Coxiella burnetii seropositivity and associated risk factors in sheep, goats, their farm workers and veterinarians in Ontario, Canada

by

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ABSTRACT

COXIELLA BURNETII SEROPOSITIVITY AND ASSOCIATED RISK FACTORS IN SHEEP, GOATS, THEIR FARM WORKERS AND VETERINARIANS IN ONTARIO, CANADA

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This thesis was conducted to investigate the seroprevalence and risk factors for *Coxiella burnetii* exposure in meat and dairy sheep and goats, their farm workers, and small ruminant veterinarians and veterinary students in Ontario. Four cross-sectional studies involving serological testing and questionnaire administration were conducted. Sera from reproductively active ewes and does were tested for *C. burnetii* specific antibodies using an enzyme-linked immunosorbent assay (IDEXX); human sera were tested using an immunofluorescence assay (Focus Diagnostics). Seropositivity was common among all groups. The individual-level seroprevalence was 14.7% (95% CI=13.3-16.2) in sheep, 32.5% (95%CI=30.6-34.5) in goats, 64.5% (95%CI=57.2-71.4) in farm workers, and 59.4% (95%CI=41.9-75.2) in veterinarians/veterinary students. Overall, 48.6% (95%CI=37.2-60.1) of sheep farms and 63.2% (95%CI=51.9-73.4) of goat farms had at least one seropositive animal, while 76.3% (95%CI=65.8-84.6) of farms that participated in human testing had at least one seropositive farm worker. Mixed logistic multivariable models of individual seropositivity, and controlling for clustering by farm, were constructed for sheep, goats and farm workers. The sheep and goat models highlighted the importance of farm hygiene and biosecurity measures. Female flock size (log\textsubscript{10} scale), lambing/kidding in a separate airspace, and failure to disinfect lambing/kidding pens were positively associated with
seropositivity in both the sheep and goat models. For goats, male herd size (log_{10} scale), and kidding outdoors in the absence of swine on farm were negatively associated with seropositivity; the presence of other sheep/goat farms within 5km was positively associated with seropositivity. For sheep, loaning sheep was positively associated with seropositivity. Workers on dairy goat farms had higher odds of seropositivity, compared to working on meat goat or dairy sheep farms. Increasing proportions of seropositive sheep/goats on farm was also positively associated with farm worker seropositivity. Veterinary students had significantly lower odds of seropositivity than practicing veterinarians in univariable exact logistic regression.
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To quote Greek philosopher Plutarch, “the mind is not a vessel to be filled, but a fire to be kindled.” This thesis is the result of effort from many remarkable individuals who I wish to acknowledge for their tremendous contributions to this work, and for inspiring my pursuit of knowledge along the way.

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I was lucky to have the help of remarkable summer students, who on a particularly long road-trip earned the name SRRGE (Small Ruminant Research Group Extraordinaire): Kelly Kozlowski, Nina Gauthier and Denise Yates, as well as my co-sampler, Cathy Bauman. In addition, I wanted to thank Ben Schlegel and David Baker for their assistance in our first summer of sampling, as well as many other student volunteers who donated their time to help out on farm. You all contributed to a very positive, efficient and fun sampling phase of the study, and I am forever grateful!

