Coxiella burnetii in Small Ruminants and Humans: Evidence-based Control and Prevention Strategies with a Model to Assess Financial Impact of Coxiellosis to the Ontario Goat Industry

By

Dr. Tyler J. O'Neill

A Thesis
presented to
The University of Guelph

In partial fulfillment of requirements
for the degree of
Master of Science
in
Population Medicine

Guelph, Ontario, Canada

© Dr. Tyler J. O'Neill, September, 2012
Infection with zoonotic *Coxiella burnetii*, the causative agent of Q fever, poses significant challenges for control and prevention in both human and small ruminant hosts. In this context, the research objectives were to systematically identify, assess the risk of systematic bias, and quantitatively synthesize available data reported on interventions to control the development of acute clinical Q fever in high-risk occupationally exposed humans and to prevent bacterial shedding from sheep and goats from routes of public health importance (e.g. vaginal, uterine, and placental secretions, urine, and feces). This study was the first to systematically synthesize and analytically describe vaccination effects in humans and small ruminants. Vaccination in humans had the potential to prevent Q fever in abattoir employees but significant heterogeneity existed in the data. In contrast, vaccination significantly reduced the prevalence of shedding from vaginal and uterine secretions in previously sensitized goats, and milk, vaginal secretions, and feces from naïve goats. The mean levels shed from placental and vaginal routes were significantly decreased in vaccinated naïve goats. No effect of vaccination was found on the prevalence of shedding or mean level of shedding in vaccinated sheep compared to control sheep. The threat to public health remains despite vaccination of livestock. Substantial herd-level financial cost was described through stochastic simulation of direct production losses and expenditures in dairy and meat-producing goat farms in Ontario. Both high and low levels of *C. burnetii* exposure were modeled. The results of the systematic reviews, meta-analyses, and financial analysis are critical to inform evidence-based decision-making regarding future Q
fever policy on prevention and control.
ACKNOWLEDGEMENTS

Few experiences arise in one’s life that cannot be disputed as unequivocally excellent decisions. My pursuit of graduate studies in the Department of Population Medicine is a testament to that fact. I am wholly grateful to the opportunity to have been taught by some of the greatest Veterinary Epidemiologists during my time at the OVC not only as a graduate student, but also as a student of Veterinary Medicine. However, it is time to pursue life beyond the University of Guelph; my home for nearly a decade.

To Dr. Jan Sargeant; your willingness to allow me to formulate, postulate, stump and stumble is a skill that, one would imagine, few possess as an advisor. Thank you for actively (and passively) illustrating to me the reality that is our profession. It is impossible to articulate my gratitude. I have appreciated your intellect, insight, and sense of humour over the past few years, and I greatly respect you as a mentor. Your unequaled passion for pragmatism ("Just the facts, ma’am") has been much appreciated throughout this process. But to say the very least, it has been an honour to call myself a student of yours.

If only more time was granted to me here in Population Medicine, as it would be Dr. Zvonimir Poljak whom I would attribute another reason it is so challenging for me to leave Guelph. Commitment, knowledge, and enthusiasm to an arm’s-length project astounded me daily. I only hope that I can partly emulate your abilities in my future. I look forward to working with you in the future Zvonimir and maintain our friendship.

And whether airport or Alps, Switzerland or Savannah, I could always rely on the solid voice of reason from afar from Dr. Esther Schelling. Thank you for your input over the course of this project. Your guidance and thoughtfulness has been an invaluable resource as we dredge through the human-animal interface swamp.

There are too many who have greatly assisted in the realization of this project, but in particular Dr. Paula Menzies and Shannon Meadows for their assistance with understanding Q fever in Ontario and beyond. As well, Devin Vriezen and Caleigh Maclachlan for your assistance in sifting through the mess of literature
for the systematic reviews. I also would like to thank Drs. Lee Weisner and Victoria 
Ng-Popa for allowing me to pose questions and pick your brains on a daily basis, and 
always having the answers! For those who kept me inline (Sally Maclachlan, Linda 
Kraemer, Karla de Uslar, Mary Elliot and Julie Tremblay-Audet); I couldn’t have 
completed without your assistance. And finally, Ashley Whiteman for keeping me 
organized and helping without ever being asked or thanked enough.

A special note of thanks to Drs. Sandra Watzin and Cheryl MacPhee of Watzin 
Veterinary Clinic for reminding me why I pursued a DVM (and for allowing me to 
finance my graduate education!).

The first day of graduate school, it was imparted that colleagues gained 
during tenure in the Faculty of Graduate Studies would be lifelong friendships. I 
strongly believe that this is true and cannot thank (the future Dr.) Evan Schneider, 
Drs. Laura Falzon and Laura Pieper enough for their friendship during our graduate 
degrees. I wish you all the best and look forward to our shared successes in the 
future.

I also need to thank Stephanie Kovacs for her continued support, 
understanding, patience, encouragement and assistance. Although I can’t conceive 
the challenges you face when you blindly jumped head first in to the deep end of my 
unrelenting educational pursuits, I cannot imagine anyone else by my side along the 
trip down. To many more late nights, early mornings, and piles of papers, 
publications, and textbooks scattered around the house.
# TABLE OF CONTENTS

## CHAPTER 1: Introduction, Study Rationale and Objectives

1. Zoonoses: Interdisciplinary approaches to health problems ................................. 1
2. Q fever ................................................................................................................. 2
   2.1 General pathobiology of *C. burnetii* .................................................................. 3
   2.2 General *C. burnetii* epidemiology .................................................................... 4
   2.3 Q fever in humans ............................................................................................... 5
      2.3.1 Clinical Q fever in humans ........................................................................... 5
      2.3.2 Diagnosis of Q fever in humans .................................................................... 7
   2.4 Small ruminant epidemiology ............................................................................ 7
      2.4.1 Coxiellosis in goats ...................................................................................... 8
      2.4.2 Coxiellosis in sheep ..................................................................................... 9
      2.4.3 Diagnosis of *C. burnetii* infection in sheep and goats .............................. 9
   2.5 Transmission epidemiology at the human-animal interface ............................. 10
   2.6 Methods of Q fever prevention and control ....................................................... 12
   3. Synthesis research ............................................................................................. 14
      3.1 Scoping studies ............................................................................................... 14
      3.2 Systematic reviews ......................................................................................... 15
   3.3 Meta-analysis and meta-regression ................................................................... 16
   4. Economic modeling of Coxiellosis ..................................................................... 17
      4.1 Financial analysis of Coxiellosis in Ontario goat herds .................................... 19
      4.2 Simulating economic models stochastically .................................................... 20
   5. Study rationale ................................................................................................... 21
   6. Study objectives .................................................................................................. 22
   References .............................................................................................................. 23

## CHAPTER 2: The Effectiveness of Q Fever Vaccines in Occupationally Exposed Populations – A Systematic Review and Meta-analysis

Abstract .................................................................................................................... 37
1. Introduction ........................................................................................................... 38
2. Methods ............................................................................................................... 41
   2.1 Search strategy .................................................................................................. 42
   2.2 Relevance screening ......................................................................................... 43
   2.3 Systematic review ............................................................................................ 44
   2.4 Methodological assessment of bias ................................................................... 45
   2.5 Data extraction ................................................................................................ 45
   2.6 Meta-analysis .................................................................................................. 48
3. Results .................................................................................................................. 48
   3.1 Study selection and description of included publications ................................. 49
   3.2 Methodological assessment of bias ................................................................... 52
   3.3 Meta-analysis .................................................................................................. 53
4. Discussion ............................................................................................................. 53
Acknowledgements .................................................................................................. 61
Funding ...................................................................................................................... 62
References ............................................................................................................... 63
LIST OF TABLES

Table 2.1. Descriptive characteristics of full publications reviewed
describing Q fever vaccination in human populations. 72

Table 2.2. Publications included in the systematic review of C. burnetii
vaccines in occupationally exposed populations to prevent clinical Q fever. 73

Table 2.3. Outcomes of randomized controlled trial and cohort studies
reporting confirmed or suspected cases of Q fever in included publications. 74

Table 2.4. Methodological quality assessment (risk of bias) of publications
included in the systematic review of the effectiveness of Q fever vaccines in
occupationally exposed populations to prevent clinical disease. 75

Table 3.1. Publications included in the systematic review of vaccinations
(reactive or preventative vaccination) to reduce shedding of C. burnetii
from sheep or goats from routes of public health importance. 114

Table 3.2. Effect of vaccination on prevalence and the mean level of
shedding of C. burnetii in sensitized and naïve populations of goats and
sheep from multiple routes of public health importance. 115

Table 3.3. Evaluation of risk of systematic error of experimental and
observational publications included in the systematic review of vaccines to
prevent shedding of C. burnetii from sheep or goats from routes of public
health importance. 117

Table 4.1. Estimates of effect for epidemiologic and financial costs
identified in the rapid evidence review (RER) and expert opinion. 149

Table 4.2. Input parameter estimations from estimates of effect to
estimate the herd level financial loss (HFL) and expenditures (HFE) in
Ontario dairy and meat goat farms. 150

Table 4.3. Epidemiological production and financial parameters used in
the model to estimate the financial cost of Coxiellosis in Ontario goat herds. 151

Table 4.4 Estimated herd-level costs (based on summation of herd-level
losses and expenditures) of exposure to C. burnetii infection and
subsequent Coxiellosis within dairy and meat goat herds in Ontario 152
LIST OF FIGURES

**Figure 2.1.** Flow diagram of the scoping study search and identified publications in the systematic review of vaccination to prevent clinical Q fever in occupationally exposed populations. 76

**Figure 2.2.** Forest plot for publications included in the meta-analysis evaluating Q fever vaccines effectiveness to prevent clinical Q fever in occupationally exposed populations. 77

**Figure 3.1.** Flow of information through the stages of the scoping review with the number of publications included and excluded at each level outlined. 113

**Figure 4.1.** Probability distributions of the total estimated cost of Coxiellosis in an Ontario dairy goat herd with (a) low level of infection exposure (mean of 12.7% of animal-level seroprevalence) and with (b) a high level *C. burnetii* exposure (mean of 61.5% of animal-level seroprevalence). 153

**Figure 4.2.** Probability distributions of the total estimated cost of Coxiellosis in an Ontario meat goat herd with (a) low level of infection exposure (mean of 24% of animal-level seroprevalence) and with (b) a high level of *C. burnetii* exposure (mean of 61.5% animal-level seroprevalence). 154
1. ZOONOSES: INTERDISCIPLINARY APPROACHES TO HEALTH PROBLEMS

Zoonoses are communicable diseases that can be naturally transmitted between vertebrate animals and humans (WHO, 2005) directly, indirectly, or through foods. At least 61% of all human pathogens are known to be zoonotic, with 75% of emerging pathogens affecting humans in the past decade being classified as a zoonosis (Taylor et al., 2001; WHO, 2005). Many zoonoses are not prioritized by most health systems globally and are neglected due to the low burden of illness compared to non-communicable chronic diseases of humans (Meslin, 2006; WHO). However, zoonotic agents can have significant impact on public health. Zoonoses of livestock can affect animal productivity and economic viability, thereby affecting the quality of both human and animal life. Prevention and control strategies should focus on evidence-based interventions that target animal reservoirs when appropriate, with collaborative efforts amongst veterinarians, physicians and other government stakeholders. Control and prevention of zoonoses are necessary for economic and public health benefit.

Transmission of pathogens from livestock to high-risk occupational groups may occur, for example, through exposure during parturition, manure and waste removal, slaughter and product handling, and the consumption of unpasteurized milk. The World Health Organization (WHO) classified occupational groups and populations at high risk of zoonotic infections, including those in contact with animals, their environment or products of animal-origin (WHO, 1982). However, the probability of effective contact depends on the health status of the animals, the type of activity of the employee, the frequency of animal contact including their products, and the preventive measures taken to minimize risk (Battelli, 2006). Q fever has been identified as an occupational zoonosis of major socio-economic importance worldwide for those involved in ruminant farming and slaughter activities (Battelli, 2006; EFSA, 2010a; Post 2010).
2. Q FEVER

*Coxiella burnetii*, the causative agent of Q fever, is a small (0.2-1.0μm long, 0.2-0.4μm wide) Gram-negative obligate intracellular zoonotic pathogen (Maurin and Raoult, 1999; Oyston and Davies, 2011). Historically, Q fever (humans) or Coxiellosis (livestock) has been an occupational hazard in persons in contact with domestic livestock such as cattle, sheep and goats (Maurin and Raoult, 1999) and occurs globally except for New Zealand (Taurel et al., 2011). In small ruminant populations it is highly contagious and may lead to economic losses from unrealized reproductive potential of livestock due to late-term abortions, stillbirths, and lost production such as reduced milk production from aborting animals. Human cases are typically sporadic but outbreaks are reported. Inhalation of *C.burnetii* from infected livestock or a contaminated environment (Maurin and Raoult, 1999) represents the most common route of exposure.

2.1 GENERAL PATHOBIOLOGY OF *C. BURNETII*

The first description of clinical Q fever (“query fever”) was in Queensland, Australia by Derrick (1937) in a population of abattoir workers with febrile illness. Burnet and Freeman (1937) and Cox and Bell (1939) independently isolated a bacterium with viral and rickettsial-like properties from samples of infected tissue from Australian abattoir workers, and ticks (“Nine Mile Strain”) from the United States, respectively. It was not until Dyer (1939) documented cross-reaction between laboratory-acquired infected blood and inoculated the agent into guinea pigs that the pathogen in the Australian abattoir workers and the tick-associated agent were identified as the same (Maurin and Raoult, 1999).

Mutational phase variation in the bacterial lipopolysaccharide (LPS) enables antigenic variability amongst *C. burnetii* (Hackstadt, 1988). Phase I, the natural phase corresponding to a smooth bacterial LPS, is isolated in acutely infected animals, humans and arthropods and exhibits highly infectious properties. Although phase I *C. burnetii* is poorly internalized by target cells, it survives, metabolizes and multiplies within the acidic cell environment as a vacuole through the fusion of phagolysosomes (Hackstadt and Williams, 1981; Akporiaye et al., 1983; Angelakis and Raoult, 2010). The bacterium rarely damages the host cell, partly due to the
slow doubling time of 20-45 h (Maurin and Raoult, 1999; Mertens and Samuel, 2007). In contrast, phase II, with rough bacterial LPS, demonstrates avirulence and is readily internalized but rapidly killed through the phagolysosomal pathway (Maurin and Raoult, 1999; Marrie and Raoult, 1999).

The complex intracellular lifecycle of C. burnetii corresponds to different developmental stages in persistently infected cells and confers environmental stability (Scott and Williams, 1990; McCaul, 1991; Mo and Mallavia, 1994; Angelakis and Raoult, 2010). The bacterium can survive for several weeks outside a host in warm, moist environments (Tissot Dupont et al., 1992; Marrie and Raoult, 1997). To date, only long-term (24 to 48 hours) exposure to ≥10% formalin will result in the killing of C. burnetii to an undetectable level (Maurin and Raoult, 1999).

Acute C. burnetii infection is mediated by the cell-mediated immune (CMI) response (Angelakis and Raoult, 2010). Despite being T-cell dependent, C. burnetii is not eliminated by the immune system (Capo et al., 2003). Following infection, immunoglobulin is secreted according to specific phase antigen exposure. Immunoglobulin G (IgG) corresponds to phase II antigen exposure, whereas Immunoglobulin M (IgM) is directed against both phase I and II (Maurin and Raoult, 1999), and is the basis of serologic diagnostic evaluation. Defective CMI may play a role in the characteristic chronic, or persistent infection (PI) by C. burnetii in humans and animals (Babudieri, 1959; Baca and Paretsky, 1983; Angelakis and Raoult, 2010).

2.2 GENERAL C. BURNETII EPIDEMIOLOGY

Growing public health concern over the risk to the human population beyond occupational exposure stems from a sustained outbreak of Q fever in the Netherlands (2007-2010). This epidemic resulted in >3,500 individuals becoming clinically ill and the national culling of pregnant animals in goat herds and sheep flocks that led to significant economic losses, particularly in the area of Noord-Brabant (Schimmer et al., 2008; Post, 2010; van der Hoek et al., 2010; Lahuerta et al., 2011). Although there is evidence of increasing incidence globally in humans and livestock (Morse, 1995; Arricau-Bouvery and Rodolakis, 2005; WHO 2006) scarce
knowledge on the dynamics of infection between infected livestock, the environment and humans exists.

Rarely is Q fever a reportable disease, making its true incidence in human and livestock populations difficult to estimate (Maurin and Raoult, 1999). The clinical manifestations of Q fever in humans are highly polymorphic such that the disease may be misdiagnosed leading to underreporting of the disease (Battelli, 2006). Sporadic cases in livestock are rarely diagnosed and asymptomatic infection is common. Therefore, estimates have to be extrapolated from outbreaks, human or livestock serosurveys, and animal or public health diagnostic laboratory data (Maurin and Raoult, 1999). Compounded by tests with low sensitivity, this leads to underestimation of the global burden of disease (Guo et al., 1998; Maurin and Raoult, 1999; Angelakis and Raoult, 2010). A total of 18 reported outbreaks in 12 different countries involving 2 to 289 people were reported between 1999-2004 (Angelakis and Raoult, 2010). The outbreaks were attributed to exposure to sheep (n=6), goats (n=3), goat manure (n=1), sheep manure (n=1), wild animals (n=1), and dogs and cats (n=1). Two of the outbreaks were of unknown source (Arricau-Bouvery and Rodolakis, 2005).

2.3 Q FEVER IN HUMANS

Q fever cases are typically sporadic. However, as rural populations continue to decrease in most developed nations, cases of Q fever are becoming more common in naïve urban populations who have occasional exposure to infected animals or their products (Maurin and Raoult, 1999; Tozer et al., 2011). Thus, outbreaks are more likely to occur in the future. From 1994 to 2002, the reported annual incident clinical Q fever cases in Ontario, Canada ranged from 0.008 to 0.16 with an average of 0.08 per 100,000 (London-Middlesex Health Unit, 2005). Acute clinical Q fever incidence rates have also been reported to be 0.15 to 0.35 cases per 100,000 population per year in the United Kingdom (Thomas et al., 1994), 0.73 per million inhabitants per year in Nova Scotia, Canada (Marrie et al., 1988), and 50 per 100,000 inhabitants per year in France (Tissot Dupont et al., 1992). Maurin and Raoult (1999) noted that Q fever incidence was highest in areas where rickettsiologists worked and clustered around certain high-risk occupations. High-
risk occupationally exposed groups include those exposed to ruminants (veterinarians, farmers, stock-breeders, livestock truck drivers, wool shearers, sausagehouse workers), medical and paramedical practitioners, laboratory technicians with *C. burnetii* exposure, rural or semi-rural residents, and gardeners using contaminated manure fertilizer (Tissot-Dupont et al., 1999; Berri et al., 2003).

Despite comparable seroprevalence and exposure history, both gender and age may have protective effects against *C. burnetii* infection (Tissot Dupont et al., 1992). Studies in mice have suggested that 17β-estradiol in females is responsible for a protective effect of infection by *C. burnetii* compared to male mice (Leone et al., 2004). The average age of infection ranges from 30 to 60 years (Maurin and Raoult, 1999). Symptomatic clinical infection is five times more likely to occur in individuals' ≥15 years compared to those <15 years (Dupuis et al., 1985; Maltezou and Raoult, 2002). Clinical Q fever is seen more commonly in men than women (Maurin and Raoult, 1999). Age and gender may be confounding as the majority of those employed in high-risk occupations were men in this age strata. However, only 8% of French study subjects infected with *C. burnetti* were considered to have high-risk occupations (farmer, veterinarian) (Raoult et al., 2002) suggesting that further epidemiologic surveillance is required. Communicability between infectious humans is rare and not considered to be a main source of infection (Anonymous, 1950; Marmion and Stoker, 1950; Anonymous, 1977; Raoult and Stein, 1994; Stein and Raoult, 1998; Maurin and Raoult, 1999; Milazzo et al., 2001).

### 2.3.1 CLINICAL Q FEVER IN HUMANS

Infection with *C. burnetii* can cause either an asymptomatic, acute, or chronic disease. Size of the inoculum, route, and duration of exposure, as well as host factors influence the duration of the incubation period and may contribute to the clinical expression of acute and chronic clinical disease (Angelakis and Raoult, 2010; Porter et al., 2011). Monocytes and macrophages are the primary target cell (Srigley et al., 1985; Marrie, 1995; La Scola et al., 1997; Marrie et al., 1997; Maurin and Raoult, 1999). Inhalation of aerosolized bacteria through the respiratory tract is the primary route of infection, with alveolar macrophages being targeted. Kupffer cells of the liver are affected through bacteremia or, rarely, digestive-route exposure.
Of those infected, only 40% develop symptomatic acute Q fever, with 60% seroconversion of those exposed (Arricau-Bouvery and Rodolakis, 2005). Only 4% of those affected with acute Q fever need hospitalization for their symptoms (Angelakis and Raoult, 2010).

The incubation period prior to acute clinical Q fever is estimated at approximately 20 days (range: 12 to 39 days)(Young, 1948; Spelman, 1982; Lopez et al., 1986; Angelakis and Raoult, 2010) depending on inoculating dose (Maurin and Raoult, 1999). The mean delay (incubation period) between the onset of clinical signs and acute Q fever diagnosis is estimated at 2 months (Raoult et al., 1986). Acute Q fever manifests as a self-limiting mild febrile illness and may be associated with headache, myalgia, arthralgia, and coughing (Tissot-Dupont and Raoult, 2007). Fever typically plateaus within 2-4 days and returns to normality within 5-14 days in treated individuals. Untreated patients may observe fever of 5-57 days duration (Derrick, 1973). Pulmonary symptoms, including atypical pneumonia, and hepatitis are commonly observed (Tissot-Dupont et al., 1992). Rarely, dermatological and neurologic manifestations occur (Maurin and Raoult, 1999; Bernit et al., 2002). The annual mortality rates in the general population associated with acute clinical Q fever range from 0.9 per 100,000 per year in the United Kingdom (WHO, 1979) to 2.4 per million per year in France (Tissot-Dupont et al., 1992). Myocarditis is the leading cause of death in acute disease and accounts for 2% of all clinical cases (Fournier et al., 2001).

Acute disease does not have to precede chronic Q fever. Chronic Q fever occurs in 5% of infected individuals (Fenollar et al., 2004) and is characterized by persistent infection beyond six months. It occurs almost exclusively in high-risk patients, including those with immunosuppression, heart valve lesions, vascular abnormalities and pregnancy (Fenollar et al., 2001; Angelakis and Raoult, 2010). In the absence of treatment, the prognosis is very poor (Botelho-Nevers et al., 2007). Clinical manifestations of chronic Q fever include osteoarticular infections (Maurin and Raoult, 1999), chronic hepatitis (Raoult et al., 2000), infection of the ventriculo-peritoneal drain (Lohuis et al., 1994), pseudotumours of the spleen and lung (Lipton et al., 1987), and chronic fatigue syndrome (Angelakis and Raoult, 2010).
2.3.2 DIAGNOSIS OF Q FEVER IN HUMANS

Diagnosis of Q fever requires review of patient history, physical exam and confirmatory laboratory tests. Due to its low infectious dose, diagnosis of Q fever involving isolation of the organism should occur at biosafety level 3 laboratories by trained personnel. Diagnostic techniques include: (i) culture of *C. burnetii* with direct observation with Gimenez or immunofluorescence staining (Angelakis and Raoult, 2010), (ii) immunodetection (Maurin and Raoult, 1999; Lepidi et al., 2006), (iii) conventional, nested (Fenollar and Raoult, 2004; Fenollar et al., 2004) and real-time (Klee et al., 2006) PCR, and (iv) serology (Maurin and Raoult, 1999). In most cases, acute and convalescent sera are used to diagnose Q fever; complement fixation (CF) being the preferred methodology but ELISA is routinely used in practice. There is no reported association between seroconversion and protection against clinical disease (Porter et al., 2011).

2.4 SMALL RUMINANT EPIDEMIOLOGY

Acute infection is typically asymptomatic in small ruminants (Lang, 1988; Berri et al., 2002; Angelakis and Raoult, 2010) compared to the chronic sequelae of uterine and placental infections of pregnant animals (Babudieri, 1959). Variable incidence of abortion (3-80%) (Palmer et al., 1983; Marrie, 2007) and low birth weights at the first parturition post-infection are the primary clinical signs (Marrie et al., 1996). There is inconclusive evidence for association between within-herd seroprevalence of *C. burnetii* exposure and history of on-farm abortion (Domenech et al., 1985; Schelling et al., 2003; Webster et al., 2009). However, susceptible or naïve animals are more common among young stock than adults suggesting that immunity develops as age increases due to an increased probability of exposure over time (Berri et al., 2007; Garcia-Perez et al., 2009). This may explain why clinical signs and high levels of shedding (10⁹ per gram placenta) are most commonly reported in primiparous animals, or animals of any age previously naïve to *C. burnetii* (Babudieri, 1959).

The bacteria are shed through various routes including fetal membranes and fluids of parturition, feces, urine, vaginal secretions, milk and aborted fetuses (Berri et al., 2001; Arricau-Bouvery et al., 2003; Guatteo et al., 2006; Maurin and Raoult,
The primary routes of shedding are from vaginal secretions and fluids of parturition from goats, and milk and parturition fluids from sheep (Porter et al., 2011). As an infective dose can be as low as a single bacterium (Webster et al., 2009), cyclical shedding maintains within-flock endemicity of infection and sustains the zoonotic risk. Endemic concentrations of *C. burnetii* in the environment may contribute to transmission and high seroprevalence within a flock or herd (Webster et al., 2009). The survival of *C. burnetii* in the environment for up to eighteen months makes control or elimination a challenge for both veterinary and public health practitioners (Astobiza et al., 2010b). Seroepidemiologic studies are limited but Lang et al. (1991) reported 20% seroprevalence of at least one reactor present on Ontario meat sheep farms, and significant associations (p≤0.05) were found between previous exposure status and overwinter indoor housing, and the introduction of new ewes to the farm. Infected reservoirs of different species may also be able to infect one another, although conclusive evidence has not yet been reported (Hatchette et al., 2002).

### 2.4.1 COXIELLOSIS IN GOATS

Goats are the most frequently identified source of human infection globally due to extensive rearing and close human contact (Berri et al., 2007). Abortions, stillbirths, and the delivery of weak-born kids are common clinical signs, with pneumonia rarely observed (Sanchez et al., 2006; Berri et al., 2007; Rousset et al., 2009). Up to 98% of females have been reported to abort if previously naïve to infection (Berri et al., 2007). Pregnant does are more susceptible to infection than non-pregnant animals due to the bacteria’s propensity for residing in uterine lymphocytes. Once infected, these individuals may only be clinically affected at the following kidding season. Bacteria colonize the uterus and mammary glands with subsequent discontinuous shedding from vaginal secretions and milk. Long-term shedding in milk, up to 32 months post-infection, is recognized as the major route of excretion (Rodolakis et al., 2007; Berri et al., 2007; Angelakis and Raoult, 2010). Persistent shedding in the urine and feces is limited to 20 days post-partum (Angelakis and Raoult, 2010).
2.4.2 COXIELLOSIS IN SHEEP

Sheep have high incidence of abortion but rarely develop chronic clinical disease (Hirai and To, 1998; Berri et al., 2007). Shedding in vaginal secretions, urine and feces are well documented up to 60 days post-partum (Hirai and To, 1998; Rousset et al., 2007; Vaidya et al., 2010) compared to the 7 to 14 days of intermittent milk shedding (Muramatsu et al., 1997; Maurin and Raoult, 1999; Berri et al., 2001; Kim et al., 2005; Rodolakis et al., 2007; Astobiza et al., 2010b). The bacteria are shed for up to six months of time from vaginal secretions and may persist to subsequent pregnancies. Multiple concurrent shedding routes are common (Marrie, 1990; Rodolakis et al., 2007; Vaidya et al., 2010).

In Newfoundland, increased *C. burnetii* seroprevalence from 3.1% in 1997 to 23.5% in 2000 has been observed (Hatchette et al., 2002). This may be attributed to their growing small ruminant industry in addition to greater provincial-level surveillance of herds and flocks. In Ontario, Lang et al. (1991) found that 1.5% of sheep were seropositive and the flock level seroprevalence was 20% with a significant clustering effect observed between farms. It was hypothesized that environmental contamination and aerosolization of the bacteria led to the spread of infection between neighbouring farms. Human cases are less frequently associated with exposure to infected sheep and are more commonly reported with exposure to *C. burnetii* contaminated sheep manure (Berri et al., 2003; Panaiotov et al., 2009).

2.4.3 DIAGNOSIS OF COXIELLOSIS IN SHEEP AND GOATS

The diagnosis of Coxiellosis is challenging. There are disagreements amongst diagnosticians in the use of diagnostic tests and no gold standard exists (Porter et al., 2011). However, The World Organization for Animal Health (OIE) recommends that the complement fixation test (CFT) serological method be used for definitive diagnosis (OIE, 2010). Low sensitivity (0.84) compared to ELISA (Kittelberger et al., 2009) and a frequent inability to identify antibodies in infected sheep and goats (Kovacova et al., 1998) render its utility in question. *C. burnetii* is also commonly observed as a co-infection with other abortive pathogens making justification of primary abortive etiology suspect without gross and histologic diagnostics. The ELISA (serology or bulk tank milk) is highly sensitive for screening *C. burnetii* at the
herd-level but is limited for individual animals (Angelakis and Raoult, 2010). Although rapid, sensitive and specific PCR kits are available for samples (Berri et al., 2003) the utility in clinical practice has yet to be evaluated.

Serum antibodies (IgM) are detectable two weeks after initial infection with \textit{C. burnetii} and reach a maximum 30 to 60 days post-infection. This is followed by a period of rapid decline in detectable antibodies (Enright et al., 1971). A convalescence of antibodies on the day of parturition occurs and remains elevated for five weeks post-partum (Berri et al., 2005). The presence of serum (ELISA) antibodies or bacteria shed in vaginal secretions (PCR) in parturient females cannot reliably predict the presence or absence of detectable serum antibodies in their offspring (Berri et al., 2005). Some infected animals never seroconvert (Behymer et al., 1977). Therefore, assessing risk of shedding with serology is of limited utility.

Berri et al. (2001, 2005) found that 57% of parturient ewes shed the organism (vaginal swab PCR) despite being seronegative (ELISA). Seroconversion is not known to protect against shedding and clinical effects of \textit{C. burnetii} infection (Porter et al., 2011).

\textbf{2.5 TRANSMISSION EPIDEMIOLOGY AT THE HUMAN-ANIMAL INTERFACE}

Infection in humans and livestock is often associated with parturition in domestic ruminants through aerosolized bacteria from contaminated secretions, urine or feces, and inhalation of in contaminated dust particles (Tiggert and Benenson, 1956; Gonder et al., 1979; Marrie et al., 1989; Tissot-Dupont et al., 1992; Maurin and Raoult, 1999; Marrie, 2000; Arricau-Bouvery and Rodolakis, 2005; Post et al., 2010). Most human cases occur in the vicinity of farms (<5km) where small ruminants are aborting due to \textit{C. burnetii} (Thomas et al., 1995; Guo et al., 1998; Schimmer et al., 2009). There is evidence that the organism is spread by wind, which has been attributed to the widespread outbreak in the Netherlands (Marrie and Raoult, 1997; Tissot-Dupont et al., 1999; Post, 2010). Thus, patients affected by Q fever may have no history of direct contact with animals or contaminated fomites (Tissot-Dupont et al., 1992). Living near infected sheep or goat farms alone has been shown to significantly increase the risk of acquiring Q fever (Tissot-Dupont et al., 1999; Maurin and Raoult, 1999; van der Hoek, 2010).
The risk of human infection does not appear to increase as the number of animals per farm increases (not a density-dependant pathogen)(Guo et al., 1998) and no association between sheep or goat density and human Q fever seroprevalence has been established (Tissot-Dupont et al., 1999). However, most Q fever incident cases and outbreaks in humans have occurred in areas with high numbers of sheep or goats (Dupuis et al., 1987; Tissot-Dupont et al., 1999; Webster et al., 2009). Further investigation in to the relationship between livestock density and human infection is ongoing in the Netherlands (van der Hoek, 2010). The risk of infection is dependent on total time in contact with a contaminated environment or infected and shedding animals (Maurin and Raoult, 1999). Environmental contamination may also drive the temporal trend observed in human cases that lag behind parturition and shearing seasons by several weeks (Montejo Baranda et al., 1985; Tissot-Dupont et al., 1992; Hellenbrand et al., 2001). In contrast, Maurin and Raoult (1999) have not observed higher incidence following the lambing season in France.

The consumption of unpasteurized milk is unlikely to be a significant source of C. burnetii transmission (Benson et al., 1963; Fishbein and Raoult, 1992; Maurin and Raoult, 1999). Evidence from experiments of volunteers consuming Q-fever contaminated raw milk lead to inconclusive results warranting further investigation (Benson et al, 1963; Krumbiegel and Wisniewski, 1970).

Other risk factors for C. burnetii infection in humans and small ruminants include percutaneous transmission from infected ticks (Eklund et al., 1947; Beaman and Hung, 1989; Ho et al., 1995; Faix et al., 2008; Christmann, 2002), exposure to infected dogs (Marrie et al., 1985; Rauch et al., 1987; Laughlin et al., 1991; Buhariwall et al., 1996) and infected parturient cats (Kosatsky, 1984; Marri et al., 1985; Langley et al., 1988; Marrie et al., 1988; Marrie and Raoult, 2002). These modes of transmission and exposure routes are uncommon and may not contribute to any significant burden of disease to either human or livestock populations (Babudieri, 1959; Maurin and Raoult, 1999).
2.6 METHODS OF *C. BURNETII* PREVENTION AND CONTROL

Prevention recommendations of Q fever or Coxiellosis are currently focused at preventing the spread of bacteria from the livestock reservoir (EFSA, 2010a), except for Australia where a human vaccine is licensed. According to Schelling et al. (2003), controlling endemic Coxiellosis in livestock may play an important role in reducing disease burden in populations who live in close contact with infected animals. Thus, controlling the disease in animals may influence the disease in humans. Furthermore, shared risk reduction with other zoonotic diseases is probable with pathogens with similar natural histories (Schelling et al., 2003).

