id if doe gets pregnant:
obs$csum <- ave(obs$pregnant, obs$id, FUN = cumsum)
obs <- obs[obs$csum <= 1,]
obs$temp <- obs$csum + obs$pregnant
obs <- obs[obs$temp %in% c(0,2),]
obs$csumDet <- ave(obs$detection, obs$id, FUN = cumsum)
obs <- obs[obs$csumDet <= 3,]
```

Appendix B

Chapter 3 supplementary material

B.1 Suplemmentary tables and figures

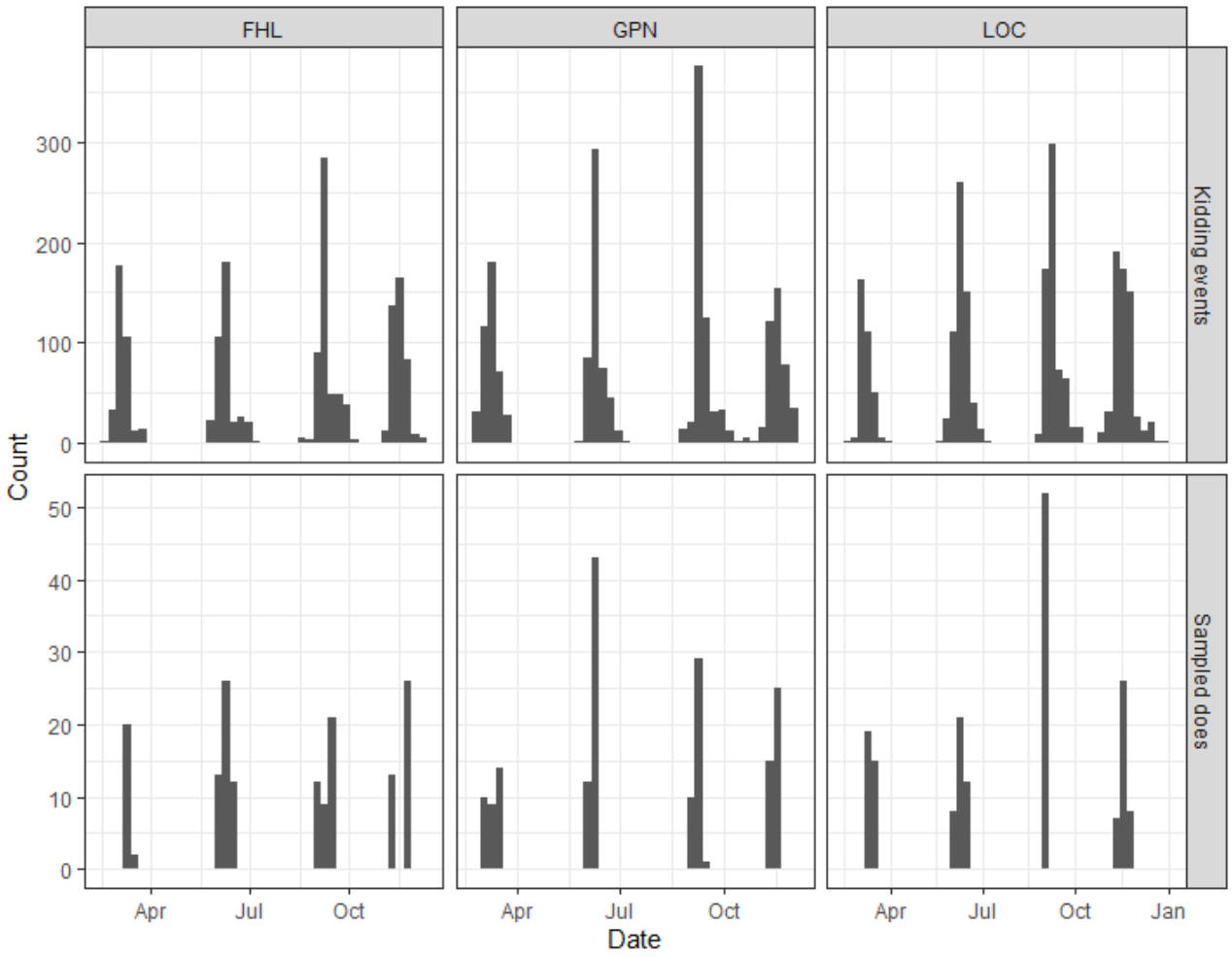

Figure B.1: Frequency histogram showing the counts of kidding events recorded in each of the three herds and four kidding seasons in the study farm in 2016.

