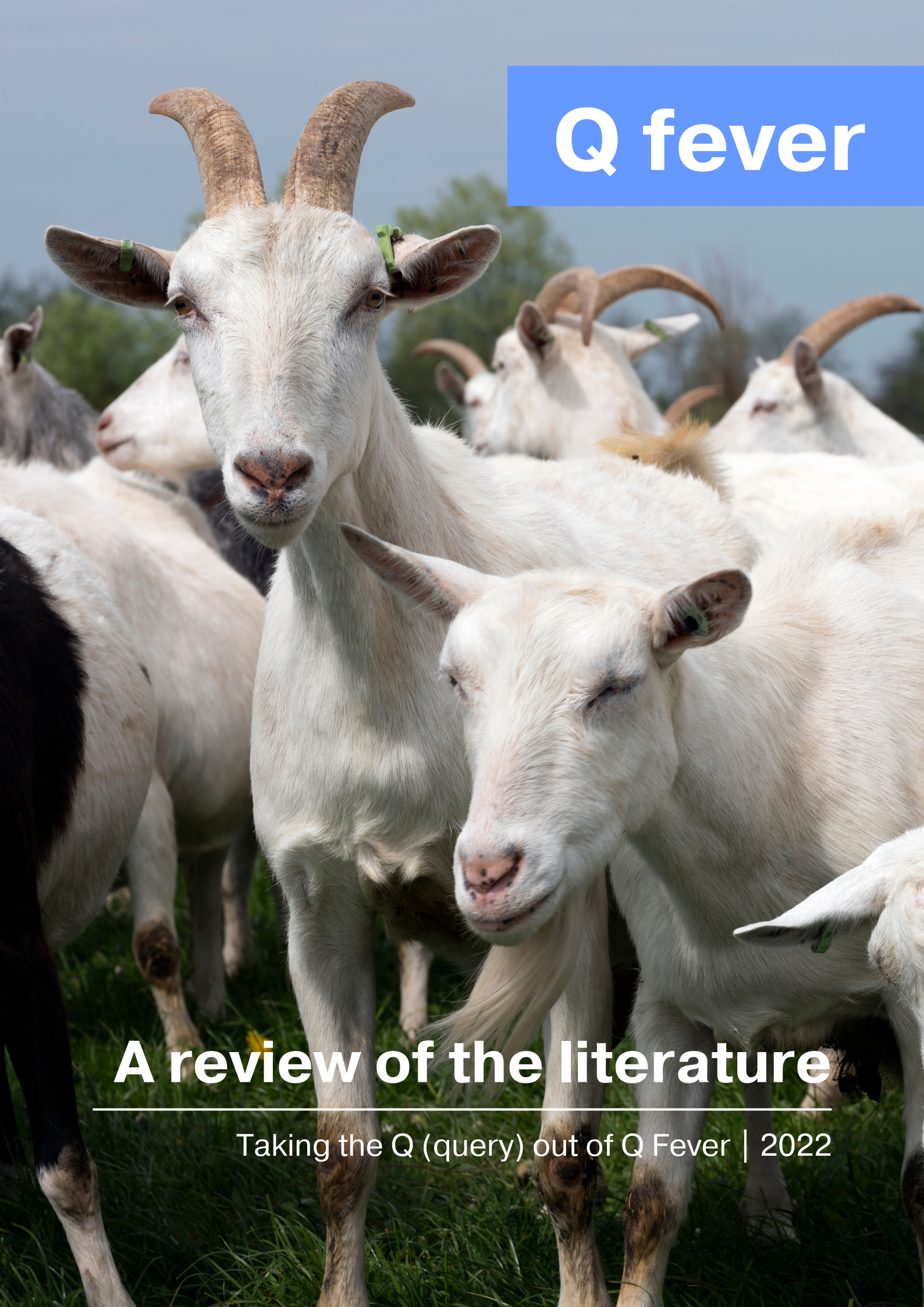

Q fever

A review of the literature

Taking the Q (query) out of Q Fever | 2022

A RESEARCH-INDUSTRY PARTNERSHIP

Taking the Q (query) out of Q Fever is a multidisciplinary research-industry group that aims to improve understanding of Q fever reservoirs, amplification and transmission pathways. Our research will help direct biosecurity resources more efficiently in Australia and more broadly. In turn this will reduce the burden of an extremely debilitating disease in rural communities and around the globe.

Our Collaborators

THE UNIVERSITY OF MELBOURNE

THE UNIVERSITY OF QUEENSLAND AUSTRALIA

THE UNIVERSITY of ADELAIDE

Charles Sturt University

Australian Rickettsial Reference Laboratory

Goat VETERINARY goat vet CONSULTANCIES

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Review of the literature on Q Fever

This literature review represents the combined work of Michael Muleme, Caitlin Wood, Tabita Tan, Jose Canevari and Hasanthi Abeykoon. It was compiled as part of the 'Taking the Query out of Q fever project' by Lynne Hayes. It is intended for personal use and not for further distribution.

Contents

1. General introduction

Coxiella burnetii is a zoonotic intracellular bacterium that has been detected in a diverse range of animals and geographic regions. Coxiellosis, the infection in animals is listed by the OIE (World Organisation for Animal Health) as a reportable disease for member countries (OIE, 2018). Infection can easily spread from animals to humans and is reported to be a re-emerging zoonosis in many countries (Arricau-Bouvery and Rodolakis, 2005). The human disease, Q fever is notifiable in most developed nations. The capacity of *C. burnetii* to survive in the environment coupled with its high infectiousness has led to its classification as a potential bioterrorism agent by the United States Center of Disease Control (CDC) (Oyston and Davies, 2011; HHS et al., 2017).

Q fever occurs on every continent of the world except for Antarctica and New Zealand (Raoult et al.; Hilbink et al., 1993; Arricau-Bouvery and Rodolakis, 2005). 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 (Hilbink et al., 1993). Across the globe, numerous sporadic human cases occur annually, and occasional outbreaks are also common (Duron et al., 2015). Q fever is predominantly reported in Europe (Great Britain, France, Italy, Switzerland, Netherlands, Spain, Bulgaria, Greece, Cyprus) and Australia, where the incidence of notification is higher than other countries (Smith, 1989; Lyytikäinen et al., 1998; Gidding et al., 2009; Banazis et al., 2010; Guatteo et al., 2011; Schneeberger et al., 2014; Bond et al., 2016; Rizzo et al., 2016). However, as the prevalence of major endemic diseases such as malaria and brucellosis decreases in developing countries, Q fever is gaining increasing recognition as an emerging or neglected cause of non-malaria febrile illness of people in these regions (Dean et al., 2013; Klaasen et al., 2014; Njeru et al., 2016; Boone et al., 2017). For all countries, reported rates of Q fever are likely to be an underestimate of true disease incidence, as majority of infections are either asymptomatic or have mild and non-specific clinical signs (Gidding et al., 2009; Chiu et al., 2010).

2. History

Q fever in humans was first described in Queensland abattoir workers and dairy farmers by Australian physician Holbrook Edward Derrick in 1934. Derrick depicts an acute febrile illness initially named “Q” (for query) fever as the cause of disease was as yet unidentified (Derrick, 1937). Derrick submitted experimentally infected guinea pig tissue to Frank Macfarlane Burnet, an Australian microbiologist, who observed a rickettsia-like organism from the spleen smears. At a similar time, Davis and Cox isolated an infectious agent from ticks, *Dermacentor*

andersoni, near Nine Mile Creek whilst investigating the cause of 'spotted fever' in the Rocky Mountains, Montana USA. It was soon discovered through an accidental laboratory acquired infection that the tick isolate produced similar clinical signs to the affected abattoir workers in Queensland and was the same causative organism as Q fever in Australia (Davis et al., 1938; Dyer, 1939; Stoker and Marmion, 1955; Marrie, 1990; McDade, 1990; Hechemy, 2012). The simultaneous and significant contributions of Herald Cox in the USA and Frank Macfarlane Burnet in Australia to identifying the Q fever agent has led to the joint naming of the rickettsia like organism as *Coxiella burnetii* (Marrie, 1990).

3. Outbreaks

3.1. Overseas

Between 2007 and 2011, the Netherlands experienced the largest recorded human Q fever outbreak to date (Delsing et al., 2010). Over 4000 cases of acute Q fever were reported with many patients requiring hospitalisation. The outbreak was traced back to dairy- goat and sheep farms infected with coxiellosis (Roest et al., 2011a; Ladbury et al., 2015). The intensive, small-ruminant dairy industry had been steadily increasing and herds had experienced episodes of abortions due to *C. burnetii* for some years without known human cases (Roest et al., 2011a). From 2008 to 2009 over 2300 cases were reported at the peak of the epidemic, with seasonal cases subsequently decreasing. Many cases had no direct contact to the infected animals, however, resided within a 5 km vicinity of infected farms (Roest et al., 2011a).

In response to the outbreak, in June 2008, it was legislated in the Netherlands that coxiellosis become notifiable in small ruminants. This was the first of many animal control measures implemented including mandatory vaccination of dairy sheep and goats and finally culling of all pregnant sheep and goats on Q fever positive farms (Roest et al., 2011a). Approximately 50 000 pregnant dairy-goats were destroyed in accordance with legislation.

Within the 20 years that preceded the outbreak the size of the national goat herd had approximately quadrupled. This rapid 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 in the number of dairy goat farms (Enserink, 2010). 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. It has also been hypothesized 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 (Wolff and Kouwenaar, 1954).

A Q fever outbreak of a scale comparable to that of the outbreak in the Netherlands occurred in Bulgaria with more than 2000 people suspected to have contracted Q fever in a period of 6 months in 1993. Early reports of Q fever occurrence in Bulgaria suggest *C. burnetii* had been already circulating in livestock populations from as far back as 1949. With 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 farmed which were seen by individual 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 the spread of the disease (Serbezov et al., 1999). Publications in English describing this outbreak are scarce.

These events highlight that the spread of *C. burnetii* can be a serious threat to public health, animal health and industry. Since the Netherlands outbreak epidemic, there has been an increase in published research on the epidemiology of coxiellosis in ruminants as well as the zoonotic spillover effects. These studies provide invaluable analysis and insights into the Netherlands outbreak and potential risks for surrounding areas. However, Australia has very different agricultural industries and ecosystems, therefore findings from studies in Europe may not be applicable to the Australian situation.

3.2. **Australia**

In 2012 an outbreak of Q fever linked to a goat and sheep dairy enterprise occurred in Victoria, resulting in seventeen employees and one family member confirmed with Q fever and six additional suspected cases (Bond et al., 2016). The dairy associated with the outbreak is comprised of three farms not distant one from the other and at the time of the outbreak kept an approximated total of 5,000 goats and 800 sheep. Animals are mainly kept in sheds on a deep litter floor and fed total mixed rations. Goats are managed to kid into four batches at different times of the year (i.e. autumn, winter, spring and summer) to achieve a sustained supply of milk. A cheese factory is located within the main of the three farms. The entry date of infection into the herd could not be determined. A perceived increase of abortions since 2004 was reported by farm staff members which could potentially be associated with disease entry. Another hypothesis pointed to the importation of a group of goats from Queensland, an Australian state known to have comparatively high Q fever report rates in humans in 2011. 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. The same practices put in place during the outbreak in the Netherlands for management of manure and aborted foetuses were recommended. Selling of kids under 2 months of age was not allowed and, for older animals, the buyer had to be notified about the Q fever status of the herd. Whilst no further human cases were reported, the disease remained endemic among goats (Muleme et al., 2017). Importation of an existing animal vaccine from Europe was not permitted due to biosecurity concerns and works towards the development of an autogenous animal vaccine were initiated.

4. Coxiella burnetii bacteriology

4.1. Classification and morphology

Coxiella burnetii was first classified as a *Rickettsia* in the class *Alphaproteobacteria,* due to its recovery from ticks and its inability to be grown axenically (Omsland et al., 2009). However, recent 16S rRNA sequence analysis has resulted in the *Coxiella* genus being reclassified into the class *Gammaproteobacteria* within the order Legionellalales (Maurin and Raoult, 1999; van Schaik and Samuel, 2012). *C. burnetii* is the only species recognised in the *Coxiella* genus, although a second highly homologous species has been proposed, *Coxiella cheraxi*, that was isolated from Australian crayfish (Cooper et al., 2007).

Coxiella burnetii is a small pleomorphic, obligate intracellular bacterium. It has been described as coccoid, bacilli and granular in appearance, depending on the stage of its developmental cycle (McCaul and Williams, 1981). While it is often reported to be gram negative, *C. burnetii* is not easily nor consistently stained using the Gram method (McCaul and Williams, 1981)). It does however, possess a cell membrane similar to gram negative bacteria and can also be visualised using the Gimenez stain (McCaul and Williams, 1981; Samuel and Hendrix, 2009).

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). Scott and Williams (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. Indeed, the high stability of the SCV of *C. burnetii* led to an increase in pasteurization temperatures in the 1950s (Enright et al., 1957). Evstigneeva 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.