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STATEMENT OF WORK DONE

Drs. Andria Jones-Bitton, Paula Menzies, and Jocelyn Jansen designed the study, prepared grant applications and secured funding for this project. I prepared and mailed out, the farm worker recruitment letters with the help of Dr. Jocelyn Jansen. I, along with Drs. Andria Jones-Bitton, Paula Menzies and Jocelyn Jansen, created the animal management and human questionnaires. I pre-tested the animal management and human questionnaires with producers and industry leaders in each small ruminant sector (meat sheep, dairy sheep, meat goats, dairy goats). I contacted producers and organized farm visits for all 148 farms visited for this research. I was present at all 148 farm visits and administered all questionnaire data collected on the farm visit. I also organized human blood collection from 172 participants, which involved either: organizing a farm visit to have a human blood sample collected by Helen Litchfield, a certified phlebotomist, or finding the closest phlebotomy lab to the farm workers address, and sending them directions as well as a completed requisition form. Upon farm workers providing a human blood sample at a phlebotomy laboratory, I arranged for re-imbursement cheques to be sent out to cover gas costs. I collected a total of 4558 sheep and goat blood samples, along with summer students: Kelly Kozlowski, Denise Yates, Nina Gauthier, Benjamin Schlegel and David Baker. On farm visits I provided supervision and guidance to the summer students regarding animal handling, collection of blood, animal records and processing/labelling of blood samples. All sheep and goat samples were centrifuged and labelled by the students and me, and I submitted all animal serum samples to the Animal Health Laboratory in Guelph, ON where the ELISA was conducted. All human serum samples received from Helen were centrifuged, labelled by me and were submitted to Dr. Samir Patel at the Public Health Ontario Laboratory in Toronto, where the IFA was conducted. Human samples taken at phlebotomy labs were shipped by their staff
directly to Dr. Samir Patel for analysis. All sample results and questionnaire data were collected, entered and analyzed by me. I performed the statistical analysis and interpretation with supervision from my co-advisors Drs. Andria Jones-Bitton and Paula Menzies, and with assistance from Dr. David Pearl. I was responsible for preparing the manuscript, and Drs. Andria Jones-Bitton, Paula Menzies, Jocelyn Jansen and Scott McEwen provided critical input and revisions throughout.
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CHAPTER ONE

Introduction and Literature Review

1.1 Introduction

*Coxiella burnetii* is an intracellular zoonotic bacterium (Maurin and Raoult, 1999).

Disease caused by *C. burnetii* is called Q fever in humans. In animals, the disease was aptly re-coined coxiellosis (Marrie, 1995). Q fever in humans and coxiellosis in sheep and goats have been recognized as endemic in Ontario since the 1980s (Palmer et al, 1983; Simor et al., 1984; Simor et al., 1987). Sheep and goats are reservoir species of *C. burnetii*, and have been shown to be an important source of infection for Q fever in humans (Porter et al., 2011). Traditionally, large portions of human and animal health examined *C. burnetii* infections from separate perspectives. Recently, added value in examining zoonotic infections synergistically, by making cogent linkages between the two is being realized (Zinsstag et al, 2011).

1.2 Epidemiology of *C. burnetii*

1.2.1 Lifecycle information about *C. burnetii*

*Coxiella burnetii* exhibits polymorphism as it has two morphologically distinct cell variants; an intracellular and metabolically active large cell variant (LCV) and a spore-like small cell variant (SCV) (McCaul and Williams, 1981). SCVs are shed by infected animals, and when introduced into a host’s body, attach to the cell membrane of phagocytic cells (Maurin and Raoult, 1999). After phagocytosis, the phagosome containing the SCV fuses with the lysosome (Williams and Thompson, 1991). The SCVs are metabolically activated in the acidic phagolysosome, and can undergo vegetative growth to form LCVs (Maurin and Raoult, 1999).
LCVs and the activated SVCs can both divide by binary fission (McCaul and Williams, 1981), and the LCV can also undergo sporogenic differentiation (Angelakis and Raoult, 2010). The spores that are produced can undergo further development to become metabolically inactive SCVs (Williams and Thompson, 1991), and both spores and SCVs can then be released from the infected host cell by either cell lysis or exocytosis (Maurin and Raoult, 1999). The entire development cycle of metabolically active *C. burnetii* takes place in acidic phagolysosomes; *C. burnetii* are resistant to microbicidal activities in the host macrophages (Mege et al., 1997). The acidic environment also protects *C. burnetii* from the effects of antibiotics, as the efficacy of antibiotics is decreased in the acidic pH (Mege et al., 1997). SCV and spore forms are more difficult to denature than LCVs (Scott and Williams, 1990), possibly due to differences in cell wall composition and thickness, as well as water content (Scott and Williams, 1990).