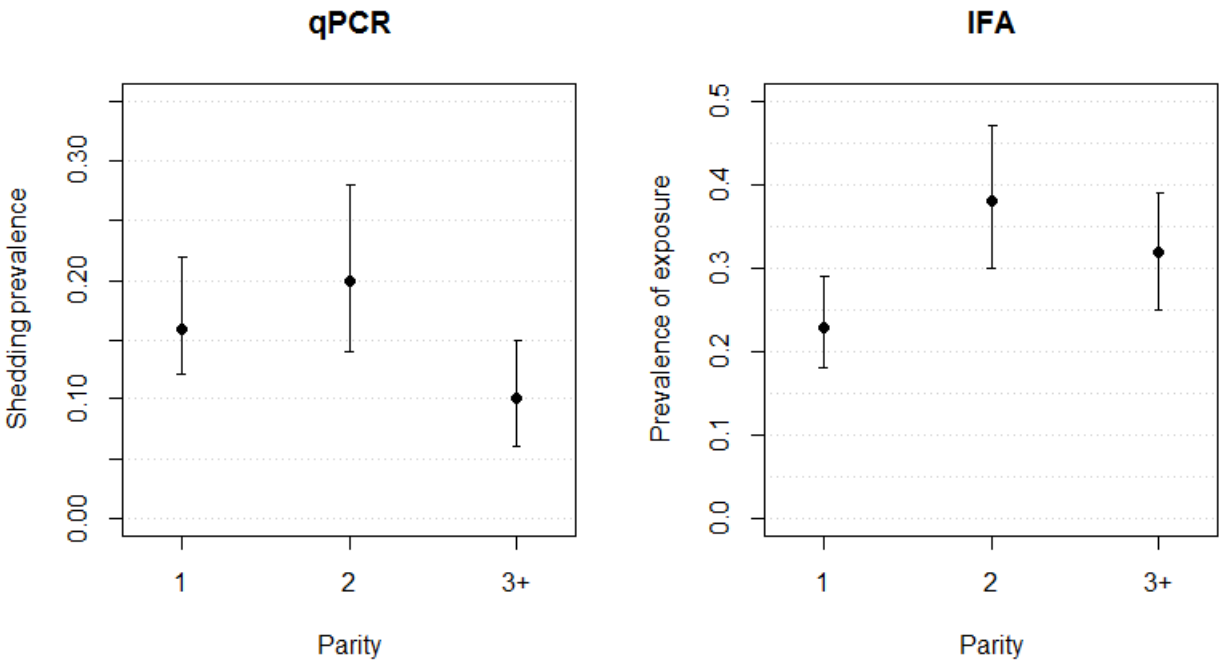

Figure B.2: Error bar plots showing the prevalence of *C. burnetii* shedding at kidding (left) and prevalence of exposure at kidding (right) as determined by qPCR in vaginal swabs and IFA in blood, respectively. The error bars show 95% CI. These plots show estimates from the aggregated results obtained from three herds that are part of an endemic dairy goat enterprise located in Victoria, Australia, 2016.