C. burnetii has a spore-like small cell variant (SCV) which can survive harsh environmental conditions such as desiccation; and a metabolically active large cell variant (LCV) (Arricau-Bouvery et al., 2005; Gidding et al., 2009; Schimmer et al., 2011). SCVs are produced when

the LCV is lysed after exposure to environments outside the host (Aitken et al., 1987). The SCV is minute with a diameter of 0.2 to 0.5 μm and can be carried in dust particles and fluid aerosols thus facilitating infection of humans and animals via inhalation (Tigertt et al., 1961; Coleman et al., 2004; Jones et al., 2006; Angelakis and Raoult, 2010). Upon transmission and phagocytosis by host cells, SCV develop into the 31 metabolically active replicative LCV through a lag phase that is characterized by production of more than 48 structural proteins within the acidic vacuoles of phagocytic host cells (van Schaik and Samuel, 2012; Norris et al., 2013). Multiplication of the LCV occurs from 2 to 6 days following infection under the acidic environment in phagolysosomes of host cells which may explain the 1-3-week period between infection and the first detection of antibodies (Kazar, 2005; van Schaik and Samuel, 2012).

There have been suggestions of an intermediate extra-cellular “Small Dense Cell” variant which is unresponsive to pressure and facilitating infection across host-cells instead of pressure-sensitive SCV (Kazar, 2005). However, further studies are needed to confirm the existence of this intermediate variant.

4.2. Phase variation in C. burnetii

Phase variation has been reported in *C. burnetii* since 1956 when the ability to react with complement was observed in the Nine Mile strain only after the 8th passage in embryonated egg yolk (Stoker and Fiset, 1956; Bobb and Downs, 1962). This characteristic was later described as a change from phase 1 (unable to react with complement) to phase 2 (able to react with complement) (Stoker and Fiset, 1956; Bobb and Downs, 1962) and has been attributed to permanent chromosomal deletions in the clone 4 Nile Mile strain (Arricau-Bouvery et al., 2005; Kazar, 2005).

The LCV form is able to change into the SCV form prior to excretion from the host. The SCV is spore-like and is resistant to changes in osmotic pressure, high ambient temperatures and ultra-violet radiation enabling its survival in relatively harsh environmental conditions, permitting transmission of viable bacteria in dust from the environment (Toman et al., 2012). When inhaled or possibly ingested, the SCV is able to enter host phagocytic cells and then turn back into the LCV to enable replication (Roest et al., 2013a).

In other strains of C. burnetii, this phase variation seems to be reversible with phase 2 organisms obtained after passage in embryonated eggs having been shown to revert to their phase 1 properties after re-introduction in guinea pigs (Stoker and Fiset, 1956). The permanent change from phase 1 to phase 2 in the clone 4 Nine Mile strain has been utilised to create phase 2 organisms with only phase 2 antigens for use in serological tests to detect antibodies against phase 2 antigen in susceptible hosts. Similarly, the change from phase 2 to phase 1 upon reintroduction into mammalian hosts has been used to make phase 1 antigen

for detection of antibodies against phase 1 antigen in infected or vaccinated hosts (Stoker and Fiset, 1956).

Naturally occurring phase 1 *C. burnetii* organisms have been shown to possess both phase 1 and 2 antigens whereas phase 2 organisms contain only the phase 2 antigen (Stoker and Fiset, 1956). Phase 2 antigens are accessible on the surface of the organisms (Bobb and Downs, 1962; Kazar, 2005) and elicit early 32 immune responses during infection. The phase 1 antigen is presented to the immune system much later following structural changes in the lipopolysaccharide (LPS) structure triggering the late appearing antibodies (Stoker and Fiset, 1956; Bobb and Downs, 1962; Cutler et al., 2007; Delsing et al., 2012). Thus, the appearance of antibodies against phase 2 in previously seronegative animals and humans is a marker of recent infection.

It has been suggested that the delay in production of antibodies to phase 1 antigen results from the LPS being inaccessible to immune recognition due to an extended carbohydrate structure that shields it from components of the immune system (Hackstadt, 1990; Fournier et al., 1998). This leaves only immune recognition of surface proteins and thus producing a phase 2 seroreactivity (Hackstadt, 1990). Other studies have attributed the delayed immune response to the poor uptake of phase 1 organisms via the phagolysosomal pathway resulting in a lag in exposure of the concealed phase 1 antigen to the immune system and delayed production of antibodies against it (Raoult et al.; Waag, 2007).

5. Coxiella burnetii genome and virulence

The *C. burnetii* genome is circular and variable in size, ranging from 1.5 to 2.4 Mb (Willems et al., 1998; van Schaik and Samuel, 2012). Although this bacterium is often referred to as homogenous, molecular studies have identified and described six different genomic groups (van Schaik and Samuel, 2012). The first whole genome sequence of *C. burnetii* (Nine Mile strain) was published in 2003 (Seshadri et al., 2003). The AuQ01 strain was the first to be completely sequenced in Australia after isolation from an acute Q fever patient (Walter et al., 2014). Since then, many *C. burnetii* strains have been successfully sequenced and researchers have been able to compare strains and draw correlations between genomic groups of *C. burnetii* and the development of varying human disease (Glazunova et al., 2005; van Schaik and Samuel, 2012). However, there are still gaps in the knowledge of specific *C. burnetii* virulence factors and studies focused on identifying and describing strain virulence factors will no-doubt add significant knowledge to the field (Million and Raoult, 2015).

There are two antigenic phase variations of *C. burnetii*, phase I and phase II as determined primarily by the composition of cell wall LPS (Hackstadt, 1990). The phase I antigen is

phenotypically smooth and can be identified on *C. burnetii* isolated directly from animals and human patients; it is then converted to the rough, phase II antigen through multiple passage of embryonated eggs or an immunocompentent host (Narasaki and Toman, 2012). The smooth phase I antigen appears to be the virulent phase, which can invade immunocompetent hosts and is protected from the hosts immune system. The phase II antigen has an incomplete LPS due to a genetic deletion and is therefore, an avirulent variation that is unable to survive immunocompetent host responses (Williams and Thompson, 1991; Hoover et al., 2002).

5.1. Host cell/tissue invasion

It is well documented that host monocytes and macrophages are target cells for *C. burnetii* invasion in vertebrates (Amara et al., 2012). The bacterium has adapted to enable multiplication in the acidic vacuoles of eukaryotic cells without being destroyed, thus it is able to invade host phagocytes and survive and replicate inside phagolysosomes (Maurin and Raoult, 1999; Flannagan et al., 2009; van Schaik and Samuel, 2012). Once replication occurs in local lymph nodes, the intracellular bacteria may circulate to peripheral sites where optimal survival will ensue. A published case report from Australia found *C. burnetii* could persist in the bone marrow following "successful" treatment of acute Q fever (Harris et al., 2000). Adipose tissue and the placenta appear to be tissues where *C. burnetii* can also survive concealed from the host's immune response (Amara et al., 2012). Although the exact mechanisms are not completely understood, changes in the host's immune response during pregnancy may allow the infection to persist with recrudescence of shedding at the time of parturition. The bacteria have been found in placental trophoblasts of animals and humans and within placental macrophages (Amara et al., 2012; Roest et al., 2013a).

5.2. Molecular epidemiology

Although *C. burnetii* is the only species in the genus *Coxiella*, strain variations have been identified from different human, animal and environmental sources globally (Jado et al., 2012; Ceglie et al., 2015). Conventional serological methods cannot discriminate between exposures to different bacterial strains. Characterisation of *C. burnetii,* using molecular genotyping methods, is advancing rapidly and appears to be an ideal tool to help unravel the complex epidemiology of infection across multiple host species and geographic regions (Roest et al., 2011b; Massung et al., 2012). Studies in Spain, Portugal, Italy, France and the Netherlands have observed genotype clustering within animal species (livestock and wildlife) and within geographical locations (Astobiza et al., 2012; Piñero et al., 2015; González-Barrio et al., 2016). In particular it has been found that cattle genotypes are highly species-specific; *C. burnetii* within these "cattle-clusters" have not commonly been associated with human Q fever cases (Ceglie et al., 2015; Piñero et al., 2015; Joulié et al., 2017). Genotypes of

C. burnetii isolated from goats and sheep were more likely to be identified in human Q fever cases, however these small ruminant genotypes may occasionally be found to infect cattle (Astobiza et al., 2012).

During the Q fever outbreak in the Netherlands, molecular genotyping techniques were successfully utilised to trace the probable source of human infection (Roest et al., 2011b). This enabled control measures to be implemented at the apparent origin (infected dairy goats) thus reducing human Q fever cases. Currently, no data are available on specific genotypes of *C. burnetii* found in cattle or other reservoir animals in northern Australia (Vincent et al., 2016). In Victoria, the *C. burnetii* genotype responsible for a human Q fever outbreak in 2015 was successfully isolated from goat and human samples (Bond et al., 2016). The identification of bacterial genotypes present in a range of Australian animals is crucial to ascertain the likely sources of human disease and to aid in potential outbreak investigations.

There have been recent advances in isolating novel genotypes from human Q fever cases in Australia (Tozer, 2015; Vincent et al., 2016). In one study, 42 C. burnetii isolates from acute Q fever patients were found to be genetically distinct compared to more than 300 C. burnetii strains from patients of other countries (Vincent et al., 2016). These findings support the theory that Australian strains have evolved to produce a unique phylogenetic clade of C. burnetii most likely due to geographical isolation (Vincent et al., 2016). To extend the current knowledge of molecular epidemiology within Australia, it is necessary to compare isolates from diverse hosts and locations across Australia.

6. Epidemiology (human disease)

6.1. Worldwide

Livestock are commonly implicated as the source of human infection, and the number of studies investigating prevalence in this reservoir has been increasing. A literature review of Q fever publications across the globe was conducted by (Guatteo et al., 2011) on the apparent prevalence of *C. burnetii* infection at the animal-level, herd-level and within-herd-levels for three main domestic ruminant species: cattle, sheep and goats (Guatteo et al., 2011). Great variation was found among prevalence data at all three prevalence levels for all three species. Overall, the animal and herd level seroprevalences, regardless the species, were at least 15–20% in many countries.

Apparent prevalence values slightly higher in cattle (20.0% and 37.7% of mean apparent prevalence at animal and herd level, respectively) than in small ruminants (around 15.0% and 25%, respectively for animal and herd level in sheep and goat) (Guatteo et al., 2011).

Most of these studies have investigated seroprevalence rather than shedding prevalence. Their focus was at the animal level and there was little data available on herd and within-herd prevalence. Studies involving cattle were the most common followed by sheep and then goats. Information about *C. burnetii* in Oceania (including Australia) is increasing but still limited, especially in goats (Guatteo et al., 2011).

Very few of the studies reviewed had a well-designed sampling strategy to collect reliable prevalence data, and none provided clear informative values about the tests used. For these reasons, the apparent prevalence results should be interpreted with caution (Guatteo et al., 2011).

6.2. **Australia**

Since its initial discovery in Brisbane, Queensland, in the 1930s, *C. burnetii is* now accepted as being endemic and widespread across Australia. In fact, Australia has the highest rate of Q fever notifications for humans than anywhere else in the world, with the incidence of disease being 2, 3 and 6 times higher than in France, European Union and the United Kingdom, respectively (Gidding et al., 2009). Notification rates in Europe for countries with high incidence (France, Germany, the UK, Spain, Bulgaria, Greece, Cyprus) were <0.5 per 100,000 (2005 to 2007), compared to Australia where the annual Q fever notification rates between 1991 and 2006 ranged from 1.7 to 4.9 per 100,000 (Gidding et al., 2009; Chiu et al., 2010).

The eastern states of Australia, Queensland and New South Wales account for the majority (82%) of cases notified each year, with southwest Queensland and adjacent northwest NSW reporting the highest notification rates at >50 cases per 100,000 population (Garner et al., 1997; Gidding et al., 2009; Bond et al., 2017).

Unlike the occurrence of Q fever in Europe where peak seasons of disease reporting aligned with the small ruminant reproductive cycle, no seasonal prevalence patterns are apparent in the Australian notification data. This might reflect the different epidemiology of *C. burnetii* in Australia, involving various transmission routes, environment of reported cases, relative contribution of sporadic cases and alternative sources of infection, such as native wildlife (Gidding et al., 2009; Chiu et al., 2010).

Q fever is a nationally notifiable disease of humans in Australia, but Coxiellosis is not a notifiable disease of livestock. This makes it necessary to survey the livestock population to ascertain the amount and distribution of *C. burnetii* infection in animals. Independent studies investigating the prevalence of *C. burnetti* in animal sources have been conducted across most of Australia. When comparing prevalence of infection in cattle, Queensland has a much higher seroprevalence relative to the other states. Interestingly, Queensland also has the

highest rate of human Q fever notification. A very high seroprevalence has been recorded in feral goats from western NSW and South Australia, although it is not known why this is the case.

Most Australian studies have involved cattle in Australia rather than small ruminants. Recent studies have used an ELISA test rather than the far less sensitive CFT, and most have reasonable sample sizes (>1000 samples). Samples were mostly collected by convenience, and no study clearly included information regarding the ability of the serological test used to correctly identify infected and uninfected animals. These characteristics of Q fever prevalence literature in Australia are similar to what was described in the review of Q fever literature worldwide (Guatteo et al., 2011). In majority of these studies, samples were obtained in a way that was not completely representative of the target population. Nonetheless, they still provide a rough estimate of the level of infection in the population which is better than no information at all.

6.2.1. Victoria

Compared to Queensland and New South Wales, Victoria has a much lower 10 year average annual Q fever notification rate at 0.5 cases per 100,000 population (Australian Government Department of Health, 2015; Bond et al., 2018). Prior to the implementation of the national vaccination program, most cases were abattoir associated, with pre-existing immunity to *C. burnetii* in Victorian farmers being 5%, compared with 15% and 21% for farmers in NSW and Queensland respectively (Bond et al., 2018). The assumption that the low number of human cases of Q fever in Victoria were acquired from livestock being moved interstate has prompted some authors to suggest that Q fever is not endemic in Victoria (Bond et al., 2018). However, structured surveys demonstrating freedom of disease in the animal sources would be required to prove the absence of infection in this state and the single study of Victorian cattle in 1972 did find a low seroprevalence using the CFT (Hore and Kovesdy, 1972).