Several control and prevention strategies in small ruminants are currently recommended. Firstly, antimicrobial therapy has been suggested to control clinical outbreaks of Coxiellosis despite a lack of evidence for its effect (EFSA, 2010). The addition of in-feed tetracycline or injectable oxytetracycline pre-partum has been no reported utility to prevent *C. burnetii* shedding (milk, feces, vaginal secretions) in two descriptive cross-sectional studies (Berri et al., 2007; Astobiza et al., 2010b). Secondly, controlling environmental contamination through the control of infected ticks and biosecurity measures may reduce introduction of *C. burnetii* to naïve farms (Angelakis and Raoult, 2010). Biosecurity measures include the appropriate burying, composting, or incineration of fetal fluids and membranes, aborted fetuses and contaminated bedding. Infected manure and bedding waste should be treated with lime or 0.4% calcium cyanide before being spread on fields. Spreading manure should occur only on days with an absence of wind to reduce the risk of spread through aerosolization (Tissot-Dupont et al., 1999; Tissot-Dupont et al., 2004). Allocating dedicated areas for parturition is also recommended. Lastly, a program to identify and cull shedders to reduce the overall herd-level prevalence has been recommended, despite its expense and impact on genetics within a herd or flock. However, limited scientific evidence has been offered to support these recommendations (EFSA, 2010a).

For human populations, those with high-risk occupations should be aware of the risks of Q-fever and take preventive measures to protect themselves (e.g. protective clothing, face masks, hand hygiene). Promoting the consumption of
Pasteurized milk and reducing exposure to parturient livestock by high-risk populations is also recommended (Angelakis and Raoult, 1999).

Vaccines for *C. burnetii* in humans and livestock are produced. Experimental evidence has supported the use of a phase I organism vaccine because it is 100 to 300 times more effective at eliciting an antibody response in guinea pigs compared to a phase II vaccine after aerosol challenge (Zhang and Samuel, 2004). Vaccination of livestock has been suggested to limit within and between herd spread, reduce clinical disease, and limit spillover from animals to human (Arricau-Bouvery et al., 2005; van den Brom and Vellema, 2009; EFSA, 2010a). An inactivated Nine Mile strain phase I vaccine (*Coxevac*, CEVA Sante Animale, France) is provisionally licensed for cattle and goats within the European Union (E.U.) and has been proposed as a long-term control strategy (EFSA, 2010a). Although not a label claim, the vaccine formulation has also been used in sheep (Sadecky and Brezina, 1977; Astobiza et al., 2010a). Several studies have presented conflicting results on the vaccine’s effect on reducing bacterial shedding (Porter et al., 2011). Therefore, the risk of transmission to humans through environmental contamination may persist.

The goal of human vaccination is to reduce the risk of clinical Q fever in vaccinated individuals who were previously naïve to infection. A Henzerling strain phase I whole-cell formalin inactivated vaccine (*Q-vax*, CSL Biotherapies Ltd, Australia) is licensed for human use in Australia and has been recommended for seronegative high-risk occupationally exposed individuals (Maurin and Raoult, 1999; Arricau-Bouvery and Rodolakis, 2005; Chiu and Durrheim, 2007). Immunocompromised persons, pregnant women and those with pre-existing vascular or cardiac valvular defects may also benefit from vaccination (Zhang and Samuel, 2004) providing confirmation of no previous exposure. A CMI skin test is required to establish previous sensitization to *C. burnetii* and reduce the risk of subsequent severe local and occasional systemic reaction to the vaccine, making vaccination costly and time consuming (Zhang and Samuel, 2004). A meta-analysis by Gefenaite et al. (2011) reported a pooled vaccine effectiveness of 97% (95% CI: 94 to 99%). However, the authors reported several biases were present in the study, making the validity of the conclusion limited. No further details on the biases were
provided. A scoping study by Chiu and Durreheim (2007) reported similar vaccine effectiveness based on similar publications. No assessment of systematic bias was presented for the scoping study making the results difficult to interpret. Both studies acknowledged the lack of external validity of current vaccine publications, with the majority restricted to Australian abattoir employees.

3. SYNTHESIS RESEARCH

3.1 SCOPING STUDIES

Scoping studies, a systematic form of literature review, are useful tools for broad, rapid mapping of relevant literature in a given field of interest (Arksey and O’Malley, 2005). A specific research question and the assessment of methodological quality are not components of a scoping study, and limited literature on their design is available (Jepson et al., 2001; Katz et al., 2003; Arksey and O’Malley, 2005; Anderson et al., 2008). Key concepts in a field of research, the sources and types of available evidence, and identification of gaps in research are typical outcomes of a scoping study (Mays et al., 2001; Arksey and O’Malley, 2005). The scoping study approach may assist researchers and policy makers in understanding the characteristics of the available evidence, and through increased transparency and replication of the review process, contribute to the overall evidence base for public health decision making. Scoping studies are often a method component of a full systematic review (SR) (Katz et al., 2003; Anderson et al., 2008) to refine the study question and may be useful for identifying literature available on interventions to control zoonotic infections at the human-animal interface.

The European Food Safety Authority (EFSA) published a systematic narrative literature review (EFSA, 2010a) on Q fever to scope potential control and prevention strategies to mitigate C. burnetii spread within livestock populations and limit the zoonotic potential. However, the estimates of effectiveness were primarily based on expert opinion, derived from observation rather than rigorous scientific assessment of the limited body of available literature. Thus, methods for assessing risk of systematic bias of included Q fever publications were lacking.
3.2 SYSTEMATIC REVIEWS

Traditional literature reviews in zoonotic public health lack structure and transparency. In contrast, SR in zoonotic public health have been shown to be an effective means of structurally and transparently addressing intervention questions using globally available primary literature sources (Waddell et al., 2009). SR is an approach providing a structured and standardized methodology for the transparent identification, selection, and summarizing of results of large quantities of research with critical assessment of systematic error (Cook et al., 1997; AHRQ, 2002; USDA, 2005). This methodology allows for conclusions with limited bias based upon a review of the quality of methods used to conduct primary studies (e.g. internal validity) thereby improving the reliability and accuracy of conclusions (Mulrow, 1994; CRD, 2001; USDA, 2005; Sargeant et al., 2006; EFSA, 2010b).

SR utilizes a predefined and documented step-wise search strategy to identify all relevant literature based on specific inclusion criteria as directed by a focused a priori study question (USDA, 2005; EFSA, 2010b; Cochrane, 2011). This method is both comprehensive and explicit in order to minimize selection bias through the process of critical appraisal and quantitative synthesis of data (meta-analysis)(CRD, 2001; Sargeant et al., 2006; Borenstein et al., 2009). Studies are objectively reviewed by at least two independent qualified individuals for relevance, quality and report findings (Sargeant et al., 2006). SR often leads to quantitative synthesis of reported statistical data from multiple studies. However, due to variability in veterinary medical literature, SR may also summarize the results qualitatively if the variability in the research screened does not support a pooled data analysis (EFSA, 2010b). Determining the quality of literature available for C. burnetii interventions, in addition to data synthesis, is a valuable contribution to the scientific community when considering the long-term, evidence-based programs and policies resulting from the research. Current knowledge on C. burnetii interventions may be synthesized and increase the credibility of findings in the field; or alternatively, highlight areas of insufficient evidence to support claims of interventions for control and prevention and to provide input on future research directions.
The use of SR has been widely accepted in human health, with limited application of this method in veterinary medical research (Sargeant et al., 2006; EFSA, 2010b). Thus, reviews of epidemiological evidence-based intervention decisions are lacking in veterinary medicine. Reviews performed thus far have often highlighted knowledge gaps that can direct future research and target funding, rather than answering the original question posed by the systematic review (Exponent, 2000; Sargeant et al., 2006). Differences in veterinary medical systematic reviews compared to those published in human medical literature are due to the nature of research conducted in these fields. Sargeant et al. (2006) claim that the reasons for this are two-fold. Firstly, challenge and observational studies may be more common than randomized controlled trials (RCTs) in animal health in providing species-specific evidence and conclusions. Secondly, animal density within some trials may be non-representative of commercial operations due to financial and logistic limitations, and the statistical effect of clusters must be taken into account. As such, SR methods developed in the field of human medicine must be modified to account for the differences of veterinary medical research (USDA 2005; Sargeant et al., 2006).

Both assessment of systematic error and data extraction may need to be altered to include research from multiple study designs while considering the statistical effects of clustering. This will allow for the evaluation of the validity of measures of variability (e.g. confidence intervals). The method must also take into consideration the differences in agricultural intensity and husbandry observed internationally when deciding to synthesize data from multiple regions (e.g. external validity). Researchers in veterinary medicine must be aware that, for many questions posed by systematic reviews, there will simply be a lack of evidence from which an answer may be determined (Sargeant et al., 2006).

### 3.3 META-ANALYSIS AND META-REGRESSION

Understanding the effects of \textit{C. burnetii} interventions can be aided by synthesizing epidemiological data. Meta-analysis (MA) is a quantitative method of combining the results of independent studies, exploring heterogeneity, and synthesizing results (Egger et al., 2001; Cooper et al., 2009). The benefit of MA lies in
the statistical power to detect an effect with greater than one study as the estimation of two or more parameter values (e.g. odds ratio) leads to a combined effect more accurate on average than the parameters’ estimates independently (Egger, 2001). Weighting of studies is dependent upon sample size and precision in an included study (Borenstein et al., 2009). Therefore, larger studies with greater precision will contribute a greater weight to the overall effect size. Through the complete coverage of relevant studies, the identification and exploration of heterogeneity, and the use of regression to explore the robustness of the pooled estimate where appropriate, precise and transparent pooled outcomes can be estimated and interpreted (Borenstein et al., 2009; Crombie and Davies, 2009).

If statistical heterogeneity among studies is found to be significant ($I^2 > 30\%$), suggesting variability is due to the included studies rather than chance alone, it can be explored through stratified MA or meta-regression of potentially relevant predictors, confounders, or interacting variables (Sutton et al., 2000). Meta-regression allows for potential associations between the study design, methodological soundness characteristics, and the reported effect size to be evaluated (Deeks et al., 2001; Cooper et al., 2009; Crombie and Davies, 2009).

The validity of the MA depends upon the quality of the SR upon which it is based. A high quality SR, with comprehensive critical appraisal of the included studies, will generate valid data outcomes (Egger, 2001; Borenstein et al., 2009; Cooper et al., 2009). To date, the SR and MA for Q fever in high-risk occupationally exposed groups (Chiu and Durrheim, 2007; Gefenaite et al., 2011) have not reported a weighted pooled effect estimate of vaccination. To the author’s knowledge, no SR or MA has been conducted on C. burnetii vaccination in sheep or goats.

4. ECONOMIC MODELING OF COXIELLOSION

The field of economics is concerned with how individuals, governments, and private organizations make decisions regarding the distribution of scarce resources (CCA, 2012). A range of economic modeling techniques exists to evaluate the effects of animal health diseases and their control (e.g. deterministics, probabilistic) (France and Thornley, 1984). Regardless of the method, economic evaluation in health care attempts to address both the economic and philosophical aspects of health care for
livestock infectious diseases. Thus, our understanding of the monetary implications of veterinary medical decision-making for *C. burnetii* is maximized when economic approaches are applied to animal health problems. In animal health economics, the merits of different approaches to improve the health of a population are evaluated to make the best decisions on the allocation of disease control measures.

Evaluating disease costs from clinical and policy perspectives is a multi-step process (Detsky and Naglie, 1990). The first is to demonstrate that the intervention(s) provide greater benefit than harm when utilized under routine circumstances. If the cost of disease is deemed sufficiently important to the sheep and goat industries, then it may be worthwhile to consider the cost-effectiveness of implementing on-farm intervention programs to mitigate the spread of *C. burnetii*. In this sense, we would be interested in determining the effectiveness of preventing *C. burnetii* shedding from sheep and goats. However, the cost (sum of both production losses and expenditures) of disease must be first established.

No formal Coxiellosis financial analysis exists for Ontario goat farms to model the direct impact of disease to on-farm profitability. Determining and then controlling the costs associated with Coxiellosis may be valuable to inform and contribute to sustainable and competitive farming practices. Thus, decisions regarding animal health preclude the independent importance of the individual farm in favour of determining optimal cost-effective policy directives for all producers (Dijkhuizen et al., 1995). A systematic and objective framework of costing can assist decision makers to make informed Q fever policy. Initial recommendations for integrating simple animal health economics for veterinary decision-making were suggested by Morris (1969) and Ellis (1972). Both suggested an equimarginal approach to veterinary economics, such that the costs of the additional intervention are at least equivalent to the additional output gained (Dijkhuizen et al., 1995). Health economics are increasingly being used to inform decisions to adopt, reimburse or issue guidance on the use of health care technologies (Hjelmen et al., 2001).
4.1 FINANCIAL ANALYSIS OF COXIELLOSIS OF ONTARIO GOAT HERDS

Financial analysis, or production economics, is a formal comprehensive economic evaluation whereby the losses and expenditures of Coxiellosis are examined to help set health intervention priorities over time (James, 1987; Dijkhuizen et al., 1995; Rushton et al., 1999; Drummond et al., 2005). The use of this method maximizes potential disease control and prevention strategies in the target population based on available fiscal and management resources.

The livestock capital approach to financial analysis is one in which individual animals are regarded as equivalent to capital equipment through ownership by an individual. If the direct value of clinical outcomes during the period of time examined is equal to the value of an individual animal, then the losses attributed to being diseased can be measured in terms of the future income that has been forgone because of the clinical effects of Q fever. The direct cost of Coxiellosis due to decreased productivity is therefore a summation of the decreasing value of outputs (losses) due to disease and the higher level of inputs required to achieve a given level of output (expenditures). Thus, reproductive losses, veterinary (including treatment and diagnostics) expenditures, and lost financial opportunity regarding meat, milk, hide, and live animals sales are considered.

The productivity of the Ontario dairy and meat goat industries is important as immigrant ethnic demand for goat products has continued to increase over the past decade (OMAFRA, 2011). Therefore, increasing profitability of the production system is desirable. Estimating the cost of Coxiellosis within a herd may provide necessary information for on-farm disease control prioritization and policy in Ontario. The cost of Coxiellosis may vary between production systems. In Ontario, 252 registered goat milk shippers with an average herd size of 152 animals on dairies have been reported. In comparison, an estimated 1915 meat producing goat farms are in the province with only 19.2 goats per farm on average. Following trends similar in other livestock sectors, although the number of herds continues to decrease, the total animal population for both production sectors has steadily rose over the past decade (OMAFRA, 2011). There are no reported data available on fibre
producing goats. Limited fiscal data is available to quantify the proportion of provincial agricultural gross domestic product attributed to the goat industry.

4.2 SIMULATING ECONOMIC MODELS STOCHASTICALLY

Estimates of model parameters in animal health and veterinary economics have been traditionally deterministic. However, many of these estimates are associated with a high degree of uncertainty. Sensitivity analysis has been suggested as a means to respond to the stochastic nature of economic output results. One variable is considered at a time in sensitivity analyses, thus ignoring combinations of measures of errors in different variables (Valle et al., 2005). When many variables are uncertain, sensitivity analysis on the effect on outcomes becomes cumbersome and a challenge to interpret. It has also been suggested that sensitivity analysis does not indicate the likelihood of a particular result being achieved (Hardaker et al., 2004).

Stochastic models offer a solution to the challenges presented with sensitivity analysis of multivariable estimates with uncertainty. This method accounts for some uncertainty in the evaluation and provides an indication of the distribution of outcomes. Thus, individual variables can be expressed with probability distributions and multiple combinations of variable values can be analyzed concurrently to provide a range of outcomes (Vose, 2000; Hardaker et al., 2004; Valle et al., 2005). Monte Carlo sampling techniques have been used to model variability in parameters due to lost production for other diseases of livestock (Roth et al., 2003; Schweizer et al, 2005). This method relies on the simulation of random processes using random numbers to decide whether or not a particular event occurs (Thrushfield, 1995; Vose, 2000). This method is used to estimate median values of a range of possible financial costs, and estimates the upper and lower bounds within which the true losses to the Ontario agricultural economy likely exist. The multifactorial effects of *C. burnetii* on sheep and goat production suggest that such an approach may be useful for the economic analysis of the effects of Q fever.

Economic information may provide important data in determining the priorities for future *C. burnetii* control options. There have been no attempts to quantify the financial losses due to *C. burnetii*, despite the within herd (1.5%) and
herd-level seroprevalence (20%) of exposure on Ontario sheep and goat farms as reported by Lang et al. (1991). A financial analysis of the associated losses would be justified and useful for future cost-benefit analyses of intervention strategies, and for suggesting the degree of investment necessary for further C. burnetii research.

5. STUDY RATIONALE

Outbreaks of Q fever in the Netherlands in small ruminants and subsequently humans led to increased consideration regarding the risks posed by C. burnetii as a zoonotic agent. In order to quickly mitigate further spread of disease, the public health authorities in the Netherlands mandated vaccination of dairy goats, monitoring of bulk tank milk every two weeks to detect C. burnetii-infected herds and monitor C. burnetii-negative herds, restricting sheep and goat movement, and a national culling program of all pregnant dairy goats or sheep on infected farms (van der Hoek et al., 2010; ECDC, 2010; Hogerwerf et al., 2011). These measures led to significant negative economic impacts (e.g. loss of production sales) on the Dutch small ruminant industry, and many suffered psychosocial stress similar to behavior observed during the national culling of livestock during the British foot-and-mouth outbreak in the 1990’s (Schimmer et al., 2008; Lahuerta et al., 2010; 2009; Post, 2010; van der Hoek et al., 2010).

Evidence-based decision-making in public health should carefully incorporate the best available scientific evidence through a transparent, validated, and documented method with considerations of values, perceived needs and resources in the given context (ECDC, 2010). Evidence-based public health control strategies should be sought to reduce disease risk to humans and shedding from small ruminants. There are limited robust quantitative evaluations published about animal-based control and prevention of spread between animals and from animals to humans. For almost all control options recommended, medium to high levels of uncertainty about the estimates of effectiveness were suggested (EFSA, 2010a). Current estimates of effectiveness are based on expert opinion derived from previous experience rather than rigorous and repeatable scientific approaches. In the absence of accurate assessments of available literature on C. burnetii interventions, it has not been possible to accurately estimate the effectiveness of
specific individual control options based on literature review as presented by EFSA (2010a).

6. STUDY OBJECTIVES

As C. burnetii remains an important contagious bacteria from small ruminants, spreading rapidly through a flock or herd in a naïve population, and represents a zoonotic risk, critical evidence-based interventions must be explored to reduce the risk of disease spread in populations. Research may also benefit from focusing on improving knowledge concerning the costs associated with Coxiellosis in goats. If knowledge on disease costs is available, this becomes an opportunity to target disease interventions based on cost effectiveness and evidence-based efficacy and effectiveness data.

The aim of this thesis is to describe the current body of literature, assess the bias in the published methodology, summarize, and synthesize where appropriate, current interventions in sheep, goats and high-risk occupationally exposed groups (Chapter 2 and 3). The efficacy and effectiveness of vaccination in high-risk occupationally exposed populations will be presented to provide a scientifically objective body of evidence for human C. burnetii vaccination policy (Chapter 2). The quantitative reduction in bacterial prevalence and load shed from routes of public health importance from small ruminant populations is described (Chapter 3). In conclusion, a financial analysis of Coxiellosis will be presented to describe the financial losses associated with variable levels of exposure to C. burnetii and subsequent clinical disease within Ontario dairy and meat goat herds (Chapter 4). It is the intention to provide sheep and goat producers with a comprehensive public health evidence base to direct C. burnetii policy and decision making in the province of Ontario.
REFERENCES


Cox HR, Bell EJ. The cultivation of Rickettsia diaporica in tissue culture and in the tissues of chicken embryos. Public Health Rep 1939;53;2270-6.


Crombie IK, Davies HTO. What is meta-analysis? London: Hayward Medical Communications, 2009. What is...? Series available at: www.whatisseries.co.uk


Ellis P. An economic evaluation of the swine fever eradication programme in Great Britain: Study no. 11, 1972. Reading: University of Reading.


Marrie TJ. Coxiella burnetii (Q fever) pneumonia. Clin Infect Dis 1995:21(S.3);5253-64.


Meslin FX. Public health impact of zoonoses and international approaches for their detection and containment. Veterinaria Italiana 2006:44(4);583-90.


Sadecky E, Brezina R. Vaccination of naturally infected ewes against Q fever. Act Virol 1977:21(1);89.


Stein A, Saunders NA, Taylor AG, Raoult D. Phylogenetic homogeneity of *Coxiella burnetii* strains as determined by 16S ribosomal RNS sequencing. FEMS Microbiol Lett 1993;113;339-44.


Tozer SJ, Lambert SB, Sloots TP, Nissen MD. Q fever seroprevalence in metropolitan samples is similar to rural/remote samples in Queensland, Australia. Eur J Clin Microbiol Infect Dis 2011:Epub 2011 Apr 16.


Young FQ. Q fever in Artesia, California. Calif Med 1948:69;89-90.

Zhang G, Samuel J. Vaccines against Coxiella infection. Expert Rev Vaccines 2004:3(5);577-84.
CHAPTER 2:
THE EFFECTIVENESS OF C. BURNETII VACCINES IN OCCUPATIONALLY EXPOSED POPULATIONS – A SYSTEMATIC REVIEW AND META-ANALYSIS

ABSTRACT
Methods of acute Q fever prevention in occupationally exposed populations have not been well evaluated. To estimate the effect of vaccination at preventing Q fever in individuals occupationally exposed to C. burnetii, a systematic review and meta-analysis was undertaken of controlled trials and observational studies. Relevant publications were obtained through a scoping study of English and non-English articles. Reviewing citations of included publications and searching bibliographies of recent literature reviews on Q fever identified additional publications. Publications reporting the use of a commercially licensed or licensable C. burnetii vaccine compared to an unvaccinated or placebo control group in occupationally exposed individuals were included in the review. Two authors using predefined tools performed independent assessment of risk of systematic error and data extraction. All pooled analyses were based on a random-effects meta-analysis. One controlled trial (n=200) and five cohort publications (n=11613) met the inclusion criteria. All trials used a Henzerling phase I vaccine. Significant heterogeneity among publications was observed. Univariate meta-regression of selected explanatory variables could not explain the statistical heterogeneity and may reflect the uncertainty provided by data reported in the cohort publications. Vaccination significantly prevented laboratory confirmed acute Q fever in abattoir workers responsible for livestock care, slaughter, or processing of meat (pooled RR, 0.07; 95% confidence interval [CI], 0.02 to 0.22) compared to the control individuals. In individuals with rare or sporadic contact with high-risk activities in the abattoir a significant benefit of vaccination was also found (pooled RR, 0.06; 95% CI, 0 to 0.93). Overall, the vaccine effectively prevented acute Q fever in individuals responsible for handling animals or their products and those working in the abattoir but not directly exposed to animals (overall pooled RR, 0.06; 95% CI 0.02-0.18). Caution
must be taken when interpreting the effect of *C. burnetii* vaccination as systematic biases were present in the small number of reviewed publications, and evidence included in this study may not be sufficiently robust to extrapolate the effect of vaccination to occupationally exposed groups beyond the population of abattoir employees in Australia where all included studies occurred.

1. INTRODUCTION

Q fever is an important, debilitating, and poorly understood zoonotic disease with an endemic global distribution, except in New Zealand (Guatteo et al., 2011). The small, facultative intracellular bacterium *Coxiella burnetii* is the necessary cause of acute and chronic Q fever (Maurin and Raoult, 1999). *C. burnetii* is highly infectious, with one organism capable of causing clinical infection (Madariaga et al., 2003). In susceptible human populations with direct exposure to infected ruminants or a contaminated environment, infection may lead to a severe, acute febrile illness (40% of exposed individuals) associated with significant direct financial burden and reduced quality of life (Babudieri, 1959; Hilbink et al., 1993; Marmion, 1996; Garner et al., 1997; Kermode et al., 2003). Despite a low estimated global disease burden (Marrie, 1988; Tissot-Dupont et al., 1992; Thomas et al., 1995) a re-emergence of public health concern has developed from recent sustained outbreaks observed in the Netherlands (Schimmer et al., 2008; ECDC, 2009; Post, 2010; Van der Hoek et al., 2010; Lahuerta et al., 2011).

The World Health Organization (WHO) has classified Q fever as an Occupational Zoonosis due to exposure to infected livestock or infected products of animal origin (WHO, 1979; Schwabe, 1984; Battelli et al., 2006; Battelli, 2009). Q fever occurs primarily in occupationally exposed groups such as workers from the livestock and meat industries. Sporadic cases are rare (Maurin and Raoult, 1999). Infection is primarily through inhalation of aerosolized contaminated particulate matter shed from infectious livestock, or through environmental contamination and subsequent aerosolization (Marrie, 1990; Fishbein and Raoult, 1992; Maurin and Raoult, 1999; Norlander, 2000; McQuiston and Childs, 2002). The bacterium is shed
from ruminants in fluids of parturition, milk, urine and feces, and can be spread from infected carcasses at the time of slaughter (Porter et al., 2011). Because *C. burnetii* is extremely resistant to desiccation, and to chemical and physical agents, it can survive in the environment for greater than 20 days (Babudieri, 1959; van Woerden et al., 2004; Kazar, 2005).

The acute clinical manifestations of Q fever are non-specific and flu-like (fever, pneumonia, headache, and general malaise) leading to misdiagnosis and underreporting. Only 60% of infected individuals develop acute clinical symptoms. Of those clinically affected in the Netherlands, 20% seek medical attention, with 2-3% being admitted to a hospital (Delsing and Kullberg, 2008). The development of chronic Q fever can occur up to 20 years after initial infection and may be characterized by a valvular endocarditis (Fenollar et al., 2004). Severe granulomatous hepatitis and osteomyelitis are rare outcomes (Snyder, 2003; Arricau-Bouvery and Rodolakis, 2005; Angelakis and Raoult, 2010). Mortality associated with Q fever is low (1-2%) in treated patients (Tissot-Dupont et al., 1992), but is higher in those affected with chronic form leading to *C. burnetii*-attributable death (65%) (Raoult et al., 2005).

Q fever is rarely a notifiable disease in few countries, making its true incidence in the human population difficult to estimate (Maurin and Raoult, 1999). Estimates of disease burden have to be extrapolated from outbreaks, serosurveys, and public health diagnostic laboratory data (Maurin and Raoult, 1999). Insufficient epidemiological data likely leads to underestimation of the global burden of disease compounded by tests with low sensitivity, and variable clinical signs in humans (Guo et al., 1998; Maurin and Raoult, 1999; Angelakis and Raoult, 2010). In Australia, it has been estimated that a Q fever case’s Quality Adjusted Life Years (QALY) is $6,294-7,984 (AUD) (Kermode, 2003).

High-risk occupationally exposed groups include those exposed to ruminants (veterinarians, farmers, stock-breeders, livestock truck drivers, wool shearers, slaughterhouse workers), medical and paramedical practitioners, and laboratory
technicians with *C. burnetii* exposure (Tissot-Dupont et al., 1999; Berri et al., 2003), making vaccination a potential method of clinical Q fever prevention (Maurin and Raoult, 1999; OIE, 2005). Angelakis and Raoult (2010) also propose the use of the vaccine in populations high-risk of developing chronic Q fever including immunocompromised persons, pregnant women and those with pre-existing vascular or cardiac valvular defects. Those individuals living in a community with a high Q fever prevalence in animal populations may also benefit from vaccination due to an ability for *C. burnetii* to become airborne and travel by wind up to distances of 5 kilometers (Tissot-Dupont et al., 2004). Upon introduction of targeted vaccination programs in Australia to abattoir employees and the greater rural population, Q fever notification rates have declined by 50% between 2002 and 2006 (Government of Australia, 2009).

Vaccines have been produced from either phase I or phase II antigens. A formalin-killed whole-cell vaccine prepared from phase I Henzerling strain of *C. burnetii* (Q-vax, CSL Group Biotherapies Limited, Parkville, Victoria, Australia) is licensed in Australia. Screening is required prior to vaccination to reduce the risk of vaccine-site reactions in previously exposed individuals (Bell et al., 1964(a); Marmion et al., 1990). Gefenaite et al. (2011) published a meta-analysis on *C. burnetii* vaccine effectiveness (VE) and included publications reporting serologic titers in addition to clinical outcomes. However, serology may not adequately reflect the true status of protection against acute Q fever (Healy et al., 2011) and may not be an appropriate end-point to evaluate protection against clinical disease. Recent studies on inactivated influenza vaccines have suggested that the use of serological outcomes to evaluate vaccine effectiveness may have biased in favour of overestimating the vaccine’s protective effect (Ohmit et al., 2006; Ohmit et al., 2008; Monto et al., 2008). Thus, only physician diagnosed clinical cases with or without laboratory confirmation was included in this review.

The objectives of this systematic review were to (i) identify comparative studies of licensed *C. burnetii* vaccines or potentially licensed formulations, (ii) assess the risk of bias (RoB) in reporting, and (iii) synthesize a pooled estimate of
risk ratio (RR) to estimate VE within and across studies in occupationally exposed populations. To assess the pooled effect of Q fever RR to prevent clinical disease, we performed a meta-analysis of observational and controlled trials that used clinical and diagnostic outcomes to confirm \( C.\ burnetii \) infection. This review was a component of a larger synthesis research study to determine evidence-based public health methods to prevent \( C.\ burnetii \) infection, spread and clinical disease at the human-animal interface.

2. METHODS

To assess the quality of available evidence and pool estimates of Q fever RR, we performed a scoping study, systematic review and meta-analysis of randomized controlled trials (RCT), controlled trials (CT) and observational studies that reported outcomes of clinically diagnosed Q fever symptoms, with or without laboratory confirmation in occupationally exposed populations (confirmed and suspected cases, respectively). Observational studies considered for inclusions were case-control (with test-negative controls), retrospective or prospective cohorts, and longitudinal studies. We defined Q fever VE as the relative reduction of clinical (confirmed or suspected) Q fever symptoms after vaccination. Methods of data extraction, analyses and inclusion criteria were specified \( a\ priori \) and documented in a protocol (Arksey and O’Malley, 2005; Cochrane Collaboration, 2011).

2.1 SEARCH STRATEGY

A scoping study was conducted to describe the quantity, scope, characteristics and knowledge gaps pertaining to primary research on strategies to prevent clinical disease in occupations exposed to Q fever, and captured human and livestock publications concurrently (Chapter 3). The review question was refined given the results of scoping. In consultation with an information scientist, a specific search strategy was developed that combined thesaurus terms for Q fever, occupations at-risk of Q fever exposure, and interventions (ECDC, 2010; EFSA, 2010)(Appendix 1).
An iterative pragmatic approach was adopted to ensure that all relevant quantitative studies examining Q fever interventions were captured. To identify primary intervention studies, grey literature, conference abstracts, and theses, published from January 1937 to the end of June 2011 in any language, we searched the following electronic databases available at the University of Guelph (Guelph, Canada): Agricola (from 1970), ISI Web of Knowledge (BIOSIS Previews Biological Abstracts, from 1969), CAB Direct (from 1990), Dissertation Abstracts and Theses, Medline (from 1993), PUBMED (from 1950), Web of Science, Scirus, Academic OneFile (from 1980), Biomed Central (from 1997), Cumulative Index to Nursing and Allied Health Literature (from 1981), Current Research @ (Dissertations and Theses, ProQuest Full Text), Environmental Sciences and Pollution Management (from 1967), Popline (from 1937), Health and Wellness Resource Centre, and Google Scholar (from 1937). Non-English abstracts were considered for relevance through the use of an online open-source statistical machine-translation tool (Google Translate, Google Inc.). Publication citations were imported into RefWorks-COS© (ProQuest, LLC, Cambridge Information Group) and de-duplicated. Subjects and indexes were removed prior to relevance screening.

2.2 RELEVANCE SCREENING

Two levels of relevance screening (RS) were applied. The purpose of the relevance screening was to rapidly remove non-primary literature and articles not relevant to the a priori research topic of Q fever interventions in human and livestock populations. Two unblinded reviewers (TJO and JMS) independently screened non-randomized titles and abstracts (RS-1), and full publications (RS-2) according to pre-tested RS tools (Appendix 2). The reproducibility and validity was tested in the initial phase of each RS (N=500, RS-1; N=20, RS-2). Cohen’s Kappa (κ) (Cohen, 1960) was estimated for agreement of study selection at each stage or screening, and RS tool modifications was made when necessary until adequate agreement was achieved (κ ≥0.6). Both reviewers applied RS inclusion criteria for the final decision regarding publication selection. Publications were only excluded if both reviewers responded “no”. In cases where the two reviewers disagreed,
discussions were held to resolve the disagreement until consensus was achieved (Arskey and O’Malley, 2005).

The first level of RS evaluated citations and abstracts in the reference database using the questions: (i) “Does the abstract or title describe published primary research or grey literature?” and (ii) “Does the abstract or title investigate the effect of a Q fever intervention in human or livestock populations?”. Potentially relevant publications were obtained in full-text format. The second level was applied to full publications: “Does the publication evaluate and report the effect of an intervention for C. burnetii in humans to change the probability of transmission, infection, or clinical symptoms?”. Relevant publications were categorized by intervention as a component of RS-2. The reviewers prioritized specific interventions for rigorous systematic review based on number of publications.

To identify additional potential publications, we reviewed the citations of articles passing RS-2, and hand-searched the reference lists of recent Q fever literature reviews (Raoult and Maurin, 1999; Angelakis and Raoult, 2010; Porter et al, 2011). These were retrieved and the same criteria for inclusion were applied. If non-peer reviewed publications such as theses or conference proceedings were identified, a first author search was conducted in the aforementioned databases with no language or date restrictions. Peer reviewed publications identified by the first author search were included in place of the non-peer reviewed material initially identified. We also contacted CSL Group-Biotherapies Limited, makers of the commercially available vaccine (Q-vax), informing them of the review and inviting them to offer additional unpublished data.