SCVs are likely to account for the prolonged infectivity of *C. burnetii* in adverse environmental conditions, as SCVs can persist for weeks or months in dairy products, meat products and surface water, or many years in dust or soil (McCaul and Williams, 1981; Hanczaruk et al., 2012). Strong resistance to desiccation enhances survival of *C. burnetii* in the environment (Maurin and Raoult, 1999) and favours the spread of contaminated aerosols (Tissot-Dupont et al., 1999; Tissot-Dupont et al., 2004). Survival of *C. burnetii* is attributed to its stability in acidic environments up to pH 4.5 (Hackstadt and Williams, 1981; Baca and Paretsky, 1983), ultraviolet light (Little et al., 1980), and osmotic shock (Amanot and Williams, 1984). *Coxiella burnetii* is also resistant to high temperatures. In fact, the temperature and time requirements for milk pasteurization were based on evidence that *C. burnetii* is the most heat-resistant pathogen of public health significance in milk (Enright et al., 1957; Cerf and Condron, 2006). To adequately eliminate viable *C. burnetii* from raw whole milk, the milk must be
pasteurized at either 63°C for 30 minutes, or with high temperature short time pasteurization at 72°C for 15 seconds (Enright et al., 1957).

Various chemical disinfectants tested in laboratory settings have been found to be ineffective in inactivating *C. burnetii*, as liquid suspensions of $10^8$ *C. burnetii* in 2% Roccal, and 5% formalin were infective after 24 hours at 25°C (Scott and Williams, 1990). However, similar suspensions in 70% ethyl alcohol, 5% chloroform, or 5% Enviro-Chem (mixture of N-alkyl dimethyl benzyl (2.25%), and ethylbenzal (2.25%) ammonium chlorides) resulted in inactivation of *C. burnetii* within 30 minutes (Scott and Williams, 1990). Formaldehyde gas has also been tested and inactivated *C. burnetii* in a small sealed chamber, but failed to consistently inactivate more than $10^{4.6}$ *C. burnetii* in a larger room (5600 feet$^3$) without humidity control (Scott and Williams, 1990).

There are three disinfectants recommended in the literature to inactivate *C. burnetii* for animal research laboratories: a 5% solution of hydrogen peroxide, a 1:100 dilution of chlorine bleach (containing a final solution of 0.0525% sodium hypochlorite), or a 1:100 dilution of Lysol® (5% final solution) (0-phenylphenol, 2.80%; 0-benzyl-p-chlorophenol, 2.70%; alcohol, 1.80%; xylenols, 1.5%; isopropyl alcohol, 0.90%) (Bernard et al., 1982; Welch, 2003; Eibach et al., 2012). However, the efficacy of 5% Lysol® or 0.05% chlorine bleach is questionable, as research has indicated that liquid suspensions of $10^8$ *C. burnetii* were still infective after 24 hours in contact with even stronger than the above recommended concentrations of Lysol and sodium hypochlorite at 25°C (Scott and Williams, 1990). As such, more research is needed to confirm whether or not these common disinfectants are efficacious in killing *C. burnetii* in laboratory settings.
The efficacy of disinfection procedures for preventing the spread of *C. burnetii* infection remains largely unproven in farm settings (Rodolakis, 2009). Treatment of infective slurry with lime or 0.4% calcium cyanide was shown to be an effective method of disinfection (Arricau-Bouvery et al., 2001; Rodolakis, 2009); hence, the authors recommended infective slurry and manure be treated on infected farms prior to spreading on fields (Rodolakis, 2009). Research identifying other disinfection protocols for animal environments is lacking, particularly lambing/kidding pens, and needs to be examined.