A review of human Q fever notification data in Victoria from 1994 to 2013 found that 30% of cases were acquired from within the state, providing further evidence that Q fever is endemic in Victoria. Cases were presumed locally acquired after excluding for associations with abattoirs and related industries, travel interstate or overseas and contact with livestock from interstate. More than 60% of locally acquired cases were attributed to exposure on farms. Counts of acute Q fever cases amongst dairy farmers were increasing by 14.9% per annum, suggesting that this group in particular might be at increased risk (Bond et al., 2018).

Outside of abattoir-associated outbreaks, several foci of local acquisition were identified in Gippsland, Wodonga and in the Golden Plains shire. Most of the outbreaks (≥2 epidemiologically linked cases) in these areas have been linked to exposure to livestock. A

community outbreak in Wodonga in 2006 was attributed to a local abattoir. The Golden Plains shire outbreak associated with a dairy goat farm is the largest in Australia, so far with 18 people directly affected. Seven small (≤3 human cases) farm-associated outbreaks (out of 10 in the last 20 years) have been reported in Gippsland. Of these, six were associated with cattle farms and one with a goat farm (Bond et al., 2016; Bond et al., 2018). The epidemiological and spatiotemporal analyses from the study suggests a low level of endemic transmission within the state, with multiple foci of increased zoonotic transmission (Bond et al., 2018). Ruminants are presumed to be the main source of infection for these regions, although wildlife may also play a role. Serological surveys of livestock and wildlife in Victoria would be of benefit to clarify the public health and production risks from both of these sources (Bond et al., 2018).

There has been only one previous study addressing the prevalence of *C. burnetii* infection in Victoria's livestock population. A serological survey published on Victorian dairy cattle in 1972 found only 0.5% of the 1,576 animals sampled to be positive with the complement fixation test (CFT) (Hore and Kovesdy, 1972). However, the CFT is known to have a poor sensitivity and so this study may have considerably underestimated the true prevalence of infection. To date, there have been no published prevalence studies in small ruminants. Consequently, the prevalence of *C. burnetii* infection in Victoria's domestic ruminant population remains uncertain, and it is unknown if this situation has changed in the last 45 years.

6.3. Animal reservoirs

Coxiella burnetii has been identified in a wide range of vertebrate and invertebrate reservoirs, including mammals, some birds, Australian marsupials and ticks (Babudieri, 1959; Cooper et al., 2012). From an ecological perspective*, C. burnetii* is maintained in two well-recognised, effectively independent reservoirs: wildlife and domestic ruminants. Spread to any number of accessory hosts can occur from these two reservoirs (Aitken et al., 1987; Hilbink et al., 1993), establishing a zoonosis that has a human– livestock–wildlife interface.

Australia's earliest clusters of Q fever cases were associated with working at an abattoir processing a high number of pregnant dairy cattle, an outbreak in sheep shearers and an outbreak involving a family with no obvious animal exposure, only a history of picking pineapples likely contaminated by bandicoot faeces (Derrick, 1937; Derrick et al., 1959; Derrick, 1961).

6.3.1. Ruminants

It is commonly reported that cattle, sheep and goats are the most frequent source of human Q fever (Maurin and Raoult, 1999; Porter et al., 2011; Million and Raoult, 2015; Eldin et al., 2017). High numbers of bacteria are shed in milk and the placenta and associated fluids and therefore, zoonotic spillover is expected when humans are in close contact with parturient and

lactating livestock (Guatteo et al., 2006). It is likely that between countries and regions, different ruminant species may be more commonly associated with human infection (Babudieri, 1959). In the Netherlands pregnant dairy-goats have been implicated as the most significant source of human infections (Roest et al., 2013c). In the United States of America (USA), although high seroprevalence and PCR positive bulk talk milk samples have been reported in dairy-cattle, the predominant strain of *C. burnetii* identified in cattle has not been found in human disease (Pearson et al., 2014). However, a study in Minnesota, USA, found a significant association between the number of sheep flocks in a region and incidence of human Q fever. No association was evident with cattle or goat properties (Alvarez et al., 2018).

In Australia, Q fever cases have been commonly associated with working at abattoirs that slaughter cattle, sheep and goats (Gidding et al., 2009). However, a recent non- abattoir outbreak occurred at a dairy-goat farm in Victoria (Bond et al., 2015). A significant outbreak (involving 25 notified cases) has also been associated with attending a sheep saleyard on a dry and windy day and another (involving 4 notified cases) was traced back to working at a cosmetics supply factory that used ovine-derived products (O'Connor et al., 2015; Wade et al., 2006).

6.3.2. Companion animals

It has long been known that cats and dogs have the potential to spread *C. burnetii* (Babudieri, 1959; Kosatsky, 1984; Pinsky et al., 1991). In Australia, two recent outbreaks have been associated with parturient cats (Kopecny et al., 2013; Malo et al., 2018). Seroprevalence studies have also identified exposure in several populations of domestic dogs, domestic cats and breeding cats (Shapiro et al., 2016, 2015). However, the prevalence rates in companion animals in Australia is low (Shapiro et al., 2015; Shapiro et al., 2016) and cases of canine and feline Q fever cases are infrequent especially considering the abundant and widespread ownership of these animals (Norris et al., 2013). Owning a dog or cat has not been shown to increase the risk of infection with *C. burnetii* (Skerget et al., 2003). Horses are susceptible to infection as shown by positive serological and PCR testing, however it is not clear if they are a common source of human infection (Marenzoni et al., 2013; Tozer et al., 2014).

6.3.3. Wildlife

In Australia there are reports of high seroprevalence and PCR detection of *C. burnetii* in native marsupials, dingos, foxes and feral cats (Cooper et al., 2013; Shapiro et al., 2015), with evidence of active shedding in their excreta (Potter et al., 2011; Stevenson et al., 2015). The exact role that native wildlife and feral animals may play in the spread of Q fever to humans and in maintaining infection within livestock populations is unknown. However, it seems likely that they are a potential source of *C. burnetii*. Human cases of Q fever have been linked to

recent direct contact with kangaroos or wallabies (Stevenson et al., 2015; Flint et al., 2016) and a few cases in New South Wales were postulated to have been acquired from kangaroo faeces aerosolised by wind or lawn mowing (Archer et al., 2017). From a 10 year review of Q fever cases in a hyper-endemic region of North Queensland, 69.8% of cases reported exposure to macropods compared to only 23.8% which reported exposure to cattle (Sivabalan et al., 2017).

In Guyana, South America, repeated human Q fever outbreaks have been linked to the three toed sloth, a reservoir of a highly virulent strain of *C. burnetii* (Million and Raoult, 2015). The European rabbit is a reservoir of *C. burnetii* in Spain and has been reported to be associated with human Q fever pneumonia cases (Gonzalez-Barrio et al., 2015; Marrie et al., 1986). This species of rabbit was introduced to Australia in 1879 and has since become one of Australia's major feral-animal pests (Fenner, 2010).

6.3.4. Ticks

Ticks are able to carry and transmit *C. burnetii*, however, they do not appear to have an essential role in the maintenance of infection within animal or human populations (Woldehiwet, 2004). *C. burnetii* has been identified in the microbiome of native Australian ticks (Pope et al., 1960; Tozer et al., 2014; Graves and Stenos, 2017) that infest native wildlife naturally and domestic animals opportunistically. The bacterium multiplies in the gut and the salivary glands of its arthropod host and transmission to animal hosts occurs through direct contact, blood sucking, and tick faeces (Hilbink et al., 1993). It has been suggested that infection is perpetuated in a sylvatic tick-wildlife lifecycle that can spill over into domestic animals, then continue to cycle within herds and flocks (Pope et al., 1960; Aitken et al., 1987). Although ticks have been shown to be competent vectors in some situations, percutaneous transmission through tick bites is limited to domestic mammals and wildlife (Graves and Stenos, 2017). Under natural conditions, Q fever infection in humans is far more frequently airborne than vector-borne. Virtually all human cases occur via infected aerosols (Graves et al., 2006; Duron et al., 2015) and ticks are not considered to be a significant source of infection for humans (Astobiza et al., 2011c; Sprong et al., 2012; Duron et al., 2015).

Recent genetic studies suggest that research relying on the PCR detection of *C. burnetii* from ticks may have high false positive results due to a "coxiella-like" endosymbiont (Duron, 2015; Elsa et al., 2015). It has been suggested that the pathogenic *C. burnetii* bacteria may have evolved from such a tick endosymbiont, however for now this is simply a hypothesis (Duron et al., 2015).

7. Transmission

7.1. Environmental transmission

The spore-like form of *C. burnetii* can survive for long periods in the environment and direct contact with shedding animals is not necessarily required for transmission of the organism. In Queensland, *C. burnetii* DNA has been detected in urban soil, the atmosphere and dust samples from vacuum cleaners. General environmental contamination could account for surprisingly high seroprevalence rates in Queensland's metropolitan regions, which approaches that of rural areas (Tozer et al., 2011; Tozer et al., 2014). Urban communities are usually considered as low risk, but there is growing concern over increases in the number of notified cases in non-farming rural and metropolitan populations, including human cases with no direct animal contact (Maurin and Raoult, 1999; Tozer et al., 2014; Archer et al., 2017).

Indirect transmission through carriage on fomites and, more importantly, in dust or aerosols, also occurs as the small-cell variant of the organism is highly resistant and can survive in a desiccated form for several months (Hilbink et al., 1993; Kersh et al., 2013). People can be infected in the absence of ruminants by the inhalation of contaminated dust from wool, manure or clothes soiled with faeces (Garner et al., 1997; Arricau-Bouvery and Rodolakis, 2005). *C. burnetii* is highly infectious for humans with the minimum infectious dose via inhalation being as little as one to ten organisms. This suggests that even low amounts of environmental contamination can lead to an outbreak (Wedum et al., 1972; Sawyer et al., 1987; Archer et al., 2017).

In pregnant mammals, the organism multiplies extensively in the trophoblasts of the placental villi and is shed in large numbers during both abortions and normal delivery (Aitken et al., 1987; Roest et al., 2012). The largest numbers are found in the placenta, foetal membranes and birthing fluids, with fewer in the urine, faeces and milk of affected animals (Hilbink et al., 1993). Aborted material can contain as many as 1,000,000,000 *C. burnetii*/g of placenta in the goat, and excretion in vaginal mucus and faeces can continue for several months (Bouvery et al., 2003). Massive environmental contamination occurs during parturition and the highest concentrations of environmental *C. burnetii* has been found in goat birthing areas (Kersh et al., 2013; Duron et al., 2015). Epidemics typically occur following a birth or abortion where the environment becomes contaminated with infected birthing material. Infectious dust from contaminated environments can also be dispersed by wind for some distances (Aitken et al., 1987). Outbreaks of Q fever in European villages, towns and cities have been attributed to airborne spread and community-wide dispersal of the bacterium up to 5 km from nearby source farms (Smith, 1989)

In Australia, the occurrence of drought is associated with higher incidences of human Q fever (Graves et al., 2006). *C. burnetii* survives well in dry weather conditions and dry periods may

promote the formation and propagation of infectious dust and aerosols (Lyytikäinen et al., 1998; Gilsdorf et al., 2008). Drought conditions also cause increased stock movements and, in conjunction with dry, dusty conditions, this favours the dispersal of *C. burnetii* in dust. This increased risk for infection is supported by observations that peak rates of Q fever notifications in Australia occurred during severe drought periods (2002-2004) (Gidding et al., 2009; Lowbridge et al., 2012). Links between dry weather, strong wind, and the spread of infection in dust from animal sources has also been reported in other Q fever outbreaks in Europe (Aitken et al., 1987; Lyytikäinen et al., 1998; Gilsdorf et al., 2008).

7.1.1. Sampling methods used to detect C. burnetii in the environment

Environmental sampling, in combination with advanced molecular techniques such as real-time PCR, can identify the source of Q fever outbreaks by detecting *C. burnetii* DNA in the environment. This has informed risk assessment and guidelines for Q fever prevention and control (O'Connor et al., 2015). Merits and limitations of environmental sampling methods should be considered carefully in order to choose the best sampling strategy for a given context (Thorne et al., 1992; Lemmen et al., 2001).

A systematic review was recently completed by Abeykoon et al. (2021). In this review it was found that *C. burnetii* was detected in dust, air, soil and liquids from a variety of outbreak and endemic settings. Dust was analysed most frequently while air, soil and liquids were sampled in descending frequency, in many countries around the world. Dust was the sample type that demonstrated the highest bacterial load. Detection of *C. burnetii* in a given environmental sample type seems to be related to the source of the organism and the timing of sampling. Limited systematic sampling across spatiotemporal gradients and standardized or validated sampling methods were identified.