### 2.3 SYSTEMATIC REVIEW

The review question was refined from the quantity of available literature identified in the scoping study: “What is the effect of vaccination in occupationally exposed individuals with any commercially available Q fever vaccine or vaccine product given independently, in any dose, preparation, or time schedule, compared with a placebo, another vaccine, or an unvaccinated group, with any means of
exposure in any geographical location on the prevention of clinical acute Q fever (confirmed or suspect incident cases)?”. No \textit{a priori} case definition was defined for inclusion due to the limited number of available publications. To account for the total body of literature assessing Q fever vaccines, non-case outcomes, including antibody (IgG to phase I antigen, and IgM to phase II antigen) and lymphoproliferative assays were reported for descriptive purposes. In addition, non-commercially viable vaccine types were captured by the review, but were excluded (e.g. M-44). We included randomized and non-randomized study designs with defined control groups to enhance the relevance of our review to evidence-based public health decision-making.

\textbf{2.4 METHODOLOGICAL ASSESSMENT OF BIAS}

Two independent reviewers (TJO and JMS) performed risks of bias (RoB) assessment on all publications included in the SR. Reviewers were not masked to the title, author or journal of publication. The quality at the design and analysis stages as reported by the included publication’s authors were used as a means of interpretation of the validity of reported results. An aggregate quality score per publication was not reported. There is sufficient evidence for limited validity of summary scores in meta-analysis as means of assessing overall publication quality (Emerson et al., 1990; Greenland, 1994; Stroup et al., 2008).

Publications were reviewed for potential bias and strength of evidence provided by controlled trial (CT) publications according to the Cochrane Collaboration (Verhagen et al., 2001; Cochrane Collaboration, 2011). RoB was assessed in domains including: generation of the random allocation sequence of subjects, concealment of the allocation sequence, blinding of participants and outcome assessors, incomplete data outcomes including loss to follow-up, and selective reporting (Appendix 3). The Risk of Bias of Non-randomized Studies (RoBANS) tool, validated for observational study designs, was used to assess the included cohort publications (Park et al., 2011). Participant selection, confounders, measurement of intervention, blinding of outcome assessment, incomplete outcome
data, and selective outcome reporting were reviewed (Appendix 4). Confounders were selected according to recent reviews on Q fever risk factors (McQuiston and Childs, 2002; Angelakis and Raoult, 2010; Porter et al., 2011).

2.5 DATA EXTRACTION

We developed an electronic data extraction sheet, pre-tested it on all included studies because of the limited number of publications, and refined it accordingly by reviewer consensus. Two review authors (TJO and JMS) extracted the following data from included studies: study population (age, occupation(s), vaccination status, exposure status, number in the vaccinated and control groups), study environment (geographical location, study location(s)), study methods (design), intervention (formulation, dose, dosage, route of administration, follow-up length), control intervention (formulation, dose, dosage, route of administration), outcome(s) (type of outcome, measurement tool (diagnostic method(s)), timing and frequency of assessment, length of follow-up), and clinical outcome results (case definition, number of confirmed or suspect incident clinical Q fever cases in study and control populations). Raw data were extracted from CT and cohort publications as reported by the authors of the included publications.

2.6 META-ANALYSIS

We extracted reported individual-level clinical Q fever case data for control and vaccinated groups into an electronic spreadsheet. Single publications reporting multiple study locations were pooled if the same occupationally exposed groups were reported. We considered publications reporting multiple occupations or phases of vaccine implementation as unique data sets. This was to account for variable risks of different occupational Q fever exposures over time (Raoult et al., 2005; Whitney et al., 2009). Studies reporting clinical cases within the incubation period for Q fever were included in the case population to produce a more conservative VE estimate. Where confirmed and suspected Q fever cases were reported independently, we pooled the data to represent one category for all cases.
We estimated the crude risk ratio (RR) and standard error (SE) of developing clinical Q fever for enrolled vaccinated and unvaccinated participants. The RR is defined as the protective effect of the vaccine in the vaccinated individuals for preventing clinical disease (Halloran et al., 2010). The RR was assumed to be equivalent to the odds ratio (OR) of CT publications as Q fever cases were rare. Incidence risk differences (ID) and SE were estimated for publications reporting only ID at the population level (Rothman et al., 2008). When publications reported no clinical cases in a cell of the 2x2 table, a continuity correction of 0.5 was used to estimate the measure of association (Sweeting et al., 2004; Cochrane Collaboration, 2011). Publications were excluded from the pooled analysis if the measure of association or effect and SE could not be extracted or estimated. All analyses were performed in the statistical package Stata V11 (StataCorp., 2009).

Abattoir workers with direct exposure to infected ruminants are known to have a higher risk of \textit{C. burnetii} exposure compared to those not routinely exposed to livestock (Whitney et al., 2009; Battelli, 2009). We classified the study populations based on risk of occupational exposure to \textit{C. burnetii}. Abattoir workers were those individuals responsible for handling, slaughtering, or processing meat; and non-abattoir workers were employed by the abattoir, but not directly exposed to livestock.

Given the \textit{a priori} assumption that the between trial vaccination effect was not homogeneous, a DerSimonian and Laird (D-L) random effects meta-analysis stratified on occupational risk of exposure was performed (DerSimonian and Laird, 1986). A random-effects model is recommended to give the least biased results when a continuity correction is applied to sparse data (Sweeting et al., 2004). This method yielded a pooled effect size (ES) of vaccination using the natural logarithm (ln) of the generated measure of association (lnRR) and standard error (lnSE); that is, the relative effect of vaccination at preventing clinical Q fever between vaccinated and control groups (Harbord and Higgins, 2008; Halloran et al, 2010). Transformation to the logarithmic scale was used because the small sample size of studies precluded applicability of the central limit theorem (Bland and Altman
1996; Harbord and Higgins, 2008) to comply with the model assumptions of normally distributed measures of association, and to reduce dispersion of the data (Breslow and Day, 1987). Results for the pooled ES and confidence intervals were back-transformed to the risk scale (RR) and a summary ES was presented. Study ES were weighted in proportion to their total sample sizes to estimate the overall ES in each occupational category. This summary ES indicated the risk of a vaccinated individual being diagnosed (confirmed or suspected) with symptoms consistent with clinical Q fever compared with the risk of a non-vaccinated or placebo-treated individual being diagnosed.

The level of statistical heterogeneity between publications was quantified with the $I^2$ test statistic (Higgins et al., 2003). Due to the limited number of studies included in each occupational stratum, $I^2$ was estimated for each occupational subgroup and the summary ES measure (Song et al., 2001).

If results showed substantial heterogeneity ($I^2 > 60\%$), heterogeneity was explored with step-wise study exclusion. The sources of any unexplained variance were further explored with univariate random effects meta-regression; a weighted regression of the lnRR outcome variable. Explanatory variables considered were based on descriptive characteristics thought to be a source of variation that might influence the response of subjects to the effects of the vaccine (Lean et al., 2009; Borenstein et al., 2009). The potential explanatory variables were: (i) study design (CT or cohort study), (ii) publication year (1980-1989, 1990-1999, ≥2000), (iii) follow-up length (days), (iv) sample size, (v) population (abattoir employees, supporting firms, mechanics/electricians, sporadic contact), (vi) provision of a case definition (yes/no), and (vii) the number of locations included in the study.

The presence of study-level publication bias was assessed using funnel plots of SE plotted against RR. When the plot suggested asymmetry, statistical significance was tested by Egger's linear regression tests. Publication bias was considered to be present if the test statistic was significant ($P<0.1$)(Egger and Smith, 1999).
For studies included in the MA where RR was estimated, we reported the absolute effect of the vaccine against clinical Q fever and 95% CI expressed as a proportion of confirmed or suspected Q fever cases, using the formula:

\[
VE = 1 - \frac{(c_i / N_1)}{(c_o + 1)/(N_o + 1)} \\
95\% CI: \left[ 1 - e^{(\hat{\beta} + z\hat{\sigma})}, 1 - e^{(\hat{\beta} - z\hat{\sigma})} \right]
\]

where \( c \) represents the number of cases (\( c_i \)) or controls (\( c_o \)); \( N_1 \) is the sample size in the vaccinated group; \( N_o \) is the sample size in the control group; \( \hat{\beta} \) is the partial likelihood estimate of the lnRR in the meta-analysis; \( z \) is the (1-\( \alpha \)) of the standard normal distribution quantile; \( \hat{\sigma} \) is an estimate of the variance of \( \hat{\beta} \). This estimation accounts for the correction of bias when Q fever cases are rare in both the vaccinated and unvaccinated groups and assumes no loss to follow-up and no censoring of data (Katz et al., 1978; Halloran et al., 1998; Chick et al., 2001). We interpreted the effectiveness of the Q fever vaccine as “no effect” when \( VE < 0 \).

3. RESULTS

3.1 STUDY SELECTION AND DESCRIPTION OF INCLUDED PUBLICATIONS

The scoping study was conducted to determine the available literature for interventions for Q fever prevention and control in humans and livestock. In humans, we were concerned with literature regarding public health interventions that could prevent clinical Q fever in those exposed to \( C. burnetii \). Citations from 12,939 titles were captured by our initial database search (Figure 1). Twenty-four (0.19%) publications were identified as meeting the scoping study’s inclusion criteria for Q fever interventions in humans. Contact with the pharmaceutical company of a licensed Q fever vaccine did not yield any additional data.

All 24 publications reviewed for possible inclusion in the systematic review assessed Q fever vaccination (Table 1). No other interventions to prevent clinical Q fever were identified. All publications were reported in English. The vaccine
formulations identified included formalin-killed whole-cell (i.e. Q-vax), acellular extracted (i.e. chloroform methanol residue (CMR)), and attenuated live vaccines (i.e. M-44). Multiple outcomes were reported to evaluate the effect of a given vaccine in the study population. Antibody assays included complement fixation (CF) (n=12) (Meiklejohn and Lennette, 1950; Luoto et al., 1963; Vivona et al., 1963; Bell et al., 1964; Sterkhova, 1965; Genig and Gameleya, 1966; Morris et al., 1967; Kazar et al., 1981; Ascher et al., 1983; Worswick and Marmion, 1985; Izzo et al., 1988; Krutitskaya et al., 1996), immunofluorescence (IF) (n=3) (Ascher et al., 1983; Worswick and Marmion, 1985; Krutitskaya et al., 1996), microagglutination (MA) (n=5) (Luoto et al., 1963; Bell et al., 1964; Kazar et al., 1981; Kazar et al., 1982; Kazar et al., 1987) and ELISA (n=3) (Fries et al., 1993; Krutitskaya et al., 1996; Camacho et al., 2000;). However, there was limited consistency in assay preparation, sensitivity, specificity or cut-off interpretation. Whole blood evaluations of cell-mediated immunity (CMI) to C. burnetii were measure by in vitro proliferative responses of peripheral lymphocytes to multiple mitogens (n=5) (Ascher et al., 1983; Gajdosova and Brezina, 1988; Izzo et al., 1988; Fries et al., 1993; Waag et al., 2008). Delayed-type hypersensitivity was used as a proxy measure of CMI in one study (Kazar et al., 1987).

Vaccine trials without controls were excluded from the SR (n=15) (Meiklejohn and Linnette, 1950; Luto et al., 1963; Vivona et al., 1963; Sterkhova, 1965; Genig and Gameleya, 1966; Kazar et al., 1981; Kazar et al., 1982; Ascher et al., 1983; Worswick and Marmion, 1985; Kazar et al., 1987; Gajdozova and Brezina, 1988; Izzo et al., 1988; Camacho et al., 2000; Waag et al., 2008), as was an assessment of a non-relevant vaccine strain in a RCT (n=1) (Krutitskaya et al., 1996). Two CT publications were excluded because vaccine efficacy was assessed with serological outcomes not relevant to the review question (n=2) (Morris et al., 1967; Fries et al., 1993). Six publications were included in the systematic review with eleven unique vaccine-to-control comparisons (Table 2). All included studies were conducted in Australia from 1984 to 2009.
Adults were studied in all publications but one cohort study (Marmion et al., 1990) that involved occupationally exposed individuals but did not describe population demographics. All publications reported study participant occupations. Abattoir employees (n=6), sheep shearers (n=1), farmers and their families or employees (n=1), and abattoir support staff such as mechanics and electricians (n=1) were studied. All unexposed individuals were unvaccinated or placebo vaccinated subjects selected from the same population as those vaccinated. Shapiro et al. (1990) vaccinated unexposed individuals with an alternative vaccine. Two publications (Marmion et al., 1984; Marmion et al., 1990) evaluated similar populations, but it could not be determined if they were identical datasets based on the reporting in both publications. The publications were therefore assessed independently. Follow-up length varied from 6 months to 8 years.

A case definition and method of clinical diagnosis were reported by three publications (Ackland et al., 1994; Gilroy et al., 2001; Gidding et al., 2009)(Table 3). Shapiro et al. (1990) obtained cases actively from the attending physician, and state or private pathology laboratories, or retrospectively through a review of local and statewide communicable disease unit records linked to abattoir employees. Individuals with Q fever symptoms were confirmed by serologic assays (CF or IF) in all publications but one (Gilroy et al., 2001), who accepted cases as either laboratory confirmed or physician diagnosed suspect cases. Two publications did not report a method for case acquisition or specific case definition (Marmion et al., 1984; Marmion et al., 1990). Sufficient details for replication regarding implementation of the screening and vaccination components of the study were provided for all publications except for Gilroy et al. (2001).

All included publications used Henzerling phase I formalin inactivated \textit{C. burnetii} strains in their vaccines. Of those, four specifically reported the use of \textit{Q-vax} (Marmion et al., 1984; Shapiro et al., 1990; Ackland et al., 1994; Gidding et al., 2009). Preventative vaccination was assessed in all studies but one publication (Gilroy et al., 2001) that prospectively evaluated the effectiveness of vaccination during an outbreak. According to Gilroy et al. (2001), vaccination during an outbreak of Q
fever or ≥10 days post-exposure to an infectious dose of *C. burnetii* was significantly efficacious at protecting against confirmed clinical symptoms of Q fever compared to pre-exposure preventative vaccination (RR 0.005 (95%CI: 0.002 to 0.18))(Table 3). Raw data for the unvaccinated cohort could not be extracted for post-exposure vaccination effect and this publication was therefore excluded from the meta-analysis.

The Australian National Q fever Vaccination Programme was evaluated before-and-after implementation by Gidding et al. (2009). This program targeted occupationally exposed individuals. After the vaccination of abattoir workers (Phase I), the ID of Q fever notification cases and hospitalizations decreased by 0.65 per 100,000 compared to the ten years prior to the national vaccination campaign. The trend was repeated after the expansion of the program to Phase II compared to Phase I with a decrease of incident cases by 1.69 per 100,000. The ID was excluded from the meta-analysis and no VE was reported as the denominator for the exposed population could not be estimated or extracted.

### 3.2 METHODOLOGICAL ASSESSMENT OF BIAS

The risk of bias was assessed using the RoBANS tool in all publications but one (Shapiro et al., 1990) where the Cochrane RoB framework was used (Table 4). There was low risk of bias for all design categories for the RCT (Shapiro et al., 1990). Variable risk of bias across design categories in the cohort studies was found.

Variable risk of bias due sequence generation and allocation concealment was assessed (Table 4). The random generation of participant selection for either vaccination or control was either not reported or performed, and if reported, may have been performed inadequately. Similarly, few publications reported the use of a method to prevent selection bias by concealing the allocation sequence from those assigning participants to intervention groups until the moment of assignment.

All cohort studies except one (Gidding et al., 2009) were assigned a high risk of confounding, as they did not report control of potential confounders (age, sex,
duration of employment, and previous *C. burnetii* exposure). Selection bias may be present in these studies if participant selection, or inadequate confirmation or consideration of confounding variables were reported. All studies but one (Gidding et al., 2009) did not report blinding of those assessing the outcome of clinical Q fever. However, we could not assess whether blinding would reduce detection bias based on reported data. Therefore, the risk of bias was categorized as “unclear”. The risk of bias was considered low in Gidding et al. (2009) as clinical diagnosis was based on a retrospective review of objective hospital codes for Q fever paired with serological confirmation. The absence of blinding would likely not affect the outcome, and detection bias was unlikely. There was a high risk of bias assigned to Marmion (1984) due to incomplete outcome data, as a discrepancy of reported data between text and tables was identified. Similarly, Gilroy et al. (2001) reported limited demographic details over time leading to suspected incomplete reporting of the primary outcome of clinical Q fever in the study population.

### 3.3 META-ANALYSIS

A meta-analysis was conducted on publications reporting a formalin-killed whole-cell Henzerling phase I vaccine in occupationally exposed populations. Occupations were stratified according to occupational risk. Five comparisons were included in abattoir workers from five separate publications. One publication (Marmion et al., 1990) with three separate reported occupations contributed to the non-abattoir employee strata. The ES (95% CI) included all reviewed studies and data sets in Table 3 with the exception of post-exposure vaccination as presented by Gilroy et al. (2001) and ID reported by Gidding et al. (2009). The effective sample size was reduced in Ackland et al. (1994) as we excluded 298 unvaccinated individuals from the unvaccinated control group based on confirmation of previous exposure.

The pooled ES favoured the vaccine irrespective of occupation (Figure 2; Table 3). The risk of occupationally exposed individuals receiving the vaccine and developing clinical Q fever was significantly lower than the risk in occupationally
exposed individuals not receiving the vaccine or receiving a placebo (RR=0.06 (0.02 to 0.18)) with a VE of 92.1% (78.4 to 97.2%). The vaccine was significantly associated with a protective effect in abattoir employees reported in four publications, while in only two publications (Shapiro et al., 1990; Marmion et al., 1984) were the result not statistically significant. In the single publication reporting lower-risk occupations, two of the three study populations statistically significantly favoured vaccine effectiveness.

We detected statistically significant heterogeneity ($I^2=65.2\%, p=0.005$). No individual data set was identified to affect the statistical heterogeneity through step-wise exclusion. There were no significant predictors in the univariate meta-regression model to explain the heterogeneity ($p>0.05$). There were insufficient data to assess the population demographics that may have contributed to the observed statistical heterogeneity.

There was no visual suggestion from a funnel plot or statistically significant evidence of publication bias (Egger’s test, $p=0.697$). However, limited publications were included in the review to make this conclusion.

4. DISCUSSION

Our results from a meta-analysis of one randomized controlled trial and five cohort publications suggest that a Henzerling phase I strain vaccine significantly reduces clinical Q fever in occupationally exposed individuals. With variable systematic biases present and an inability to explain significant statistical heterogeneity in the small number of reviewed publications, caution must be taken when interpreting RR and the Q fever vaccination effectiveness. The vaccine may also protect unexposed individuals in the face of an outbreak based on a single study (Gilroy et al., 2001). However, the evidence included in this study is not sufficiently robust to extrapolate the effect of vaccination against Q fever to occupationally exposed groups beyond the population of abattoir employees in Australia.
Systematic reviews in zoonotic public health are an effective means of structurally and transparently addressing intervention questions using globally available primary literature sources (Waddell et al., 2009). Determining the quality of literature available for Q fever vaccination, in addition to the synthesis of its effect, is valuable when considering the long-term, evidence-based programs and policies resulting from the research. Evidence-based decision-making is best served with integration of all available evidence using meta-analytic methods (Stroup et al., 2000; Sargeant et al., 2007; Golder et al., 2011). A diverse range of study designs encompassing experimental and observational studies may potentially capture the true effect of vaccination and provide useful data for systematic review and meta-analysis.

In this study, both controlled trials and observational study designs were reviewed. Both types of study methodologies were included because, while providing different levels of evidence for VE, the two provide complimentary data on efficacy or effectiveness, and captured the total body of literature available to determine this effect (Weinberg and Szilagyi, 2010). No causal relationship can be inferred without controls, which lead to the exclusion of some experimental publications. The potential for error in data extraction in this review was minimized by concurrent extraction by two independent reviewers.

Controlled trials allow us to test the effect of a vaccination within a target population allocated to either a vaccinated or control group. A controlled trial represents the most reliable form of scientific evidence influencing healthcare interventions yet may lack external validity (Rothman et al., 2008). However, without appropriate randomization and allocation to vaccination, the controlled study design does not reduce the possibility of confounding between study groups. Follow-up time may be too limited to identify Q fever cases in controlled trials. Ethical considerations when exposing individuals to C. burnetii and withholding vaccination should be considered for vaccine study design.
The randomized controlled trial by Shapiro et al. (1990) was a phase IIb clinical proof-of-concept trial. It demonstrates the challenge associated with designing studies to determine the effect of the Q fever vaccine due to ethical considerations of an unvaccinated population in the face of exposure to *C. burnetii*. This type of controlled trial is designed to establish initial data on the safety of the vaccine in the target population and explore the relationship between the dose and desired activity, as measured by serological conversion to *C. burnetii*. Sequential analyses led to trial termination after 15 months when a one-sided 95% statistical significance (equivalent to a 2-sided 90% CI test) (Piaggio et al., 2008) was reached as evidence of the vaccine’s effect to prevent clinical disease. This non-inferior trial design pre-states the effect of the vaccine as being equally effective at preventing clinical Q fever as not vaccinating (Piaggio et al., 2008). The development of clinical disease is thus censored from reporting after the follow-up period ends. Although the authors acknowledge the ethical necessity for this methodology and the increased risk of a Type I error based on its design (erroneous acceptance of an inferior vaccine), the use of a 95% CI demonstrated that the vaccine that was not significantly different from controls at preventing clinical Q fever based on a 2-sided hypothesis test.

Observational studies are an accepted, yet controversial, alternative to investigate the effect of vaccination using meta-analytic methods (Blettner et al., 1999). Controversy around their inclusion in meta-analyses comes from the potential biases, relative to bias present in RCTs. This can make the estimation of a single summary ES potentially misleading and its interpretation problematic (Stroup et al., 2000). The cohort study design allows for the vaccination of eligible subjects based on lack of previous *C. burnetii* exposure, rather than through a pre-specified allocation method. Owing to the lack of randomization, cohort studies are afflicted by an increased risk of bias (particularly confounding) and may therefore be a weaker study design for establishing causation (true effect of vaccine protection against clinical disease). Nevertheless, observational study designs are the primary source of data currently available for Q fever vaccines in occupationally
exposed populations. It has also been argued that confounding is less likely to occur when an outcome is unintended (e.g. development of Q fever in the vaccinated group), and therefore does not influence the decision to vaccinate or not vaccinate (Halloran et al., 2010). Thus, any difference in risk of clinical Q fever may be due to inherent differences in the population being vaccinated or the rare outcome of clinical Q fever, and not the vaccine itself.

In cohort studies, there is a threat to the validity of the results if residual confounding arises from a possible confounding variable cannot be measured with sufficient precision or when it is unaccounted (Sackett, 1979). The inclusion of observational study designs in a MA can lead to increased bias due to unaccounted for confounding. Statistical power of a study does not indicate bias, or the presence or absence of confounding. Therefore, poor quality methods, analyses, or reporting of observational studies may lead to a conclusion of an ineffective vaccine regardless of statistical significance. Spurious associations may lead to results due to bias rather than a true association between protection against clinical Q fever due to vaccination of seronegative individuals and exposure to $C.\ burnetii$.

The analysis agrees with the conclusions of previous reviews of Q fever vaccination. However, our systematic methods of documented inclusion criteria to minimize bias and use of confirmed and suspect clinical cases as the primary outcome resulted in a more conservative estimate of the VE. A review by Chiu and Durrheim (2007) examined the efficacy of registered human Q fever vaccines in Australia. Although our findings of an effect of vaccination are similar to their estimates, they provided some summary of quantitative outcomes; there was no defined systematic review or meta-analysis.

A recent meta-analysis of Q fever vaccines reported protective effects on definitive and presumptive Q fever in abattoir workers and healthy adult men (Gefenaite et al., 2010). The findings in this study agreed with that of Gefenaite et al. (2010) that vaccination was associated with a reduced risk of clinical disease in vaccinated individuals occupationally exposed to Q fever. However, they included
publications reporting serologic titers in addition to clinical outcomes to assess the effect of the vaccine and excluded clinical cases if disease was reported within 14 days post-immunization. Individuals in publications reporting serologic outcomes were classified by vaccination status and followed up to measure an immune response. However, of those exposed to *C. burnetii*, only 60% will seroconvert (Arricau-Bouvery and Rodolakis, 2005) thereby underestimating the true incidence of exposure. There are no publications to our knowledge that identify vaccine-induced immune response as a predictor of protection against clinical disease after challenge or natural Q fever exposure. Therefore, serology may not adequately reflect or be an effective surrogate for the true status of protection against acute or chronic Q fever infection (Healy et al., 2011). In addition, post-exposure incubation in acute Q fever is estimated at approximately 20 days but ranges from 12 to 39 days (Young, 1948; Spelman, 1982; Lopez et al., 1986; Angelakis and Raoult, 2010; Porter et al., 2012) depending on inoculating dose (Maurin and Raoult, 1999). Marmion et al. (1990) argued that individuals infected prior to vaccination would bias against the true effect of vaccination leading Gefenaite et al. (2010) to include those subjects vaccinated and developing clinical Q fever within 14 days post-immunization as non-cases. The inclusion of serologic outcomes and exclusion of clinical cases may have biased the VE away from the null of no effect and explain their point estimate of 97% (95% CI: 94 to 99%). In addition, the authors did not report an assessment of the potential for systematic error in the reviewed publications.

This systematic review and meta-analysis had a number of limitations. There was considerable heterogeneity between studies, suggesting that differences may be specific to a particular population. The $F$ statistic describes the percentage of total variation across studies that is due to heterogeneity rather than chance alone, and is based on an iterative non-central chi-squared distribution method (Hedges and Pigott, 2001). Statistical heterogeneity may either arise from systematic differences between studies or random differences between effect sizes, or both. The test is considered unstable with a low number of studies as the case here, due to the
limited statistical power leading to wide confidence intervals (Paul and Donner, 1992; Ioannidis et al., 2007). In addition, individual studies with few participants or few cases of Q fever have low statistical power and precision to detect a difference between groups if one truly exists (Borenstein et al., 2009). The observational nature of the evidence from included publications may have also contributed to the significant heterogeneity across studies. With a greater risk of bias, observational studies may show a bias from the null, and greater heterogeneity (DerSimonian and Laird, 1986). This has been observed in literature reporting influenza vaccination, where cohort studies have shown large reductions in morbidity, in contrast to the results from randomized controlled trials (Jefferson et al., 2005a; Jefferson et al., 2005b).

Reported demographic differences between studies could not explain the extensive heterogeneity and as a result, interpretation of estimates of effect of vaccination that are based on a few cases with wide CI is difficult. The random effects ES may accurately capture the true effect in occupationally exposed populations, but the small number of studies limited our power to detect a contribution of any one individual study to the overall heterogeneity.

Significant statistical heterogeneity was not unexpected as the studies occurred at multiple locations over time with possibly different population demographics. Given our inability to identify significant explanatory variables as an external cause for heterogeneity, it is possible that the vaccine itself is heterogeneous. The efficacy may be different for each individual study subject, and may be protective at the group-level but not within a group. We reported the effect size results with high statistical heterogeneity to demonstrate the gaps in knowledge and limited reporting in studies of Q fever vaccines as a directive for policy, decision-making, and future research.

Empirical evidence and theoretical consideration support the notion that trials of higher methodological quality will provide results closer to the true effect than lower quality trials (Cochrane Collaboration, 2011). The observed
heterogeneity could be explained by selection bias, if individuals in the study population were at a lower risk of clinical Q fever than those not included in the study and if the extent to which selection bias occurred varied among studies. Behavioural differences have been observed in individuals with Q fever; individuals who are sero-negative when acutely sick are unlikely to re-test when they are healthy once again. This behavior increases when individuals live in rural areas, as demonstrated in Western Australia (Mak et al., 2003). It is therefore possible that individuals with a history of Q fever are less likely to be included in the sampling frame, biasing the effect of vaccination towards from the null, and overestimating the effect of vaccination. Infected people may have been included in the meta-analysis despite the inclusion of only seronegative individuals since the use of acute and convalescent serology is often non-contributory in early disease stages due to the absence of antibodies (Fournier and Raoult, 2003).

There were significantly fewer males, mean age was lower, and length of service in the abattoir was shorter, as was the length of exposure to livestock (in and out of the abattoir) in vaccinated volunteers as compared with the unvaccinated cohort as reported by included publications (Marmion et al., 1984; Marmion et al., 1990; Ackland et al., 1994). Individuals with a shorter length of service in the abattoir were less likely to be immune, and therefore may have been overrepresented in the vaccinated group. Unvaccinated subjects not included in the meta-analysis were more likely to have a past history of Q fever, and worked in higher risk areas of the plant (i.e. slaughtering and processing of animal products)(Marmion et al., 1984; Marmion et al., 1990). Younger, newly employed individuals were vaccinated due to their initial seronegative status, yet would have experienced a higher incidence of clinical Q fever if unvaccinated. This may have biased away from the null, and towards a protective effect.

The observed heterogeneity may be due to an unmeasured underlying risk of developing Q fever; a risk that differs amongst vaccinated and control individuals. Higher risk individuals (seronegative individuals) in high-risk occupations within the abattoir were more likely to benefit from Q fever vaccination than those in lower
risk jobs, in whom vaccination may not have any additional protective effect. However, this interpretation may be attributed to a regression dilution bias (Bland and Altman, 1994a; Bland and Altman, 1994b); possibly due to variable risk of bias assessment. There was insufficient reported data to explore or correct for the possible structural relationship. Therefore, VE may be interpreted as having an artificially negative relationship between the observed risks in the vaccinated group compared to the unvaccinated cohort biasing towards the null.

Underlying risk of Q fever exposure leading to clinical disease as a cause of heterogeneity may have been more effectively explored if infection prevalence was known in the slaughtered animals or environment. Of the included publications, only Gilroy et al. (2001) reported that cattle were the only species slaughtered in the abattoir, but did not report the presence or absence of infection or shedding of those animals entering the facility. Inconsistent clinical case definitions and variable follow-up times across publications may have also contributed to observed variability in vaccine performance across publications.

A constraint of our review is that we included information as reported by the authors. We did not attempt to source additional, potentially missing, or discrepant data. For instance, we relied on the authors’ case definitions of clinical Q fever despite discrepancies of reported case definitions amongst publications. All case definitions including suspect cases we included to capture the current scope of evidence reported in the studies.

The phase I vaccine evaluated in this review (Q-vax) can only be used safely in seronegative individuals due to risk of sterile injection site sarcomas (Ascher et al., 1983; Marmion et al., 1990). Alternative vaccines should be investigated further for both effect of protection against Q fever as well as safety for those previously exposed. CMR vaccines have been proven safe and as effective as Q-Vax in cynomolgus monkeys challenged by aerosol C. burnetii (Waag et al., 2003). The use of this vaccine may also preclude the necessity to screen individuals for prior immunity, leading to compelling reasons to adapt CMR as the next generation of Q
fever vaccines. It may be of utility in low-income or developing countries where budgets for screening are limited, despite the high burden of disease. Like any vaccine, it should induce long lasting humoral and cell-mediated immune responses that can activate quickly with the production of IFN-γ upon exposure to *C. burnetii* (Waag, 2007). However, no publications evaluating this response met our inclusion criteria.

Overall, our review and meta-analysis provided valuable evidence of a statistically significant protective effect of vaccination to prevent clinical Q fever in those occupationally exposed to *C. burnetii*. A logical next-step would be the validation of an individual or population-based correlate of vaccine protection that accurately predicts protection against clinical disease. Otherwise, those diagnostics currently used to diagnose infection (e.g. immunoglobulins) should be validated for protection. The benefits of trying to understand why differences in the effect of vaccination occurred across studies and populations should also be investigated further. Advantages to the use of experimental trials without the use of unvaccinated controls may subvert the ethical considerations with Q fever vaccine trials (e.g. unvaccinated and exposed individuals). However, a large double blind, multi-centre randomized controlled trial may better help to establish the clinical effect of vaccination amongst occupationally exposed groups. This is of particular interest to Veterinary professionals, ruminant producers, and abattoir employees who are viewed to have a higher risk of zoonotic disease (Battelli, 2009; Baker and Gray, 2009). Exploring further demographic data in the study population may also explain heterogeneity more clearly.

**ACKNOWLEDGEMENTS**

The authors acknowledge the assistance of Devin Vriezen and Caleigh Machlachlan for assistance with publication acquisition; Shannon Meadows (Ph.D. candidate, Department of Population Medicine, Ontario Veterinary College) and Dr. Paula Menzies D.V.M., M.P.V.M., Dip. ECSRHM (Associate Professor, Small Ruminant Research Co-ordinator, Ruminant Health Management Group, Ontario Veterinary
College) for Q fever biology consultations; Dr. Lee Weisner D.V.M., M.Sc. (Veterinary Epidemiologist, Centre for Public Health and Zoonoses, Department of Population Medicine, Ontario Veterinary College) for guidance in systematic review and meta-analysis methods; Jane Burpee (Librarian, University of Guelph) for assistance with scoping study thesaurus and database. Editorial recomendations from Drs. Andria Jones-Bitton D.V.M., M.Sc., Ph.D. (Assistant Professor, Department of Population Medicine, Ontario Veterinary College) and Olaf Berke M.Sc., Ph.D. (Associate Professor of Statistical Epidemiology, Department of Population Medicine, Ontario Veterinary College) was greatly appreciated.

FUNDING

A Canadian Institute of Health Research Institute of Population and Public Health/Public Health Agency of Canada Applied Public Health Research Chair supported Dr. Jan M. Sargeant D.V.M., M.Sc., Ph.D.

Dr. Tyler J. O’Neill B.Sc., D.V.M., M.Sc. was a Graduate Veterinary Fellow funded by the Ontario Veterinary College Graduate Fellowship, developed for Canadian or permanent resident veterinarians to pursue graduate research training at the University of Guelph.
REFERENCES


Battelli G. Zoonoses as occupational diseases. Veterinaria Italiana 2009:42(4);369-79.