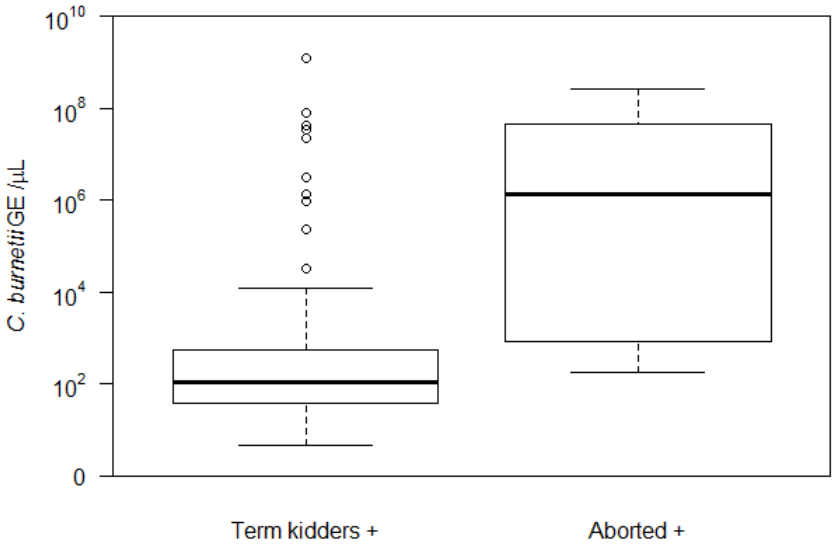

Figure B.3: Box and whisker plot showing the concentration of *C. burnetii* GEs in vaginal swabs (log scale) found in qPCR positive does that were full-term kidders or that aborted. Does that aborted were shedding comparatively higher quantities of *C. burnetii* than full-term kidders. A relatively small number of full-term kidders were shedding *C. burnetii* quantities comparable with aborted does. A similar shedding pattern has been described by de Cremoux et al. (de Cremoux et al., 2012).

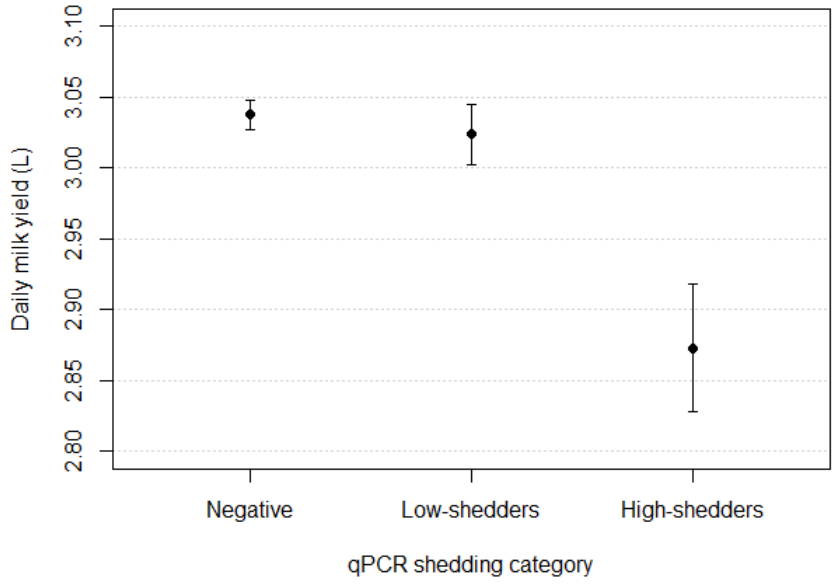

Figure B.4: Error bar plot showing mean daily milk yields in litres and 95% CI for different *C. burnetii* shedding levels as determined by qPCR in vaginal discharges of does sampled within 24 hours after kidding.