C. burnetii was detected in environmental samples mostly in endemic compared to outbreak settings. This could be a result of multiple factors including persistent release of the organism into the environment, sampling and detection methods and the timing of sampling. In endemic settings, all sample types have returned positive results, which indicates that *C. burnetii* is circulating in the air as well as being deposited. Dust, soil and liquids contain particles accumulated over time and thus represent a broader time window in one sample. Most positive air samples in endemic settings were linked to an animal reservoir (ruminants) which is likely due to continuous excretion of the organism into the environment in contaminated birth fluids (Bouvery et al., 2003). An outbreak investigation at a waste-sorting plant, on the other hand, detected *C. burnetii* in dust, but not in air (Alonso et al., 2015), despite using the MD8 air sampler, which was successfully used in other studies. The article reported an increase in the volume of waste processed concurrently with the start of the outbreak. This might have led to

quicker waste processing meaning that *C. burnetii* was only detectable for a short period in the air, but for longer in dust. In settings with a sustained bacterial source, such as a ruminant farms, the organism is more likely to be detectable in the environment for extended periods (Hackert et al., 2012). However, even in such situations, sampling strategy and timing could have an impact on detection. In an outbreak at an intensive small ruminant farm, air sampling yielded inconclusive results even though animal samples were positive (Bond et al., 2016). Overall, dust, soil and liquids can be used to detect deposited *C. burnetii* over time, and air samples can be used if a source is present at the time of sampling to detect circulating *C. burnetii* in air. Obtaining multiple environmental sample types appears advantageous, especially when the source and timing of the release is unknown as at least one type of sample might detect the organism as evidenced.

It is challenging to assess the factors behind non-detection in the four articles (Woerden et al., 2004; Wilson et al., 2010; Naranjo et al., 2011; Medić et al., 2012). All non-detections sampled either dust, air or did not mention the sample type. Sampling was less intensive than in other studies, with many details on sampling and laboratory methods lacking. Detection of *C. burnetii* in environmental samples depends on many other factors as well. Meteorological factors such as temperature and humidity have been shown to play a role (de Rooij et al., 2016). Weather conditions can influence particle settling indoors as well as outdoors and consequently impact sampling and detection. Moreover, successful detection is likely associated with proximity to high numbers of animals (de Rooij et al., 2016), which may imply more intensive holdings and potentially more infected animals shedding *C. burnetii*. Non-detections in this review were outbreak investigations without any direct livestock involvement. Overall, non-detection could be related to a combination of setting, poor choice of sampling strategy and timing of sampling.

Detection of *C. burnetii* DNA does not equate to viability and infectivity of the organism, and therefore bears no direct correlation to infection risk. Performing viability studies for *C. burnetii* is challenging due to the requirement of a Biosafety Level 3 laboratory. All sample types have been shown to harbour viable *C. burnetii*, however, only for a limited period such as after abortion waves or parturition. *C. burnetii* has shown to survive in soil under laboratory conditions for up to 20 days (Evstigneeva et al., 2007). In the field, it can remain viable in soil during the lambing period and for 1 month thereafter.

Collection of dust, soil and liquid samples is restricted by the availability of these substrates. For example, dust samples are present only if there is a surface to accumulate dust. If the sample is standing water on the ground, it might not be reliably available in outbreak situations which are often linked to dry, dusty and windy weather conditions (Hawker et al., 1998; Tissot-

Dupont et al., 2004). However, collection of dust, soil and liquid samples is more convenient than air sampling due to the minimal need for specialised equipment and therefore suitable for large scale sampling with minimal resources. In contrast, collecting air samples requires a suitable device, which is typically expensive and can be fragile. Further, an air sampler needs to operate for set periods of time to test specific volumes of air, so collecting a large number of samples either requires multiple air samplers used simultaneously or takes long periods of time. The efficiency of the most commonly used apparatus (MD8 Airport/Airscan, Sartorius) has not been compared against other air samplers nor against differing concentrations of airborne *C. burnetii* like for other bacterial species and fungi (Bonadonna and Marconi, 1994; Tissot-Dupont et al., 2004).

It was recently demonstrated that PM10 is a strong risk factor for Q fever in humans (Reedijk et al., 2013). Three articles reported on sampling of PM10 with a 28% and 16% success in demonstrating *C. burnetii* in these samples (Leski et al., 2011; Hogerwerf et al., 2012; de Rooij et al., 2016). However, it should be noted that even with the 2 µm pore size filters used in these studies, capturing the SCV form of *C. burnetii* would be challenging due to its small size (0.2–0.5 µm) unless they are attached to larger dust particles. Soil and slurry samples present difficulties that influence the efficiency of detection due to their complex nature compared to other sample types. The choice of DNA extraction method is therefore important when processing these samples. Among the articles reviewed, the only article that validated DNA extraction kits for processing soil samples for subsequent detection of *C. burnetii* is important to highlight (Fitzpatrick et al., 2010). Most articles published after this followed the same protocol and reported successful results (Kersh et al., 2010; Hong et al., 2013; Kersh et al., 2013; Tozer et al., 2014). Based on the reviewed evidence, this validated method can be DNA. Only one recent article (Carrié et al., 2019) followed a validated method for quantification of *C. burnetii* (Rousset et al., 2012). Other reviewed articles did not perform validations or standardizations of laboratory or sampling methods. Given that *C. burnetii* possesses unique characteristics, only validated environmental sampling methods will accurately indicate the bacterial burden present in the environment.

Decision-making around location and depth of obtaining a soil sample is important and was not extensively discussed in the articles reviewed. Soil from locations where animal carcasses, parturition materials, or contaminated manure or bedding material have been buried or distributed would be more likely to be positive compared to locations in which these activities had not occurred. While choosing sample sites based on convenience in screening studies might be more feasible, those samples are inadequate to assess the overall risk for the general community. Instead, random selection of sampling sites within the target area would be desirable and provide more powerful results. Moreover, the type of soil has not been taken

into consideration by any of the reviewed articles even though the type of soil may affect the presence of *C. burnetii* (Evstigneeva et al., 2007). Therefore, it is important to report the type of soil together with the results to allow comparison with other studies. There was limited opportunity to assess repeatability and reproducibility of methods presented. The level of detail reported on environmental sampling methods, equipment used and laboratory protocols was sufficient only in some of the articles. Therefore, the validity of the evidence presented in many studies is hard to assess (Bustin et al., 2009; Haddaway and Verhoeven, 2015). Several other articles failed to report the methods followed for sampling processing and DNA extractions. It is recommended that authors explicitly report methods to enable complete evaluation of the evidence and reproducibility of the science. The multi-copy transposable element IS1111 was the most common target used for PCR detection and was sometimes used to quantify *C. burnetii* DNA. If this target is used for quantification, the number of organisms present may be overestimated and results will not be accurate unless the exact number of copies present in the sample strain is known (Jones et al., 2011). Recent research demonstrated that IS1111 was abundantly present in Coxiella-like endosymbionts of ticks (Duron, 2015; Jourdain et al., 2015) therefore, the target is not specific to *C. burnetii*. However, the risk of ticks being present in environmental samples is low except for soil. Use of *C. burnetii* specific PCR assays and SNP genotyping methods could ultimately provide confirmation of *C. burnetii* in samples (Pearson et al., 2016).

7.2. Other sources of transmission

Other sources and routes of transmission are far less significant. For example, infection can potentially occur through the ingestion of unpasteurised dairy products, since mammals also shed *C. burnetii* in milk (Maurin and Raoult, 1999; Angelakis and Raoult, 2010). However, whilst the few cases of infection confirmed via the oral route have resulted in seroconversion, these have seldom produced clinically overt Q fever disease (Aitken et al., 1987; Barandika et al., 2019). Person-to-person transmission is rare. Most cases have been confined to direct contact with patients in hospitals and those attending autopsies. *C. burnetii* has been found in semen and sexual transmission of infection has been reported (Aitken et al., 1987; Angelakis and Raoult, 2010).

8. Vaccine

8.1. Human vaccine

In Australia, a vaccine (Q-VAX®, Seqiris, Australia), is licensed for use in humans (Gidding et al., 2009). Q-VAX® is a formalin-inactivated whole-cell vaccine produced with the Henzerling phase I strain of *C. burnetii.* The vaccine was first trialled in 6000 abattoir workers between 1981 and 1989, before it was rolled out to the farming community and eventually to all risk

groups in 2000-2003 through the National Q fever management program (Marmion et al., 1990). The vaccine appears to be effective in preventing Q fever in “high risk” occupations such as abattoir workers and veterinarians (Marmion et al., 1990).This vaccine is reported to cause adverse reactions and cannot be used in children under the age of 15 years, although a review has recently been published describing some paediatric use of the vaccine (Armstrong et al., 2019). Vaccination requires a pre-screening serologic and skin prick test to reduce the chance of local and systemic reactions associated with prior exposure. The necessity of pre-screening and occurrence of side-effects means that this vaccine is not suitable if fast delivery of vaccination is necessary in the face of a large outbreak.

9. Clinical disease in humans

9.1. Presentation

Acute Q fever can present with a variety of clinical signs including fever, nausea, headaches and fatigue, following an incubation period of 19 - 21 days (Gunaratnam et al., 2014). There are reports that suggest clinical illness in Australia appears unique from disease in other countries (Parker et al., 2006). While in Australia, Q fever patients commonly present with a classical “flu like illness” including fever, headache, night sweats and fatigue (Eastwood et al., 2018), hepatitis is more common in southern Spain, Ontario and France (Tissot Dupont et al., 1992); and pneumonia in areas of the Netherlands, Switzerland and Crete (Maurin and Raoult, 1999; Wielders et al., 2014). It is common in Australia for acute Q fever cases to be misdiagnosed as influenza and not immediately recognised or notified (Eastwood et al., 2018). Of the acute Q fever cases, 2% require hospitalisation and the case fatality rate is approximately 1 - 2% (Parker et al., 2006; Raoult et al., 2005). Although rare, a severe life-threatening case of Q fever sepsis syndrome was reported in Brisbane in 2015 (Stevenson et al., 2015). A 28-year-old woman, who had been exposed to kangaroos, initially presented with flu-like illness which then developed into sepsis with multiple organ failure and progressive respiratory failure requiring intubation for 7 days.

Approximately 10 – 15% of acute Q fever cases develop a debilitating “Post-Q fever fatigue syndrome”. An additional 1 - 5% of acute cases progress to chronic *C. burnetii* infections, including vascular and osteo-articular infections and gestational complications (Gunaratnam et al., 2014; Million and Raoult, 2015). A recent review by experts in the field have discouraged use of the term “chronic Q fever” as this oversimplifies the diagnosis and clinical implications; the term “persistent focalised *C. burnetii* infections” has been recommended (Eldin et al., 2016; Million and Raoult, 2015). Several cases of paediatric osteomyelitis have also been reported in Australia as a result of persistent infection (Britton et al., 2015; Nourse et al., 2004). Without treatment, endocarditis from persistent focalised *C. burnetii* infection can be fatal. In

Australia, even with treatment, *C. burnetii* endocarditis has a 10% mortality rate (Gunaratnam et al., 2014).

Even though most Q fever outbreaks involve a limited number of people and mortality rates are very low (<1-3%), the disease can be highly incapacitating with potential for large and widespread outbreaks to occur (Engelthaler and Lewis, 2004), as highlighted by the 2007-2009 epidemic in The Netherlands. Public health measures to control Q fever are difficult to implement as it is difficult to diagnose, is extremely infective, able to survive harsh conditions for long periods, is aerially transmitted and can be dispersed by wind (Duron et al., 2015)

Q fever is recognised in Australia as a zoonotic disease of public health significance and case incidence is monitored through the Communicable Disease Network Australia, Office of Health Protection (health.gov.au/cdna). Although Q fever is a nationally notifiable disease, it is still considerably underdiagnosed and underreported because of its normally subclinical course in humans, variability and non-specific clinical signs, low awareness among clinicians and current testing practices are sporadic and imprecise.

10. Diagnostics

10.1. Serology

10.1.1. Detection of the organism

Microscopic examination of *C. burnetii* organisms in placental tissues is done using Stamp-Macchiavello coloration in which heat-fixed smears are stained with basic fuschin, before decolourisation with citric acid, followed by counter-staining with methylene blue. *Chlamydia spp., C. burnetii* and *Rickettsia spp.* stain red under a blue background (Bildfell et al., 2000).The organism can also be detected by examining its cytopathic effects when cultured in cells, embryonated hen eggs or cell-free media (Guatteo et al., 2006; Porter et al., 2011). Microscopy and culture are expensive and require Biosafety Level 3 facilities. Furthermore, microscopic examination of stained tissues for *C. burnetii* detection is reported to have poor specificity because *C. burnetii* can be confused with other organisms like Chlamydia and Brucella (Porter et al., 2011). The culturing of *C. burnetii* is slow and has been reported to be unsuccessful from some individuals despite them being positive by PCR, serology and microscopy, suggesting culturing is an unreliable method for *C. burnetii* detection (Million et al., 2014).

10.1.2. Advantages and limitations of serological diagnosis

Antibodies are produced within a short timeframe of usually 2-3 weeks after infection with *C. burnetii* in animals (Roest et al., 2013b). Thus, detecting antibodies is useful for timely diagnosis of new C. burnetii infections. Antibodies to *C. burnetii* in ruminants and humans

have also been reported to remain in circulation for long periods thus making serological diagnosis a reliable method of detecting exposure to the organism (Teunis et al., 2013). Antibodies following acute Q fever in human patients in the Netherlands were reported to persist for at least 1 year after the initial diagnosis (Teunis et al., 2013). A seroprevalence study in humans reported that IgM antibodies lasted for 6 months after the onset of natural infections, however they did not specify whether the antibodies were against phase 1 or 2 antigens (Guigno et al., 1992). Limitations of serology for the diagnosis of coxiellosis include the 2-3 week delay between exposure and seroconversion, when antibodies against *C. burnetii* cannot be detected in blood (Roest et al., 2013b; Wielders et al., 2013).