Bell JF, Lackman DB, Meis A, Hadlow WJ. Recurrent reaction at site of Q fever vaccination in a sensitized person. Mil Med 1964(a);7;591-5.


Bland JM, Altman DG. Regression towards the mean. BMJ 1994(a):308;1499.

Bland JM, Altman DG. Some examples of regression towards the mean. BMJ 1994(b):309;188.


Camacho MT, Outschoorn I, Kovacova E, Tellez A. Distribution of immunoglobulin G (IgG) and A (IgA) subclasses following Q fever vaccination with soluble phase I Coxiella burnetii extract. Vaccine 2000:18;1773-77.


European Food Safety Authority (EFSA). Application of Systematic Review Methodology to Food and Feed Safety Assessments to Support Decision Making. EFSA J 2010:8(6);1637-1727.


Golder S, Loke YK, Bland M. Meta-analyses of adverse effects data derived from randomized controlled trial as compared to observational studies: methodological overview. PLoS Med 2011:8(5); e1001026. doi:10.1371/journal.pmed.1001026


Hedges LV, Pigott TD. The power of statistical tests in meta-analysis. Psych Methods 2001;6;203-17.


Ioannidis J, Patsopoulos N, Evangelou E. Uncertainty in heterogeneity estimates in meta-analyses. BMJ 2007;335(7626);914-6.


Sargeant JM, Amezcu M, Rajic A, Waddell L. Pre-harvest interventions to reduce the shedding of E.coli O157 in the faeces of weaned domestic ruminants: a systematic review. Zoonoses Pub Health 2007:54(6-7);260-77.


StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP.


Song F. Exploring heterogeneity in meta-analysis: Is the L’abbe plot useful?. J Clin Epidemiol 1999:52(8);725-30.


Verhagen AP, de Vet HCW, de Bie RA, Boers M, van den Brandt, PA. The art of quality assessment of RCTs include din systematic reviews. J Clin Epidemiol 2001:54(7);651-54.

Waag DM, England MJ, Rammariello RF, Byrne WR, Gibbs P, Banfield CM, Pitt MLM. Comparative efficacy and immunogenicity of Q fever chloroform:methanol residue (CMR) and phase I cellular (Q-vax) vaccines in cynomolgus monkeys challenged by aerosol. Vaccine 2002;20(19-20);2623-34.

Waag DM. *Coxiella burnetii*: Host and bacterial responses to infection. Vaccine 2007;25;7288-95.


Waddell L, Rajic A, Sargeant JM, Parker J, Deckert A, McEwen S. The methodological soundness of literature reviews addressing three potential zoonotic public health issues. Zoonoses Pub Health 2009:56(9-10);477-89.


Young FQ. Q fever in Artesia, California. Calif Med 1948;69;89-90.
<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Vaccine type</th>
<th>Control</th>
<th>Outcome(s) studied</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uncontrolled trial</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camacho et al. (2000)</td>
<td>Slovakia</td>
<td>Acellular extracted</td>
<td>-</td>
<td>Antibody assay</td>
</tr>
<tr>
<td>Luoto et al. (1963)</td>
<td>Czechoslovakia</td>
<td>Acellular extracted</td>
<td>-</td>
<td>Antibody assay</td>
</tr>
<tr>
<td>Kazar et al. (1987)</td>
<td>Czechoslovakia</td>
<td>Acellular extracted</td>
<td>-</td>
<td>Antibody assay</td>
</tr>
<tr>
<td>Kazar et al. (1982)</td>
<td>Czechoslovakia</td>
<td>Acellular extracted</td>
<td>-</td>
<td>Antibody assay</td>
</tr>
<tr>
<td>Kazar et al. (1981)</td>
<td>Czechoslovakia</td>
<td>Acellular extracted</td>
<td>-</td>
<td>Antibody assay</td>
</tr>
<tr>
<td>Waag et al. (2008)</td>
<td>U.S.A.</td>
<td>Killed whole-cell vaccine</td>
<td>-</td>
<td>T-cell assay</td>
</tr>
<tr>
<td>Meiklejohn and Lennette (1950)</td>
<td>U.S.A.</td>
<td>Killed whole-cell vaccine</td>
<td>-</td>
<td>Antibody assay</td>
</tr>
<tr>
<td>Ascher et al. (1983)</td>
<td>U.S.A.</td>
<td>Killed whole-cell vaccine</td>
<td>-</td>
<td>Antibody assay, T-cell assay</td>
</tr>
<tr>
<td>Izzo et al. (1988)</td>
<td>Australia</td>
<td>Killed whole-cell vaccine</td>
<td>-</td>
<td>Antibody assay, T-cell assay</td>
</tr>
<tr>
<td>Bell et al. (1964)</td>
<td>U.S.A.</td>
<td>Killed whole-cell vaccine</td>
<td>-</td>
<td>Antibody assay</td>
</tr>
<tr>
<td>Worswick and Marmion (1985)</td>
<td>Australia</td>
<td>Killed whole-cell vaccine</td>
<td>-</td>
<td>Antibody assay</td>
</tr>
<tr>
<td>Vivona et al. (1964)</td>
<td>U.S.A.</td>
<td>Killed whole-cell vaccine</td>
<td>-</td>
<td>Antibody assay</td>
</tr>
<tr>
<td><strong>RCT/CT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morris et al. (1967)</td>
<td>U.S.A.</td>
<td>Killed whole-cell vaccine</td>
<td>Placebo</td>
<td>Antibody assay</td>
</tr>
<tr>
<td>Fries et al. (1993)</td>
<td>U.S.A.</td>
<td>Acellular extracted</td>
<td>Placebo</td>
<td>Antibody assay, T-cell assay</td>
</tr>
<tr>
<td>Shapiro et al. (1990)</td>
<td>Australia</td>
<td>Killed whole-cell vaccine</td>
<td>Placebo</td>
<td>Clinical cases</td>
</tr>
<tr>
<td>Krutitskaya et al. (1996)</td>
<td>Russia</td>
<td>Killed whole-cell vaccine*</td>
<td>Placebo</td>
<td>Antibody assay</td>
</tr>
<tr>
<td><strong>Cohort study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ackland et al. (1994)</td>
<td>Australia</td>
<td>Killed whole-cell vaccine</td>
<td>Placebo</td>
<td>Clinical cases</td>
</tr>
<tr>
<td>Marmion et al. (1984)</td>
<td>Australia</td>
<td>Killed whole-cell vaccine</td>
<td>Concurrent control</td>
<td>Clinical cases</td>
</tr>
<tr>
<td>Marmion et al. (1990)</td>
<td>Australia</td>
<td>Killed whole-cell vaccine</td>
<td>Concurrent control</td>
<td>Clinical cases</td>
</tr>
<tr>
<td>Gidding et al. (2009)</td>
<td>Australia</td>
<td>Killed whole-cell vaccine</td>
<td>Concurrent control</td>
<td>Clinical cases</td>
</tr>
<tr>
<td>Gilroy et al. (2001)</td>
<td>Australia</td>
<td>Killed whole-cell vaccine</td>
<td>Concurrent control</td>
<td>Clinical cases</td>
</tr>
</tbody>
</table>

*Whole-cell formalin inactivated vaccine (phase I Luga strain)
Table 2. Publications included in the systematic review of *C. burnetii* vaccines in occupationally exposed populations to prevent clinical Q fever.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population*</th>
<th>Occupation*</th>
<th>Vaccine description</th>
<th>Control description</th>
<th>Follow-up length</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shapiro et al. (1990)</td>
<td>Adults</td>
<td>Abattoir employees</td>
<td>Henzerling phase I strain (Q-vax) [30μg, SQ, 1 dose]</td>
<td>Influenza A vaccine 0.5mL, SQ, 1 dose</td>
<td>15 months</td>
</tr>
<tr>
<td>Cohort study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ackland et al. (1994)</td>
<td>Mean age: 27.7</td>
<td>Abattoir employees</td>
<td>Henzerling phase I strain (Q-vax) [30μg, SQ, 1 dose]</td>
<td>Unvaccinated employees</td>
<td>5 years</td>
</tr>
<tr>
<td>Marmion et al. (1984)</td>
<td>Adults</td>
<td>Abattoir employees</td>
<td>Henzerling phase I strain [30μg, SQ, 1 dose]</td>
<td>Unvaccinated employees</td>
<td>18 months</td>
</tr>
<tr>
<td>Marmion et al. (1990)</td>
<td>Not reported</td>
<td>Abattoir employees Supporting firms on-site Mechanics/Electricians Sporadic abattoir visits</td>
<td>Henzerling phase I strain (Q-vax) [30μg, SQ, 1 dose]</td>
<td>Unvaccinated employees</td>
<td>8 years</td>
</tr>
<tr>
<td>Gidding et al. (2009)</td>
<td>Adults Male and female</td>
<td>Abattoir employees Sheep shearers Sheep and cattle farmers, employees or families</td>
<td>Henzerling phase I strain (Q-vax) [30μg, SQ, 1 dose]</td>
<td>Unvaccinated employees</td>
<td>Phase I: 4 years Phase II: 3 years</td>
</tr>
<tr>
<td>Gilroy et al. (2001)</td>
<td>Median age: 33.5</td>
<td>Abattoir employees</td>
<td>Henzerling phase I strain [30μg, SQ, 1 dose]</td>
<td>Unvaccinated employees</td>
<td>6 months</td>
</tr>
</tbody>
</table>

*As reported in the publication by authors.
Table 3. Outcomes of RCT and cohort studies reporting confirmed or suspected cases of Q fever in included publications.

<table>
<thead>
<tr>
<th>Study</th>
<th>Case acquisition and definition</th>
<th>Level of observation (cases/group)</th>
<th>Measure of Association (95% CI)</th>
<th>VE (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RCT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shapiro et al. (1990)</td>
<td>Active surveillance of physician diagnosed and laboratory confirmed cases (phase I and II CF*)</td>
<td>Abattoir employees (N&lt;sub&gt;1&lt;/sub&gt;=0/98; N&lt;sub&gt;0&lt;/sub&gt;=7/102)^</td>
<td>RR 0.07 (0.004-1.33)</td>
<td>92.1% (-37 to 100%)</td>
</tr>
<tr>
<td><strong>Cohort study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ackland et al. (1994)</td>
<td>Retrospective abattoir and statewide physician-reported and laboratory confirmed disease records [Case definition: Q fever clinical symptoms (Marrie, 1990) and serologically laboratory confirmed]</td>
<td>Abattoir employees (N&lt;sub&gt;1&lt;/sub&gt;=2/2553; N&lt;sub&gt;0&lt;/sub&gt;=55/1067)</td>
<td>RR 0.02 (0.005-0.08)</td>
<td>95.9% (93.6 to 97.6%)</td>
</tr>
<tr>
<td>Marmion et al. (1984)</td>
<td>Case definition not provided</td>
<td>Abattoir employees (N&lt;sub&gt;1&lt;/sub&gt;=4/934; N&lt;sub&gt;0&lt;/sub&gt;=13/998)</td>
<td>RR 0.32 (0.108-1.005)</td>
<td>67.4% (0 to 89.7%)</td>
</tr>
<tr>
<td>Marmion et al. (1990)</td>
<td>Case definition not provided</td>
<td>Abattoir employees (N&lt;sub&gt;1&lt;/sub&gt;=3/2713; N&lt;sub&gt;0&lt;/sub&gt;=52/1960) Supporting firms on-site (N&lt;sub&gt;1&lt;/sub&gt;=3/269; N&lt;sub&gt;0&lt;/sub&gt;=24/140) Mechanics/Electricians (N&lt;sub&gt;1&lt;/sub&gt;=1/3; N&lt;sub&gt;0&lt;/sub&gt;=2/5) Sporadic abattoir visits (N&lt;sub&gt;1&lt;/sub&gt;=0/524; N&lt;sub&gt;0&lt;/sub&gt;=19/48)</td>
<td>RR 0.04 (0.01-0.14)</td>
<td>95.8% (94.1 to 97.5%)</td>
</tr>
<tr>
<td>Gidding et al. (2009)</td>
<td>Retrospective data extracted from National Notifiable Disease Surveillance System (NNDSS) and Australian Institute of Health and Welfare Hospital Morbidity Database [Case definitions: phase II seroconversion (years: &lt;2004), detection of organism by culture or nucleic acid detection (years: ≥2004); Discharge codes: ICD-9 code 083.0 (years: 1993-1998), and ICD-10-AM code A78 (years: ≥1999)]</td>
<td>Phase I: abattoir employees Phase II: all high risk occupationally exposed populations</td>
<td>RD -0.65 per 100,000</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Gilroy et al. (2001)</td>
<td>Active surveillance during outbreak of physician diagnosed and laboratory confirmed cases [Case definition: Acute primary Q fever clinical symptoms and serological laboratory confirmed case (CF or IF)]</td>
<td>Abattoir employees Pre-exposure (N&lt;sub&gt;1&lt;/sub&gt;=0/19; N&lt;sub&gt;0&lt;/sub&gt;=37/68) Post-exposure</td>
<td>RR 0.005 (0.002-0.18)</td>
<td>95.2% (94.8 to 95.5%)</td>
</tr>
</tbody>
</table>

*Complement fixation (CF); ^Clinical cases reported in the vaccinated group (N<sub>1</sub>); Clinical cases reported in the control group (N<sub>0</sub>)
Table 4. Methodological quality assessment (risk of bias) of publications included in the systematic review of the effectiveness of Q fever vaccines in occupationally exposed populations to prevent clinical disease.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sequence generation</th>
<th>Allocation concealment</th>
<th>Patient blinded</th>
<th>Outcome assessor blinded</th>
<th>Incomplete outcome data addressed</th>
<th>Selective reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RCT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shapiro et al. (1990)</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td><strong>Cohort</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ackland et al. (1994)</td>
<td>High risk</td>
<td>High risk</td>
<td>Low risk</td>
<td>Unclear</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td>Marmion et al. (1984)</td>
<td>Unclear</td>
<td>High risk</td>
<td>Low risk</td>
<td>Unclear</td>
<td>High risk</td>
<td>High risk</td>
</tr>
<tr>
<td>Marmion et al. (1990)</td>
<td>Low risk</td>
<td>High risk</td>
<td>Low risk</td>
<td>Unclear</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td>Gidding et al. (2009)</td>
<td>High risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td>Gilroy et al. (2001)</td>
<td>High risk</td>
<td>High risk</td>
<td>Low risk</td>
<td>Unclear</td>
<td>Low risk</td>
<td>High risk</td>
</tr>
</tbody>
</table>

Figure 1. Flow diagram of the scoping study search and identified publications included in the systematic review of vaccination
to prevent clinical Q fever in occupationally exposed populations.

Figure 2. Forest plot for publications included in the meta-analysis evaluating Q fever vaccines effectiveness to prevent
clinical Q fever in occupationally exposed populations. The subtotal effect size (ES) for each occupation and overall ES represents the log of the risk ratio (RR), back-transformed and expressed as a RR.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Occupation</th>
<th>RR [ ES (95% CI) ]</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abattoir</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shapiro</td>
<td>1990</td>
<td>Abattoir employees</td>
<td>0.07 (0.00, 1.33)</td>
<td>8.23</td>
</tr>
<tr>
<td>Ackland</td>
<td>1994</td>
<td>Abattoir employees</td>
<td>0.02 (0.00, 0.08)</td>
<td>15.58</td>
</tr>
<tr>
<td>Marmion</td>
<td>1984</td>
<td>Abattoir employees</td>
<td>0.32 (0.11, 1.00)</td>
<td>17.38</td>
</tr>
<tr>
<td>Marmion</td>
<td>1990</td>
<td>Abattoir employees</td>
<td>0.04 (0.01, 0.14)</td>
<td>17.12</td>
</tr>
<tr>
<td>Girroy</td>
<td>2001</td>
<td>Abattoir employees</td>
<td>0.05 (0.00, 0.84)</td>
<td>8.35</td>
</tr>
<tr>
<td>Subtotal</td>
<td></td>
<td>(I-squared = 63.7%, p = 0.026)</td>
<td>0.07 (0.02, 0.22)</td>
<td>66.66</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Occupation</th>
<th>RR [ ES (95% CI) ]</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-abattoir</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marmion</td>
<td>1990</td>
<td>Supporting firm on-site</td>
<td>0.07 (0.02, 0.22)</td>
<td>16.78</td>
</tr>
<tr>
<td>Marmion</td>
<td>1990</td>
<td>Mechanics/Electricians</td>
<td>1.17 (0.06, 21.35)</td>
<td>8.14</td>
</tr>
<tr>
<td>Marmion</td>
<td>1990</td>
<td>Sporadic contact</td>
<td>0.00 (0.00, 0.04)</td>
<td>8.41</td>
</tr>
<tr>
<td>Subtotal</td>
<td></td>
<td>(I-squared = 77.7%, p = 0.011)</td>
<td>0.06 (0.00, 0.93)</td>
<td>33.34</td>
</tr>
</tbody>
</table>

| Overall   |       |                      | 0.06 (0.02, 0.18)  | 100.00 |

NOTE: Weights are from random effects analysis
CHAPTER 3:
A SYSTEMATIC REVIEW AND META-ANALYSIS OF PHASE I INACTIVATED VACCINES TO REDUCE SHEDDING OF COXIELLA BURNETII FROM SHEEP AND GOATS FROM ROUTES OF PUBLIC HEALTH IMPORTANCE

ABSTRACT

Vaccination against *C. burnetii* shedding from routes of public health importance from sheep and goats was evaluated using systematic review and meta-analytic techniques to provide evidence for policy direction to prevent the potential zoonotic spread. Publications reporting shedding of *C. burnetii* in vaginal and uterine secretions, milk, placenta, and feces were included. A single cohort (1 goat) and seven experimental (4 goat and 3 sheep) vaccine publications were included in the review from a scoping study. No publications on other interventions meeting inclusion criteria were identified in the literature. Random effects meta-analyses were performed for the prevalence of shedding in individuals in the control and vaccinated groups, and for the mean difference in level of bacterial shedding in sheep and goats stratified by age and previous exposure status. Significant heterogeneity was found in all stratified comparisons. Limited data were available for further analytic evaluation. An inactivated phase I vaccine significantly reduced the prevalence of shedding from vaginal (odds ratio (OR): 0.29; 95%CI 0.20 to 0.42) and uterine (OR: 0.08; 95%CI 0.04 to 0.16) secretions in previously sensitized goats, and milk (OR: 0.03; 95%CI 0 to 0.25), vaginal secretions (OR: 0.39; 95%CI 0.21 to 0.74) and feces (OR: 0.78; 95%CI 0.63 to 0.96) from naïve goats. The mean levels of bacteria shed from placental (mean difference (MD): -5.24 Log_{10}; 95%CI -6.75 to -3.7) and vaginal (MD: -1.78 Log_{10}; 95%CI -2.19 to -1.38) routes were significantly decreased in vaccinated naïve goats compared to controls. Shedding through all other routes from vaccinated goats was not significantly different than shedding from control goats. No effect of vaccination was found on the prevalence of shedding or mean level of shedding in vaccinated sheep compared to control sheep. Our conclusions are based on a limited amount of data with variable risk of systematic error that warrants further research to improve our understanding of Coxiellosis.
biology and reduce the public health risk of spill-over from small ruminants to humans.

1. INTRODUCTION

Coxiellosis, caused by *Coxiella burnetii*, is a zoonotic disease found in ruminants globally excluding New Zealand (Guatteo et al., 2011). Its status as a relevant emerging disease impacting livestock and human health concurrently has been highlighted from recent outbreaks in Europe (Schimmer et al., 2008; Van der Hoek et al., 2010). Traditionally viewed as a low-risk pathogen from a public health perspective, the Q fever outbreak in the Netherlands led to 3,525 notified cases between 2007 and 2009 in humans and the subsequent national cull of carrying goat herds lead to significant attention to this zoonosis (van der Hoek et al., 2010). Intensification of goat production and the high human population density were considered as contributors to the outbreak. (Wouda and Dercksen, 2007; Van Steengergen et al., 2007; Schimmer et al., 2008, 2009; van den Brom and Vellema, 2009; Klaassen et al., 2009; van der Hoek et al., 2010). However, any individual in contact with goats and sheep is at risk of contracting infection, irrespective of location. Small ruminant infections are clinically manifested as abortions, and are associated with shedding considerable amounts of bacteria in placentas, birth fluids, feces and milk (Sanchez et al., 2006; Webster et al., 2009). Human infections are derived from inhalation of dust particles contaminated with bacteria shed from infected animals (Marrie et al., 1988; Tselentis et al., 1995; Armengaud et al., 1996; Lyytikainen et al., 1996; Schelling et al., 2003). Efforts to prevent the spread of *C. burnetii* to human populations from sheep and goats, the main reservoirs for human infections, have been stagnated by the lack of effective interventions in livestock to prevent zoonotic spread (Norlander, 2000; Arricau-Bouvery and Rodolakis, 2005; Angelakis and Raoult, 2010; EFSA, 2010).

Controlling endemic Coxiellosis in small ruminants may play an important public health role in reducing disease burden in populations who live in close contact with infected sheep or goats (Schelling et al., 2003). Evidence suggests that Slovakia’s reported decrease Q fever incidence in humans may be due to the
national vaccination campaign of cattle over a 10-year period, in conjunction with strict veterinary control and mitigation strategies (Serbezov et al., 1999). Through expert opinion and critical evaluation of available literature, the European Food Safety Authority (EFSA) Panel on Animal Health and Welfare (AHAW), and Panel on Biological Hazards (BIOHAZ) recommended the implementation of a range of long-term control options in the domestic ruminant population if the public health risk was assessed to be substantial (EFSA, 2010). Zoonotic transmission of \textit{C. burnetii} was considered a high risk to public health. The interventions focused on livestock to mitigate transmission included: preventive and reactive vaccination of small ruminants, antibiotic therapy to treat clinical disease, culling of pregnant females, breeding ban in positive herds or flocks, control of mammalian reservoirs, shearing management, tick control, localized disease elimination, general biosecurity measures (including segregated areas of parturition, removal of risk material (i.e. placentas, aborted fetuses), regional and farm-level control of animal movement) and manure management (treating slurry with cyanamide calcium, deep litter composting). The proposed interventions were to reduce the impact of an outbreak by altering within-herd transmission, reduce between herd spread, and the risk of spillover from small ruminants (sheep and goats) to humans.

A commercially killed Phase I vaccine of highly purified corpuscular \textit{C. burnetii} antigen Nine Mile strain inactivated by formaldehyde without adjuvant (\textit{Coxevac}, CEVA Santé Animal, France) is available for goats and cattle in Europe, but has been used off-label in sheep (Hogerwerf et al., 2011). It is indicated in goats to reduce both abortion caused by \textit{C. burnetii} and shedding via milk, vaginal mucus, feces and placenta (EMA, 2010). Primary vaccination is targeted in all goats >3 months of age for the completion of 2-doses 4 weeks apart at least 3 weeks prior to breeding (EMA, 2010). Booster vaccines are recommended every 280 days, but the exact duration of immunity has yet to be determined.

Accurate assessment of the public health implications of vaccination of sheep and goats is essential and relies on comprehensive understanding of the effect of vaccination to allow reasoned choices between alternative strategies. Utility of
vaccination has been identified to reduce the risk of future outbreaks and in the face of an outbreak. The immunogenicity of the commercially available vaccines in open does and ewes may be more effective than in those pregnant for long-term control of within-herd *C. burnetii* spread (Porter et al., 2011). However, the efficacy to prevent shedding from infectious animals needs to be assessed if vaccination is to be a method of public health intervention to reduce the spread to humans.

The objectives of this study were to use scoping methods to systematically evaluate the evidence for specific control options as presented by EFSA (2010) to prevent the spillover of *C. burnetii* from small ruminants to humans. Systematic review and meta-analysis was used to evaluate the effect of vaccination with commercial vaccines against Coxiellosis on the prevalence of *C. burnetii* shedding from routes of public health importance, and the change in level of shedding in vaccinated sheep or goats.

2. METHODS

2.1 SCOPING STUDY

2.1.1 SEARCH STRATEGY

A scoping review was undertaken to survey the extent of available published evidence on *in vivo* interventions concurrently in small ruminants (sheep and goats) and humans. Interventions were defined according to recommendations for Q fever prevention and control as described by the Scientific Opinion on Q fever (EFSA, 2010). Search terms were developed with the assistance of an information scientist to capture relevant publications. The search terminology was divided into population, intervention, and outcome to identify primary intervention studies, grey literature, conference abstracts, and theses, published from 1937 to the end of February 2012 (Appendix 1). The search for publications captured human (Chapter 2) and livestock publications concurrently (Chapter 2). Studies were identified by searching the following databases: Agricola (from 1970), ISI Web of Knowledge (BIOSIS Previews Biological Abstracts, from 1969), CAB Direct (from 1990), Dissertation Abstracts and Theses, Medline (from 1993), PUBMED (from 1950),
Web of Science, Scirus, Academic OneFile (from 1980), Biomed Central (from 1997), Cumulative Index to Nursing and Allied Health Literature (from 1981), Current Research @ (Dissertations and Theses, ProQuest Full Text), Environmental Sciences and Pollution Management (from 1967), Popline (from 1937), Health and Wellness Resource Centre, and Google Scholar (from 1937) with no restriction by language or study design. Abstracts in languages other than English and French were considered for screening using an online open-source translation tool (Google Translate™). All located citations were imported into a reference management program (RefWorks-COS©, Proquest, LLC, Cambridge Information Group) and de-duplicated via the automated RefWorks de-duplication process and subsequent manual de-duplication. Subjects and indexes were removed prior to review.

2.1.2 RELEVANCE SCREENING

To rapidly remove non-primary and other publications non-relevant to the a priori research topic of *C. burnetii* interventions in small ruminants, two levels of relevance screening (RS) were applied. Two unblinded reviewers (TJO and JMS) independently screened titles and abstracts (RS-1), and full publications (RS-2) according to pre-tested RS tools (Chapter 2, Appendix 2). The reproducibility and validity were tested in the initial phase of each RS (N=500, RS-1; N=20, RS-2). Cohen’s Kappa (κ)(Cohen, 1960) was estimated for agreement of study selection at each screening, and RS tool modifications were made when necessary until adequate agreement was achieved (κ ≥ 0.6). Both reviewers applied RS inclusion criteria for the final decision regarding publication selection. Publications were only excluded if both reviewers responded “no”. In cases where the two reviewers disagreed, discussions were held to resolve the disagreement until consensus was reached (Arksey and O’Malley, 2005).

The first level of RS evaluated citations and abstracts in the reference database using the questions: (i) “Does the abstract or title describe published primary research or grey literature?” and (ii) “Does the abstract or title investigate the effect of Q fever or *C. burnetii* interventions in small ruminant or human
populations?”. Potentially relevant manuscripts were obtained in full-text format. The second level of RS question was applied independently: “Does the publication evaluate and report the effect of an intervention for C. burnetii in small ruminants or humans to change the probability of transmission, infection, or clinical symptoms?”. The second level of RS identified the number of relevant publications by intervention. The reviewers prioritized interventions for systematic review based on number of publications.

To identify publications not present in the master database, we reviewed the citations of articles passing RS-2, and hand-searched recent literature reviews of C. burnetii interventions (Maurin and Raoult, 1999; Angelakis and Raoult, 2010; Porter et al., 2011). Additional publications were retrieved and the same criteria for inclusion (RS-1 and RS-2) were applied. If non-peer reviewed publications such as theses or conference proceedings were identified, a first author search was conducted in the aforementioned databases with no language or date restrictions. Peer reviewed publications identified by the first author search were included in place of the non-peer reviewed material when available; otherwise the non-peer reviewed publication was retained. We also contacted CEVA Santé Animale, makers of the commercially available Coxevac, informing them of the review and inviting them to offer additional unpublished data for inclusion.

2.2 SYSTEMATIC REVIEW

From the publications identified in the scoping study, the systematic review (SR) question was refined: “What is the effect of vaccination in a commercially viable, single antigen formulation in any dose, preparation or time schedule on the prevalence or level of shedding of Coxiella burnetii from routes of public health importance from previously exposed or unexposed small ruminants under any condition, in any geographical location compared with a placebo, another vaccine, or an unvaccinated population?”.
2.2.1 INCLUSION CRITERIA

Strict inclusion criteria for the review question were developed *a priori* and applied to publications identified as relevant from RS-2. Both natural exposure field trials and challenge studies were considered for review. Only those publications reporting a vaccinated and control population (placebo, alternative vaccine, or unvaccinated) were included. Small ruminants included sheep (ovine) and sheep flocks, and goats (caprine) and goat herds. Routes of public health importance included vaginal secretions, milk, feces, uterine fluids, and placenta as these represent the most common direct routes of exposure to humans (Porter et al., 2011). There was no restriction on the method of outcome measurement or analysis, but these data were captured during data extraction. Animals or herds/flocks reported as previously sensitized were those classified as having prior exposure to *C. burnetii* (individual-level or herd-level diagnostically positive, assuming an underlying within-herd disease prevalence). Those unexposed were individual animals or herds/flocks that tested diagnostically negative for previous *C. burnetii* exposure. All direct identification and serologic diagnostic methods confirming exposure to *C. burnetii* listed by the OIE (2010) were accepted for inclusion.

Studies of non-licensed vaccines, diagnostic tests for *C. burnetii* exposure or infection in small ruminants, and literature investigating only other aspects of the effect of vaccination (i.e. serologic conversion, clinical disease) were not included in the SR as they were deemed non-relevant to the review question.

2.2.2 METHODOLOGICAL QUALITY ASSESSMENT

Two reviewers (TJO and JMS) assessed the risk of systematic error using two separate methods to account for the different study designs included. The quality at the design and analysis stages was used as a means of interpretation of the validity of reported results. All discrepancies between reviewers for risk of bias (RoB) evaluation were resolved by consensus. An aggregate quality score was not reported on individual publications because of limited validity of summary scores as a means
of assessing overall publication quality (Emerson et al., 1990; Greenland, 1994; Stroup et al., 2008).

Experimental studies (randomized controlled trials (RCTs), controlled trials (CT), and challenge studies (CS)) were evaluated using six quality criteria: random sequence generation, allocation concealment, blinding to vaccine or control administration, outcome assessor blinded, addressing of incomplete outcome data, and evidence of selective reporting (Verhagen et al., 2001; Higgins and Altman, 2008; Cochrane Collaboration, 2011). Each criterion was scored “low risk” if the principle was entirely respected and its execution was adequately reported, “high risk” if the principle was not adequate or inadequately reported, or “unclear” if the principle was not applied or reported (Chapter 2, Appendix 3).

Observational studies were evaluated with a validated risk of bias assessment tool for non-randomized studies (RoBANS) (Park et al., 2011). Six design categories were evaluated: participant selection, control of confounding variables, measurement of C. burnetii exposure (e.g. laboratory evaluation confirms diagnosis), blinding of outcome assessment, incomplete outcome data, and selective outcome reporting. The criterion were scored “low risk” if the publication adequately reported details to assess the category, “high risk” if the publication did not adequately report or perform the given category, or “unclear” if it was not possible for the reviewer to assess the category based on inadequate or unclear reporting (Appendix 4). Confounders were selected from risk factors for shedding C. burnetii by small ruminants as reported in recent reviews on Q fever (Maurin and Raoult, 1999; Angelakis and Raoult, 2010; Porter et al., 2011).

2.3 DATA EXTRACTION

We developed an electronic data extraction tool, pre-tested it on all included studies because of the limited number of included publications, and refined it accordingly by reviewer consensus. Two review authors (TJO and JMS) extracted the following data for vaccinated and control groups: (i) study design; (ii) geographical location; (iii) description of the vaccine and control interventions,
including dose and type of vaccines, timing and route of administration; (iv) characteristics of the species, production stage, and commodity group; (v) exposure status (sensitized or naïve) prior to vaccination and method of evaluation of previous exposure; (vi) number of subjects in each vaccination and control group; (vii) description of disease challenge if applicable; (viii) follow-up length; (ix) number of animals shedding *C. burnetii* by route and parity; (x) arithmetic mean and standard deviation of mean level of shedding by route and parity; (xi) diagnostic tests used to evaluate shedding; (xii) other interventions applied concurrently with vaccination. All raw data were extracted from publications as reported by the authors. No attempt was made to contact individual investigators for additional clarification or data.

### 2.4 STATISTICAL ANALYSES

It was hypothesized *a priori* that there would be a differential effect of vaccination on *C. burnetii* shedding between those previously exposed and those susceptible to infection. Aggregation of data across studies into comparison populations was dependent on the species (sheep or goat), route of shedding (vaginal, milk, uterine, placental, and fecal), and reported age of the animals. Age was divided according to parity: (i) young animals were those classified as kids, lambs, nulliparous or primiparous individuals, (ii) multiparous were ewes or does that have had more than one parturition, and (iii) mixed populations included animals of multiple age groups including multiparous, primiparous, nulliparous, and kids or lambs where the publication did not report them separately.

When publications reported only population level exposure status with the possibility of a mixture of exposed and naïve individuals (herd/flock-level data), all individuals were assumed to be equivalent in exposure status to that of the population. If individual animal data were reported for prior exposure status, these data were used for exposure status regardless of a reported population-level exposure classification.
Publications reporting more than one location with vaccinated and control populations at each unique location were treated as independent studies within a publication in the stratified analysis (Borenstein et al., 2009). In contrast, for publications that reported multiple locations, with vaccinated animals and control animals at separate locations, data were aggregated across all vaccinated and control locations to develop a single population of vaccinated and control animals (Borenstein et al., 2009). The raw data were entered into an electronic spreadsheet and a dataset was constructed for each summary measure.

2.4.1 SUMMARY MEASURES

Two outcomes of interest were estimated for each study by comparison group: (i) the unweighted odds ratio (OR), as well as the standard error (SE), of the odds of *C. burnetii* shedding, and (ii) the mean difference (MD) in bacterial load (log$_{10}$) including the standard deviation (SD) or SE. Both outcomes were estimated from reported raw data even if the outcome of interest was reported in the publication. Clustering was accounted for when studies reported herd, pen or group-level data. The heteroskedasticity-robust estimator was used to adjust the SE of the binary outcomes (Nichols and Schaffer, 2007). Continuous outcomes were not adjusted for clustering. All analyses were performed in the statistical package Stata V11, StataCorp, College Station, TX.