1.2.2 Routes of transmission

The pathogenesis of coxiellosis is characterised by replication of the agent in regional lymph nodes, followed by bacteremia (Babudieri, 1959). The bacteraemic phase allows for detection of *C. burnetii* in blood via Polymerase Chain Reaction (PCR) (Schneeberger et al., 2010). This stage is followed by persistent localisation of *C. burnetii* in the mammary gland and uterus, especially in periparturient animals, as *C. burnetii* displays high tropism for these organ systems (Babudieri, 1959; Mege et al., 1997; Roest et al., 2012). Experimental infection of pregnant goats demonstrated that the chorioallantoic membrane are the first target cells in the placenta, and after a substantial delay (approximately 44 days), the placenta was colonized and placentitis separated the fetal cells from the maternal epithelium, resulting in abortion (Sánchez et al., 2006). DNA from *C. burnetii* has also been identified in tissues from the abomasum, intestines, vagina, liver, spleen, kidney and lungs (Cantas et al., 2011; Porter et al., 2011; Reusken et al., 2011). Subsequently, *C. burnetii* may be shed by animals in the placenta, vaginal secretions, manure, milk, and urine (Berri et al., 2001; Guattéo et al., 2006).

Observational studies suggest that inhalation of aerosolised *C. burnetii* is the major mechanism whereby *C. burnetii* is transmitted to humans (Gonder et al., 1979; Marrie et al.,
1989; Williams et al., 1991; Stein et al., 2005). Research has demonstrated that *C. burnetii* DNA exists in air samples, as detected by PCR. Aerosolized *C. burnetii* has been detected a month after lambing (Aztobiza et al., 2010), although the rate *C. burnetii* settles out of the air in controlled or turbid animal environments is unestablished. It is hypothesized that transmission to animals is primarily achieved via inhalation as well (Maurin and Raoult, 1999), as coxiellosis transmission can occur between animals in the same airspace with no direct contact (Welsh et al., 1945). In addition, screening for aerosolized *C. burnetii* on dairy goat and sheep farms in the Netherlands using a qualitative multiplex PCR identified higher average levels of *C. burnetii* DNA in the air on farms with a history of abortions related to *C. burnetii* in 2008 or 2009 and/or bulk milk tank positive than control farms which were bulk tank negative and had no history of *C. burnetii* abortions (de Bruin et al., 2012).

Sporadic cases have also been attributed to the ingestion of infected materials, but the oral route is considered less efficient than inhalation (Welsh et al., 1945; Benson, Brock, and Mather, 1963; Fishbein and Raoult, 1992). In humans, *C. burnetii* infection via the oral route is usually attributed to the consumption of contaminated unpasteurized dairy products (Hatchette et al., 2001; Fishbein and Raoult, 1992), while in animals, exposure may occur by ingesting contaminated pastures, hay, straw, or placentas (Willeberg et al., 1980; Woldehiwet, 2004).

### 1.3 Reservoir species and other hosts

*Coxiella burnetii* has been recovered from a vast array of mammals, birds and arthropods world-wide, except in New Zealand (Enright et al., 1971; Astobiza et al., 2011; Thompson et al., 2012; Maurin and Raoult., 1999). Most of these species are considered to be accidental or spill-over hosts, and rarely transmit infection to humans. The most common species implicated in human Q fever cases are goats, sheep and cattle (Maurin and Raoult, 1999), and occasionally
cats (Marrie et al., 1988; Marrie et al., 1989; Kopecny et al., 2013), and dogs (Buhariwalla et al., 2010). However, many species have not been methodically evaluated. New research suggests that rodent species, such as rats (in the Netherlands), and squirrels (in Canada) may be capable of maintaining transmission cycles independent of livestock contact, although their respective roles in the transmission of C. burnetii to other animals and humans remains unclear (Reusken et al., 2011; Thompson et al., 2012).

1.4 C. burnetii infection

1.4.1 C. burnetii infection in sheep and goats

Infection in sheep and goats is often subclinical, but it can cause abortion in late gestation, as well as the delivery of stillborn, unviable or weak lambs/kids (Rodolakis, 2006; Rodolakis et al., 2007), frequently without preceding symptoms (Schimmer et al., 2011). Up to a billion copies of C. burnetii have been found per gram of placenta in ewes, during an abortion or normal delivery (Berri et al., 2001; Masala et al., 2004; Berri, Rousset et al., 2005). Consequently, during the lambing or kidding period, the risk of disease transmission is high due to heavy bacterial loads contaminating the birthing environment (Schulz et al., 2005; McQuiston et al., 2006; Astobiza et al., 2010; Maurin and Raoult, 1999). However, the risk of exposure extends beyond the birthing period, as intermittent or persistent shedding may also occur in the feces, urine and milk for several weeks or months following parturition (Berrie et al., 2000; Berri et al., 2001). Also contributing to the extended period of transmission, is the ability of C. burnetii to remain infective for months in aerosols or contaminated dust, which continue to be released as the placental/fecal material desiccates (Woldehiwet, 2004).