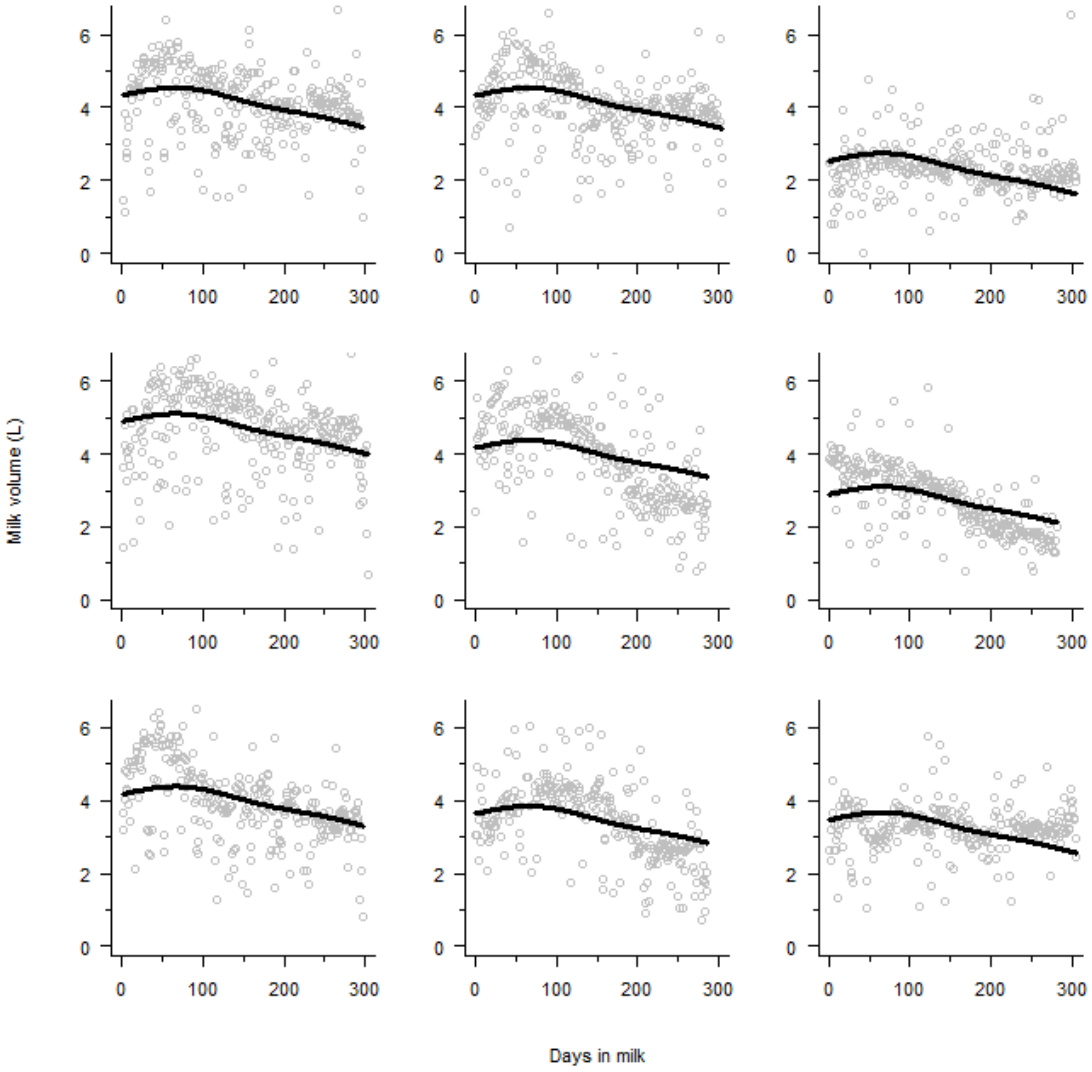

Figure B.5: Scatter plots showing observed daily milk yields (litres) for nine does as a function of days in milk. Superimposed on each plot are predictions (black line) using the regression model described in this chapter to compare daily milk yields among does classified as non-shedders (1^{st} column), low-shedders (2^{nd} column) or high-shedders (3^{rd} column).

B.2 Bulk tank milk testing

Testing of bulk tank milk (BTM) by PCR can be used for monitoring *C. burnetii* infection in dairy goat herds (Kim et al., 2005; Muskens et al., 2011; van den Brom et al., 2012). As part of this thesis BTM samples were obtained monthly throughout one year from three herds known to be Q fever endemic. BTM samples were tested using two different qPCR assays targeting the *com1* gene and the IS*1111* insertion sequence, respectively. Positive results in BTM were obtained for the three herds for almost all sampling points using the IS*1111* qPCR assay (i.e. 11 out of 12 sampling points for two herds and 9 out of 12 sampling points for the remaining herd). On the other hand, BTM samples were negative to *com1* qPCR assay at several sampling points (Table B.1). These results suggest that the use of the IS*1111* assay should be favoured over *com1* for Q fever monitoring strategies based on BTM testing. BTM testing offers a low-cost monitoring tool because it requires only one sample per herd to be tested. However, the frequency of testing should be relatively high (e.g. monthly) as intermittent *C. burnetii* shedding patterns in milk, dilution effects and sampling time relative to kidding could lead to false negatives. This is supported by the fact that even using the highly sensitive IS*1111* assay infection was missed at some sampling points during the study period. Still, regular testing of BTM is a monitoring tool of relatively low cost to farmers and its enforcement in commercial-scale dairy goat farms should be considered by government public health agencies, particularly in farms where access is granted to a non-vaccinated public.