From extracted data on the prevalence of shedding, we estimated the crude unweighted OR and SE of shedding *C. burnetii* for vaccinated and control animals in each comparison group. The OR was defined as the protective effect of the vaccine in the vaccinated individuals at preventing shedding (Halloran et al., 2010). When publications reported no clinical cases in a cell of a 2x2 table, a continuity correction of 0.5 was added to that cell to estimate the measure of association (Sweeting et al., 2004; Cochrane Collaboration, 2011). Publications were excluded from the analyses if the OR and SE could not be estimated based on reported data.

The second outcome of interest was estimated from the mean load (log$_{10}$) of bacteria, as well as the SD or SE between the vaccinated and control populations.
The mean difference (MD) and SD in bacterial load shed was reported on the log_{10} scale and stratified according to species, previous exposure status, and age as previously described. When results were reported as ordinal data for vaccinated and control groups, a central tendency to estimate the mean ($\bar{x}$) and standard deviation ($\bar{S}$) of small samples sizes was calculated from the formulas according to Hozo et al. (2005):

$$\bar{x} \approx \frac{a + 2m + b}{4} \quad \bar{S} \approx \sqrt{\frac{1}{12} \left( \frac{(a - 2m + b)^2}{4} + (b - a)^2 \right)}$$

where $a$ is the minimum value in the dataset, $m$ is the median value, and $b$ is the maximum value. When results were reported graphically or in a form that did not allow for comparison of level of shedding (i.e. Hazard Ratio), the data were excluded from the analysis.

### 2.5 META-ANALYSIS

When two or more studies were available within the same comparison category, a meta-analysis was performed. The effect of vaccination was analyzed using the natural logarithm of the odds of shedding (lnOR) with associated SE (lnSE), and mean difference (lnMD) in load of bacterial shedding with the SD (lnSD) between the vaccinated and control groups in each stratified comparison. To reduce data heteroscedasticity and comply with the assumption of normal distribution of means, this transformation was required (Bland and Altman, 1996a, 1996b). The transformation was applied to each unweighted OR ($\pm$SE) and MD ($\pm$SD) prior to each comparison’s estimation of lnOR or lnMD, where appropriate (Higgins et al., 2008). The resulting pooled effect size (ES) and the corresponding 95% CI were back-transformed to the OR and MD (log_{10}) scales, respectively.

A random-effects model was used to represent the mean of a distribution, given the a priori assumption that statistical heterogeneity existed (Carlin, 1992). Binary (OR) and continuous (MD) data were empirically weighted to estimate the between-study variance with restricted maximum likelihood (REML) methods. The
Knapp-Hartung variance of the ES was estimated using the $t$-distribution due to the small number of publications (Smith et al, 1995; Sutton and Abrams, 2001; Knapp and Hartung, 2003). Under the normality assumption, this method has been shown to be more robust to the normality assumed in random effects models and produces a more conservative estimate of the pooled stratum ES (DerSimonian and Kacker, 2007; Borenstein et al., 2009; Hedges and Olkin, 1985). Since the model assumes that effects are independent with a small number of publications, this model is formally equivalent to an empirical Bayes meta-analytic technique (Carlin, 1992).

The absolute vaccine efficacy and effectiveness (VE) were estimated as $1 - \text{crude unweighted OR}$ for comparisons with a single study. When possible, $1 - \text{backtransformed lnOR}$ was used to estimate the VE expressed as a percentage in each comparison for comparisons where an ES was estimated (Halloran et al., 2010). Effect of vaccination was interpreted according to study design. Vaccine effectiveness is the vaccine’s ability to reduce the prevalence of shedding of *C. burnetii* from vaccinated individuals in an observational study (Weinberg and Szilagyi, 2010; Halloran et al., 2010). We defined vaccination efficacy as the relative reduction of shedding of *C. burnetii* after vaccination as established by a RCT, CT or CS. A negative efficacy or effectiveness estimate ($VE < 0$) was interpreted as no-effect of vaccination to reduce the prevalence of shedding of *C. burnetii*.

For every comparison’s pooled estimate, the effect of statistical heterogeneity between publications was quantified with the $I^2$ test statistic (Higgins et al., 2003). This test describes the percentage of total variation across studies that is due to heterogeneity rather than chance alone, and is based on an iterative non-central $\chi^2$ distribution method (Hedges and Pigott, 2001). Statistical heterogeneity may either arise from systematic differences between studies or random differences between effect sizes, or both. When $I^2$ is less than 30%, there is limited concern about statistical heterogeneity not due to sampling error (Cochrane Collaboration, 2011)
3. RESULTS

3.1 SCOPING STUDY AND DESCRIPTIVE DETAILS OF PUBLICATIONS

The scoping study was conducted to determine the extent of literature available on interventions for Coxiellosis prevention and control. Of those publications concerning small ruminants, we were interested in publications evaluating the public health effect of interventions to prevent the spillover of *C. burnetii* from sheep or goats to humans. The procedure of study selection is outlined in Figure 1. A total of 12,939 titles were initially identified by the scoping study. The most frequent reason for exclusion after RS-2 was a lack of independent control in experimental study designs (n=69), and non-relevant interventions (n=56). Seven publications in small ruminants were retrieved for inclusion in the systematic review. Citations of included publications were reviewed and relevance screening (RS-1 and RS-2) was applied to the additional manuscripts. One additional publication was selected for review inclusion.

Vaccination had a sufficient number of publications with independent control groups to support a full SR (n=8 independent publications; n=6 English, n=2 French). There were no trial reports or additional data provided by *CEVA Santé Animale*. In addition to vaccination, only Hogerwerf et al. (2011) reported additional interventions being applied concurrent with the use of vaccination. Interventions for control of Q fever were applied during the outbreak in the Netherlands and included the culling of pregnant females, movement restrictions and the implementation of a temporary breeding ban during an outbreak of Q fever.

Table 1 provides a descriptive summary of the three sheep (Sadecky and Brezina, 1977; Brooks et al., 1986; Astobiza et al., 2010) and five goat (de Cremoux, 2009; Arricau-Bouvery et al., 2005; Rousset et al., 2008, 2009; Hogerwerf et al., 2011) publications included in this review. There were 26 different comparisons by previous exposure, parity, and route of shedding for which vaccination was compared with a control or placebo, and for which relevant results were reported.
Phase I inactivated vaccines with the preparation (Nine-mile strain), dosage and vaccination schedule equivalent to that of Coxevac were compared to unvaccinated controls in two sheep controlled trials (CT) reporting three separate comparisons (Sadecky and Brezina, 1977; Astobiza et al., 2010). One sheep challenge study (CS) with six comparisons evaluated a Henzerling phase I inactivated vaccine compared to a chloroform-methanol residue (CMR) vaccine or Chlamyvax FQ (Merial, France), a combination vaccine to protect against the clinical effects of Chlamydia abortus and C. burnetii as a phase II preparation (Brooks et al., 1986).

In the five publications reporting goats as the species of interest, all named Coxevac as the vaccine used (Arricau-Bouvery et al., 2005; Rousset et al., 2008, 2009; de Cremoux, 2009; Hogerwerf et al., 2011). The single cohort publication had a total of 5 separate herd-level exposures with clustered within-herd data reported (Hogerwerf et al., 2011). Two CT with a total of six comparisons of clustered data were reported in an English (Rousset et al., 2009) and French (Rousset et al., 2008) publication. Additional data and further analysis was offered in Rousset et al. (2009), but we included both in the review and deemed the publications two reports of the same study. A RCT commissioned by CEVA and the Association Nationale Interprofessionnelle Caprine reported five relevant comparisons (de Cremoux, 2009). A single CS publication of goats was included, with two separate vaccination comparisons to an unvaccinated population and to those vaccinated with Chlamyvax FQ (Arricau-Bouvery et al., 2005).

Publications reported outcomes measured at two (Astobiza et al., 2010), three (de Cremoux, 2009) and twelve (Hogerwerf et al., 2011) independent locations and were treated as independent studies within a publication. Of these, control and vaccinated animals were maintained on the same farm in two studies (Astobiza et al., 2010; de Cremoux, 2009) while clustered data were reported as vaccinated or unvaccinated groups in another (Hogerwerf et al., 2011). All other publications reported only one study location (farm, group in separate pens, or animals housed in one pen).
Both CS reported populations of *C. burnetii* naïve sheep (Brooks et al., 1986) and goats (Arricau-Bouvery et al., 2011) unexposed to any other individuals during the study duration. In contrast, one study reported the use of a completely sensitized population of sheep prior to vaccination (Sadecky and Brezina, 1977). Two publications assessed the individual exposure status of animals co-mingled at the same locations leading to a mixed population of previously sensitized and naïve sheep (Astobiza et al., 2010) and goats (de Cremoux, 2009). Goat farms were classified as previously sensitized in three publications as evaluated by pooled bulk milk tank samples (Rousset et al., 2008, 2009; Hogerwerf et al., 2011). The numbers of individuals in the vaccinated and control groups were reported, but we assumed all to be previously exposed based on herd-level exposure status. No publications reported the inclusion of male goats or male sheep.

All publications but one (Hogerwerf et al., 2011) reported preventative vaccination exclusively to reduce the risk of shedding or clinical effects of *C. burnetii* infection following vaccination. As Hogerwerf et al. (2011) retrospectively reported during the Q fever outbreak in the Netherlands, some included farms vaccinated reactively to reduce shedding and clinical effects whereas others were vaccinated to prevent infection or subsequent clinical effects.

Shedding was evaluated with PCR in all studies but one where complement fixation (CF) was reported (Sadecky and Brezina, 1977). The prevalence of shedding of *C. burnetii* from vaccinated compared to control groups was reported as an outcome of interest in all but one study (de Cremoux, 2009)(Table 2). In five goat and one sheep publication, vaginal shedding was reported (Arricau-Bouvery et al., 2005; Rousset et al., 2008, 2009; de Cremoux, 2009; Astobiza et al., 2010; Hogerwerf et al., 2011). Other reported routes of shedding were milk or colostrum in three publications (Sadecky and Brezina, 1977; Arricau-Bouvery et al., 2005; Hogerwerf et al., 2011), uterine from two publications (Arricau-Bouvery et al., 2005; Hogerwerf et al., 2011), and fecal shedding (Arricau-Bouvery et al., 2005), amnion and placental shedding (Brooks et al., 1986) each reported once.
Mean load (log$_{10}$) of bacteria was reported in five publications (Rousset et al., 2008, 2009; Astobiza et al., 2010; Arricau-Bouvery et al., 2005; de Cremoux, 2009) (Table 2). The mean load (log$_{10}$) of C. burnetii in vaginal secretions was an outcome measure for one sheep (Astobiza et al., 2010) and one goat publication (de Cremoux, 2009), in goat milk for one study (Rousset et al., 2008, 2009), and placenta in parturient goats in another (Arricau-Bouvery et al., 2005).

3.2 RISK OF BIAS ASSESSMENT

Publications were reviewed for risk of systematic error at the methods and analysis stages using the Cochrane RoB tool in all publications but one (Hogerwerf et al., 2011) where the RoBANS framework was used (Table 3). In the single cohort study herds, flocks and animals within each farm were convenience sampled for inclusion leading to a high risk of selection bias. Investigators controlled for confounding (age, vaccination status) during the analysis, but did not report measurement of C. burnetii exposure or whether laboratory personnel were blinded to the farm vaccination status. Missing data due to attrition of study subjects throughout the study period may have had an effect on the outcome. The authors did not report sufficient detail to account for the loss and we could not judge whether the incomplete outcome data would bias the effect towards or away from the null.

For those publications classified as experimental, the RCT (de Cremoux, 2009) reported both adequate random sequence generation to vaccine and control groups and allocation concealment. The potential for selection bias in CT and CS publications was due to inadequate or unclear methods of generation of a random sequence and concealment of allocation (Sadecky and Brezina, 1977; Astobiza et al., 2010). Of those publications that reported an adequate sequence generation, neither had appropriate allocation concealment (Brooks et al. 1986; Rousset et al., 2008, 2009). Potential performance bias was identified in three publications with animal handlers/vaccine administrators not blinded to treatment status (Sadecky and Brezina, 1977; Rousset et al., 2008, 2009; Astobiza et al., 2010). However, laboratory confirmation may reduce the risk of bias. No experimental publication
reported blinding of laboratory personnel responsible for assessing the outcome. Selective reporting was not detected and incomplete outcome data was properly addressed in all experimental publications including reporting reasons for loss to follow-up.

Clustering at the farm level may have been present in three publications (Rousset et al., 2008, 2009; Hogerwerf et al., 2010). This was accounted for in the meta-analysis. Reported sequence generation was by individual animal to intervention groups in the other publications.

### 3.3 Meta-analysis & Descriptive Summary of Single Study Comparisons

Data synthesis was restricted to publications reporting phase I inactivated Nine-mile strain vaccines. To account for between-study variability, we performed random-effects meta-analyses on seven comparisons where sufficient data existed (Table 2). The OR were estimated post-hoc from reported raw data. Due to scarcity of publications, only seven meta-analyses were performed on comparisons within the population strata: sensitized sheep with vaginal shedding (comparisons 1b and 5), sensitized goats with uterine and vaginal shedding (comparisons 3b, 3c, and 6), and naïve goats with placental and vaginal shedding (comparisons 7a and 7b). All other comparisons in population strata were presented for descriptive purposes. No study reported level of shedding in naïve sheep. As the small number of publications examining each outcome of interest precluded sub-analysis of predictor variables, we examined individual comparisons in each publication to identify potential reasons for between-study variability.

Both goat herds and sheep flocks were reported by Hogerwerf et al. (2010). One unvaccinated sheep flock with the potential for clustering within a flock was reported but the data were not suitable for our analysis as no comparable vaccinated sheep population was reported. In addition, the publication reported the rate at which unvaccinated control goats had a positive PCR sample compared to the
vaccinated cohort, as expressed by a hazard ratio (HR). This could not be extracted further to include in our statistical analysis.

3.3.1 OUTCOME 1: ODDS RATIO (OR) EFFECT SIZE (ES)(95%CI) FROM SHEDDING C. BURNETII

Table 2 outlines the descriptive results and meta-analysis of the effect of vaccination on prevalence of bacterial shedding in sheep and goats in both sensitized and naïve individuals. The phase I inactivated vaccine in sensitized multiparous sheep had no statistically significant effect on reducing the prevalence of C. burnetii shedding in milk (comparison 1a), although only one study contributed to this estimate. No data were reported for lambs or primiparous ewes. The overall effect of vaccination on vaginal shedding in sensitized sheep was also not significant compared to the unvaccinated cohort (comparison 1b). In a mixed population of young and multiparous sheep, the phase I inactivated vaccine was no more effective at reducing vaginal shedding in naïve vaccinated sheep compared to controls based on one small study (Astobiza et al., 2010). In comparison 2 the vaccine did not significantly reduce vaginal shedding in the mixed-age vaccinated group compared to the control reported in one publication (Astobiza et al., 2010).

In goat farms identified as previously sensitized to C. burnetii, the vaccine Coxevac demonstrated 71% effectiveness at reducing shedding in milk from multiparous goats (comparison 3a) in one publication that had clustered farm-level data (Hogerwerf et al., 2011). In a single publication of two independent studies (Hogerwerf et al., 2011), the prevalence of uterine shedding was reduced by the use of the Coxevac vaccine in multi- and primiparous goats (comparison 3b) with an overall effectiveness of 92%. Vaginal shedding prevalence in vaccinated nulli-, primi- and multiparous goats in two publications was not statistically different than those unvaccinated overall (comparison 3c). In naïve goats, vaginal shedding was reduced in multiparous goats challenged with C. burnetii (comparison 4b) resulting in 61% overall vaccine efficacy from one publication reporting two separate trials (Arricau-Bouvery et al., 2005). Similarly, there was 97% efficacy in reducing milk shedding in those vaccinated compared to goats unvaccinated or vaccinated with
*Chlamyvax* (comparison 4a). This comparison in multiparous naïve goats also had an efficacy of 22% of fecal shedding reduction in those vaccinated with *Coxevac* (comparison 4c). No significant heterogeneity was found in our meta-analyses of binary outcomes ($I^2<30\%$).

### 3.3.2 OUTCOME 2: MEAN DIFFERENCE (MD) EFFECT SIZE (ES)(95%CI) FROM SHEDDING *C. BURNETII*

The effect of vaccination on the MD in bacterial load ($\log_{10}$) estimated from the cycle threshold ($C_t$) values of quantitative PCR per swab sampled from routes of interest is reported in Table 2. In comparison 5, vaccination in two studies from one publication (Astobiza et al., 2010) of mixed age sensitized sheep led to an overall non-significant mean decrease in vaginal shedding compared to those unvaccinated and housed on the same farms. In previously sensitized goats of all age groups (comparison 6) mean bacterial shedding was numerically decreased, but was not significant compared to the vaccinated cohort overall. Vaccination of multiparous does (comparison 6) by de Cremoux (2009) did not significantly reduce vaginal shedding compared to control animals (MD 0.12 $\log_{10}$ (95% CI: 0.08 to 0.16)). The vaccination of naïve multiparous goats with *Coxevac* compared to unvaccinated controls or *Chlamyvax* was effective at reducing shedding overall from placenta in two studies from one publication (comparison 7a). The vaccine also performed significantly better at reducing vaginal shedding in naïve vaccinated goats (comparison 7b) overall compared to the unvaccinated cohort, despite significant heterogeneity ($I^2=98.4\%$) in a single publication reporting two independent study locations.

### 4. DISCUSSION

The results of the scoping review suggest that limited evidence is available in the literature on interventions besides vaccination to reduce the prevalence or level of *C. burnetti* shedding from sheep or goats from routes of public health importance. In the systematic review, a killed phase I *C. burnetii* vaccine significantly reduced the prevalence of shedding from uterine secretions of previously sensitized goats, and
mean difference of shedding in vaginal and placental secretions from naïve goats. Individual studies reported an effect of vaccination at reducing the prevalence of shedding from milk of sensitized goats, and milk, vaginal secretions and feces from naïve goats. Vaccination was not found to be effective in sheep. However, our conclusions are based on a little over 1000 individual observations from one observational and six experimental publications with variable risk of within-study systematic error based on quality assessment criteria. To our knowledge this is the first systematic review and meta-analysis evaluating the public health effect of vaccination with a *C. burnetii* vaccine in small ruminants.

Evidence-based decision-making on interventions to prevent the zoonotic spread of *C. burnetii* is complicated by the absence of reliable publications on the effect of interventions in small ruminants, and by uncertainties about the interventions. EFSA (2010) reported a narrative review of available literature identifying risk factors for *C. burnetii* spillover from small ruminants to humans with a high degree of uncertainty around the effect of recommended interventions. From the results of our comprehensive scoping study, no publications assessed non-vaccine interventions independent of vaccination in small ruminants to reduce the prevalence or bacterial load shedding of *C. burnetii*.

All available databases were searched to avoid sampling bias. We acknowledge that potentially relevant peer and non-peer reviewed publications may have been missed due to the sensitivity of search terms used. However, publication bias is still probable as there has been a trend to publish only positive results (Rosenthal, 1979; Ahmed et al., 2012). It is difficult to assess unpublished studies, and investigators of included publications were not contacted to add supplementary unpublished data as this approach is time intensive and can result in little additional information (Sargeant et al., 2007). However, the provision of explicit inclusion criteria provided a transparent method upon which publications were identified and included in the review. Our attempts to identify non-peer reviewed literature, inclusion of studies in languages other than English, and a detailed assessment of factors that influence validity of reported results across
study designs adds necessary information to further our understanding of Q fever prevention.

Multiple study designs were evaluated to provide different, yet complimentary evidence regarding vaccine efficacy or effectiveness and to account for the totality of available literature. The study designs included in the SR were randomized controlled, controlled, challenge, and observational studies. Challenge studies, involving deliberate infection with a pathogen, allow for the vaccine to be evaluated in a highly controlled environment where the difference in shedding from individual animals and mean levels of shedding between vaccinated and controls can be precisely measured. However, CS may not be an accurate model of how a vaccine will perform in a field setting. Deliberate infection allows for an understanding of vaccine efficacy based on the bacterial level of challenge. *C. burnetii* is classified as a level-3 pathogen (Waag, 2007) making CS expensive and stocking density limited due to space availability and animal welfare concerns within research facilities. Thus, small sample sizes are common, as reported in both sheep and goat CS included here (Brooks et al., 1986; Arricau-Bouvery et al., 2005). This may reduce the likelihood of detecting a statistically significant difference in shedding between vaccinated and control groups. In addition, the level and route of bacterial exposure may not accurately reflect natural exposure. While CS may be necessary prior to regulatory approval of vaccination, the strength of evidence conducted under field conditions should also be sought.

In contrast to CS, publications reporting natural exposure (RCT, CT, cohort studies) provide necessary information on the effect of vaccination in a real-world setting. This is true when considering the inability of a CS to replicate the effect of management on exposure to *C. burnetii* and subsequent shedding. However, publications reporting natural exposure were conducted on commercial or working research farms in Europe and may not reflect the effect of vaccination in all situations. On-farm management that may influence shedding include high stocking density within pens, exposure to neighboring infected farms and other carriers of *C. burnetii* (e.g. felids, bovines), and aerosolized bacteria from manure. Again, small
sample sizes present the risk of not finding a statistically significant effect due to power limitations. Power limitations may explain why Sadecky and Brezina (1977) reported a reduction in shedding in milk when 50% of control subjects continued to shed compared to 0% of the vaccinated. Only twelve sheep were included in total, resulting in a non-significant OR in our study. The authors (Sadecky and Brezina, 1977) did not offer statistical assessments of the outcome.

High-quality evidence from RCT on *C. burnetii* will aid in understanding the effect of vaccination in different populations. However, methodological concerns were identified for several experimental publications. Randomization requires both appropriate sequence generation and allocation concealment to occur (Schulz et al., 1994; Schulz and Grimes, 2002). Lack of randomization, randomization but failure to report how sequence was generated, or if randomization was reported the description of sequence generation suggested otherwise leading to possible biased estimates of vaccination effect (Chalmers et al., 1983; Kunz and Oxman, 1998; McKee et al., 1999). Compared with RCTs that used adequate concealment, those with inadequate or unclear allocation concealment may have produced ES up to 40% greater than the true effect (Schulz et al., 1995; Schulz and Grimes, 2002). The ES reported in this meta-analysis may have overestimated the effect of vaccination in controlled trials due to improper or unclear methods of randomization. In addition, poorly concealed trials tend to yield greater heterogeneity in results. Adherence to the recently published Randomized Clinical Trials for Livestock and Food Safety (REFLECT statement) (O’Connor et al., 2010) reporting guidelines may improve the quality of reporting and aid in *a priori* study design considerations for future publications.

Observational studies can supplement evidence of experimental studies by contributing data about specific populations and offer information on vaccine effectiveness. The investigator has no control over allocation to treatment group (Dohoo et al., 2009) leading to greater chance of external validity. Hogerwerf et al. (2011) reported significant effects with a high degree of precision. Controlling for other explanatory variables and adjusting for clustering using the
heteroskedasticity-robust estimator as suggested by Nichols and Schaffer (2007) may explain why our results on the prevalence of shedding goats were conservative compared to the VE estimated from multivariate logistic regressions reported in Hogerwerf et al. (2011). Animals within a particular farm, pen, or group may respond similarly to vaccination, and therefore their data are no longer independent. Statistical dependency based on common environmental exposure (Dohoo et al., 2009) may have resulted in underestimated variance due to small sample sizes in those publications that reported clusters of animals housed at different farms (Rousset, 2008, 2009; Hogerwerf et al., 2011). Future observational studies should minimize selection bias, be prospectively designed permitting data collection for all relevant prognostic factors with sufficient power, and report standardized and validated assessments of vaccination that can be summarized in systematic review.

In contrast to our findings, Rousset et al. (2008, 2009) and Hogerwerf et al. (2011) concluded that vaccination significantly reduced the prevalence and level of shedding overall. However, after transforming ordinal data and adjusting for clustering, no significant overall effect of vaccination from the meta-analysis was found in exposed goats.

Hogerwerf et al. (2011) also reported that vaccinated herds had histories of Coxiellosis, compared to no history in those unvaccinated but exposed. If a herd was classified as exposed with pooled bulk tank milk PCR according to Netherland’s Ministry of Agriculture (2010), but the unvaccinated herd had no previous exposure (Hogerwerf et al., 2011), then it is possible that the precision of the diagnostic test at the animal-level misclassified when the prevalence of infection was low (Cowling et al., 1999). In contrast, in those herds previously exposed to *C. burnetii*, it is unknown if the vaccine was responsible for the reduction in shedding or natural immunity over time.

In addition to comparing effect to *Coxevac*, three publications independently assessed *Chlamyvax* compared to a control and reported a reduction in the level of shedding (Brooks et al., 1986; Fishbein and Raoult, 1992; Arricau-Bouvery et al., 2005). Thus, when considering the placebo effect when compared to *Coxevac*, fewer
individuals in the control group (*Chlamyvax*) may have shed bacteria compared to a population that received a placebo biasing vaccination towards no effect if the control group had reduced shedding because of *Chlamyvax*. However, publications that reported unvaccinated controls may be a continuous source of *C. burnetii* replication, leading to a possible underestimation of effect of vaccination (Guatteo et al., 2008). The findings of this study are in general agreement with publications of phase I vaccines in cattle reporting no significant differences in the level of shedding between previously seronegative vaccinated and unvaccinated animals (Biberstein et al., 1977; Schmeer et al., 1987).

Only two publications considered vaccine coverage (Rousset et al., 2008, 2009; de Cremoux, 2009;) by reporting seroconversion due to vaccination rather than exposure to *C. burnetii*. Those that did not seroconvert according to an *a priori* titre (% OD) were excluded from the trial. Therefore, only those who developed antibodies necessary for protection due to the vaccine were included in the vaccinated group. This may have lead to outcomes with greater precision for vaccine efficacy estimation than those publications that assumed 100% coverage post-vaccination. However, not all animals vaccinated seroconvert (Rousset et al., 2009; Astobiza et al., 2010) thus decreasing the effective sample size of controls at risk of shedding if it truly reduces the prevalence or level.

Although there were insufficient data to determine if vaccination varied by age, previous studies have shown that shedding is greatest at the first and second parturition after infection in both goats and sheep, with most animals becoming infected in the first year of life (Berri et al., 2002; Hatchette et al., 2003; Rousset et al., 2008, 2009; de Cremoux, 2009; Hogerwerf et al., 2010; Porter et al., 2011). Primiparous sheep and goats also have a significantly higher titre post-vaccination compared to most multiparous animals (Porter et al., 2011). Therefore, if vaccination reduces shedding, it may be more effective in young animals leading to an estimate closer to the true effect. However, both sheep and goats may continue to shed despite having a protective titre (Rousset et al., 2009; Astobiza et al., 2010). Further evaluation between seroconversion and shedding should be conducted. It is
also possible that a proportion of infected and shedding sheep or goats of any age group may have been non-differentially misclassified exposure if they were not shedding from a sampled route. This would lead to the conclusion that the vaccine is less effective at preventing shedding than the unvaccinated or placebo vaccinated controls.

Although we did not review the effect of timing of vaccination on protection, vaccination of young stock in the first year of life (> 3 months of age) prior to natural exposure and with a second dose at the time of breeding to improve immunity may be beneficial regardless of herd-level exposure status (Porter et al., 2011). From a public-health perspective, this was only beneficial to reduce the mean level of vaginal shedding from naive young stock as reported by one publication (de Cremoux, 2009). In contrast, the probability of finding a statistically significant difference between the vaccinated and control groups of sensitized multiparous animals should be biased away from the null of no vaccine effect due to the low prevalence of shedding expected, thus increasing the risk of a Type I error when sufficient power is not present.

Goats frequently become chronically infected with persistence of the bacteria in the mammary glands and uterus (Rodolakis et al., 2007). In addition, shedding of C. burnetii has been shown to be discontinuous in vaginal secretions and milk, but can be sustained for long periods of time from milk post-partum (Berri et al., 2007). In contrast, sheep shed the bacteria predominately from vaginal secretions, urine and feces, and less commonly and less constantly from milk (Rodolakis et al., 2007). The methods used to evaluate the presence and level of shedding varied across publications. In the absence of published data on the sensitivity of one diagnostic method compared to another, we did not consider one method to provide a more accurate assessment of shedding. Moreover there is no consensus about antigen types and cut-off values used to evaluate C. burnetii shedding (OIE 2010). Variable test sensitivity may have contributed to the reported outcomes in the included publications. Vaccine-derived C. burnetii DNA is shed in goat milk and can be detected through quantitative PCR (Hermans et al., 2011). Although this has not
been evaluated in other routes of excretion, it is possible that vaccinated individuals were shedding only DNA and not live bacteria. Thus, the reported data from all studies using quantitative methods relying on bacterial DNA may have reported underestimated results. Multiple sampling times over multiple lambing or kidding periods needs to be examined to evaluate the issue of intermittent shedding and its impact on vaccination evaluation. Further research needs to evaluate the effect of vaccination on misclassification of vaccine effect.

Limitations require consideration for both interpreting the results and conducting future research. The general methodological quality of included publications was variable and reduced the confidence in the estimates of effect. We do not have evidence to determine whether methodological quality was due to reporting rather than conducting the studies. As well, estimates reported may be affected by biases in the primary studies. However, if the biases are not systematic these estimates are likely to be conservative (Rothman and Greenland, 1998). Low statistical heterogeneity in pooled ES suggest that the estimates are reliable except for one publication reporting young naïve goats at separate locations (de Cremoux, 2009). In this context, heterogeneity relates to the variation observed in results that goes beyond what is expected from random variation, and would suggest that there may be a difference in the effect of the same vaccine in a different study. However, meta-regression was not used to explore the source of heterogeneity due to the limited power from the small number of included publications.

A producer's decision to vaccinate is often based on practical outcomes such as effects on production through reduced abortions (Courcoul et al., 2010). The benefits of the vaccine must therefore outweigh the financial and practical costs involved. Astobiza et al. (2010) reported significant reduction in abortions in vaccinated ewes and yearlings compared to an unvaccinated animals located at the same farm, despite a continuation of bacterial shedding. Evaluating the clinical effects of a C. burnetii vaccine was not the purpose of this review, but should not negate the importance for consideration in future studies. Quantifying the decline in incidence of clinical disease in the unvaccinated animals in which a vaccination
program is instituted may lead to further understanding of how *C. burnetii* vaccines function. However, the lack of effect from a public health perspective limits justification for licensing on this premise alone.

Due to its sustained environmental viability and range of susceptible species, *C. burnetii* prevention and control must employ multi-modal approaches to control and prevention as recommended by EFSA (2010). Control of Q fever from a public health perspective cannot rely solely on the efficacy of a vaccine and may benefit from adjunctive on-farm interventions such as controlled manure handling, screening of animals for *C. burnetii* infection, and biosecurity measures despite the lack of evidence to support the activities. There is a need for further research in the field of control methods from an on-farm animal health and welfare, economic viability, and public health perspective. Good farm-management practices may also benefit by reducing in other pathogens of animal or public health importance.

In summary, there is some evidence for the effect of vaccination in goats to eliminate shedding of *C. burnetii*. However, there were limited publications and considerable variability in the effect of vaccination. Vaccination may be most effective at significantly reducing prevalence and level of shedding from goats, with no evidence of effect in sheep. Multiple study designs contributed to the evaluation of vaccination, but further development of evaluative tools for *C. burnetii* vaccines is required. Although a growing body of evidence shows the effect of Q fever on human health, we found no publications directly assessing interventions other than vaccination that could change the risk of transmission from sheep or goats to susceptible humans.

**ACKNOWLEDGEMENTS**

The authors acknowledge the assistance of Devin Vriezen and Caleigh Machlachlan for assistance with publication acquisition; Shannon Meadows (Ph.D. candidate, Department of Population Medicine, Ontario Veterinary College) and Dr. Paula Menzies D.V.M., M.P.V.M., Dip. ECSRHM (Associate Professor, Small Ruminant Research Co-ordinator, Ruminant Health Management Group, Ontario Veterinary
College) for Q fever biology consultations; Dr. Lee Weisner D.V.M., M.Sc. (Veterinary Epidemiologist, Centre for Public Health and Zoonoses, Department of Population Medicine, Ontario Veterinary College) for guidance in systematic review and meta-analysis methods; Jane Burpee (Librarian, University of Guelph) for assistance with scoping study thesaurus and database; Dr. Renée de Cremoux D.V.M., Ph.D. (Epidemiologist, Insitute de l'Elevage, Département Techniques d'Elevage et Qualité Service Bien-Etre animal, Santé, Traçabilité et Hygiène, France) for the provision of the full manuscript of “Evaluation de l'incidence de la vaccination par un vaccine de phase I sur la dynamique de l'infection a Coxiella burnetii dans les élevages caprins en situation de fièvre Q clinique: Rapport Final”. Editorial recomendations from Drs. Andria Jones-Bitton D.V.M., M.Sc., Ph.D. (Assistant Professor, Department of Population Medicine, Ontario Veterinary College) and Olaf Berke M.Sc., Ph.D. (Associate Professor of Statistical Epidemiology, Department of Population Medicine, Ontario Veterinary College) was greatly appreciated.

FUNDING

A Canadian Institute of Health Research Institute of Population and Public Health/Public Health Agency of Canada Applied Public Health Research Chair supported Dr. Jan M. Sargeant.

Dr. Tyler J. O’Neill was a Graduate Veterinary Fellow funded by the Ontario Veterinary College Graduate Fellowship, developed for Canadian or permanent resident veterinarians to pursue graduate research training at the University of Guelph.
REFERENCES


European Food Safety Authorities (EFSA). Scientific opinion on Q fever. EFSA Journal 2010;8;1595.