The 2-3 week delay in seroconversion during suspected infections can be controlled for by testing paired samples collected at least 4 weeks apart to ensure seronegative animals are diagnosed appropriately (Fournier et al., 1998; Field et al., 2000). The collection of paired samples is also important in identifying re-infection in endemic herds where a certain base antibody titre may be present. In such circumstances, collecting paired sera to demonstrate seroconversion or a four-fold raise in titres and the detection of IgM antibodies to phase 2 have been reported to be important diagnostic pillars (Baranda et al., 1985; Cutler et al., 2007).

10.1.3. Performance of serological tests

The World Organization for Animal Health (OIE) recommends the complement fixation test (CFT) for serological diagnosis of coxiellosis in animals (OIE, 2018) despite this assay being widely reported to have a very low diagnostic sensitivity (Rousset et al., 2007) and to have non-specific reactions on some samples leading to uninterpretable results. The indirect immunofluorescence assay (IFA) is the human reference test and has been reported to have a diagnostic sensitivity (DSe) ranging from 98% to 100%, and a diagnostic specificity (DSp) of 95% (Fournier et al., 1998; Meekelenkamp et al., 2012) for human sera. The indirect ELISA is reported to have similarly high specificity but a lower sensitivity than the IFA in detecting antibodies against *C. burnetii* antigen when diagnosis of human Q fever is done using serum samples (Slaba et al., 2005; Meekelenkamp et al., 2012).

A number of studies have reported that the IFA and the indirect ELISA are more sensitive than CFT for diagnosis of coxiellosis in ruminants (Rousset et al., 2007; Kittelberger et al., 2009; Natale et al., 2012; Niemczuk et al., 2014). For instance, a study that compared serological positivity in 50 aborting and 70 non-aborting ruminants demonstrated the IFA and ELISA detected higher numbers of infected animals in the aborting goats (45/50 for ELISA and 45/50 for the IFA) compared to the CFT (34/50) (Rousset et al., 2007). Key published diagnostic specifications for the ELISA, IFA and CFT show that the CFT is less sensitive than the ELISA or the IFA despite the test being standardised across laboratories and not being species-

specific. There is thus need to validate more sensitive tests that could be used to accurately estimate the prevalence of disease and identify infected animals in herds.

The reported sensitivities of the ELISA and IFA in ruminants are based on testing a few samples from affected populations and were computed based on relative sensitivities using imperfect tests as gold standards or on results obtained from presumably infected and non-infected animals (Kittelberger et al., 2009; Natale et al., 2012; Niemczuk et al., 2014). One study estimated DSe and DSp using maximum likelihood methods although there were no reference positive and negative samples for goats and cattle (Horigan et al., 2011). Maximum Likelihood methods are known to provide a consistent unbiased estimate of DSe and DSp if large sample sizes are present, and the distribution of test results are approximately normal. Bayesian latent class analysis have also been reported to provide reliable estimates to DSe and DSp in situations where the reference test (gold standard) is imperfect; as is the case with diagnosis of coxiellosis where the reference test (CFT) is known to have poor DSe (Pepe and Janes, 2007; Rousset et al., 2007; Böttcher et al., 2011). Bayesian latent class methods in addition to analytical methods to estimate and ascertain the characteristics of more sensitive tests like the IFA and ELISA for detection of *C. burnetii* in livestock herds.

All these serological tests can be used in Australia. The CFT is available through submission to State government diagnostic laboratories but testing using the CFT could also be carried out by independent laboratories. The IDEXX CHEKIT ELISA is commercially available in Australia for use by certified laboratories, and the IFA has only been implemented by certain research groups.

10.2. **Diagnosis using cell-mediated immunity to C. burnetii infections**

Infection with *C. burnetii*, like other infections by intracellular pathogens, elicits cell-mediated immunity (Ben Amara et al., 2010). The detection of components of cell-mediated immunity, for instance, cytokines, resulting from *C. burnetii* infection could be important in the diagnosis of infections in human and livestock (Schoffelen et al., 2014a). Assays based on cell-mediated immunity include the whole-blood interferon-gamma (IFN-γ) production assay (IGPA) and the enzyme-linked immunospot (ELISPOT) assay, and are commercially available for the diagnosis of tuberculosis in humans and livestock but are not yet commercially available for diagnosis of *C. burnetii* in livestock and humans (Marassi et al., 2010; Diel et al., 2011; Waters et al., 2011; World Health Organization, 2011). A few studies have reported the use of reactions to intra-dermal injection of *C. burnetii* antigen in cattle as a measure of cellmediated immunity but this method is likely to have very low specificity as reported in tuberculin tests used in the diagnosis of tuberculosis (Rodolakis et al., 2009; Trajman et al., 2013).

The IGPA and ELISPOT are highly specific and implore the fact that T-lymphocytes infected with *C. burnetii* will release IFN-γ if they are re-exposed to *C. burnetii* antigens (Trajman et al., 2013; Schoffelen et al., 2014a). The IGPA measures the amount of IFN-γ released in plasma while ELISPOT depends on visualization of cells producing IFN-γ (Schoffelen et al., 2014a) (Pomorska-Mol and Markowska-Daniel, 2010; Schoffelen et al., 2013; Schoffelen et al., 2014b; Schoffelen et al., 2015). The ELISPOT is thought to be more specific than the IGPA as reported by a recent study that compared the performance of the tests using samples from known *C. burnetii* infected patients (Schoffelen et al., 2014a). The ELISPOT returned a positive test and negative test for 88% of the samples (n = 16) from infected *C. burnetii* patients and 100% (n = 17) from samples from the control patients, respectively, while the IGPA detected was positive on 3/17 negative control patients although it was 100% sensitive (Schoffelen et al., 2014a).

The detection of IFN-γ appears to have been validated for in-house use by some research laboratories for diagnosis of *C. burnetii* infection in humans (Schoffelen et al., 2014a) (Pomorska-Mol and Markowska-Daniel, 2010; Schoffelen et al., 2013; Schoffelen et al., 2014b; Schoffelen et al., 2015) but there appears to be limited commercialisation of such assays for use in other laboratories. The 56 methods seem to be similarly less common for diagnosis of *C. burnetii* in livestock; as there seems to be only few studies where the assays have been used for detection of *C. burnetii* infection in livestock (Roest et al., 2013b). The production of IFN-γ also appeared to have been downregulated during pregnancy in experimentally infected goats and substantial amounts were detected only after parturition which could limit its use to only non-pregnant animals. The performance of the methods appears to be affected by the nature of the antigen used, as described by a study in which stimulation using the Nine Mile strain of *C. burnetii* resulted in more false positives (3/17) compared to stimulation using the Henzerling strain (1/17) of *C. burnetii* (Schoffelen et al., 2014a). These results point out serious limitations surrounding the diagnosis of *C. burnetii* using assays targeting cell-mediated immunity.

10.3. **Genomic detection of C. burnetii**

Polymerase Chain Reaction (PCR) is used for detection of DNA of the organism in tissues and in secretions like birth fluids and milk (Rousset et al., 2007). These reactions target DNA sequences known to exist in the *C. burnetii* genome and considered to be absent from the genomes of other organisms. Some of the Coxiella genome sequences that have been targeted by PCR reactions include; the highly conserved single copy com1 and htpB, plasmid QpH1 and QpRs genes as well as the multiple copy transposase IS1111 element (Harris et al., 2000; Klee et al., 2006).

PCR methods of detecting *C. burnetii* DNA are considered to be highly sensitive (Malou et al., 2012). PCR has been shown to detect *C. burnetii* DNA in peripheral blood cells within days of exposure in humans, before antibodies can be detected in blood samples although this can only be done within a 2-3 week window following infection (Roest et al., 2013b; Wielders et al., 2013). This early detection of *C. burnetii* DNA in peripheral blood before seroconversion has however not been reported in any of *C. burnetii* infection studies in ruminants that were selected for this review. In one experimental infection of goats with *C. burnetii*, the earliest PCR positive blood samples 57 were obtained 28 days after exposure much later after antibodies to *C. burnetii* were mounted (Roest et al., 2012). The limitations of PCR assays in the diagnosis of *C. burnetii* include their dependency on the shedding of the organism which occurs for a relatively short period of time in ruminant faeces, milk, vaginal mucus and urine (Bouvery et al., 2003; Rodolakis et al., 2009). This limits the use of PCR assay for the detection of *C. burnetii* infection to only the peripartum period when the organism is shed in faeces, milk, vaginal fluids and urine (Woldehiwet, 2004).

10.3.1. Diagnostic performance of C. burnetii PCR assays

PCR assays targeting the multi-copy genes (e.g. IS1111) are important in detecting *C. burnetii* but may not be suitable for quantifying the concentration of *C. burnetii* present in the original samples, whereas single-copy genes like com1 are important in quantifying the number of *C. burnetii* organisms present as every copy of the gene detected corresponds to a single organism (Lockhart et al., 2011). Specificity can also be improved and definitive diagnosis achieved by testing samples using a multiplex PCR in which genes of other similar bacterial species like Chlamydia and Brucella have been included (Berri et al., 2000).

10.4. Discrepancy between serology and PCR results

Discrepancies, such as seronegative animals shedding *C. burnetii,* could be due to the timing of the infection, or to the lack of sensitivity of the serological tests rather than a true absence of antibodies in infected animals (Berri et al., 2000; Rodolakis et al., 2007). Indeed, some of these studies have used a mixed antigen enzyme linked immunosorbent assay (ELISA) that is reported to lack sensitivity for IgG phase 2 antibodies (Emery et al., 2012). However, it would be expected to detect seropositive animals that may have earlier but are no longer shedding *C. burnetii* since antibodies last long after the animals are exposed, which further highlights the importance of considering a fourfold rise in antibody titre to ascertain recent infection.

10.5. Factors to consider when selecting diagnostic tests for use in livestock

Although the selection of diagnostic tests for detection of *C. burnetii* infection or exposure is affected by several factors, some of which may be logistical, close consideration should be

given to the presence of analytes (*C. burnetii*, antibodies to *C. burnetii* or components of cell-mediated immunity) in the samples selected as well as the availability of suitable assays for detecting the analytes.

11. Livestock disease

11.1. Impacts on production

Coxiellosis 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 sheep (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 seem to be at higher risk of having a *C. burnetii* associated abortion than other ruminants (Lang, 1990). Moreover, *C. burnetii* is one of the main causes of abortion in goats in the USA and Europe (Moeller Jr, 2001; Chanton-Greutmann et al., 2002). The proportion of goats showing reproductive symptoms can vary significantly in natural infections; abortion risks from 3% to 90% of pregnant goats have been reported (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 reasons behind these differences can only be hypothesized. 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 be a potential explanation for 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 direct and indirect impacts of this on the efficiency with which replacements are reared is 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. Epidemiological studies in which removal rates, removal causes and disease incidence in young animal categories are compared between Q fever positive and negative herds while accounting for potential confounders would be required to assess whether there is a relationship between Q fever status of the herd and these variables.

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. Also, viable *C. 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. (2017). The authors found seroconversion to phase 1 antibodies was associated with an extra 0.276 L of milk per day (95% CI: 0.010 to 0.543) and hypothesized 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 often unapparent as evidence by the fact that often diagnosis follows the occurrence of human cases (Berri et al., 2005; Bond et al., 2016). However, large abortion outbreaks due to Q fever have been observed in both sheep and goat herds. Abortions can also occur at relatively low rates and therefore not capture the attention of farmers. *C. burnetii* can be detected in milk of all cows, sheep and goats (Rodolakis et al., 2007). However, studies assessing the potential impact of *C. burnetii* infection on either milk yields or milk quality are scarce. Also, the extent to which Q fever in livestock can affect the efficiency with which replacements are reared due to low birth weights and a resulting increase in removal rates in young animals or increased susceptibility to other diseases (e.g., respiratory diseases or gastrointestinal parasites) is unknown. The fact that impacts on production are often subtle underscores the importance of the use of consistent methods for recording and reporting herd performance allowing the early detection of changes in herd health that could be indicative of coxiellosis. This could in turn reduce the risk of human Q fever occurrence.

The increasing uptake by farmers of technologies like electronic identification systems and systems that automate the recording of variables like animal weights and individual daily milk yields have 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). Further, 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. To assess the impact of Q fever and other health disorders standardized methods for measuring herd performance are needed.

11.2. Epidemiology of coxiellosis in cattle

Minimal studies have investigated *C. burnetii* exposure or infection in beef cattle. When dairy and beef cattle are reported in the one study, there are consistent findings of lower prevalence in beef than dairy herds (Alvarez et al., 2012; McCaughey et al., 2010; Paul et al., 2014). In the Madrid region of Spain an overall apparent prevalence of 6.76% (95% CI 5.42, 8.41) was reported. When stratified for production types beef cattle showed 1.89% (95% CI 1.34, 2.54) seropositive and dairy cattle 2.73 % (95% CI 2.04, 3.52) seropositive (Alvarez et al., 2012). Although, when analysed for herd level prevalence, beef cattle were found to have only 24.3% of herds positive compared to dairy cattle, where 75.0% of herds tested positive. A similar pattern was identified in France; the between-herd seroprevalence was significantly higher in dairy cattle (n = 176, mean: 64.9%, 95% CI = 58.9, 70.6) compared to beef cattle (n = 87, 18.9%, 95% CI 15.4, 22.8) (Gache et al., 2017). Overall, there have been reports of animal-level prevalence in beef cattle ranging from 1.7% (in Korean native cattle) to 6.6% (in semi-extensive grazing systems in northern Spain; Ruiz-Fons et al., 2010).