Table B.1: Presence of *C. burnetii* DNA in BTM samples obtained at three farms that are part of a Q fever endemic dairy goat enterprise in Victoria, Australia. March 2016 – February 2017.

Farm	**Test**	**Date**											
		Mar-16	Apr-16	May-16	Jun-16	Jul-16	Aug-16	Sep-16	Oct-16	Nov-16	Dec-16	Jan-17	Feb-17
A	IS*1111*	+	-	-	+	+	+	-	+	-	+	+	+
	com1	-	-	-	-	-	-	-	NA	-	+	+	+
B	IS*1111*	+	+	-	+	+	+	+	+	+	+	+	+
	com1	-	-	-	-	-	+	-	-	-	-	+	-
C	IS*1111*	+	+	-	+	+	+	+	+	+	+	+	+
	com1	-	+	-	-	-	-	-	-	-	+	+	+

Appendix C

Chapter 4 supplementary material

C.1 Suplementary tables and figures

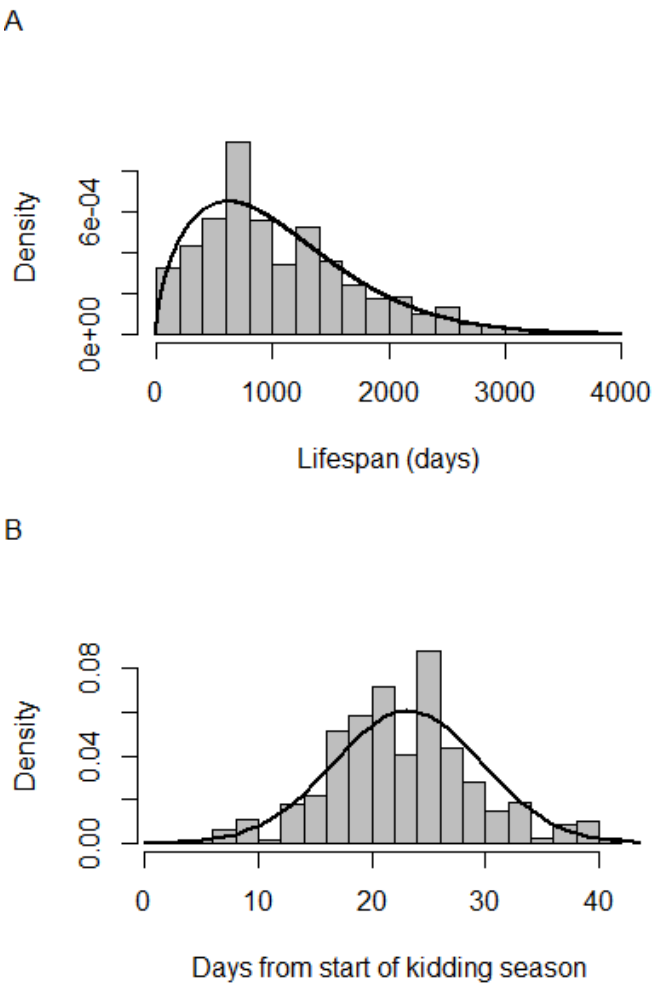

Figure C.1: A. Density histogram showing the distribution of observed lifespan (in days). Superimposed on this plot is a Weibull distribution fitted to the data (shape = 1.9, scale = 944.5). **B.** Density histogram showing the distribution of kidding dates as a function of the number of days from the planned start of kidding. Superimposed on this plot is a Normal distribution fitted to the data (mean = 23, SD = 6.6).