Hogerwerf L, van den Brom R, Roest HJJ, Bouma A, Vellema P, Pieterse M, Derksen D, Nielen M. Reduction of *Coxiella burnetii* prevalence by vaccination of goats and sheep, the Netherlands. EID 2011:17(3);379-86.


Rodolakis A, Berri M, Hechard C. Comparison of Coxiella burnetii shedding in milk of dairy bovine, caprine, and ovine herds. JDS 2007;90(12);5352-60.

Rosenthal R. The file drawer problem and tolerance for null results. Psych Bull 1979;86(3);638-41.


Sargeant JM, Amezcue MR, Rajic A, Waddell, L. Pre-harvest interventions to reduce the shedding of *E.coli* 0157 in the faeces of weaned domestic ruminants: a systematic review. Zoonoses Public Health 2007:54;260-77.


Serbezov VS, Kazar J, Novkirishkis V, Gatcheva N, Kovacova E, Voynova V. Q fever in Bulgaria and Slovakia. EID 1999:5(3);388-94.


Sterne JC, Egger M. Funnel plots for detecting bias in meta-analysis: guidelines for choice of axis. JCE 2001:10(54);1046-55.


Verhagen AP, de Vet HCW, de Bie RA, Boers M, van den Brandt, PA. The art of quality assessment of RCTs include din systematic reviews. J Clin Epidemiol 2001:54(7);651-54.

Waag DM. Coxiella burnetii: Host and bacterial responses to infection. Vaccine 2007:25(42);7288-95.


Wouda W, Derksen DP. Abortion and stillbirth among dairy goats as a consequence of Coxiella burnetii. Tijdschr Diergeneeskd 2007:132(23);908-11.
Figure 1. Flow of information through the stages of the scoping review with the number of publications included and excluded at each level outlined.
**Table 1.** Publications included in the systematic review of vaccinations (reactive or preventative vaccination) to reduce shedding of *C. burnetii* from sheep or goats from routes of public health importance.

<table>
<thead>
<tr>
<th>Species</th>
<th>Publication</th>
<th>Study location</th>
<th>Study design</th>
<th>Population (stage at sampling)</th>
<th>Vaccine (purpose)</th>
<th>Control</th>
<th>Route of shedding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>Sadecky and Brezina (1977)</td>
<td>Czechoslovakia</td>
<td>Controlled</td>
<td>Multiparous (gestating)</td>
<td>Nine-mile strain inactivated</td>
<td>Unvaccinated</td>
<td>Milk</td>
</tr>
<tr>
<td></td>
<td>Astobiza et al. (2010)</td>
<td>Spain</td>
<td>Controlled</td>
<td>Primiparous (lactating)</td>
<td>Nine-mile strain inactivated</td>
<td>Unvaccinated</td>
<td>Vaginal</td>
</tr>
<tr>
<td></td>
<td>Brooks et al. (1986)</td>
<td>United States</td>
<td>Challenge</td>
<td>Multiparous (lactating)</td>
<td>Henzerling strain inactivated</td>
<td>i. Chlamyvax*</td>
<td>Placental</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ii. CMR^</td>
<td>Amnion</td>
</tr>
<tr>
<td></td>
<td>Hogerwerf et al. (2011)</td>
<td>Netherlands</td>
<td>Cohort</td>
<td>Nulliparous</td>
<td>Coxevac®</td>
<td>Unvaccinated</td>
<td>Uterine</td>
</tr>
<tr>
<td></td>
<td>de Cremoux (2009)</td>
<td>France</td>
<td>Randomized</td>
<td>Nulliparous</td>
<td>Coxevac®</td>
<td>Placebo</td>
<td>Vaginal</td>
</tr>
<tr>
<td></td>
<td>Rousset et al. (2008, 2009)</td>
<td>France</td>
<td>Controlled</td>
<td>Primiparous</td>
<td>Coxevac®</td>
<td>Unvaccinated</td>
<td>Vaginal</td>
</tr>
<tr>
<td></td>
<td>Arricau-Bouvery et al. (2005)</td>
<td>France</td>
<td>Challenge</td>
<td>Multiparous (gestating, lactating)</td>
<td>Coxevac®</td>
<td>i. Unvaccinated</td>
<td>Fecal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ii. Chlamyvax*</td>
<td>Milk Placental</td>
</tr>
</tbody>
</table>

*Chlamyvax (Merial, France) is a combination vaccine containing a killed phase II *C. burnetii* and *Chlamydia abortus* bacterins.

^Chloroform-methanol residue (CMR) vaccine.
**Table 2.** Effect of vaccination on the odds and mean level of shedding of *C. burnetii* in sensitized and naïve populations of goats and sheep from multiple routes of public health importance.

### Odds of Shedding

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Route</th>
<th>Parity groups</th>
<th>Nobserved</th>
<th>Summary Measure Results</th>
<th>Weight (%)</th>
<th>F (%)</th>
<th>VE (%)</th>
<th>Publications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR [95% CI]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Sensitized sheep</td>
<td>(a) Milk</td>
<td>Young Multiparous Mixed</td>
<td>12</td>
<td>0.14 [0.01 to 2.28]</td>
<td>NA</td>
<td>NA</td>
<td>[86%]*</td>
<td>Sadecky and Brezina (1977)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Young Multiparous Mixed</td>
<td>47</td>
<td>0.81 [0.55 to 1.2]</td>
<td>0.83 [0.58 to 1.17]</td>
<td>78.8%</td>
<td>&lt;0.1%</td>
<td>17%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Young Multiparous Mixed</td>
<td>185</td>
<td>0.83 [0.38 to 1.83]</td>
<td>2.46 [0.14 to 44.8]</td>
<td>19.8%</td>
<td>1.5%</td>
<td></td>
</tr>
<tr>
<td>2. Naïve sheep</td>
<td>Vaginal</td>
<td>Young Multiparous Mixed</td>
<td>47</td>
<td>0.76 [0.47 to 1.23]</td>
<td>NA</td>
<td>NA</td>
<td>[24%]*</td>
<td>Astobiza et al. (2010)</td>
</tr>
<tr>
<td>3. Sensitized goats</td>
<td>(a) Milk</td>
<td>Young Multiparous Mixed</td>
<td>411</td>
<td>0.29 [0.2 to 0.42]</td>
<td>NA</td>
<td>NA</td>
<td>[71%]*</td>
<td>Hogerwerf et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Young Multiparous Mixed</td>
<td>474</td>
<td>0.12 [0.05 to 0.3]</td>
<td>0.08 [0.04 to 0.19]</td>
<td>42%</td>
<td>7%</td>
<td>92%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Young Multiparous Mixed</td>
<td>286</td>
<td>0.53 [0.41 to 1.15]</td>
<td>0.69 [0.38 to 1.2]</td>
<td>49.7%</td>
<td>13%</td>
<td>31%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Young Multiparous Mixed</td>
<td>252</td>
<td>0.03 [0 to 0.25]</td>
<td>NA</td>
<td>NA</td>
<td>[97%]*</td>
<td>Arricau-Bouvery et al. (2005)</td>
</tr>
<tr>
<td>4. Naïve goats</td>
<td>Vaginal</td>
<td>Young Multiparous Mixed</td>
<td>43</td>
<td>0.03 [0 to 0.25]</td>
<td>NA</td>
<td>NA</td>
<td>[61%]*</td>
<td>Arricau-Bouvery et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Young Multiparous Mixed</td>
<td>43</td>
<td>0.39 [0.21 to 0.74]</td>
<td>NA</td>
<td>NA</td>
<td>[22%]*</td>
<td>Arricau-Bouvery et al. (2005)</td>
</tr>
</tbody>
</table>

### Mean Difference of shedding

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Route</th>
<th>Parity groups</th>
<th>Nobserved</th>
<th>Log10 MD [95% CI]</th>
<th>Effect Size [95% CI]</th>
<th>Weight (%)</th>
<th>F (%)</th>
<th>VE (%)</th>
<th>Publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>5. Sensitized sheep</td>
<td>Vaginal</td>
<td>Young Multiparous Mixed</td>
<td>34</td>
<td>0.19 [-0.5 to 0.88]</td>
<td>-0.082 [-0.69 to 0.52]</td>
<td>53.04%</td>
<td>46.95%</td>
<td>&lt;0.1%</td>
<td>Astobiza et al. (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Young Multiparous Mixed</td>
<td>13</td>
<td>-0.37 [-1.72 to 0.98]</td>
<td>-0.3 [-4.32 to 3.72]</td>
<td>42.68%</td>
<td>3.72%</td>
<td>48.79%</td>
<td>de Cremoux (2009), Rousset et al. (2008, 2009)</td>
</tr>
<tr>
<td>6. Sensitized goats</td>
<td>Vaginal</td>
<td>Young Multiparous Mixed</td>
<td>286</td>
<td>-0.51 [-0.56 to -0.46]</td>
<td>-0.63 [-2.85 to 1.58]</td>
<td>42.68%</td>
<td>9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>-5.84 [-7.54 to -4.14]</td>
<td>-5.84 [-7.54 to -4.14]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(b) Vaginal</td>
<td>Mixed</td>
<td>Young</td>
<td>225</td>
<td>6</td>
<td>-1.81 [-2.31 to -1.31]</td>
<td>-1.78 [-2.21 to -1.38]</td>
<td>64.8%</td>
<td>98.4%</td>
<td>de Cremoux (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multiparous</td>
<td></td>
<td></td>
<td>-1.3 [-2.19 to -0.41]</td>
<td>-1.78 [-2.21 to -1.38]</td>
<td>35.2%</td>
<td>98.4%</td>
<td></td>
</tr>
</tbody>
</table>

* Adjusted for herd-level clustering (Nicholas and Schaffer, 2007)
* Based on a single study or publication and not a pooled result from meta-analysis
Table 3. Evaluation of risk of systematic error of experimental and observational publications included in the systematic review of vaccines to prevent shedding of *C. burnetii* from sheep or goats from routes of public health importance§.

<table>
<thead>
<tr>
<th></th>
<th>Sequence generation</th>
<th>Allocation concealment</th>
<th>Laboratory technician blinded to vaccine or control administration</th>
<th>Outcome assessor blinded</th>
<th>Incomplete outcome data addressed</th>
<th>Selective reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RCT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>de Cremoux (2009)</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Uncl ear</td>
<td>Uncl ear</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td><strong>CT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sadecky and Brezina (1977)</td>
<td>High risk</td>
<td>High risk</td>
<td>High risk</td>
<td>Uncl ear</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td>Astobiza et al. (2010)</td>
<td>Uncl ear^</td>
<td>Uncl ear</td>
<td>High risk</td>
<td>Uncl ear</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td>Rousset et al. (2008, 2009)</td>
<td>Low risk</td>
<td>High risk</td>
<td>High risk</td>
<td>Uncl ear</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td><strong>CS</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arricau-Bouvery et al. (2005)</td>
<td>Low Risk</td>
<td>High risk</td>
<td>Uncl ear</td>
<td>Uncl ear</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td>Brooks et al. (1986)</td>
<td>High risk</td>
<td>High risk</td>
<td>Uncl ear</td>
<td>Uncl ear</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Participant selection</th>
<th>Confounding variables</th>
<th>Measurement of <em>C. burnetii</em> exposure</th>
<th>Blinding of outcome assessment</th>
<th>Incomplete outcome data addressed</th>
<th>Selective reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cohort</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hogerwerf et al. (2011)</td>
<td>High risk</td>
<td>Low risk</td>
<td>Uncl ear</td>
<td>Uncl ear</td>
<td>High risk</td>
<td>Uncl ear</td>
</tr>
</tbody>
</table>

§Both Cochrane Collaboration (2011) risk of bias (RoB) and the risk of bias of non-randomized studies (RoBANS) are shown.

*Randomized controlled trial (RCT) **Controlled trial (CT) ***Challenge study (CS)

^Systematic sampling reported for multiparous animals; random sampling of young animals; did not report how sample was generated.
CHAPTER 4:

ESTIMATING HERD-LEVEL FINANCIAL COSTS OF COXIELLOSIS
IN ONTARIO GOAT FARMS

ABSTRACT

In dairy and meat goats in Ontario, the prevalence of exposure to *Coxiella burnetii*, the causative agent of Coxiellosis, has been considered persistent within herds and clinical disease may induce significant reproductive effects including abortions, stillbirths, and weakborn kids. Subsequent direct effects include loss of milk production, live animal sales, and expenditures to replace culled animals, diagnose Coxiellosis, and treat clinical disease. Herd-level losses and expenditures in meat and dairy goat herds with low and high levels of exposure to *C. burnetii* were modeled. Variability and uncertainty in each within-herd parameter estimate was introduced in the analysis by specifying a PERT distribution for selected parameters. The within-herd parameter estimates for PERT distributions were informed from literature or by expert opinion. A Monte Carlo simulation based on 10,000 iterations was then used to provide the distribution of financial cost for each unique herd type and exposure combination. The estimated financial costs of Coxiellosis in a typical Ontario dairy herd in one year ranged from approximately $2,670 to $9,200CAD in those with low and high levels of *C. burnetii* exposure, respectively. Comparatively, the average cost ranged from approximately $1,400 in meat goat herds with low exposure to $3,500CAD in herds with evidence of high exposure. Production losses due to reduced herd-level milk production in dairies and loss of live-animal sales at weaning due to aborted or stillborn kids in meat goat herds led to the most important parameter estimates to the financial cost of *C. burnetii* exposure and subsequent Coxiellosis.
1. INTRODUCTION

Q fever, a zoonotic disease with global occurrence caused by the intracellular bacterium *Coxiella burnetii* and is capable of infecting several animal species, including goats (Kaplan and Bertagna, 1955; Maurin and Raoult, 1999; Kovacova and Kazar, 2002; Porter et al., 2011). Ruminants are the primary reservoir for *C. burnetii* and are the most frequent source of human infection (Lang, 1990). Q fever is regarded as an underrated and widely neglected public health problem in North America (Marrie et al., 1985; Lang, 1989; Sanford et al., 1994; Hatchettet et al., 2001; Porter et al., 2011), and is considered a significant clinical problem in the Ontario goat population (Lang, 1990). Although associated economic impact has been suggested (Arricau-Bouvery and Rodolakis, 2005), no quantified analysis of its effects on production in any species has been published globally.

Epidemiologic and experimental evidence have shown that inhalation of desiccated particles is the primary route of infection through contact with infected animals, their reproductive tissues, or other animal products (Maurin and Raoult, 1999; Arricau-Bouvery and Rodolakis, 2005; ECDC, 2010). Clinical disease is not necessarily associated with seroconversion or concomitant shedding of the bacteria (Berri et al., 2005; Guatteo et al., 2007; Rousset et al., 2009). Within a herd, two common antibody serological diagnostics (ELISA and indirect immunofluorescence assay (IFA)) have been poorly indicative of *C. burnetii* attributed abortion. 35% of goats not exhibiting clinical reproductive signs presented strongly positive sera and approximately 20% of those aborting had no seroconversion (Rousset et al., 2007). Shedding of *C. burnetii* has also been reported in seronegative experimentally infected goats making serological screening problematic (Arricau-Bouvery et al., 2003). However, a strong within-herd antibody response has been suggested to be associated with a high bacterial circulation (Rousset et al., 2007).

Along with most other ruminant species, goats can be persistently infected leading to high rates of reproductive losses including abortion, weakborn kids, stillbirths, and occasionally pneumonia (Palmer et al., 1983; Arricau-Bouvery and Rodolakis, 2005; Berri et al., 2005a; Berri et al., 2007; Vaidya et al., 2008; Rousset et
al., 2009). Goats can be subclinically infected and shed *C. burnetii* in products of parturition (birth fluids, placenta), vaginal mucus, feces, milk (Berri et al., 2003; Guatteo et al., 2007), and urine (Heinzen et al., 1999) leading to environmental contamination and spread. Goat herds naturally infected with *C. burnetii* and aborting represent an important direct and indirect source of bacterial exposure (Sanford et al., 1994; Kovacova et al., 1998; Hatchette et al., 2001; Schimmer et al., 2008). Long-term shedding after an outbreak after two successive parturitions has also been suspected to contribute to the persistence of environmental contamination (Berri et al., 2007). Lang (1989) reported that herds were endemically infected, but newly exposed herds may have abortion epidemics followed by persistent infection in subsequent years (OIE, 2012). Goats persistently infected and shedding are the source of infection for those previously unexposed.

With the largest goat population in Canada, the total number of goats has continued to increase over the past 15 years in Ontario (Agriculture and Agri-food Canada, 2006). This is in part due to growing domestic milk and meat consumption from increased ethnic and niche market demands for goat products. However, the Ontario goat industry remains a developing sector of provincial agriculture with limited official data and information services (Agriculture and Agri-food Canada, 2006). Therefore, production losses impacting producer income or opportunity costs limit the ability of the industry to meet the continuing demand for goat products (meat, milk, and fibre or mohair). Increased demand for goat products will lead to growth in supply.

It is hypothesized that a high level of exposure to *C. burnetii* may cause significant economic losses. On the basis of recommendations made by the European Food Safety Authority (EFSA, 2010), Rousset et al. (2009) recommended a greater understanding of the financial impact may improve the implementation of rational preventative and control measures. However, very little is known about the economic implications of Coxiellosis in the Ontario goat population. Understanding the true financial impact of Coxiellosis may be useful to producers and public policy makers. The prevalence of previous exposure to *C. burnetii* varies based on industry
(meat or dairy) in Ontario (Meadows, personal communication). Within-herd seroprevalence of *C. burnetii* in dairy goats reported 20 years ago by Lang et al. (1991) was approximately 20%. The available estimates of the seroprevalence of *C. burnetii* infection justify a financial analysis of the production losses.

Coxiellosis may cause financial losses and expenditure to producers both directly and indirectly. Direct losses include animal death, loss of production, and cost of treatment in a herd clinically affected with disease. In contrast, indirect losses include the cost of prevention and control efforts to prevent infection or Coxiellosis. Thus, even if no infection is present, costs are incurred (e.g. biosecurity measures). Although health economics has become more frequent over the last thirty years, most results remain in the grey literature of unpublished government agency reports (James and Rushton, 2002). Analytic approaches for the financial costs of disease vary considerably in health economics (Buxton et al., 1997). Deterministic estimates have historically been used in livestock health financial analyses. This is despite the high degree of uncertainty associated with these effects and estimates.

Stochastic simulation is an alternative that may overcome the methodological and analytic challenges associated with sensitivity analyses. By accounting for uncertainty about variables, a distribution of outcomes is produced and expressed as a probability distribution. This method also allows for multivariable and range of variable value analyses (Vose, 2000; Hardacker et al., 2004) to produce a full scope of outcomes for the costs of *C. burnetti* infection.

In contrast to stochastic estimates to determine a range of possible estimates, a sensitivity analysis is often conducted as a component of deterministic modelling. Traditionally, one variable is considered at a time, ignoring the effects on the performance measure of combinations of errors in different variables. When there is uncertainty in a large number of variables of interest, sensitivity analysis becomes laborious and difficult to interpret. The likelihood of a particular outcome being achieved is also not estimated with the use of sensitivity analysis (Hardaker et al., 2004).
In the present financial analysis of Coxiellosis, we aimed to estimate the total herd-level cost to producers with *C. burnetii* infected animals and the clinical effects of disease, associated prophylaxis, and treatment by summing the losses and expenditures in Ontario meat and dairy goat farms. The within-herd and herd-level parameter estimates associated with the greatest impact to the models were explored for future targeted risk assessment and intervention program consideration.

2. METHODS

For the purpose of the analyses outlined here, direct costs were defined as the value of the loss of expected output and/or of resource loss due to Coxiellosis, together with the costs incurred in prophylactically mitigating the effects of infection on clinical effects of *C. burnetii* infection. Direct costs included loss of livestock output (stillbirth, abortions, increased days to weaning, lost milk production in dairy animals) (Maurin and Raoult, 1999; Angelakis and Raoult, 2010; Porter et al., 2011) and expenditures of extra resources to address on-farm disease and infection (prophylaxis, replacing culled animals, veterinary diagnostics) (Menzies, personal communication). Direct costs are compatible with the concept of economic costs suggested by McInerney et al. (1992) under a framework for the economic analysis of livestock disease. This may be differentiated from indirect costs (e.g. impacts on human health, welfare, additional feeds required etc.). The estimated parameters (Table 1) identified from the rapid evidence review (RER), relevant government bodies, expert opinion, and a producer interest group were used to estimate direct input parameter costs (Table 2) and the total herd-level costs were modeled stochastically over one parturition season (one year).

2.1 SOURCES OF PARAMETER ESTIMATES

Data on financial and epidemiologic parameter estimates were gathered through a rapid evidence review (RER) (Ganann et al., 2010) of the literature. A RER systematically identifies research in a particular area and records the main
outcomes. A systematic search of the Ontario (Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)) or Canadian grey literature (Agriculture and Agri-food Canada, Statistics Canada)(non-peer reviewed) was also conducted. The provincial goat producer association (Ontario Goat) was solicited to add additional epidemiologic and financial data.

The extent of available peer-reviewed evidence on within-herd epidemiologic and financial parameter estimates was systematically searched in literature databases. Thesaurus terms were developed with the assistance of an information scientist ([goat* OR caprine OR herd]AND[Q fever OR Coxiell* OR C. burnetii OR Query Fever]AND[Ontario OR Canada]). Search terms were entered into the following databases: Agricola (from 1970), CAB Direct (from 1990), PUBMED (from 1950), and Google Scholar (from 1937). We used no restrictions by language or study design. An electronic database, RefWorks-COS (ProQuest, LLC, Cambridge Information Group) was used to manage publications. Manual de-duplication was performed prior to screening.

Publications identified in the RER and systematic search were screened by title, abstract and then full manuscript to establish their appropriateness for RER inclusion. The following inclusion criteria were applied to screen titles and abstracts: (i) references from peer-reviewed literature published until June 2012 (ii) in English or French (iii) with full text reporting primary within-herd data from meat or dairy goat herds. Relevant provincial and national government websites were systematically searched for grey literature with the same search terms and for matching inclusion criteria: (i) grey literature published until June 2012, (ii) in English or French, (iii) full text publications referencing Ontario or Canadian meat or dairy goats.

If the relevance of a reviewed publication was unclear from the abstract or no abstract was available, a full manuscript was retrieved for further screening. Full texts of potentially relevant manuscripts were retrieved for further evaluation for inclusion of Ontario-specific C. burnetii epidemiologic or financial data for parameter estimates of interest (Table 1). Where relevant data for Ontario goats
were not identified, data sources from other Canadian provinces, the United States, or Western Europe were considered acceptable. Data from other locations were not included in this study due to variability in herd management. Reference lists of included publications and recent literature reviews (Maurin and Raoult, 1999; Angelakis and Raoult, 2010; Porter et al., 2011) were reviewed for additional titles missed by the initial search.

An existing network of goat medicine and *C. burnetii* experts at the University of Guelph and the OMAFRA were identified. Expert opinion and the provincial goat producer association identified additional publications not captured by the initial search. If no further publications were identified, speculative knowledge using experts on Coxielllosis in goats was solicited to identify parameter estimates not reported in retrieved publications.

### 2.2 DATA EXTRACTION AND PARAMETER ESTIMATE ASSUMPTIONS

Relevant publications were reviewed and expert opinions were solicited for the purpose of identifying financial and epidemiologic parameter estimates, including baseline demographic data. Financial and epidemiologic parameters were extracted into an electronic spreadsheet database from relevant publications and expert opinion: (i) the exposure to *C. burnetii* (incidence or prevalence, differentiating where possible, between acute clinical and sub-clinical chronic disease), (ii) the range, severity, and incidence of clinical outcomes, (iii) prophylactic practices, (iv) and the associated financial costs of disease and baseline costs on Ontario goat farms (Table 1). Together, these data sources determined the parameter estimates for the model. Where appropriate and available, parameters for dairy and meat goat herds were extracted separately to reflect the difference in management.

Statistics Canada (2006) reported 2,169 goat herds in Ontario. OMAFRA (2010) reported 254 goat milk shippers with no significant change in herd numbers since 2006. Therefore, it was assumed that 1,915 herds were meat producers. No formal data has been collected on herd size or structure for either industry, but the
reported goat population in Ontario continues to grow (OMAFRA, 2011). Recent reports (OMAFRA, 2010; OMAFRA, 2011) on annual milk and live kid sales allowed us to estimate an average of 152 dairy animals and 19.6 meat goats per herd. We assumed that 60% of the entire dairy goat herd were breeding animals compared to 70% of meat goats, and therefore at risk of a *C. burnetii*-attributed reproductive event.

Any diagnostic method outlined by OIE (2012) was accepted as means of evaluating the level of *C. burnetii* infection, due to the lack of gold standard for diagnosis. Immune control of *C. burnetii* infection is T-cell dependent leading to granuloma formation and chronic infection (Raoult et al., 2005). Antibody titres (immunoglobulin G) to *C. burnetii* phase I and phase II antigens reflect acute and chronic infection, respectively (Hatchette et al., 2001; Hatchette et al., 2003). Therefore, a significant rise in phase II antibodies was assumed equivalent to the proportion of goats with recent *C. burnetii* exposure. No significant difference in proportion of shedding has been reported between aborting and non-aborting goats (Rousset et al., 2009). Similarly, a high within-herd seroprevalence antibody response has been suggested as evidence of extensive within-herd bacterial circulation (Rousset et al., 2007). Chronically infected goats routinely have seronegative titres (Hatchette et al., 2003) but may continue to shed in subsequent parturitions after initial infection (Berri et al., 2007). Thus, reports of bacterial shedding from any route (vaginal secretions, urine, feces, placental secretions) (Maurin and Raoult, 1999; Angelakis and Raoult, 2010) were also considered evidence of the number of goats with *C. burnetii* infection irrespective of reported paired serological status.

Evidence of endemic exposure to *C. burnetii* has been reported within Ontario goat herds (Lang, 1989; Lang et al., 1991). We hypothesized costs of disease differed between herds with a low and high level of *C. burnetii* infection. We extracted data on level of *C. burnetii* infection or exposure and categorized the data into high and low infection levels. We set low point estimate of animal-level infection risk to be ≤25% of the reported sampled population reflecting endemic
infection. Conversely, highly infected herds (>25%) assumingly reflected those with recent exposure to *C. burnetii* as assessed by seroprevalence or rate of bacterial shedding (Sanford et al., 1994; Berri et al., 2005; Wouda and Dercksen, 2007).

The analysis was stratified according to type of production system. Infection prevalence differs between meat and dairy populations (Lang, 1989; Meadows, personal communication). Herd structure and parameter estimates were modeled according to current dairy (OMAFRA, 2010) and meat production (Agriculture and Agri-food Canada, 2006) systems in Ontario. High and low levels of herd infection were modeled in both production systems.

Scenario analysis (Kahn and Wiener, 1967) was used to define the hypothetical sequence of clinical outcomes from herds infected with *C. burnetii*. Coxiellosis has been reported to be more common among those with antibodies than those that had been exposed and not seroconverted (Spicer et al., 1977; Crowther and Spicer, 1976; Berri et al., 2007). However, only publications or expert opinion on the within-herd risk of clinical reproductive events (abortion, stillbirth, weakborn) were considered for inclusion as a parameter estimate.

The total population lost due to abortion or stillbirth within 24 hours of parturition (Radostits et al., 2009) represented lost potential revenue in live animal sales at the time of weaning. In dairy herds, it was assumed that 50% of weakborn kids would be bucks and, due to their low financial value, these animals would be humanely euthanized at birth and contribute to the total stillbirth population. In meat herds, viable weakborns can still be marketed at the time of weaning such that they would not be euthanized at birth. For dairy doelings and all meat kids, we assumed that being weakborn led to an increased time to weaning (>70 days) or market (>90 days), respectively. These were the additional days required to reach a marketable weight. The increased days to weaning or market makes no consideration for additional days open or feed required for does with weakborn kids. Meat goats are typically pasture raised in Ontario, so no direct cost to rearing was assumed up to weaning. In contrast, dairy kids are raised in pens and bottlefed
prior to transition to a solid diet. The meat and dairy kid populations were assumed to be sold off-site at the time of weaning with no additional costs incurred.

Recent provincial herd-level estimates (OMAFRA, 2010, 2011) reported an average of 650kg per lactation in milking does. In dairies, animals that abort were assumed to never enter milk production and were sold shortly after parturition. In both meat herds and dairies, it was assumed that all animals with reproductive events due to Coxiellosis were culled for slaughter and yearling replacements were purchased at either the completion of the lactation in dairy goats or after weaning in meat goats. We discounted the expenditure of replacement values by an assumed 15% to reflect the gain in profit from cull animal sales. This is the average value of a culled mature goat for slaughter (Ontario Goat, 2010).

In both meat and dairy herd models it was assumed that all animals in both high and low level of infection and at risk of clinical disease (e.g. breeding age animals) were prophylactically treated twice with oxytetracycline prior to parturition to control Coxiellosis (Radostits et al., 2009). Direct veterinary care is not required for the purchase of oxytetracyline in Ontario as long as the producer has established a valid veterinary-client-patient-relationship (VCPR). Therefore, no cost of veterinary care was included in prophylaxis. Dosage (0.7 m.g./k.g.) was calculated according to Plumb (2011) for average adult meat (126 k.g.) and dairy goat (77 k.g.) weights according to market reports (Ontario Goat, 2011). However, we assumed professional veterinary care and a herd-level diagnostic evaluation of aborted fetuses occurred one time when reproductive disease was present. This was to confirm C. burnetii as the primary etiological agent responsible for reproductive disease, which must be distinguished for appropriate disease management (Cai et al., 2010). The estimates for veterinary and diagnostic fees reflected current costs incurred by clients of the Ontario Veterinary College Ruminant Field Service. A homogeneously mixing population was assumed.

When more than one value was reported for a parameter, a weighted mean and, where appropriate, a standard error (SE) based on the number of included animals in the study was estimated prior to stochastic modeling. The parameter
estimates were programmed in Excel and simulated using the add-In program @RISK (Palisade Corp, Newfield, NY). All parameter estimates from the combination of peer-reviewed publications, grey literature, and expert opinion were fit with PERT probability distributions except for fixed parameters for days to weaning, milk production, cost of rearing (meat goats) and oxytetracycline parameters (Table 1). A version of the beta distribution, PERT distributions were necessary to account for divergent evidence with a range of uncertainty about the parameter estimate variables (Vose, 2000). The outcomes of the parameter estimate distributions, representing total uncertainty that is a combination of variation and uncertainty, were minimum, maximum, and most-likely values are defined (Table 1).

2.3 MODEL OF HERD-LEVEL COSTS

Each model-parameter’s stochastic distribution was an input to estimate herd-level parameters, representing the effects of Coxiellosis for dairy and meat herds (Equations 1-13; Table 2). Herd-level financial losses (HFL) were assumed to result from abortions, stillbirths, increased days to weaning of weakborn kids, reduced milk, and live-animal sales. Similarly, we assumed herd-level expenditures (HFE) to include prophylactic control of clinical disease in all pregnant females pre-partum with antibiotics (oxytetracycline), the purchase of yearling replacement animals from individuals culled due to Coxiellosis, and diagnostic and veterinary expenses. The HFL and HFE were calculated for both low and highly infected herds:

\[ HFL_{ij} = \sum_{i=1}^{n} X_{ij} \nu_j \]  
\[ HFE_{ij} = \sum_{i=1}^{n} \Upsilon_{ij} \nu_j \]

where \( X_{ij} \) and \( \Upsilon_{ij} \) are the input parameters on each herd-level loss or expenditure parameter estimate (\( i \)) in meat or dairy herds (\( j \), respectively. These parameters were multiplied by the corresponding financial parameter estimate, \( \nu_j \) (Table 3).

Total cost (\( C \)) for dairy and meat herds with high and low infection prevalence were estimated assuming overhead and variable costs to be constant.
were a summation of losses \( (L) \) and expenditures \( (E) \). Mathematically, this is represented as: \( C = L + E \) (Otte and Chilonda, 2000). The external market or consumer effects on goat production were also ignored. The estimated direct herd level financial cost of exposure to \( C. \ burnetii \) and Coxiellosis in herds was therefore:

\[
C_{ij} = HFL_{ij} + HFE_{ij} \quad \text{[Equation 16]}
\]

All financial parameters, \( HFL_{ij} \), and \( HFE_{ij} \), were reported in Canadian dollars (CAD).

During the model simulation procedure to estimate total herd-level cost \( (C) \), \( HFL_{ij} \), and \( HFE_{ij} \), Monte Carlo Analysis (MCA) simulations were used for a total of 10,000 iterations. The number of iterations was chosen to achieve stability in the simulation results (Greenland, 2001). On each iteration, a value from each parameter estimate probability distribution was sampled and entered in to Equations 1-13 (Table 2) to give the estimated herd level input parameter for expenditure, loss, and total cost estimates from Equations 14-16. The most-likely value of the probability distribution of the 10,000 iterations and the 2.5\(^{th}\) and 97.5\(^{th}\) percentiles represented the \( HFL_{ij} \), \( HFE_{ij} \), and \( C_{ij} \), with corresponding 95\% confidence intervals (95\% CI) (Doubilet et al., 1985), for each herd structure modeled were reported.

A sensitivity analysis was performed with @RISK using a regression plot for each modeled herd's \( HFL_{ij} \) and \( HFE_{ij} \) on parameter and estimated herd-level input estimates. The relative contributions of the different estimates were expressed as dimensionless, normalized regression coefficients \( (R^2) \). This was to determine the most important estimates contributing to the total cost in each herd model.

3. RESULTS

The direct costs of Coxiellosis at the herd level in Ontario varied based on production sector and within-herd infection using stochastic distribution parameters for uncertain parameter estimator. A limited number of peer-reviewed and grey literature publications, in addition to expert opinion, resulted in high degree of uncertainty based on the distributional spread about the most-likely
estimate of the cost of exposure and subsequent clinical Coxielosis in Ontario goat herds (Figure 1, 2; Appendix 5).