11.2.1. Experimental infection in cattle

Early publications have described the experimental infection of cattle with *C. burnetii* (Bell et al., 1949; Derrick et al., 1942). Although sample sizes were small, this early research provide great insight into possible routes of infection and pathogenesis of coxiellosis. Derrick et al. (1942), experimentally infected calves via subcutaneous inoculum derived from infected guinea-pigs. Two out of four calves experienced a mild febrile condition 3 days after inoculation, bacteraemia was present by the 4th day and antibodies to *C. burnetii* were detected from 11 to 29 days post exposure. Results from these experiments demonstrated that cattle are susceptible to infection with some experiencing asymptomatic acute infection.

In the United States of America, a controlled experimental studies was performed in heifers, lactating cows and male calves (Bell et al., 1949). Treatment groups were inoculated with *C. burnetii* infected yolk sac via teat canal, intranasal, intravenous, vaginal tract or alimentary tract through ingestion of milk. Results indicated that *C. burnetii* infection could be produced in cattle infected via teat canal, intranasal, intravenous, vaginal tract routes, although results were inconclusive regarding ingestion of infected milk, as it was not conclusively determined if calves became infected. Of the other treatment groups, bacteraemia was identified within the first 5 days and urine tested positive for up to 8 days post inoculation in some animals.

C. burnetii antibodies were detected at high levels in the serum of experimentally infected cows (using complement-fixation test) for up to 191 days after inoculation. Lactating cows, inoculated via teat canal and cervical canal, developed systemic infections. Milk and blood became infective, and an acute mastitis developed, following a noted systemic reaction including marked pyrexia, serous nasal discharge, severe depression, inappetence, decreased rumination, moderate tachycardia and moderate polypnea. The cows recovered spontaneously without antimicrobial treatment, however *C. burnetii* continued to be shed in the milk for over 200 days in some cases. Sacrificed infected cows were examined postmortem on days 5, 11, 22 and 63 post inoculation. Bacteria was isolated from many tissues including liver, spleen, lung, lymph nodes, intestinal tract and mammary glands, however, pathological changes were not obvious in these tissues with the exception of oedematous mastitis and local lymphadenopathy.

The above-mentioned experiments did not include pregnant cows, therefore observations could not be made on the effect of experimental infection of *C. burnetii* on pregnancy outcomes in cattle. A controlled experimental study published in French was translated to English for this literature review (Plommet et al., 1973). This study aimed to investigate acute and chronic pathogenesis of *C. burnetii* infection in cattle, including pregnancy outcomes (Plommet et al., 1973). The treatment group consisted of 12 heifers that were inoculated with an intradermal suspension of *C. burnetii*, and a control group of 98 heifers of the same age and source. Eleven cattle from the treatment group and 54 from the control group were artificially inseminated 8 months following the inoculation date and observed longitudinally for their entire pregnancy or until abortion occurred.

Cattle became seropositive between 6 and 13 days after inoculation in all treatment group animals and titres progressively decreased, except at parturition or time of abortion when serological titres increased markedly. All inoculated animals developed pyrexia, inappetence and acute respiratory signs within 24 – 36 hours. Acute symptoms began to self-resolve after 7 days. Chronic non-reproductive disease is suggested in this study, as one animal died of heart failure 6.5 months after inoculation. Four out of the 11 inseminated treatment heifers had a normal pregnancy and delivered live calves. Two were slaughtered during gestation and were found to have normal foetuses. Two heifers aborted dead foetuses and 3 heifers may have suffered early embryonic loss. Overall, this experimental study identified a fertility rate in the inoculated group of 73% compared to the control group of 93%; abortion rate respectively was 37% (treatment) compared to 1.7% (control) and full-term delivery of a live healthy calf was observed in 55% of the treatment group compared to 81% in the control group.

11.2.2. Transmission and pathogenesis in naturally infected cattle

Inhalation and possibly ingestion (through the oropharynx) of the SCV form of *C. burnetii*, seems to be the natural route of transmission in animals (Porter et al., 2011; Woldehiwet, 2004). The highly resilient, "spore-like" form of the bacteria can be aerosolised during shedding directly from infected animals (other livestock and wildlife) and can survive in the environment. There is a single report that suggests venereal transmission in cattle (Kruszewska and Tylewska-Wierzbanowska, 1997); however, more research is required to substantiate this as a common route of infection. It has been shown through experimental studies that ticks can transmit infection between animals; however, it does not appear to be a necessary invertebrate host for infection to survive in livestock populations (Derrick et al., 1942; Derrick, 1961; Maurin and Raoult, 1999). The SCV bacterium has an affinity towards host macrophages and monocytes and can survive phagocytosis, the LCV then replicates within phagolysosomes. Multiplication in regional lymph nodes occurs early in the infection with a short bacteraemia for 7- 21 days (Woldehiwet, 2004). The placenta and mammary tissue are the primary target organs for infection and multiplication of *C. burnetii* in pregnant ruminants (Agerholm, 2013; Brom et al., 2015; H. Roest et al., 2013). Although there is not much information on the pathogenesis of *C. burnetii* infection in non-pregnant animals, the bacteria has been identified in inflamed cardiac valves of cattle going to slaughter (Hansen et al., 2011).

There is little current research on "acute" infection and the intrauterine spread of *C. burnetii* in cattle. A published review on the clinical effect of coxiellosis in domestic ruminants has described the complexity of possible outcomes of an intrauterine *C. burnetii* infection in a pregnant animal (Agerholm, 2013). After infection is established in the placenta, *C. burnetii* may follow two main routes: a latent infection or an active infection. Likely outcome then depends on infection remaining localised to the placenta or spreading vertically to the foetus through transplacental or haematogenous spread. These two main routes can lead to two likely outcomes; firstly, normal offspring or secondly an "abortion, premature delivery, stillbirth and weak offspring (APSW Complex)" (Agerholm, 2013). Agerholm (2013) proposed this model for natural infection in pregnant cows which may explain conflicting reports on the clinical effect of coxiellosis in pregnant cattle. Some research indicates mostly asymptomatic infection, others found coxiellosis to be associated with sporadic abortion, subfertility, placentitis, retained foetal membranes and metritis (Agerholm, 2013; Bildfell et al., 2000; Cabassi et al., 2006; López-Gatius et al., 2012). It is reported extensively in literature that cattle can then remain chronically infected with *C. burnetii* without showing overt signs of disease (Agerholm, 2013; Guatteo et al., 2006; Lang, 1990). Cattle are therefore more likely to have asymptomatic (latent) infections than small ruminants.

11.3. **Disease occurrence and risk factors**

Several global studies have investigated risk factors for exposure or infection to *C. burnetii* through observational studies. Most utilise a cross-sectional study design with serological antibody response to anti- *C. burnetii* IgG (phase I and/or phase II) as the outcome variable, however some studies also used PCR testing as a criteria for infection (Agger and Paul, 2014; Paul et al., 2014, 2012). Additional data at both animal and herd level have been used to assess risk factors for previous exposure, or active infection. Most studies focused on dairy cattle (Agger and Paul, 2014; Boroduske et al., 2017); however, there are a few seroprevalence surveys including dairy, beef, bullfighting and mixed-use cattle (**Error! Reference source not found.**) that performed further analysis to identify putative risk factors. There have been no studies primarily focused on identifying risk factors for coxiellosis in beef cattle.

A *C. burnetii* sero-survey of cattle going to slaughter in Denmark assessed risk factors using Bayesian methods to account for diagnostic test uncertainties (Paul et al., 2012). They identified that the number of animal movements, age and breed groups (cattle raised for milk production vs meat production) were risk factors for seropositivity (Paul, 2013). This finding is consistent with a report from Spain that identified production type (dairy, beef, bullfighting) and herd size as significantly associated with seropositive results at bivariate analysis (Alvarez et al., 2012). However, in the final multivariable model, production type was not found to be significantly associated with herd-level test results.

The hypothesis that dairy cattle are at a higher risk of *C. burnetii* infection could be explained by genotypic differences between cattle breeds. Within commercially used dairy cattle, it was identified that Danish Holstein breeds had an increased risk of seropositivity compared to Jersey breeds (Paul et al., 2012). Holstein breeds are typically higher yielding milk producers; therefore this association could be correlated with the metabolic stress experienced during lactation, enabling *C. burnetii* infection to thrive. However, the putative association of prevalence and production-type could be just as likely due to different management systems implemented across cattle industries. Intensive housing, controlled mating and synchronised calving periods tend to be more common in dairy production compared to beef and bullfighting production. These management factors may increase the risk of transmission of infection at the time of parturition and lactation in closely confined animal sheds.

In Southern Italy, farm management practices were assessed to identify if different cattle housing practices could be identified as potential risk factors for increased *C. burnetii* seropositivity (Capuano et al., 2001). A cross-sectional study was performed using 1188 cattle from 53 farms. Serology was performed with an IFA test and animal-level proportion positive used as the outcome variable of interest. The farms were categorised into three groups

according to housing: permanently housed cows (n = 21), cattle housed in winter and moved to graze in spring (n=26), cattle not housed (n=6). Results indicated that cattle housed in winter after grazing in spring had a higher seroprevalence of coxiellosis (19.6%) and non-housed cattle had the lowest seroprevalence (1.9%). Although this study did find a statistically significant difference between seropositivity and specific housing systems, there are limitations in the analysis that reduces the validity of the findings. The animal-level prevalence estimates do not take into consideration the clustered nature of the data (animals grouped within farms) and no other putative risk factors were included in the analysis. This study would benefit from multilevel, multivariable analysis that includes data on animal and herd level variables such as breed, age, size of herd and production type, to ensure associations are accurately interpreted.

A well-designed cross-sectional investigation focused on cattle in the main milking region of Ecuador identified some interesting results (Carbonero et al., 2015). Serological testing was performed on a sample of dairy and mixed (dairy-beef) cattle (animal n=2668, herd n = 386) using an ELISA test with known diagnostic sensitivity and specificity. Individual cattle data (age, breed and sex) were collected, and farm surveys competed to assess additional explanatory variables related to nutrition, farm facilities, general farm biosecurity and animal health. Univariable analysis was performed, and all explanatory variables significant at $p > 0.2$ by a Chi-square test were then included in multivariable analysis. Generalised estimating equations (GEE) modelling was used to determine factors associated with seropositivity. From 26 variables retained for the multivariable analysis, only four showed significance at $p < 0.05$. *C. burnetii* seropositivity was found to be significantly associated with increasing age, feeding calves milk replacer and the presence of bovine respiratory syncytial virus. The fourth factor, disinfection of the calves' umbilical cord, was identified as a protective factor. Cow age or parity have been associated with seropositivity in several other studies (Böttcher et al., 2011; McCaughey et al., 2010; Paul et al., 2012). Feeding calves milk replacer and the presence of bovine respiratory virus are novel and may be worth following up with prospective cohort studies to elucidate if these factors could be considered causal.

In Denmark, a cross-sectional study identified a decreased risk of *C. burnetii* seropositivity in dairy-cows from herds where the quarantine of newly purchased animals exists and where veterinarians took higher hygienic precautions (e.g. biosecurity and infection control measures; Paul et al., 2012). Another study focused on dairy-cattle in Latvia and identified purchasing cattle from abroad and the increasing number of cattle in milking sheds as associated with increased seropositivity and PCR positive milk (Boroduske et al., 2017). The movement of latently infected cattle without standard quarantine practices and increased stocking density, seem plausible factors to increase the risk of spread of *C. burnetii* into

uninfected herds and to increase transmission between infected and susceptible animals. These studies highlight the importance that simple quarantine practices and not overstocking could have in reducing the potential spread of *C. burnetii* between and within cattle populations.

Currently, there have not been any studies that have investigated potential risk factors for coxiellosis in beef or dairy cattle within Australia. There would be great value in investigating recent or past C. burnetii infections across different cattle industries and geographical regions to identify risk factors that may link to animal, environmental or agent factors.

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 et al.

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. (2014), found presence of ticks on dairy cattle doubled the odds of a farm being PCR positive to *C. burnetii* in bulk tank milk (BTM). 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 (González-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 (Canislatrans), 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. Further, some of these marsupials (e.g. bandicoots and possums) are relatively abundant not only in rural areas but also 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; Cooper et al., 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* was detected in 33.5% and 24.1% 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). However, there is not clear evidence of disease transmission occurring via artificial insemination or natural service either in cattle or other livestock species. *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).

The stability of the SCV form of *C. burnetii* can theoretically result in viable organism remaining 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.

Results from an observational study by the same research group (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 et al. 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 *C. burnetii* 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 bulk 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. The death rate 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. 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).

The role of wind in between-farm transmission of Q fever has been the subject of numerous studies. Nusinovici et al. (2017) assessed the risk of dairy cattle farms changing their Q fever status from negative to positive in between two four-month separated sampling points against the cumulative environmental detection of *C. burnetii* in farms located upwind each farm within a 5 Km radius. A statistically significant association was found between the *C. burnetii* environmental burden of upwind farms and the risk of a farm becoming Q fever positive. The introduction of new animals into the herds under study throughout the study period was not recorded and could be a potential source of bias in the analysis.