1.4.2 C. burnetii infection in people
In people, *C. burnetii* infection may be asymptomatic or present with acute or chronic clinical manifestations (Maurin and Raoult, 1999). Approximately 60% of cases are asymptomatic, and 38% experience mild symptoms without the need for hospitalization (Maurin and Raoult, 1999). Of the remaining 2% that require hospitalization, 1.8% have acute Q fever and 0.2% have chronic Q fever (Maurin and Raoult, 1999). Symptomatic acute Q fever manifests primarily as a self-limiting febrile illness associated with severe headaches, atypical pneumonia, or granulomatous hepatitis, while endocarditis is the most common presentation of chronic Q fever (Maurin and Raoult, 1999). The most frequent clinical presentations varies by area (Maurin and Raoult, 1999), but is unclear as to why these geographical differences occur. There are however, some experimental rodent models that suggest some factors that influence clinical expression, which may vary across areas. Experimental infection of mice and guinea pigs demonstrated that inoculation in the intraperitoneal route is associated with hepatitis (La Scola et al., 1997), while infection via the respiratory route is associated with pneumonia (Marrie et al., 1996). Direct inferences to natural infections in humans cannot be made, but results suggest that route of infection may influence clinical presentation (Raoult et al., 2005). Additionally, a sex hormone, 17β-estradiol, has been shown to influence host response to *C. burnetii* in mice, and may account for differences in clinical presentations of Q fever between males and females (Leone et al., 2004). Therefore, if this holds true for humans as well, the distribution of clinical disease in each area may be depend on the proportion of males and females infected with *C. burnetii*. In Ontario between 2006 and 2011 the most common reported symptoms were: fever (80%, 35/44 cases), weakness (52%, 23/44 cases), and chills and malaise (45% each, 20/44 cases) (PHO, 2012). The non-specific nature of the symptoms associated with Q fever likely
leads to under-diagnosis and under-reporting (Marrie and de Carolis, 2002; PHO, 2012; de Valk, 2012).

There is evidence of long-term persistence of *C. burnetii* in human hosts, in acute and chronic cases, as well as in asymptomatic individuals (Harris et al., 2000; Marmion et al., 2009). *C. burnetii* DNA was found in bone marrow aspirates, liver biopsies, and blood mononuclear cells up to five years following acute Q fever incidents, although it was unclear whether or not the patients with detected *C. burnetii* were infective to others (Harris et al., 2000). This may have important ramifications explaining the reactivation of *C. burnetii* infection in asymptomatic cases in times of challenged immunity such as pregnancy, or during immunosuppressive therapy treatment (Harris et al., 2000).

1.4.3 *Dynamics of antibodies through time in humans and animals*

In humans, seroconversion is usually detected 7 to 15 days after the onset of clinical symptoms (Scola, 2002). A longitudinal study of 344 acute Q fever patients demonstrated that both the magnitude and the shape of the serum antibody response varied strongly between individuals (Teunis et al., 2013). IgM and IgG against phase II tended to reach higher levels than the corresponding phase I responses, while IgG antibodies tended to be more persistent than IgM (Teunis et al., 2013). Estimated decay rates are very slow for IgG, and half times up to several years are common (Teunis et al., 2013). Data on antibody persistence in asymptomatic individuals is not available.