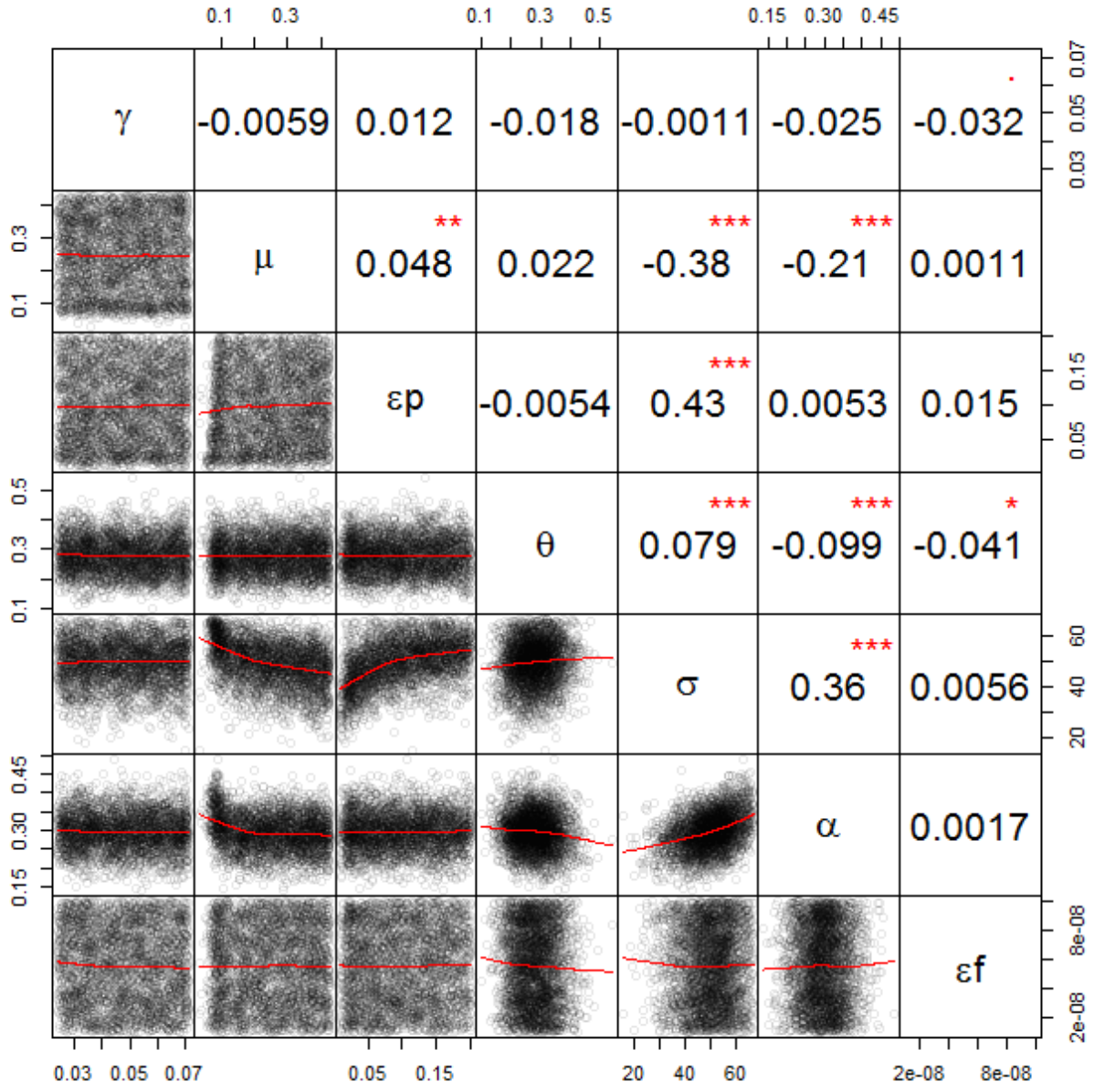

Figure C.2: Correlation matrix of parameter values accepted by the ABC rejection-sampler.