From the RER, 246 publications were identified as potentially relevant after de-duplication. From screening the titles and abstracts, 22 full manuscripts were retrieved for further review. Only five descriptive cross-sectional peer-reviewed publications met the full inclusion criteria. Exposure to *C. burnetii* was reported as seroprevalence at the animal level within goat farms in four publications (Lang, 1988, 1989; Hatchette et al., 2001, 2002) and a single publication reported the range and severity of Coxiellosis (Sanford et al., 1994). All peer-reviewed publications included Ontario specific data except two (Hatchette et al., 2001, 2002) where data from Newfoundland was reported. One publication (Delgado-Pertinez et al., 2009) was identified from reference review and reported cost of raising dairy kids in Florida as no Ontario was available. Two additional peer-reviewed publications that reported incidence of clinical reproductive events in French goats were included (Berri et al., 2005, 2007) as no Ontario or Canadian data were identified for parameter estimates. One publication (Lang et al., 1991) from reference review reported meat sheep seroprevalence and we assumed it to be equivalent to that in meat goats. Additional publications offering epidemiologic and financial parameter estimates were identified in a systematic search of grey literature (Agriculture and Agri-food Canada, 2006; OMAFRA, 2009, 2010, 2011; Ontario Goat, 2010)(Table 1). No publications explored the financial or production effects of *C. burnetii* infection or clinical disease. Parameter estimates were extracted and stochastic distributions were modeled from relevant publications (Table 1).

For each herd modeled, total cost (*C*) was estimated from the sum of HFL*ij* and HFE*ij* (Table 4) in one year. In dairy herds with low level of exposure to *C. burnetii* (12.7% (95%CI: 11.8 to 30%)), expenditures were greater than losses amounting to a total cost of $2,667 (95%CI: $2,363 to 2,983)(Table 4; Figure 1a). The largest contribution to cost (39.3%) was loss of milk production ($1,049 (95%CI: $850 to 1,262)). Expenses from the replacement of culled animals ($896 (95%CI: $807 to 988)) represented 33.6% of the total cost.
The cost to producers on dairy farms with a high level of *C. burnetii* (61.5% (95%CI: 55.4 to 67.6%)) was estimated at $9,197 (Table 4; Figure 1b). Although losses accounted for 58.7% of the total cost, 55.3% ($5,088 (95%CI: $4,078 to 6,186)) was due to lost milk production. Similarly, the herd level expenditures from the replacement of culled animals ($3,167 (95%CI: $2,452 to 4,000)) contributed 34.4% to the total cost.

In meat herds with a low level of exposure estimated at 24% (95%CI: 21.6 to 26.4%), the cost at the herd level was estimated at $1,418 (Table 4; Figure 2a). The expenditures with diagnosing Coxiellosis were 41.6% ($590) of the total cost of disease. Further expenses to replace culled adults ($327 (95%CI: $290 to 367), in addition to estimates of total losses due to abortions ($263 (95%CI: $207 to 321) represented 30.2% and 18.5%.

In contrast, a meat goat herd with a high level of *C. burnetii* exposure (61.5% (95%CI: 57.8 to 62%)) would most-likely cost a producer $3,487 (Figure 2b). The potential revenue lost due to stillbirth ($1,295 (95%CI: $839 to 1,960)) and abortion ($673 (95%CI: $431 to 983)) accounted for 37.1% and 19.3% of total estimated costs. However, 24.1% of costs were due to expenditures to replace culled does ($839 (95%CI: $695 to 1,027).

The cost of diagnosing Coxiellosis with an attending veterinarian and subsequent submission of samples (including placenta and fetuses from abortions, and pooled bulk tank milk (BTM) or serum samples) was constant across all herds modeled ($590.20 (95%CI: $553.20 to 627.34)).

The most important (highest regression coefficients) components in the sensitivity analysis were milk production losses and the total number of breeders in expenditure estimations for both high and low prevalence dairy herds. The average number of kids born per doe per year was most important in estimating losses in meat goat herds. The number of veterinary visits for expenditures in those with low prevalence had the highest regression coefficient, whereas the impact on
expenditures from the number of meat doe breeders in the herd was most important for those with a high prevalence of infection (Appendix 5).

4. DISCUSSION

This is the first study on the herd-level direct financial costs associated with *C. burnetii* infection and subsequent effects of Coxiellosis in Ontario dairy and meat goat farms. Our results estimated the substantial financial burden with variable degrees of uncertainty about the most-likely estimates of losses and expenditure outcomes. This may be reflective of the limited availability of data, or the heterogeneity of sources and types of data for parameter estimates used in the models.

There was limited data available on Coxiellosis within Ontario to substantiate input parameter estimates. Inferences to the provincial or national goat herd should be guarded. Parameter estimates may have biased the reported financial losses and expenditures due to an over or underestimation of the true effect in the Ontario goat population. A recent qualitative critical review (Guatteo et al., 2011) identified two publications reporting within-herd data from Ontario goats included in this study (Lang, 1989; Hatchette et al., 2002). Guatteo et al. (2011) suggested that these publications had methodological deficiencies leading to questionable results due to the method of study population selection and lack of random sampling. It is possible that the allowance of sample selection bias may have increased the uncertainty of seroprevalence estimates (Copas and Li, 1997), and therefore the external validity of the data. Under- or overestimation of the seroprevalence could not be determined based on reported data. However, a current seroprevalence survey of meat and dairy goat farms in Ontario has estimated within-herd seroprevalence of *C. burnetii* exposure to be at least equivalent to the results previously reported (Meadows, personal communication).

To supplement publications and grey literature, expert opinion contributed additional parameters based on experience and knowledge of market conditions. In a field such as livestock health economics, with limited published data, the use of
expert information is a rapid and cost effective means of making an informed prediction about the cost of disease (Martin et al., 2005). The addition of expert knowledge in stochastic analyses has been shown to be sufficient for outcome prediction with lower variability about estimates (Jackson et al., 1999; Martin et al., 2005).

As with any financial analysis, we are aware that the overall estimated costs and their associated losses and expenditures, distributions used, references, and time scale can be questioned. However, we attempted to reduce bias of data entry into the analysis by the application of inclusion criteria specified a priori to publications and grey literature to identify parameter estimates following a systematic and transparent methodology. Given the data and resources available to quantify *C. burnetii* exposure in Ontario goats, only direct costs were considered; not the wider economic impacts. Even when the effects of infection and subsequent clinical disease can be quantified with explicit market values (e.g. value associated with a k.g. of meat, or litre of milk), the prices may be distorted to some degree (e.g. seasonal demand for goat products) and therefore not represent true economic values. Markets and associated prices can also be affected by external factors (e.g. international trade) having distributional implications within the industry.

Data to inform key parameter estimates required for the financial assessment were commonly lacking, inadequate, or unreliable. In particular, disease incidence, the magnitude and distribution of disease effects (e.g. the percentage of animals affected with one form of reproductive effects), and the prophylactic measures on-farm were challenging to identify. Methods in the literature were heterogeneous, being based on different time periods, livestock populations, geographical areas, and methods of diagnosis. Case reports were more common than random sampling of animals or herds (Sanford et al., 1994; Hatchette et al., 2002; Berri et al., 2002, 2005a) and publications reporting seroprevalence were published 20 to 25 years ago (Lang et al., 1988, 1989; Sanford et al., 1994). Expert opinion and grey literature with no reported methods of data collection (OMAFRA 2008, 2009, 2010, 2011; Agriculture and Agri-food Canada, 2006, 2011; Ontario
Goat, 2010) were also available. Therefore, assumptions had to be made. The uncertainties of reported data for both epidemiologic and financial parameter estimates led to the use of stochastic methods. This method also accounted for the complexities of disease impact on production with limited data.

Not all published data were suitable for inclusion in the model or were unavailable. This led to the inclusion of two publications reporting sheep-specific data in the parameter estimates (Lang et al., 1991; Berri et al., 2005b). Small ruminants are commonly grouped for disease evaluation due to their similar interactions with, and immunologic response to *C. burnetii*, despite differences in clinical disease expression (Palmer et al., 1984; Lang, 1990; Berri et al., 2007). Further data in goats are required to refine these estimates.

The stochastic nature of the estimates is more sophisticated than deterministic models as it relies on various data sources to construct probability distributions of financial deficits. The nature of this analysis relied on various data inputs to construct probability distributions and model the variability about the parameter estimates. By using Monte Carlo methods, it was possible not only to quantify herd-level costs of Coxiellosis on Ontario goat farms, but also, by including variability, provide a reasonably accurate range within which true costs may exist. Although the modeled variability implicit in the experimental and observational data used represents an important methodological advance over deterministic methods, the disadvantage of a stochastic approach are the sources of data.

Methodological differences of *C. burnetii* diagnostics and reporting of data made estimating the true level of exposure challenging. The relationships amongst exposure to *C. burnetii*, seroconversion, and expression of clinical disease are complex (Hatchette et al., 2001, 2002; Rousset et al., 2007). Evidence of exposure to infection does not always relate to the development of clinical disease. Conversely, the expression of clinical signs does not indicate that antibodies are present (Hatchette et al., 2001; Arricau-Bouvery et al., 2005; Berri et al., 2005; Rousset et al., 2008, 2009; de Cremoux, 2009; Hogerwerf, 2011) due to the cell mediated immune dependency. However, a high seroprevalence is suggestive of recent infection
(Rousset et al., 2007; Raoult et al., 2005) and the risk of clinical disease is higher in newly infected herds as opposed to those that have persistent (low) level of exposure due to chronic shedding (Berri et al., 2007; Rousset et al., 2007). Within newly infected herds experiencing reproductive problems due to *C. burnetii*, most animals may be shedding high levels of bacteria whether or not they have developed signs of Coxiellosis (e.g. abortion)(Rousset et al., 2009). Persistent shedding in heavily infected herds may exhibit a highly positive serologic profile (Guatteo et al., 2007). Thus, the total quantity of *C. burnetii* within a farm with a high level of exposure is greater than within a herd with a low level of exposure to infection (OIE, 2012). A high within-herd exposure risk significantly increases the probability of shedding from multiple routes (vaginal secretions, milk, feces)(Rousset et al., 2009).

Once infection is present within a herd, animals can remain chronically infected, become seronegative, but persistently shed the bacteria thereby spreading infection to the remaining seronegative animals in a herd (e.g. young stock)(Wisniewski and Krumbiegel, 1970; Berri et al., 2007). Our assumption that high seroprevalence was associated with recent infection leading to clinical disease may have resulted in an underestimation of the burden of infection in herds. However, lack of research on the relationship between prevalence of infection and clinical effects precludes our ability to accurately model the relationship between seroprevalence, disease prevalence, and exposure to infection resulting in substantial model assumptions.

If the reported seroprevalence overestimated the true risk of infection in goats in Ontario, it is reasonable to conclude that the estimates of costs incurred would be no greater than the total uncertainty distribution assigned to each herd level parameters and associated total cost, with all other parameters remaining constant. Conversely, if the seroprevalence estimates used, including the variability around the estimates, represented a reasonably accurate range under which true substantial costs lie, then further investigations into the benefits of Coxiellosis interventions to reduce clinical disease should be sought. In either case, observed
Expenditures and losses would provide more accurate assessment of the financial burden to the industry.

The lack of appropriate data for estimation of direct disease cause has identified important and specific areas where further information and data collection should be sought. This is also highlighted by the difference in costs between dairy and meat herds, and between those with high and low seroprevalence of infection. An initial understanding of the potential cost of Coxiellosis in the Ontario goat industry may also add value to the magnitude of the benefits that could be gained by eliminating or reducing Coxiellosis (Howe, 1991; McInerney, 1996). Models presented in this study provide an understanding of the financial impacts of Coxiellosis in goats. In addition, areas where data were deficient have been highlighted, and preliminary estimates of direct disease costs and associated uncertainty have provided information on the magnitude on potential benefits of disease control. The study can provide an important basis upon which higher quality disease information and parameter estimates can be imputed if higher quality data comes available.

The difference in estimated costs observed between dairy and meat herds may be explained by the low and high-level exposure to \textit{C. burnetii} used to estimate the level of infection within a herd. Although the most-likely estimate for dairies was less than that of meat herds when exposure was low, the variability about the point estimate was much greater despite being right-skewed. Similar distributions were observed for both industries exposure was high. The estimated herd size may also contribute to the substantial differences reported and may explain the parameter’s high regression coefficient in all models (Appendix 5). Despite the differences, the greatest contribution to total cost for dairies, regardless of level of exposure, was lost revenue from fluid milk sales in lactating does.

In contrast, the greatest contributions to costs in meat herds were loss of potential income from unrealized animal sales at weaning from stillbirths and abortion when the \textit{C. burnetii} exposure was high, and diagnostic expenses on those farms with exposure was low. Irrespective of the type of industry or the prevalence
of disease, the assumed expenses incurred to replace culled animals due to clinical Coxiellosis represented the second greatest contribution to overall cost at the herd level. This may be attributed to the purchase of yearling does which, on average, cost more than their mature counterparts (OMAFRA, 2010, 2011; Ontario Goat, 2010) due to the increased longevity and production potential within the herd. However, if naïve to C. burnetii infection, these animals may lead to greater clinical disease in the year subsequent to herd entry (Berri et al., 2002; Berri et al., 2005). This may be a reason for sustained clinical disease at an endemic level in Ontario. Replacement costs may be lower if all clinically affected animals are not culled and replaced, but if shedding persists in those with clinical signs, significant environmental contamination may persist (Rousset et al., 2009) as was potentially reflected in herds with low exposure to infection.

The entry of naïve animals into an infected herd (dairy or meat) represents a significant challenge to the control and prevention of C. burnetii infection and clinical signs of Coxiellosis. As no effective method of intervention for goats has been reported in the literature to eliminate shedding of the bacterium (O’Neill et al., to be published), antimicrobial therapy may be the only means of within-herd control of clinical disease. However, limited evidence supports this recommendation (EFSA, 2010). In a single case report of a sheep flock experiencing an outbreak of Coxiellosis, oxytetracyline was reported to suppress abortions and shedding, but not eliminate either (Berri et al., 2005b). If culled goats have persistent shedding despite prophylaxis, without notification of disease status to other buyers at auction there is potential to spread the infection throughout the provincial herd. Thus, as financial losses in dairy and meat herds have been shown to be substantial, priority should be paid to reduce the risk of exposure to C. burnetii and alternative control methods should be sought.

Abortion is substantially more important as a clinical manifestation of infection compared to weakborn kids (Palmer et al., 1983). This is supported by evidence from financial losses associated with raising weakborn kids to marketable weaning weights contributing little to the overall cost of infection. The average cost
of rearing per day to weaning weight was estimated to be low on both dairy and meat operations. Costs incurred were attributed to additional feed required to grow kids to marketable weaning weights. In dairies, this may include both grain products and additional milk after 70 days for weakborn doelings. The cost of rearing was assumed to have no direct financial impact up to 90 days in meat kids. However, additional feeds to reach market weight may be used to supplement pasture beyond this time. Further epidemiologic data on the impact of Coxiellosis to production parameters would greatly contribute to the accuracy of estimated financial costs. Expenditures for treatment of the breeding animals with oxytetracyline, an inexpensive antimicrobial, were also limited despite the unaccounted for time capital required to treat all animals at risk of clinical effects (e.g. those at risk of parturition).

The model structure contained simplified representations of the main effects of Coxiellosis and did not account for complexities of the disease within animals and herds. The seasonal effects of price fluctuation in the market driven by cultural and holiday demands for goats and goat products in Ontario (OMAFRA, 2010) were not modeled. Lastly, alternative approaches to goat production such as small-scale production, organic farming, and accelerated breeding programs were not identified independent of the stratified production system (meat or dairy) analyzed in this study. In addition, as McInerney et al (1992) recognized, a true measure of economic costs associated with disease should also account for the true economic value of disease impact (e.g. effect of disease on human health). These were not explicitly modeled and may represent unknown or hidden costs associated with Coxiellosis. Due to data limitations for Q fever in humans in Ontario, conceptual and practical difficulties preclude our ability to consider the total economic value of the disease.

This study reports the financial costs attributed to Coxiellosis in Ontario goats, not avoidable losses or costs that could be saved as a result of appropriate intervention strategies, and therefore only a part of a complete economic analysis has been presented. Although total costs attributed to losses and expenditures were estimated, only inclusion of avoidable costs would provide sufficient information
about the financial burden of disease. However, the interventions to prevent clinical disease in goats are limited (EFSA, 2010). A vaccine (Coxevac, CEVA Sante Animale, France), not licensed in Ontario, does not eliminate shedding from goats based on limited data, maintaining a high risk of transmission within and between herds (O’Neill et al., to be published).

Prevention of clinical disease may be achieved with appropriate interventions, but will not eliminate costs even if the herd is free of infection. Although a disease negative reference herd was not modeled, if prevention strategies (e.g. biosecurity, manure management, vaccination) are to be modeled in the future it will be necessary to report a counterfactual population to those herds infected. However, Bennet (2003) suggested that the presence of disease in the total population might result in lower market prices, thereby impacting herd-level income regardless of disease status. Greater market effects in a more complex model (e.g. cost-benefit analyses) when further data are available would be beneficial to explore this possibility. The estimates presented should be sufficient evidence for policy makers in the area of animal health to recommend further research in to C. burnetii in Ontario goats.

Cost-effective means of control should be achievable. The additional benefits of reduced disease and increased productivity may help to offset the additional costs of control and prevention. C. burnetii infection is suspected to have a clustered distribution of herd level infection (Lang, 1989). Therefore, some farms would require little or no control or prevention, with a correspondingly greater return on the investment potential on the farms with higher than average infection risks. In contrast, the general level of infection on certain farms may be relatively low, so control and prevention may only produce an economic return if it were targeted at high prevalence groups such as young stock (Berri et al., 2005). For example, if the total costs of preventative measures are greater than the total cost of disease, it may not be financially viable to eliminate within-herd infection. Reducing exposure to infection may also be challenging due to the breadth of species infected with C.
burnetii capable of transmission (Arricau-Bouvery and Rodolakis, 2005; EFSA, 2010; Lang, 1990; Maurin and Raoult, 1999).

Goats infected with C. burnetii and shedding are commonly associated with disease in humans (Kovacova et al., 1998; Serbezov et al., 1999; Hatchette et al., 2001, 2003), disease control for public health purposes should be considered. The total cost of C. burnetii as a zoonotic agent may be a necessary component of further public health decision making for disease prevention and control. Reported estimates on the total average cost of human Q fever to society was $1 million Australian dollars (1991-1994), and amounted to 1,700 weeks of work time annually (Garner et al., 1997). Quantifying the total cost of disease to both humans and livestock will further aid in the understanding of financial benefits to all sectors affected with Q fever and may better inform targeted surveillance and prevention programs (Simor, 1987). Reductions in the epidemic zoonotic risks associated with goats in Ontario may be subsequently realized. The benefits of reduced disease and increased productivity of animals and humans may offset the total cost of prevention. However, the costs of an intervention program, such as a vaccination scheme, will depend on the initial prevalence of disease. Further baseline epidemiologic data on goat production, and costs associated with C. burnetii infection and clinical disease will aid in understanding the financial burden Coxiellosis imparts on the Ontario goat industry. Knowledge of the financial effects of Coxiellosis within a herd may also lead to targeted risk assessments, which could impact decisions to implement, or not implement a given control or prevention program (Guatteo et al., 2011).

The results of this study provide a potentially useful indication of the substantial financial impact of infection and Coxiellosis in Ontario dairy and meat goat herds with high or low level of exposure to C. burnetii infection and associated reproductive effects. The models have provided an output that is easily communicated to veterinarians, producers, and policy makers. Although the parameter estimates were based on multiple sources, uncertainty was modeled with stochastic measures. For a more comprehensive assessment of the impact of disease,
further within and herd-level data needs to be collected in Ontario. This data will also help design appropriate and cost-effective control programs designed to reduce the disease burden within individual farms. Additional epidemiologic and financial data on the Ontario goat industry structure, herd size and structure, level of individual infection within a herd, possible differences in *C. burnetii* virulence, method of animal or herd selection, impact of control programs, and more accurate financial and epidemiologic parameters will assist to refine parameter estimates, thereby reducing uncertainty around outcomes. This should provide a more accurate financial assessment of Coxiellosis on Ontario goat farms.

**ACKNOWLEDGEMENTS**

The authors acknowledge the assistance of Anita O’Brien, Ontario Ministry of Agriculture, Food and Rural Affairs for assistance with publication acquisition and expert consultation; Shannon Meadows (Ph.D. candidate, Department of Population Medicine, Ontario Veterinary College) and Dr. Paula Menzies D.V.M., M.P.V.M., Dip. ECSRHM (Associate Professor, Small Ruminant Research Co-ordinator, Ruminant Health Management Group, Ontario Veterinary College) for Q fever biology consultations; Jane Burpee (Librarian, University of Guelph) for assistance with scoping study thesaurus and database. Editorial recommendations from Drs. Andria Jones-Bitton D.V.M., M.Sc., Ph.D. (Assistant Professor, Department of Population Medicine, Ontario Veterinary College) and Olaf Berke M.Sc., Ph.D. (Associate Professor of Statistical Epidemiology, Department of Population Medicine, Ontario Veterinary College) was greatly appreciated.

**FUNDING**

Dr. Tyler J. O’Neill was a Graduate Veterinary Fellow funded by the Ontario Veterinary College Graduate Fellowship, developed for Canadian or permanent resident veterinarians to pursue graduate research training at the University of Guelph.
A Canadian Institute of Health Research Institute of Population and Public Health/Public Health Agency of Canada Applied Public Health Research Chair supported Dr. Jan M. Sargeant.
REFERENCES


Greenland S. Sensitivity analysis, Monte Carlo risk analysis, and Bayesian uncertainty assessment. Risk Analysis 2001:21(4);579-84.


Hogerwerf L, van den Brom R, Roest HJ, Bouma A, Vellema P, Pieterse M, Derksen D, Nielen M. Reduction of *Coxiella burnetii* prevalence by vaccination of goats and sheep, the Netherlands. EID 2011;17(3);379-86.


Martin TG, Kuhnert PM, Mengersen KL, Possingham HP. The power of expert opinion in ecological models using Bayesian methods: impact on grazing birds. Ecological Applications 2005:15(1);266-80.


Meadows S, personal communication, 15 July 2012.


Sanford SE, Josephson GK, MacDonald A. Coxiella burnetii (Q fever) abortion storms in goat herds after attendance at an annual fair. Can Vet J 1994:35(6);376-8.


Wouda W, Derksen DP. Abortion and stillbirth among dairy goats as a consequence of *Coxiella burnetii*. Tijdschr Diergeneesk 2007:132(23);908-11.
Table 1. Parameter estimates for epidemiologic and financial costs of Coxiellosis in Ontario goat herds identified in the rapid evidence review (RER) and expert opinion.

<table>
<thead>
<tr>
<th>Epidemiologic parameter estimates</th>
<th>Minimum</th>
<th>Most-likely or Mean</th>
<th>Maximum</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of breeders, dairy herd (N&lt;sub&gt;d&lt;/sub&gt;)</td>
<td>74.72</td>
<td>91.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.5</td>
<td>OMAFRA, 2009, 2010, 2011</td>
</tr>
<tr>
<td>Number of breeders, meat herd (N&lt;sub&gt;m&lt;/sub&gt;)</td>
<td>12.35</td>
<td>13.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.09</td>
<td>OMAFRA 2009, 2010, 2011</td>
</tr>
<tr>
<td>Low within dairy herd C. burnetii exposure (γ&lt;sub&gt;low&lt;/sub&gt;)</td>
<td>0.12</td>
<td>0.13</td>
<td>0.3</td>
<td>Lang et al., 1988, 1989; Hatchette et al., 2001; Meadows, personal communication</td>
</tr>
<tr>
<td>High within dairy herd C. burnetii exposure (γ&lt;sub&gt;hi&lt;/sub&gt;)</td>
<td>0.55</td>
<td>0.62</td>
<td>0.68</td>
<td>Lang et al., 1988, 1989; Hatchette et al., 2001; Meadows, personal communication</td>
</tr>
<tr>
<td>Low within meat herd C. burnetii exposure (γ&lt;sub&gt;m,low&lt;/sub&gt;)</td>
<td>0.21</td>
<td>0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.26</td>
<td>Sanford et al., 1994; Lang et al., 1991; Hatchette et al., 2002; Meadows, personal communication</td>
</tr>
<tr>
<td>High within meat herd C. burnetii exposure (γ&lt;sub&gt;m,hi&lt;/sub&gt;)</td>
<td>0.58</td>
<td>0.62&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.62</td>
<td>Sanford et al., 1994; Lang et al., 1991; Hatchette et al., 2002; Meadows, personal communication</td>
</tr>
<tr>
<td>Abortion incidence (α)</td>
<td>0.24</td>
<td>0.26</td>
<td>0.29</td>
<td>Berri et al., 2005, 2007; Meadows, personal communication</td>
</tr>
<tr>
<td>Stillbirth incidence (ε)</td>
<td>0.14</td>
<td>0.15</td>
<td>0.17</td>
<td>Berri et al., 2005</td>
</tr>
<tr>
<td>Weanborn prevalence (ω)</td>
<td>0.07</td>
<td>0.08</td>
<td>0.088</td>
<td>Berri et al., 2005, 2007; Meadows, personal communication</td>
</tr>
<tr>
<td>Average kidding per year (β)</td>
<td>1.4</td>
<td>1.9</td>
<td>2.4</td>
<td>OMAFRA, 2010</td>
</tr>
<tr>
<td>Weaning weight dairy kid (kg) (σ&lt;sub&gt;d&lt;/sub&gt;)</td>
<td>10.35</td>
<td>11.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.65</td>
<td>Menzies, personal communication; OMAFRA, 2010</td>
</tr>
<tr>
<td>Weaning weight meat kid (kg) (σ&lt;sub&gt;m&lt;/sub&gt;)</td>
<td>16.58</td>
<td>18.34</td>
<td>19.21</td>
<td>Ontario Goat, 2010&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Days to weaning dairy kid (t&lt;sub&gt;d&lt;/sub&gt;)</td>
<td>NA</td>
<td>70</td>
<td>NA</td>
<td>Menzies, personal communication</td>
</tr>
<tr>
<td>Days to weaning meat kid (t&lt;sub&gt;m&lt;/sub&gt;)</td>
<td>NA</td>
<td>90</td>
<td>NA</td>
<td>OMAFRA, O’Brien, personal communication</td>
</tr>
<tr>
<td>Average annual milk production per doe (ρ)</td>
<td>NA</td>
<td>650</td>
<td>NA</td>
<td>OMAFRA, 2010</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Financial parameter estimates</th>
<th>Minimum</th>
<th>Most-likely or Mean</th>
<th>Maximum</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$ per kg at weaning, dairy</td>
<td>1.01</td>
<td>1.12</td>
<td>1.23</td>
<td>OMAFRA, 2008</td>
</tr>
<tr>
<td>$ per kg at weaning, meat</td>
<td>4.46</td>
<td>4.96</td>
<td>1.23</td>
<td>Agriculture and Agri-food Canada, 2011</td>
</tr>
<tr>
<td>Cost of rearing per day until weaning, dairy kids ($)</td>
<td>0.46</td>
<td>0.51</td>
<td>0.56</td>
<td>OMAFRA, 2008; Delgado-Pertinez et al., 2009</td>
</tr>
<tr>
<td>Cost of rearing per day until weaning, meat kids ($)</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>OMAFRA, O’Brien, personal communication</td>
</tr>
<tr>
<td>Value of replacement per animal, dairy ($)</td>
<td>153.25</td>
<td>170.28&lt;sup&gt;e&lt;/sup&gt;</td>
<td>187.31</td>
<td>Agriculture and Agri-food Canada, 2011</td>
</tr>
<tr>
<td>Value of replacement per animal, meat ($)</td>
<td>180</td>
<td>200&lt;sup&gt;f&lt;/sup&gt;</td>
<td>220</td>
<td>OMAFRA, 2010; Ontario Goat, 2010</td>
</tr>
<tr>
<td>$/L milk shipped</td>
<td>0.71</td>
<td>0.79</td>
<td>0.87</td>
<td>Agriculture and Agri-food Canada, 2006; OMAFRA, 2009, 2010</td>
</tr>
<tr>
<td>Cost of oxytetracycline/mL</td>
<td>NA</td>
<td>0.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Oxytetracycline dosage (mL), dairy (κ&lt;sub&gt;d&lt;/sub&gt;)</td>
<td>NA</td>
<td>11&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Oxytetracycline dosage (mL), meat (κ&lt;sub&gt;m&lt;/sub&gt;)</td>
<td>NA</td>
<td>18&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Number of DVM visits per herd level abortion events (μ)</td>
<td>0.96</td>
<td>1&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Cost of abortion diagnostics (υ)</td>
<td>553.20</td>
<td>590.20&lt;sup&gt;g&lt;/sup&gt;</td>
<td>627.34</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Assumes 60% of average herd size are breeding animals based on provincial estimate of total herd size (N=152)(OMAFRA, 2009; OMAFRA, 2011)
<sup>b</sup>Assumes 70% of average herd size are breeding animals based on provincial estimate of total herd size (N=19.6)(OMAFRA, 2009; OMAFRA, 2011)
<sup>c</sup>Ontario Red Meat Sheep Program (Lang et al., 1991) assumed to be equivalent to individual animal-level prevalence in meat goat farms due to lack of goat-specific literature
<sup>d</sup>Estimated price of doeling at time of weaning
<sup>e</sup>Weekly weighted market weight average for all provincially reported sales locations (n=5) for weaned meat kids in 2010
<sup>f</sup>Producer expenses from Ruminant Field Services, Ontario Veterinary College, University of Guelph and associated laboratory fees at the Animal Health Lab, University of Guelph
<sup>g</sup>Reflects value of animals in first lactation discounted by 15% to reflect the price of replacements less the profit from cull animals sales at slaughter
Table 2. Herd-level input parameter estimations using within-herd parameter estimates to calculate herd level financial loss and expenditures in Ontario dairy and goat farms.

<table>
<thead>
<tr>
<th>Input parameter</th>
<th>Formula</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dairy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Losses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillbirth proportion</td>
<td>$X_1 = \left( N_d \gamma_d \varepsilon \beta \sigma_d \right) + \left( N_d \gamma_d \omega (\beta/2) \right) \sigma_d$</td>
<td>[Equation 1]</td>
</tr>
<tr>
<td>Abortion proportion</td>
<td>$X_2 = N_d \gamma_d \alpha \beta \sigma_d$</td>
<td>[Equation 2]</td>
</tr>
<tr>
<td>Increased days to weaning</td>
<td>$X_3 = N_d \gamma_d \omega (\beta/2) \tau_d$</td>
<td>[Equation 3]</td>
</tr>
<tr>
<td>Lost milk production (kg)</td>
<td>$X_4 = N_d \gamma_d \alpha \rho$</td>
<td>[Equation 4]</td>
</tr>
<tr>
<td><strong>Expenditures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culling affected adults</td>
<td>$Y_1 = (N_d \gamma_d \alpha) + (N_d \gamma_d \varepsilon) + (N_d \gamma_d \omega)$</td>
<td>[Equation 5]</td>
</tr>
<tr>
<td>Treating herd</td>
<td>$Y_2 = N_d \kappa_d$</td>
<td>[Equation 6]</td>
</tr>
<tr>
<td>Diagnostic work-up</td>
<td>$Y_3 = \mu \nu$</td>
<td>[Equation 7]</td>
</tr>
<tr>
<td><strong>Meat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Losses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillbirth proportion</td>
<td>$X_5 = \left( N_m \gamma_m \varepsilon \beta \sigma_m \right) + \left( N_m \gamma_m \omega (\beta) \right) \sigma_m$</td>
<td>[Equation 8]</td>
</tr>
<tr>
<td>Abortion proportion</td>
<td>$X_6 = N_m \gamma_m \alpha \beta \sigma_m$</td>
<td>[Equation 9]</td>
</tr>
<tr>
<td>Increased days to market</td>
<td>$X_7 = (N_m \gamma_m \omega \beta) \tau_m$</td>
<td>[Equation 10]</td>
</tr>
<tr>
<td><strong>Expenditures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culling affected adults</td>
<td>$Y_4 = (N_m \gamma_m \alpha) + (N_m \gamma_m \varepsilon) + (N_m \gamma_m \omega)$</td>
<td>[Equation 11]</td>
</tr>
<tr>
<td>Treating herd</td>
<td>$Y_5 = N_m \kappa_m$</td>
<td>[Equation 12]</td>
</tr>
<tr>
<td>Diagnostic work-up</td>
<td>$Y_6 = \mu \nu$</td>
<td>[Equation 13]</td>
</tr>
</tbody>
</table>

Number of breeders, dairy herd ($N_d$)  
Number of breeders, meat herd ($N_m$)  
Individual seroprevalence in low seroprevalence dairy herd ($\gamma_{d,low}$)  
Individual seroprevalence in high seroprevalence dairy herd ($\gamma_{d,high}$)  
Individual seroprevalence in low seroprevalence meat herd ($\gamma_{m,low}$)  
Individual seroprevalence in high seroprevalence meat herd ($\gamma_{m,high}$)  
Abortion incidence ($\alpha$)  
Stillbirth incidence ($\varepsilon$)  
Weakborn incidence ($\omega$)  
Annual average milk production per doe ($\rho$)  
Average kidding per year ($\beta$)  
Weaning weight dairy kid ($\sigma_d$)  
Weaning weight meat kid ($\sigma_m$)  
Days to weaning dairy kid ($\tau_d$)  
Days to weaning meat kid ($\tau_m$)  
Oxytetracycline dosage (mL), dairy ($\kappa_d$)  
Oxytetracycline dosage (mL), meat ($\kappa_m$)  
Number of DVM visits per herd level abortion events ($\mu$)  
Cost of abortion diagnostics ($\nu$)
Table 3. Stochastic distributions of epidemiological and financial parameters used in the model to estimate the financial cost of Coxiellosis in Ontario goat herds.