The putative effect of wind, in addition to the effect of animal movements, in Q fever spread among dairy cow farms was also assessed by Nusinovici et al. (2014) and Pandit et al. (2016) using statistical and mathematical models, respectively. The conclusions from both studies are in agreement in that farms located in regions of high animal density are at a higher risk of infection than those located in low animal density areas, independent from the effect of animal movements. The estimated increase in the risk of infection was attributed to the effect of wind-born environmental contamination. Overall, there is general agreement in that wind can play a role in the spreading of *C. burnetii* infection among livestock herds making disease control challenging in the event of a large-scale outbreak in areas of relatively high farm density.

Risk factors associated with herd-level presence of antibodies against *C. burnetii* in small ruminants have been assessed by Meadows et al. (2015). In cross-sectional studies carried

out in Canada, herd size was identified as a significant risk factor for *C. burnetii* exposure in both sheep and goats. This is in agreement with the results of other studies looking at risk factors in sheep and goats (Schimmer et al., 2011; Lambton et al., 2016). The study carried out by Meadows et al. 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 management practices that involved housing of cattle had an increased risk of exposure to *C. burnetii* compared with extensive production systems (Capuano et al., 2001). 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 were hygiene practices were limited to adding bedding material and removal of birthing products (Meadows et al., 2015). Also, the frequency with which cleaning of the litter was performed was found to have a significant effect on 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 higher population turnover that occurs in meat farms compared to dairy farms, which can result in exposed animals being kept in the herd for longer. In agreement with these results, Van den Brom et al. 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 (Van den Brom et al., 2013).

11.4. **Shedding of C. burnetii**

11.4.1. Routes of C. burnetii shedding important in the transmission of infections

The risk of infection with *C. burnetii* shed by infected animals at parturition is exacerbated by management strategies that synchronise oestrous and breeding resulting in an increased number of livestock giving birth within narrow timeframes. For example, the Q fever outbreak in Australia associated with 18 cases in 2013 (Bond et al., 2016) was linked to a sheep dairy that transformed into a large intensive 5000 goat enterprise with synchronised kiddings. Q fever outbreaks in other parts of the world have also been associated with intensive ruminant farms: over 4000 human cases of Q fever occurred in the Netherlands between 2007 and 2010, where increased risk of infection was associated with living in close proximity to intensively-managed dairy goat herds (Delsing and Kullberg, 2008). Similarly, 147 human

cases in the United Kingdom were associated with lambing ewes in the West Midlands in 1992 (Smith et al., 1993), and 23% of the residents in a rural German town were considered to have contracted Q fever from a large sheep farm in 1996 (Lyytikäinen et al., 1998).

The persistence of the *C. burnetii* in the environment and its subsequent spread to humans through inhalation of *C. burnetii*-contaminated dust (Tigertt et al., 1961; Angelakis and Raoult, 2010) is also an important aspect of *C. burnetii* transmission in humans. Airborne transmission between herds has not been demonstrated in livestock but was speculated to be responsible for the widespread *C. burnetii* infections in goat herds during the Q fever outbreak in the Netherlands (Roest et al., 2011a). Control efforts to minimise the persistence of the bacterium in the environment have focussed on proper disposal of faecal material and birth products. For instance, during the Netherlands Q fever outbreak, 2007-2010, a ban on the spreading of manure was one of the control measures (Delsing and Kullberg, 2008; Karagiannis et al., 2009; Roest et al., 2011a). Also, a study carried out on two sheep farms reported that *C. burnetii* contaminated aerosols persisted for 2 years after the cessation of shedding in the flock in which there was no removal of accumulated manure while no *C. burnetii* contaminated aerosols were detected after the cessation of shedding in the flock with constant removal of 42 manure (Astobiza et al., 2011b). These examples illustrating the persistence of *C. burnetii* in the environment highlight the importance of hygienic practices in the control of *C. burnetii* in infected herds. Improper disposal of birth products may also increase the risk of transmission; as was the case in a Q fever outbreak on a mixed cattle, horse and crop farm in Victoria, Australia in 2011(Department of Health and Human Services, 2013). Infection of humans and animals on the mixed farm in Victoria was linked to a change in the management of birth products (Department of Health and Human Services, 2013). The birth products were disposed-off in an open compost for use on the organic crop enterprise that been had set-up on the farm. This example further highlights the importance of hygienic practices in the transmission of *C. burnetii*. These should however be supplemented with control strategies to reduce *C. burnetii* shedding by livestock and implementing policies to prevent susceptible humans from coming into contact with infected animals and contaminated environments (i.e. restricting access to known infected properties only to those that can demonstrate evidence of appropriate vaccination or past infection) (Bond et al., 2016).

C. burnetii is also shed in milk and the bacterium has been shown to persist in the mammary glands and uterus of infected goats (Rodolakis et al., 2007). However, *C. burnetii* shed through milk appears not to be important in the transmission of *C. burnetii* to humans as experimental infection of humans through ingestion of milk demonstrated to have viable *C. burnetii,* has been shown to be unsuccessful (Krumbiegel and Wisniewski, 1970). Although the concentration of *C. burnetii* consumed during the experimental trial was not estimated in this

experiment, the individuals consumed milk containing viable *C. burnetii* shown to infect experimental animals and embryonated chicken eggs (Krumbiegel and Wisniewski, 1970). Also, with the daily consumption of the *C. burnetii* contaminated milk for a month, no antibodies were detected in any of the 34 volunteers and no clinical signs were observed which highlights the strengths of these results (Krumbiegel and Wisniewski, 1970). It still remains unknown if *C. burnetii* can be transmitted to livestock through ingestion of contaminated milk. It is also unknown if sexual transmission through *C. burnetii* contaminated semen is possible in livestock and humans. *C. burnetii* has been identified in human and cattle semen although the issue of sexual transmission is still debatable(Kruszewska and Tylewska-Wierzbanowska, 1997; Milazzo et al., 2001) . The development of Q fever in a partner of an infected individual 15 days after coitus as well as the presence of the organism in semen has however been reported (Milazzo et al., 2001). Nevertheless, the possibility that the transmission could have occurred by any other route of infection was not ruled out. There is thus need to investigate the possibility of sexual transmission from infected breeding males.

11.4.2. Differences in the pattern and route of C. burnetii shedding in livestock

Sheep, goats and cattle have major differences in the routes and duration of *C. burnetii* shedding as well as the quantity of *C. burnetii* they shed. A study of shedding patterns in naturally infected livestock herds observed sheep and goats to have shed higher loads of *C. burnetii* than cattle (Rodolakis et al., 2007). It has also been observed that caprine and ovine infections result in more severe placentitis compared to cattle (Smith et al., 1993). This is in agreement with reports of *C. burnetii* infected cattle remaining asymptomatic unlike sheep and goats which have been shown to abort when infected with *C. burnetii* (Bouvery et al., 2003; Rodolakis et al., 2007; Joulie et al., 2015). The factors responsible for the higher loads of *C. burnetii* among sheep and goats in comparison to cattle are not known. It is also not clear whether the high quantities of *C. burnetii* shed by caprine and ovine species translates into greater risk for *C. burnetii* transmission, yet noteworthy that most of the large Q fever outbreaks have been associated with small ruminant farming.

A study in naturally infected cattle, sheep and goat herds showed that *C. burnetii* shedding by infected cattle occurred exclusively in milk, while sheep and goats shed *C. burnetii* in vaginal secretions, milk and faeces (Rodolakis et al., 2007). Another study showed that *C. burnetii* shedding via the faecal route was scarce and sporadic and reported 50% of the cows to have shed *C. burnetii* in vaginal mucus and 40% in milk (Guatteo et al., 2007). Infected pregnant goats on the 44 other hand, were observed to have *C. burnetii* in vaginal mucus, faeces and milk (Bouvery et al., 2003) while in ewes, *C. burnetii* shedding occurred in vaginal mucus and faeces after abortion or lambing (Joulie et al., 2015). From these studies, it is apparent that the patterns of *C. burnetii* shedding are varied in cattle, sheep and goats but vaginal and faecal

shedding appears to be consistent in sheep and goats while shedding in milk is consistent in cattle.

The duration of shedding in vaginal fluids, faeces and milk is also variable in sheep, goats and cattle; with much of the shedding being recorded to occur around kidding (Bouvery et al., 2003; Woldehiwet, 2004). *C. burnetii* shedding persists for longer durations in vaginal fluids than in faeces and milk of sheep. In cattle, the shedding of the organism persists for longer periods in milk and may be scarce or not present in vaginal fluids and faeces (Berri et al., 2002; Guatteo et al., 2007). A study in naturally infected ewes reported that *C. burnetii* was shed for greater than 12 days in vaginal mucus and less than 12 days in faeces and milk (Berri et al., 2002). Another study in ewes reported *C. burnetii* to have been shed in vaginal mucus and faeces at 3 weeks after abortion or lambing (Joulie et al., 2015).

Goats on the other hand, appear to shed *C. burnetii* for longer in vaginal mucus and faeces than ewes and cattle. Goats infected during pregnancy, reportedly shed *C. burnetii* in vaginal fluids for 14 days after abortion, for 52 days in milk after abortion and for a duration of 20 days in faeces, intermittently before and after abortion (Bouvery et al., 2003). Similar shedding patterns were observed in naturally infected goats at 16 weeks after parturition, when a large proportion of goats shed *C. burnetii* in milk for a longer duration compared to the duration of shedding of the bacterium in vaginal mucus and faeces (Rodolakis et al., 2007).

11.4.3. Risk of shedding across parity groupings

In infected herds, higher proportions of primiparous goats reportedly shed *C. burnetii* than in multiparous goats (Rousset et al., 2009a; Hogerwerf et al., 2011; Joulie et al., 2015). Also, primiparous ewes reportedly shed *C. burnetii* in higher quantities and for longer durations of time than multiparous goats. A study in infected goats observed larger proportions of primiparous unvaccinated goats to have shed *C. burnetii* at their first kidding than multiparous goats (Rousset et al., 2009b). Another study of goat herds in The Netherlands also showed that higher proportions of maiden unvaccinated goats were positive for *C. burnetii* in uterine fluid (55%, n = 159) and vaginal swabs (96%, n = 167) compared to multiparous goats (uterine fluid: 14%, n = 159; and vaginal swabs:54%, n = 123) (Hogerwerf et al., 2011).

Similarly, primiparous ewes reportedly shed higher concentrations of *C. burnetii* and for a longer duration than multiparous ewes (Joulie et al., 2015). In another study the proportion of primiparous sheep positive for *C. burnetii* in uterine fluid (5/17) and vaginal fluid (11/79) was lower than the proportion of multiparous sheep shedding *C. burnetii* in uterine fluid (17/17) and vaginal fluid (76/82) (Hogerwerf et al., 2011). However, the low sample sizes of less than 100, used in the latter study of sheep shedding patterns (Hogerwerf et al., 2011) are likely to have affected the result than in the former where the sample sizes were greater than 150 (Joulié et

al., 2015). Presumably high concentration of *C. burnetii* shed, high proportion of animals shedding in the herd and longer duration of shedding the bacterium in secretions of infected animals lead to increased transmission, but it is worth noting the range of environmental factors that come into play, considering persistence of the organism in the environment and the low infectious dose of the bacterium.

11.4.4. Reproductive wastage and shedding of C. burnetii

Although *C. burnetii* is known to cause abortions, still birth, fertility and weak offspring in goats and sheep (Sánchez et al., 2006; Astobiza et al., 2011a), there appears to be no clear association between shedding and occurrence of reproductive wastage in livestock. For example, a study undertaken on infected herds reported no statistically significant difference in the level of *C. burnetii* shedding in milk, faeces and vaginal swabs between aborting (milk 19/50, faeces 7/34; vaginal swabs 22/50) and non-aborting goats (milk 21/60; faeces 8/41, vaginal swabs 46 19/70). However the level of seropositivity was higher in aborting goats (45/50) compared to non-aborting goats (44/70) (Rousset et al., 2007).

12. Control measures

12.1. Animal vaccine

An animal vaccine, (Coxevac™, Ceva Sante Animale, France), is used in The Netherlands, France and other countries in Europe but not available in Australia (Hogerwerf et al., 2011). Coxevac contains phase 1 formaldehyde-inactivated *C. burnetii*. Another animal vaccine, Chlamyvax-FQ, a phase 2 C. burnetii, was commercially available in France; this was shown not to be efficacious, presumably because it contains only phase 2 antigens (Arricau-Bouvery et al., 2005). In a herd where Chlamyvax-FQ was used, the risk of abortion was 87%, a percentage similar 60 to that in unvaccinated herds (88%) while the risk of abortion was 6% in herds vaccinated with the Coxevac phase 1 vaccine (Arricau-Bouvery et al., 2005). Studies have also suggested that antibodies to phase 1 antigens provide protection against *C. burnetii*. However, it has not been established whether the protection derived following vaccination is solely due to antibodies against phase 1 antigens given that phase 1 vaccines are made up of phase 1 organisms, which contain both phase 1 and 2 antigens (Bobb and Downs, 1962). Perhaps, there could also be other antigenic components other than the phase 1 lipopolysaccharide antigen that contribute to the immunogenicity and efficacy of phase 1 *C. burnetii* vaccines (which may be switched off in phase 2 organisms). Reduction of shedding and reproductive wastage through vaccination of livestock. Many large Q fever outbreaks have been linked to farms with small ruminants and key control strategies have targeted reducing shedding of *C. burnetii* by the animals (Guigno et al., 1992; Smith et al., 1993; Lyytikäinen et al., 1998; Delsing and Kullberg, 2008). Vaccination of ruminants with inactivated

phase 1 *C. burnetii* antigen 1 month before breeding is the most commonly used strategy of controlling *C. burnetii* in infected domestic ruminant herds, as recommended by the manufacturers of the only existing livestock vaccine, Coxevac (Biberstein et al., 1977; Guatteo et al., 2008; Rousset et al., 2009b; Astobiza et al., 2011a; Hogerwerf et al., 2011; Roest et al., 2011a; Eibach et al., 2013; Piñero et al., 2014; Taurel et al., 2014).