In goats inoculated with *C. burnetii*, seroconversion was detected by a strong anti-phase II *C. burnetii* IgM and IgG response two weeks after inoculation (Roest et al., 2013). The range of longitudinal data examining persistence of antibodies in animals is more limited than in humans. The average anti phase II IgG antibody titres rapidly increased between two and four
weeks post inoculation, and then at a slower rate until 10 weeks post inoculation (Roest et al., 2013). Anti-phase I IgG antibody titres started to rise at six weeks post inoculation, and at nine weeks post inoculation the titre stabilized until the end of the experiment at 12 weeks post inoculation (Roest et al., 2013). After a natural *C. burnetii* infection in a sheep flock, half of the sheep were seropositive five weeks after lambing, and a third were still seropositive nine months after the outbreak (Berri et al., 2001). These data suggests that there is relatively long term persistence of *C. burnetii* antibodies in sheep, though more research with larger sample sizes is needed to elucidate the decay rate of *C. burnetii* antibodies in small ruminants.

**1.5 Diagnostic testing and test performance**

1.5.1 *Indirect C. burnetii tests*

There are several tools available to aid in the indirect detection of previous *C. burnetii* exposure (Sidi-Boumedine et al., 2010). These methods involve detecting either *C. burnetii* specific antibodies or markers of cell mediated immunity in the serum, which are produced after individuals were exposed to a sufficient dose of *C. burnetii*. However, a limitation of indirect methods is that they do not provide information about whether an individual is currently colonized or infectious (Sidi-Boumedine et al., 2010), which has relevant implications for disease spread.

The three common serological methods are: Enzyme-Linked Immunosorbent Assay (ELISA), Immunofluorescence Assay (IFA) and the Complement Fixation Test (CFT). The IFA and commercial ELISAs have been shown to have good agreement (Kappa=0.63, 95% CI: 0.55-0.72) (Rousset et al, 2007). Additionally, a skin test and a gamma interferon release assay have been used to detect cellular mediated immunity to *C. burnetii* antigens.

1.5.1.1 *Immunofluorescence Assay (IFA)*
IFA is the reference test for serological diagnosis in humans, and both ELISA and IFA are utilized in veterinary medicine (Tissot-Dupont et al, 1994; Maurin and Raoult, 1999; Sidi-Boumedine et al., 2010). The IFA allows for the differentiation between a suspected acute or chronic clinical infection in humans, based on the ratio of phase I and phase II IgG antibodies (Wielders et al., 2012). If the phase I titre is $\geq$ phase II, the sample is indicative of a chronic exposure, and if the phase II titre is $>$ the phase I titre, the sample is indicative of an acute exposure.

Using human sera, the sensitivity and specificity of the IFA was assessed in four different laboratories using Q fever diagnosis as the gold standard. Positive samples were collected from Q fever patients who were positive on PCR or demonstrated seroconversion, while negative control samples were collected from apparently healthy blood donors and an acute Q fever patient prior to seroconversion (Herremans et al., 2013). For acute Q fever cases, the IFA’s sensitivity of IgG Phase II *C. burnetii* antigens was 100%, while the sensitivity for IgG Phase I was 93%. The sensitivities of detection for past Q fever infections were: 100% in phase II antigens, and 81% in Phase I antigens. The specificity was determined to be 96%, for Phase II antigens, and 98% for Phase I antigens (Herremans et al., 2013).

Researchers have also examined the performance of IFA in eight goat herds with evidence of *C. burnetii* abortions at 15, 30 and 60 days after the onset of Q fever abortion (Rousset et al., 2007). All goats that were IFA-positive were also ELISA positive at least once (43/43), and 22% (6/27) goats that remained IFA-negative or dubious throughout the study were ELISA-positive at least once (Rousset et al., 2007). Researchers then concluded that the IFA had a slightly lower sensitivity relative to the ELISA (Rousset et al., 2007).
1.5.1.2 *Enzyme-Linked Immunosorbent Assay (ELISA)*

The ELISA detects both phase I and phase II antibodies, and provides a cumulative outcome of seropositive, suspect or seronegative status (Herremans et al., 2013). IDEXX evaluated their ELISA test kit for *C. burnetii* using small reference goat populations with known disease status as gold standard populations, although the method of disease status verification was not described. IDEXX reported 100% sensitivity in their ELISA kit using 21 experimentally infected goats, and 100% specificity using 44 goats from known negative herds (IDEXX, 2000). In another analysis, a panel of 69 sera from a goat herd in the Netherlands which had experienced abortions were used as the positive controls. Several, but not all goats had been diagnosed with coxiellosis (placentitis with positive immunohistochemistry). IDEXX ELISA detected 100% (69/69) as either positive (90%, 62/69) or suspicious (10%, 7/69) (Kittelberger et al., 2009).