<table>
<thead>
<tr>
<th>Input Parameters (prevalence of infection)</th>
<th>Input parameter estimates</th>
<th>Corresponding financial value (CAD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Most likely</td>
</tr>
<tr>
<td><strong>Dairy (low prevalence)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Losses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillbirth ($X_{1,low}$)</td>
<td>21.58</td>
<td>32.63</td>
</tr>
<tr>
<td>Abortion ($X_{2,low}$)</td>
<td>28.26</td>
<td>44.66</td>
</tr>
<tr>
<td>Increased days to weaning ($X_{3,low}$)</td>
<td>14.38</td>
<td>20.53</td>
</tr>
<tr>
<td>Lost milk production ($X_{4,low}$)</td>
<td>808.03</td>
<td>1329.68</td>
</tr>
<tr>
<td><strong>Expenditures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culling affected adults ($X_{5,low}$)</td>
<td>4.46</td>
<td>5.27</td>
</tr>
<tr>
<td>Treating herd ($X_{6,low}$)*</td>
<td>604.85</td>
<td>66899</td>
</tr>
<tr>
<td>Diagnostic work-up ($X_{7,low}$)*</td>
<td>0.91</td>
<td>1</td>
</tr>
<tr>
<td><strong>Dairy (high prevalence)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Losses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillbirth ($X_{1,high}$)</td>
<td>116.81</td>
<td>188.00</td>
</tr>
<tr>
<td>Abortion ($X_{2,high}$)</td>
<td>28.26</td>
<td>44.66</td>
</tr>
<tr>
<td>Increased days to weaning ($X_{3,high}$)</td>
<td>67.17</td>
<td>99.47</td>
</tr>
<tr>
<td>Lost milk production ($X_{4,high}$)</td>
<td>3841.82</td>
<td>6440.812</td>
</tr>
<tr>
<td><strong>Expenditures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culling affected adults ($X_{5,high}$)</td>
<td>15.12</td>
<td>1862</td>
</tr>
<tr>
<td>Treating herd ($X_{6,high}$)*</td>
<td>604.85</td>
<td>66899</td>
</tr>
<tr>
<td>Diagnostic work-up ($X_{7,high}$)*</td>
<td>0.91</td>
<td>1</td>
</tr>
<tr>
<td><strong>Meat (low prevalence)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Losses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillbirth ($\Upsilon_{1,low}$)</td>
<td>14.34</td>
<td>19.31</td>
</tr>
<tr>
<td>Abortion ($\Upsilon_{2,low}$)</td>
<td>33.31</td>
<td>52.90</td>
</tr>
<tr>
<td>Increased days to market ($\Upsilon_{3,low}$)</td>
<td>22.83</td>
<td>35.02</td>
</tr>
<tr>
<td><strong>Expenditures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culling affected adults ($\Upsilon_{4,low}$)</td>
<td>1.32</td>
<td>1.64</td>
</tr>
<tr>
<td>Treating herd ($\Upsilon_{5,low}$)*</td>
<td>223.64</td>
<td>246.97</td>
</tr>
<tr>
<td>Diagnostic work-up ($\Upsilon_{6,low}$)*</td>
<td>0.91</td>
<td>1</td>
</tr>
<tr>
<td><strong>Meat (high prevalence)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Losses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillbirth ($\Upsilon_{1,high}$)</td>
<td>175.10</td>
<td>260.27</td>
</tr>
<tr>
<td>Abortion ($\Upsilon_{2,high}$)</td>
<td>87.34</td>
<td>135.56</td>
</tr>
<tr>
<td>Increased days to market ($\Upsilon_{3,high}$)</td>
<td>61.54</td>
<td>89.73</td>
</tr>
<tr>
<td><strong>Expenditures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culling affected adults ($\Upsilon_{4,high}$)</td>
<td>3.65</td>
<td>4.20</td>
</tr>
<tr>
<td>Treating herd ($\Upsilon_{5,high}$)*</td>
<td>223.64</td>
<td>246.97</td>
</tr>
<tr>
<td>Diagnostic work-up ($\Upsilon_{6,high}$)*</td>
<td>0.91</td>
<td>1</td>
</tr>
</tbody>
</table>

*Herd-level parameter ^Individual-level parameter
Table 4. Estimated herd-level costs based on summation of herd-level losses and expenditures) of exposure to *C. burnetii* infection and subsequent Coxiellosis in one year within dairy and meat goat herds in Ontario.

<table>
<thead>
<tr>
<th>Herd Type</th>
<th>Level of within-herd C. burnetii infection (95%CI)</th>
<th>Herd-level financial losses (HFL) most-likely estimates ($) (95%CI)</th>
<th>Herd-level financial expenditures (HFE) most-likely estimates ($) (95%CI)</th>
<th>Cost (C) most-likely estimates ($) (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy herd</td>
<td>Low 12.7% (11.8 to 30%)</td>
<td>$1,146 ($945 to 1,364)</td>
<td>$1,321 ($1,121 to 1,620)</td>
<td>$2,667 ($2,363 to 2,983)</td>
</tr>
<tr>
<td></td>
<td>High 62% (55.4 to 67.6%)</td>
<td>$5,400 ($4,373 to 6,515)</td>
<td>$3,794 ($3,445 to 4,167)</td>
<td>$9,197 ($7,958 to 10,521)</td>
</tr>
<tr>
<td>Meat herd</td>
<td>Low 24% (21.6 to 26.4%)</td>
<td>$427 ($356 to 504)</td>
<td>$930 ($828 to 983)</td>
<td>$1,418 ($1,313 to 1,528)</td>
</tr>
<tr>
<td></td>
<td>High 61.5% (57.8 to 62%)</td>
<td>$2,043 ($1,663 to 2,451)</td>
<td>$1,443 ($1,355 to 1,534)</td>
<td>$3,487 ($3,080 to 3,922)</td>
</tr>
</tbody>
</table>
Figure 1. Probability distributions of the total estimated cost of Coxiellosis in an Ontario dairy goat herd with (a) low level of infection exposure (mean of 12.7% of animal-level seroprevalence) and with (b) a high level of C. burnetii exposure (mean of 61.5% of animal-level seroprevalence).
Figure 2. Probability distributions of the total estimated cost of Coxiellosis in an Ontario meat goat herd with (a) low level of infection exposure (mean of 24% of animal-level seroprevalence) and with (b) a high level of *C. burnetii* exposure (mean of 61.5% animal-level seroprevalence).
CHAPTER 5:
SUMMARY DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

1. DISCUSSION AND CONCLUSIONS

This thesis presented the results of studies investigating the evidence reported in the literature for interventions to reduce the effects of *C. burnetii* infection and clinical Q fever in human and small ruminants, and the financial burden on the Ontario goat population at the herd level. The process highlighted the gaps that are recognizable for many endemic or neglected zoonotic health issues: the requirement for quality health data in humans and livestock, the necessity to design and report studies appropriately in the literature, the lack of available data to evaluate the evidence for specific interventions as recommended by EFSA (2010), limited data availability of studies in ruminants identified to be the primary source of *C. burnetii* infection for humans (Lang, 1989), and the significant financial burden that infection in goat populations has on herd profitability in Ontario based on limited data.

In addressing the question of what evidence was available in the published and grey literature to support the recommendations for interventions to prevent the spread of *C. burnetii* from small ruminants to humans, or prevent clinical disease in occupationally exposed human populations exposed to *C. burnetii*, the studies proceeded in a systematic manner to identify and quantitatively synthesize available data. As a result, the identified literature sources allowed us to systematically evaluate multiple levels of data from various study designs; we adapted the Cochrane Collaboration (2011) and RoBANs (Park et al., 2011) guidelines of risk of bias assessment. With meta-analytic methods we were able to assign confidence in our quantitative results based on qualitative assessments of systematic bias in the included studies. These methods uncovered limitations in both data quality and accessibility, and illustrated concerns regarding the lack of evidence upon which policy-makers are basing decisions to influence livestock and human health alike. The impact of the results have since influenced the decisions of the American Association of Public Health Veterinarians (AAPHV) to systematically
evaluate the available evidence for prevention of clinical disease in livestock and its impact on public health risks to humans (Menzies, personal communication). The results of these studies will continue to assist in directing policy directives to improve evidence-based decision-making in veterinary public health.

A thematic overtone to this thesis has been the lack of quality or available data upon which decisions for Q fever policies can be established. In general, higher quality data across all zoonotic diseases (not just those that present epidemic or pandemic potential through significant virulence or contagion potential) will allow for prioritization of actions, program planning, and further research for disease prevention and health promotion at the human-animal interface (Zinsstag et al., 2011). Thus, a standardized method of reporting should be developed and promoted for all publication types including grey literature similar to the REFLECT statement for randomized controlled trials (O’Connor et al., 2010; Sargeant et al., 2010). It may also be used as a guideline or check-list when designing studies. This would provide synthesis researchers with the ability to analytically evaluate multiple publications quickly, effectively, and systematically without having to necessarily address the qualitative methodological deficiencies such as risk of systematic bias underlying the quality of reported data. This is a simple recommendation for improving the quality of available zoonotic disease health data.

Infection with *C. burnetii* may be endemic at both the individual and herd-level in Ontario goats (Lang, 1988a; Lang et al., 1989; Meadows, personal communication), sheep (Lang et al., 1989; Lang et al., 1991; Meadows, personal communication) and cattle populations (Lang et al., 1988b; Lang, 1989). The goat population in Ontario is the largest in Canada, in part due to continued demand from ethnic markets for goat meat and products (OMAFRA, 2009, 2010, 2011). However, any reduced production from deleterious reproductive effects such as those associated with Coxiellosis could lead to consumers looking to other markets. In addition, fear of zoonotic disease transmission may stagnant further growth.

The second stage of this thesis focused on conducting a financial analysis of *C. burnetii* infection and subsequent effects of clinical disease in goat herds in Ontario.
To minimize bias and provide estimates of variability around outcomes, a systematic search was conducted to identify parameters for stochastic economic models to determine production losses and expenditures on persistently infected dairy and meat goat herds in Ontario. Again, lack of data and low quality of data was available to estimate the cost to dairy and meat goat herds, but the evidence suggests that the financial impact is substantial. More epidemiologic data on provincial herd structure, incidence of disease, and the financial impact on production due to both infection and clinical disease are needed to reduce variability around the estimates. These additional data will provide necessary data to develop a mathematical transmission model of *C. burnetii* for the province and may allow for further spatio-temporal research to provide additional evidence to decision makers on the risk of zoonotic disease, as was observed in the Netherlands (Roest et al., 2011).

In summary, this research described the first study to systematically identify, analyse, and synthesize the evidence available for interventions for Q fever prevention and control as recommended by EFSA (2010). Empirical data on the effects of interventions are rare in the literature, despite the substantial financial burden Coxiellosis may impose at the herd level. As such, the utility of vaccination of small ruminants to prevent *C. burnetii* shedding to protect public health lacks significant evidence. Alternatively, there is some significant evidence that vaccination of humans with high risk of exposure to *C. burnetii* may be beneficial in preventing clinical disease. What became evident in this research process was that quality and standardized reporting was critical to estimate the effects of vaccination in high risk human and small ruminant populations. Further to this was the effect that high quality data imparts on the ability to understand and inform decision maker’s ability to understand the effects of recommended prevention and control strategies for managing future disease risks in both livestock and human populations.
2. RECOMMENDATIONS FROM THE RESEARCH

Based on the reviewed literature (Chapter One), the systematic reviews and meta-analyses of human (Chapter Two) and small ruminant (Chapter Three) vaccines, and the financial analysis in the Ontario goat population (Chapter Four), the following recommendations are made:

- **Further validate vaccination in humans**: Significant statistical heterogeneity was identified in the outcomes of high-risk humans vaccinated against the clinical effects of Q fever in Australia. The quality of reporting may have been responsible for the statistical heterogeneity. However, we could not discredit the possibility of biologic heterogeneity amongst study populations included in the meta-regression. In either case, expanding the target population to a larger, and perhaps more international sampling frame, would broaden our understanding of the utility of vaccinating humans at risk of *C. burnetii* exposure (e.g. abattoir employees, veterinarians, farmers, laboratory workers). As Garner et al. (1997) have shown, Q fever has a significant burden on human productivity and on the Australian health care system. Reducing this burden through scientifically informed interventions may be worth pursuing in areas where exposure is likely to occur. In addition to further research on vaccine efficacy and effectiveness, vaccine modification for humans would negate the necessity of exposure negative status prior to vaccination. Cytomolgus monkeys have been used as models for chloroform-methanol residue (CMR) vaccines that do not rely on a seronegative status prior to administration and have significantly reduced the odds of developing clinical disease when aerosol challenged compared to controls vaccinated with Q vax (Waag et al., 2002).

- **Support further evaluation of *C. burnetii* intervention research in livestock**: Only vaccine publications were identified in the scoping studies of both humans and animals to prevent clinical disease, or shedding of *C. burnetii*, respectively. All other EFSA (2010) recommendations were based on literature review rather than a critical and quantitative review of available evidence. Vaccination has not been shown to be an effective means of reducing the risk of shedding of *C. burnetii* from small ruminants beyond vaginal, uterine, and placental routes from some animals.
As such, the public health risk remains. If livestock vaccination is to be the primary means of controlling the risk of human epizootic events, such as the Netherlands outbreak (Hogerwerf et al., 2011), then advances in vaccine research must occur to eliminate shedding from routes of public health importance. Elimination is necessary given the low infectious dose of \textit{C. burnetii}. Natural and experimentally infected small ruminants should also be used to determine the effect on shedding with other EFSA (2010) recommendations independently or in combination.

- \textbf{Improve the quality of study design in veterinary medical literature:} In evidence-based medicine, the hierarchy of research design is classified such that results from randomized controlled trials (RCTs) are considered to be the highest grade of evidence (so called, “gold standard”) for intervention research (Dohoo et al., 2009). In contrast, Concato et al. (2000) noted that, traditionally, observational studies are viewed as having less validity due to the potential for overestimation of treatment effect. Although the systematic reviews and meta-analyses included in this thesis offered a test of the implicit assumptions regarding the hierarchy of study design, it may be that observational studies do not systematically over-estimate the magnitude of associations between exposure and outcome compared to results of a RCT of the same topic depending on the risk of systematic bias in the publication. Concato et al. (2000) made similar conclusions when comparing RCT and observational study results for several health topics selected from major journals of health research. Thus, future \textit{C. burnetii} intervention (e.g. vaccine) research should adhere to strict \textit{a priori} epidemiologic study design methods to minimize systematic bias in the results. This will substantiate internal validity of the studies, and provide high quality evidence to make decisions for \textit{C. burnetii} intervention and control. Increasing the scope and availability of \textit{C. burnetii} research in populations at risk will improve our understanding of intervention in target populations.

- \textbf{Improve quality of data reporting in veterinary medical literature:} Despite the lack of RCTs in the veterinary medical literature on \textit{C. burnetii} vaccination, in favour of the more commonly identified observational studies, the hierarchial quality evaluated between RCTs and observational studies will ultimately depend on the quality of reporting by the authors. Thus, authors should adhere to strict reporting guidelines
as outlined by O’Connor et al., (2010) and further elaborated by Sargeant et al. (2010). The use of standardized reporting guidelines will assist researchers with establishing appropriate protocols and reporting of data to minimize biased estimates of effect measures. The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) statement (von Elm et al., 2008) should be adapted routinely for observational studies. In addition, providing a single or discrete group of outcomes for Q fever or C. burnetii infection will also aid in synthesis research. Further diagnostic evaluation may need to occur prior to making recommendations of common outcomes to ensure that infection status can be effectively measured across different populations.

- **Enhance surveillance of C. burnetii in humans and livestock:** The lack of available literature on Q fever or Coxiellosis incidence, infection rates, and clinical or productivity outcomes may be due to lack of surveillance in both human and veterinary health fields. Although C. burnetii is listed as a Level 3 pathogen (OIE, 2012), in part due to its virulence and zoonotic potential, many governments view the bacteria as an endemic disease with limited impact to human or animal health. Limited availability of empirical data has resulted in the necessity to utilize non-peer reviewed (grey) literature and expert opinion; both viewed as lower quality evidence compared to peer-reviewed literature (Brighton et al., 2003). While these are important sources of information, researchers and governments need to improve the quality of systematic monitoring of empirical baseline infection and clinical disease data across livestock and human populations. Engaging all stakeholders in this type of research and data collection can help develop strategies to reduce the disease burden that are appropriate for local farm models. This may also reduce the likelihood of a zoonotic outbreak and subsequent cull of the national goat herd, as observed in the Netherlands after the Q fever epidemic from 2007-2010 (Roest et al., 2011; Hogerwerf et al., 2011).

- **Ensure knowledge translation and transfer (KTT) of all research findings:** KTT is the process of turning research into action, and accelerating the practical application of knowledge uncovered by research (CIHR, 2008). In addition to publishing findings of this research in academic peer-reviewed journals and presenting at conferences,
the widespread dissemination of information to stakeholders outside of the scientific audience will further encourage the sustainable application of research results. KTT is a critical aspect to any research, but perhaps moreso when multiple species (humans and animals) are involved. When considering the reliance that producers have on livestock for their livelihood, zoonotic disease impacts both their health, the health of their animals, and, as is the case for C. burnetii, the profitability of their enterprise. Not only is this an issue in developed nations affected with Q fever, but may impact those in the developing world to a greater extent where the prevalence of infection in both humans and small ruminants is even greater (Schelling et al., 2003; Schelling, personal communication). Access to public health decision-makers will also help develop and scientifically inform strategies to prevent and control disease in livestock and human populations to effectively reduce the burden of disease concurrently.
REFERENCES


Hogerwerf L, van den Brom R, Roest HJJ, Bouma A, Vellema P, Pieterse M, Derksen D, Nielen M. Reduction of Coxiella burnetii prevalence by vaccination of goats and sheep, the Netherlands. EID 2011:17(3);379-86.


Meadows S, personal communication, 15 July 2012.

Menzies P, personal communication, 17 May 2012.


Oral presentation B803. [Full instrument obtained from lead author: November, 2011].


Schelling E, personal communication, 12 December 2011.


**APPENDICES**

**Appendix 1.** Thesaurus terms for scoping study literature search* of interventions to prevent and control Q fever spread, infection, and clinical disease in livestock and humans concurrently.

<table>
<thead>
<tr>
<th>Population</th>
<th>&lt;AND&gt; Intervention</th>
<th>&lt;AND&gt; Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Livestock* &lt;OR&gt;</td>
<td>Manage* &lt;OR&gt;</td>
<td>Q fever</td>
</tr>
<tr>
<td>Animal* &lt;OR&gt;</td>
<td>Prevent* &lt;OR&gt;</td>
<td>Query fever</td>
</tr>
<tr>
<td>Sheep</td>
<td>Vacc*</td>
<td>Queensland fever</td>
</tr>
<tr>
<td>Goat</td>
<td>Immuniz*</td>
<td>Coxiella burnetii</td>
</tr>
<tr>
<td>Ovine</td>
<td>Awareness*</td>
<td>C. burnetii</td>
</tr>
<tr>
<td>Caprine</td>
<td>Campaign*</td>
<td>Coxiell*</td>
</tr>
<tr>
<td>Herd</td>
<td>Control*</td>
<td></td>
</tr>
<tr>
<td>Flock</td>
<td>Isolation*</td>
<td></td>
</tr>
<tr>
<td>Human* &lt;OR&gt;</td>
<td>Environment*</td>
<td></td>
</tr>
<tr>
<td>Occupation* &lt;OR&gt;</td>
<td>Biosecur*</td>
<td></td>
</tr>
<tr>
<td>People &lt;OR&gt;</td>
<td>Manure</td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>Treatment* &lt;OR&gt;</td>
<td></td>
</tr>
<tr>
<td>Farmer</td>
<td>Antibiotic*</td>
<td></td>
</tr>
<tr>
<td>Shear*</td>
<td>Drug therapy</td>
<td></td>
</tr>
<tr>
<td>Abattoir</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Technician</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lab*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vet*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The search combined sets of search terms using “OR” and each component using “AND” (population term AND intervention term AND outcome terms).
Appendix 2. Relevance screening (RS) tools for the initial scoping study of Q fever interventions in humans and livestock.

Relevance Tool 1 (RS-1): Relevance Criteria

1. Does this abstract or title describe published primary research\(^1\) or grey literature\(^2\)?
   
   Yes (0)    No (1)    Can’t Tell (2)

2. Does this abstract or title investigate the effect of Q fever (C. burnetii) interventions\(^3\) in sheep/goats\(^4\) and/or human populations\(^5\)?

   Yes (0)    No (1)    Can’t Tell (2)

---

\(^1\)Primary research represents a study where the author(s) collected and analyzed their own data.

\(^2\)Grey literature includes technical reports from government agencies or scientific research groups, working papers from research groups or committees, white papers and preprints, and represent primary research.

\(^3\)Interventions include: preventative or reactive vaccination, removal of risk material (placentas, aborted fetuses), control of ticks and animal reservoirs apart from livestock, culling of pregnant animals and infected males from infected farms, regular milk testing of livestock dairies, restricted movements (quarantine) of livestock, composting of manure, general biosecurity measures (personal protective devices, visitor ban, controlling animal movements), identifying and culling shedders ("test and cull"), temporary breeding ban, segregated lambing/kidding area.

\(^4\)Includes sheep (dairy, meat, other) and goats (dairy, meat, other). If only cattle (Bos taurus: dairy, meat, other) were studied, NO should be selected, unless humans were also an identified study population in the same publications.

\(^5\)Human populations include any individual or human population in any location or setting.
Relevance Tool 2 (RS-2): Relevance Criteria

1. Does the publication evaluate and report the effect of an intervention (see below) for *C. burnetii* in small ruminants or humans to change the probability of transmission\(^1\), infection\(^2\), or clinical symptoms/signs\(^3\)?

   YES  NO  CAN'T TELL

If YES, please categorize by species and intervention. Check all that apply. If NO, end.

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Sheep</th>
<th>Goats</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccination(^4)</td>
<td>1</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>Removal of risk material(^5)</td>
<td>2</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td>Reservoir(^6) control</td>
<td>3</td>
<td>15</td>
<td>27</td>
</tr>
<tr>
<td>Tick control</td>
<td>4</td>
<td>16</td>
<td>28</td>
</tr>
<tr>
<td>Culling(^7) infected males</td>
<td>5</td>
<td>17</td>
<td>29</td>
</tr>
<tr>
<td>Culling(^7) of pregnant females</td>
<td>6</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>Milk testing dairies(^8)</td>
<td>7</td>
<td>19</td>
<td>31</td>
</tr>
<tr>
<td>Movement restriction(^9)</td>
<td>8</td>
<td>20</td>
<td>32</td>
</tr>
<tr>
<td>Manure management(^10)</td>
<td>9</td>
<td>21</td>
<td>33</td>
</tr>
<tr>
<td>Biosecurity(^11)</td>
<td>10</td>
<td>22</td>
<td>34</td>
</tr>
<tr>
<td>Temporary breeding ban(^12)</td>
<td>11</td>
<td>23</td>
<td>35</td>
</tr>
<tr>
<td>Segregated parturition areas(^13)</td>
<td>12</td>
<td>24</td>
<td>36</td>
</tr>
</tbody>
</table>
Infection describes the ability of *C. burnetii* to establish an infection through vertical or horizontal transmission (shedding it in to the environment through feces, milk, vaginal discharge, and high-risk material (placentas, aborted fetuses). The answer should be YES if a study measures *C. burnetii* infection, colonization, prevalence, contamination or concentration. Outcomes must be measured in live sheep, goats or humans. Environmental samples are included if the sampling environment contains one of the target species. Studies measuring the outcome using only serology/antibody detection will also be included.

Infection by *C. burnetii* includes clinical diagnostics (PCR, CF, ELISA etc) for the bacterium or antibodies, or the clinical presentation of Q-fever in sheep/goats or humans (acute or chronic).

Clinical symptoms and signs include all those identified by Maurin and Raoult (1999) in humans or small ruminants.

Vaccination may include killed or live vaccines, adjuvanted or non-adjuvanted. Any route of administration is acceptable, with any dosage and frequency of administration.

Risk material includes placenta and aborted fetuses.

Reservoir species include wild and domestic mammals (sheep, goats, cattle, canines, felines, horses, rabbits, swine, camels, water buffalo, rats, mice) and birds (pigeons, chickens, ducks, geese, turkeys) (Babudieri, 1959).

Culling indicates removal of an individual from the herd or flock.

Regular pooled sample bulk tank or individual animal (sheep, goat, cow) testing of milk samples in dairies using complement fixation (CF), ELISA or PCR.

Movement restriction of animals, humans or fomites (including vehicles and equipment) within or between farms, districts, communities, counties, provinces/states, nations or as defined by the presiding government body responsible for imposing movement restriction to control disease.

Manure management including deep litter systems, slurry treatment with cyanamide calcium, composting with or without covering, the removal of untreated slurry for field spreading and time of spread (temporally and climatically).

Biosecurity is a general term identifying hygienic practices to reduce the risk of disease introduction, transmission and spread between animals and between animals and humans. Personal protection (masks, gloves), restricted entry to barns, and shearing management. These measures are applied to all people entering and/or leaving the premise where farm animals are kept. General guidelines are available (DEFRA, 2008).

A breeding ban is a temporary stop to breeding females to reduce bacterial shedding which is highest at parturition. It might precede subsequent repopulation with vaccinated animals.

Dedicated parturition areas (lambing, kidding, calving) include areas used for the sole purpose of giving birth separate from other individuals in the herd or flock.
**Appendix 3.** The Cochrane Collaboration Risk of Bias (RoB) tool for CT publications (Cochrane Collaboration, 2011).

<table>
<thead>
<tr>
<th>Design category</th>
<th>Low risk</th>
<th>High risk</th>
<th>Unclear risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Allocation concealment</strong>&lt;br&gt; <em>Was allocation adequately concealed?</em></td>
<td>Participants and investigators enrolling participants could not foresee assignment because one of the following, or an equivalent method, was used to conceal allocation:&lt;br&gt;- Sequentially numbered vaccine containers appeared identical&lt;br&gt;- Central allocation&lt;br&gt;Sequentially numbered, opaque, sealed envelopes</td>
<td>Participants or investigators enrolling participants could possibly foresee assignments and thus introduce selection bias, such as allocation based on:&lt;br&gt;- Using an open random allocation method&lt;br&gt;- Assignment envelopes were used without appropriate safeguards&lt;br&gt;- Alternation or rotation&lt;br&gt;- Date of birth&lt;br&gt;- Case record number&lt;br&gt;- Any other explicitly unconcealed procedure</td>
<td>Insufficient information to permit the judgment of high or low risk. If the method of concealment is not described, or not described in sufficient detail to allow judgment.</td>
</tr>
<tr>
<td><strong>Sequence generation</strong>&lt;br&gt; <em>Was the allocation sequence adequately generated?</em></td>
<td>Investigators describe a random component in the sequence generation process:&lt;br&gt;- Referring to a random number table&lt;br&gt;- Using a computer random number generator&lt;br&gt;- Coin tossing&lt;br&gt;- Shuffling cards or envelopes&lt;br&gt;- Throwing dice&lt;br&gt;- Drawing of lots&lt;br&gt;- Minimization (with or without a random element)</td>
<td>Investigators describe a non-random component in the sequence generation process leading to allocation by:&lt;br&gt;- Odd or even date of birth&lt;br&gt;- Algorithm based on hospital or clinic record number&lt;br&gt;- Date/day of admission&lt;br&gt;- Alternate allocation&lt;br&gt;- Other non-random approaches</td>
<td>Insufficient information regarding sequence generation process to permit the judgment of high or low risk.</td>
</tr>
<tr>
<td><strong>Blinding</strong>&lt;br&gt; <em>Was knowledge of the allocated intervention adequately prevented during the study?</em></td>
<td>Any one of the following:&lt;br&gt;- No blinding, but the review authors judge that the outcome and the outcome measurement are not likely to be influenced by lack of blinding&lt;br&gt;- Blinding of participants and</td>
<td>Any one of the following:&lt;br&gt;- No blinding or incomplete blinding, and the outcome or outcomes measurement is likely to be influenced by lack of blinding&lt;br&gt;- Blinding of key study</td>
<td>Insufficient information to permit judgment of high or low risk of bias, or the study did not address this outcome.</td>
</tr>
<tr>
<td>Incomplete outcome data</td>
<td>Were incomplete outcome data adequately addressed?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Any one of the following:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- No missing outcome data</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Reasons for missing outcome data unlikely to be related to true outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Missing outcome data balanced in numbers across vaccine groups, with similar reasons for missing data across groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- For dichotomous outcome data, the proportion of missing outcomes compared with the observed event risk not enough to impact to any clinically relevant extent on the intervention effect estimate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- For continuous outcome data, plausible effect size among missing outcomes not enough to impact to any clinically relevant extent on observed effect size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reporting bias</td>
<td>Authors or editors report results that are congruent with the methods described. There is no evidence of discrepancy or concealment of results from reader.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was selective outcome reporting identified?</td>
<td>Results, as reported, lead the reader to believe incongruence from the methods described. There is a potentially inappropriate reporting or analysis of data.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insufficient information to permit judgment of high or low risk of bias.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insufficient reporting of attrition/exclusions to permit judgment of high or low risk of bias, or the study did not address this outcome.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

key study personnel ensured, and unlikely that the blinding could have been broken
- Either participants or some key study personnel were not blinded, but outcome assessment was blinding and the non-blinding of other unlikely to introduce bias
participants and personnel attempted, but likely that the blinding could have been broken
- Either participants or some key study personnel were not blinded, and the non-blinding of other likely to introduce bias
### Appendix 4. The Risk of Bias assessment tool for non-randomized studies (RoBANS)(Park et al., 2011).

<table>
<thead>
<tr>
<th>Design category</th>
<th>Low risk</th>
<th>High risk</th>
<th>Unclear risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selection of participants</td>
<td>Vaccination and control groups are the same populations (identical institution and time period), and the absence of outcomes among the study participants was confirmed at the start of the study (Cohort studies).</td>
<td>Vaccination and control groups were selected from different population groups. The presence of outcomes among the study participants was not confirmed at the start of the study (Cohort studies).</td>
<td>Unclear whether the selection of participants was low or high risk.</td>
</tr>
<tr>
<td>Confounding variables</td>
<td>Major confounders were confirmed and considered adequately during the design phase (i.e. matching, restriction etc.) or analysis phase (i.e. stratification, propensity score, statistical adjustment etc.).</td>
<td>Major confounding variables not considered. Even though major confounding variables were confirmed, they were not considered adequately during the design and analysis phases.</td>
<td>Unclear whether confounding resulted in low or high risk.</td>
</tr>
</tbody>
</table>
| Measurement of exposure | If Q fever exposure methods were described by using at least one or more of the following:  
  - Data obtained from trusted sources such as medical records  
  - Data were obtained from structured interviews | If Q fever exposure methods were described by using any of the following:  
  - Self-reported data  
  - Clear evidence of interview bias  
  - Evidence of recall bias | Unclear whether the measurement of Q fever exposure resulted in low or high risk. |
| Blinding of outcome assessment | Any one of the following:  
  - The outcome assessors were blinded  
  - Although blinding is not present, its absence is judged to have no effect on the outcome measurement | Since blinding was not performed or incomplete, such blinding status is considered to have effects on the outcome measurement. | Unclear whether the blinding outcome assessment resulted in low or high risk. |
| Incomplete data outcome | Any one of the following:  
  - No missing data could be observed  
  - The causes of having missing data | The missing data could have effects on the outcome. It may be attributable to the differences of missing data between vaccinated and unvaccinated. | Unclear whether the incomplete outcome resulted in low or high risk. |
data are considered to have a relationship to the outcome.
- The number of missing data was developed similarly in both the vaccinated and unvaccinated groups, and the causes of development are similar.

**Selective outcome reporting**

*Reporting bias caused by selective outcome reporting*

<table>
<thead>
<tr>
<th>Selective outcome reporting</th>
<th>Any one of the following:</th>
<th>Any one of the following:</th>
<th>Unclear whether the selective outcome reported resulted in low or high risk.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>The pre-defined primary outcomes were not fully reported</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Outcomes were not reported as previously defined</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Primary outcomes not pre-specified in the study existed (except for outcomes with clear explanation)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Incomplete reporting on primary outcome of interest</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No report on important outcomes expected to be reported in the related field</td>
<td></td>
</tr>
</tbody>
</table>
Appendix 5.

Appendix 5.1. Distribution of herd financial losses (HFL) on Ontario goat dairies from low level of *C. burnetii* exposure and results from the regression sensitivity analysis of within-herd and herd-level parameters in the simulation model estimating HFL.
Appendix 5.2. Distribution of herd financial expenditures (HFE) on Ontario goat dairies from low level of *C. burnetii* exposure and results from the regression sensitivity analysis of within-herd and herd-level parameters in the simulation model estimating HFE.
Appendix 5.3. Distribution of herd financial losses (HFL) on Ontario goat dairies from high level of *C. burnetii* exposure and results from the regression sensitivity analysis of within-herd and herd-level parameters in the simulation model estimating HFL.
Appendix 5.4. Distribution of herd financial expenditures (HFE) on Ontario goat dairies from high level of *C. burnetii* exposure and results from the regression sensitivity analysis of within-herd and herd-level parameters in the simulation model estimating HFE.
Appendix 5.5. Distribution of herd financial losses (HFL) on Ontario meat goat herds from low level of *C. burnetii* exposure and results from the regression sensitivity analysis of within-herd and herd-level parameters in the simulation model estimating HFL.
Appendix 5.6. Distribution of herd financial expenditures (HFE) on Ontario meat goat herds from low level of *C. burnetii* exposure and results from the regression sensitivity analysis of within-herd and herd-level parameters in the simulation model estimating HFE.
Appendix 5.7. Distribution of herd financial losses (HFL) on Ontario meat goat herds from high level of *C. burnetii* exposure and results from the regression sensitivity analysis of within-herd and herd-level parameters in the simulation model estimating HFL.
Appendix 5.8. Distribution of herd financial expenditures (HFE) on Ontario meat goat herds from high level of *C. burnetii* exposure and results from the regression sensitivity analysis of within-herd and herd-level parameters in the simulation model estimating HFE.