The goal of vaccination against *C. burnetii* in livestock has previously been described as the reduction of environmental contamination by infected livestock and consequently the reduction of the risk for human and animal infection (García-Ispierto et al., 2010; López-Gatius et al., 2012; Tutusaus et al., 2013). Vaccination of livestock before breeding has been shown to reduce *C. burnetii* shedding and *C. burnetii*-associated abortions in infected herds (Rousset et al., 2009b; Astobiza et al., 2011a; López-Helguera et al., 2013; Taurel et al., 2014; Garcia-Ispierto et al., 2015). Rousset et al. (2009b) tested the efficacy of a phase 1 *C. burnetii* vaccine administered before breeding and found a lower proportion (4%) of vaccinated sheep and goats among high shedders [defined as animals with concentrations of ≥ 106 *C. burnetii* organisms per mL of uterine fluid] compared to 13% of non-vaccinated sheep and goats being identified as high 61 shedders. Similarly, Taurel et al. (2014) observed a reduction of the concentration of *C. burnetii* in samples taken at parturition in herds where > 80% of the cows were vaccinated before breeding compared to herds where ≤ 80% of the cows were vaccinated. A study involving naturally infected sheep, observed that after vaccinating animals before breeding, abortions were reduced from 6% to 2% (n = 315) in one flock and from 5% to 2% (n = 332) in another flock (Astobiza et al., 2011a).

Conversely, some studies have reported no differences in the level of shedding between livestock vaccinated 1 month before breeding and unvaccinated controls. For instance, a study of sheep vaccinated before breeding did not observe a statistically significant difference in the proportion of shedders between vaccinated animals and unvaccinated animals on testing of vaginal swabs, faeces and milk samples (Berri et al., 2002). With such large sample sizes and the detection of similarly large proportions of animals shedding *C. burnetii* in the vaccinated group [vaginal swabs (63%, n = 149), faeces (53%, n = 211) and milk (23%, n = 211)] compared with the unvaccinated groups [vaginal swabs (51%, n = 61), faeces (55%, n = 97) and milk (20%, n = 97)], it is very likely that the vaccine has limited effect when given at 1 month before breeding in already infected sheep.

This, in addition to the presence of *C. burnetii* shedding in vaccinated animals, highlights the shortcomings of vaccinating animals before breeding. On infected farms, it is possible that many animals are already infected at the time of breeding when vaccination is implemented, thus vaccination of animals before breeding would be performed with the expectation that the

vaccine not only provides protection against infection but also controls infection in already infected animals.

12.1.1. Vaccination of infected and non-infected animals

A number of studies have shown vaccination to be more effective in reducing the shedding of the organism when carried-out in seronegative animals than in 62 seropositive ones, underscoring the need to vaccinate animals before they are first infected with *C. burnetii* (Arricau-Bouvery et al., 2005; Hogerwerf et al., 2011; de Cremoux et al., 2012b; Taurel et al., 2012). In an observational study of the efficacy of vaccination in a naturally infected goat herd, seronegative dairy goats were reported to have responded better to vaccination than seropositive goats, with greater reductions in the proportion of shedders and the amount of *C. burnetii* shed per animal compared to already seropositive animals (de Cremoux et al., 2012a). Given that most seronegative goats that responded to vaccination were aged 3-4 months, it is very likely that reduction in the proportion of ruminants shedding *C. burnetii* as well as the load of *C. burnetii* shed per animal is due to the lower numbers of 3–4 month-old animals infected with *C. burnetii* compared to adult animals.

12.1.2. Vaccination of pregnant animals and non-pregnant animals

It is expected that many livestock are already infected at the time of breeding. The affinity of the bacterium for trophoblast cells of the placenta and the enormous replication of *C. burnetii* in the trophoblasts would be expected to limit the efficacy of vaccination of pregnant livestock. This hypothesis is supported by the findings of many studies that have shown vaccination of pregnant animals not to be effective in reducing the proportion of shedders and the load of *C. burnetii* shed. For instance, in cattle, the proportion of vaccinated non-pregnant heifers (1/15) and vaccinated non-pregnant cows (1/14) shedding *C. burnetii* was lower than the proportion of shedders in both vaccinated pregnant heifers (8/26) and vaccinated pregnant cows (8/31) (Taurel et al., 2012). These proportions of vaccinated pregnant cattle shedding *C. burnetii* were not statistically significantly different from those observed in unvaccinated heifers (12/40) and cows (12/34) which further highlights the lack of efficacy of the Coxevac vaccine when administered in pregnant cattle on infected herds (Taurel et al., 2012). In another study by Roussel et al, vaccination did not reduce shedding in vaginal fluids, as 87% (51) of vaccinated pregnant goats shed *C. burnetii* compared to 88% (59) of unvaccinated 63 goats; 72% of the vaccinated pregnant goats also aborted (Rousset et al., 2009b). Also, vaccination did not reduce bacterial load in pregnant cattle when compared to control animals (vaccinated 101. 83 , placebo 102.91 bacteria per swab) but reduced the quantity of *C. burnetii* shed in non-pregnant cattle (vaccinated 100.60 bacteria per swab) compared to the placebo (102.41 bacteria per swab) (Taurel et al., 2012).

The studies portraying the lack of vaccine efficacy in already infected animals and pregnant animals highlight the need to review the current *C. burnetii* control strategies with a possibility of vaccinating susceptible animals before they are first infected. Also, reduction in *C. burnetii* shedding and abortions, may have little impact on transmission of infections, especially in large intensive herds, given the low infective dose of the bacterium, its persistence in dry and dusty environmental conditions and the billions of *C. burnetii* organisms that can potentially be shed per gram of placental tissue by infected livestock (Ben Amara et al., 2010; Schimmer et al., 2011).

12.1.3. Vaccination of young versus adult livestock

The initial age of infection in goats born on infected herds is not known, but this information would help to inform the decision of the optimum age to be targeted for vaccination of nulliparous animals. Some studies provide clues to when vaccination should be carried out. A study that compared vaccination of 3-4 month old goats to vaccination of goats 1 month before breeding, observed that vaccinating young animals led to a significantly higher reduction in *C. burnetii* shedding compared to goats vaccinated 1 month before breeding (de Cremoux et al., 2012a). The study also noted that unvaccinated goats kidding for the first time excreted higher quantities of *C. burnetii* [106.53 bacteria per vaginal swab] compared to unvaccinated adult goats [103.49 bacteria per vaginal swab]. Also, in five vaccinated herds in the Netherlands, 41% (n = 248) of pregnant young nulliparous goats (~10 months of age) were PCR positive for *C. burnetii* in the vaginal swabs during a mandatory culling of 64 pregnant animals which highlights that vaccination should possibly be done earlier than a month before breeding (Hogerwerf et al., 2011); the proportion of goats shedding C. burnetii in vaccinated herds was not statistically significantly different from those in four unvaccinated herds (160/241). Thus, in goats, animals should be vaccinated not later than 3 months of age as this gave better reductions in the level of shedding compared to vaccinations done 1 month before breeding.

Results of studies of *C. burnetii* efficacy in cattle are similar to those observed in goats, where reduction of shedding and not prevention was observed in heifers (1/15) and cows (1/14) vaccinated before breeding compared to shedding in those that received the placebo (heifers = 12/40 and cows 12/46) (Taurel et al., 2012). In sheep, vaccination did not result in any statistically significantly different reduction in the proportion of shedding between yearlings and ewes (Astobiza et al., 2011a). These studies, demonstrating absence of a statistically significant difference in the proportion of vaccinated sheep shedding C. burnetii when compared to unvaccinated sheep, could be the reason why the Coxevac vaccine has not been licenced for use in sheep, but its use in goats and cattle has been authorized in the European Union, (Astobiza et al., 2011a; Taurel et al., 2012). The age at which most animals born on infected farms first seroconvert to *C. burnetii* has not been documented. These prior studies

do not indicate when the goats first seroconverted to C*. burnetii* although the results point to a time before 4 months of age. Reduction in the number of animals shedding *C. burnetii* could be much higher and elimination of infection from herds might be possible to achieve if vaccination were administered at an age before animals are infected with *C. burnetii*. Vaccination of livestock with Coxevac before 3 months of age has been previously contraindicated because it was thought that adverse reactions were likely to affect young animals and, due to reports of the immunogenicity of the vaccine being hindered by the presence of maternal antibodies (Niewiesk, 2014). Also, that some 65 lymphoid tissues do not reach full anatomical and functional maturity until several months after birth has been thought to result in lack of appropriate response to the vaccine in young animals (Corpa et al., 2000). For example, the intestinal lymphoid tissue, a source of B cells in vaccinated and challenged animals, reportedly reaches maturity at 8 weeks of age. However, a study that compared the immunogenicity of a heat inactivated vaccine against Mycobacterium paratuberculosis in 15-day old and 5 month-old sheep and goats detected humoral and cell-mediated immunity against the vaccine in both age-groups (Corpa et al., 2000). Thus, killed vaccines do appear to be able to trigger humoral and cell-mediated immunity in young animals.

12.1.4. Frequency of vaccination

In humans, no annual booster vaccinations are required before 5 years and even then the absence of both humoral and cellular immunity has to be confirmed before repeated administration of the Q-Vax vaccination is administered to prevent side effects (Rodolakis et al., 2009). Although there are no documented side effects from annual vaccination in livestock herds, 80% of vaccinated cattle in herds with a vaccine coverage of > 80% still had immune markers 1 year after vaccination, which indicates that annual boosters may not be required (Rodolakis et al., 2009; Taurel et al., 2014). Vaccination of > 80% of the herd also resulted in an overall decrease in *C. burnetii* shedding (OR 0.29, 95% CI: 0.09, 0.90) when compared to vaccinating ≤ 80% of the herd (Rodolakis et al., 2009; Taurel et al., 2014) .

The proportion of cattle with cell-mediated immunity was however dependent on age, as only 68% of the heifers had cell-mediated immunity a year after vaccination compared to >80% of adult cattle, indicating that heifers might need an annual booster in the year following vaccination (Rodolakis et al., 2009). The higher proportion of cattle with cell-mediated immunity among adult cows compared to the proportion of heifers with cell-mediated immunity a year after vaccination could be confounded by longer duration of exposure of adult animals to *C. burnetii* shed on the property compared to heifers, as repeated exposure to C. burnetii66 infection is also expected to increase both humoral and cellular immunity against the organism.

Also, herds where vaccine coverage was > 80% were three times more likely to have reduced number of shedders and concentration of *C. burnetii* at their next parturition compared to herds where ≤ 80% of the animals had been vaccinated (Taurel et al., 2014). This could imply that increasing vaccine coverage reduces effective contact between infected and susceptible animals thus decreasing the probability of transmission of *C. burnetii*.

As expected, the majority of the heifers and cows that were seronegative at the time of vaccination tested negative for cellular immunity detected 1 year later through intra-dermal inoculation with killed *C. burnetii* organisms. Conversely, the majority of the cattle that were already seropositive at the time of vaccination were still positive for cellular immunity, 1 year after vaccination (Rodolakis et al., 2009). This may imply that vaccination in uninfected herds requires an annual booster (Rodolakis et al., 2009). This may also point to the possible influence of infection on measures of cell-mediated immunity in infected herds. Also, it is not known whether immunity from exposure to infection contributes to protection of animals against *C. burnetii* or not.

It has been demonstrated that small quantities of intra-dermal treatments increased antibody levels by 111 optical densities (OD) in infected and 87 OD in uninfected cattle (Rodolakis et al., 2009). Perhaps, small intradermal quantities of the vaccine could be used during the booster vaccinations instead of a full dose of the vaccine to reduce the cost of vaccinating each animal.

12.1.5. Duration of vaccination programs

Two years of annual vaccinations in sheep resulted in cessation of shedding in vaginal samples in the third year in two separate flocks (Astobiza et al., 2011a). However, *C. burnetii* contaminated aerosols were detected after 4 years of the vaccination program in one sheep flock where the 67 manure had not been routinely removed, highlighting the importance of implementing hygienic practices in combination with vaccination programmes (Astobiza et al., 2011a). In another study, shedding of *C. burnetii* was observed to reduce from 63% (n = 87) in the first year of implementing the vaccination program to 10% (n = 99) in the second year (Astobiza et al., 2011b). The load of *C. burnetii* in the vaginal samples was also observed to have reduced from 3 bacteria per mL in the first year to 2 bacteria per mL in the second year.

12.2. **Biosecurity and hygiene measures**

Hygienic measures like proper disposal of manure and birth products as well as biosecurity measures like limiting the movement of animals from infected farms to uninfected farms, closing access of infected premises to unvaccinated individuals and the vaccination of all workers at least 15 days prior to the start of work are important components for the control of *C. burnetii* infections in livestock and humans (OIE, 2010). For example, a ban on breeding,

culling of pregnant animals and vaccination of animals before breeding were used to control the large Q fever outbreak in the Netherlands (Guigno et al., 1992; Smith et al., 1993; Lyytikäinen et al., 1998; Delsing and Kullberg, 2008).

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