Latent class analysis (LCA) has been used to estimate the sensitivity and specificity of commercial ELISAs in sheep and goats. Horigan et al. (2011), used a maximum likelihood estimation and conducted a statistical analysis of test accuracy in the absence of a gold standard test. Results indicated that the IDEXX ELISA using tick-derived antigens (Nine-mile strain) (IDEXX Laboratories) had 100% sensitivity and 99.6% specificity for sheep, and 93.1% and 91.2% for goats, respectively (Horigan et al., 2011). An LSI kit ® (Laboratoire Service International, Lissieu, France) ELISA using ovine-derived antigens had 88.8% sensitivity and 98.5% specificity in sheep, and 91.6% and 98.9% for goats, respectively (Horigan et al., 2011). However, one of the assumptions of LCA is conditional independence (Dohoo et al., 2003), and since all these tests measured the presence of *C. burnetii* antibodies (Paul et al., 2013) this assumption may be violated. Bayesian analysis can be used with LCA to account for the
conditional dependence between tests, but this methodology has not yet been utilized to evaluate serological tests for *C. burnetii* using sheep or goat sera.

In cattle sera, LCA using Bayesian methodologies estimated a sensitivity and specificity of the IDEXX ELISA as 84% and 99%, respectively (Paul et al., 2013). In the future, it would be beneficial to evaluate the sensitivity and specificity of both the IDEXX ELISA and ovine-antigen ELISA using Bayesian methodologies in sheep and goats, to account for the lack of a gold standard serological test and conditional dependence among tests.

Using human sera, the overall sensitivity and specificity of two commercial ELISAs were assessed in four different laboratories using Q fever diagnosis as the gold standard (Herremans et al., 2013). The two commercial ELISA kits used were: Iverness Medical Innovations (Waltham, MA, USA) and Verion/Serion (Würzburg, Germany). For acute Q fever cases, the overall ELISA sensitivity for IgG Phase II *C. burnetii* antigens was 100%, while the sensitivity for IgG Phase I was 67%. The sensitivity of detection for past Q fever infections were: 13% with phase II antigens, and 0% with Phase I antigens, although researchers did not indicate how far in the past *C. burnetii* infection occurred. The specificity was determined to be 100% for both Phase II and Phase I antigens (Herremans et al., 2013). Therefore, overall these ELISAs show very poor test performance in human sera, relative to the IFA.

1.5.1.3 Complement fixation test (CFT)

The CFT was the reference test used in much of the animal *C. burnetii* literature prior to the 2000s; however its use is now infrequent, as it has displayed a lower sensitivity than the ELISA (Herremans et al., 2013). Using a panel of 69 goat sera from the Netherlands in which all samples were identified as either positive or suspicious by ELISA, the CFT detected 90% (62/69) of these samples as seropositive (Kittelberger et al., 2009).
Latent class analysis (LCA) has been used to estimate the sensitivity and specificity of the CFT (Horigan et al. 2011). The CFT had 56.4% sensitivity and 98.5% specificity for sheep, and 20.6% and 97.3% for goats, respectively. Therefore, the sensitivity was poor compared to ELISAs which were tested using this same population, as described in Section 1.5.1.2.

The test accuracy of the CFT has also been assessed with human sera. For acute Q fever cases, the sensitivity of IgG Phase II *C. burnetii* antigens was 100%, while the sensitivity for IgG Phase I was 61% (Herremans et al., 2013). The sensitivity of detection for past Q fever infections were: 67% in phase II antigens, and 17% in Phase I antigens. The specificity was determined to be 95% for Phase II antigens, and 95% for Phase I antigens (Herremans et al., 2013). Therefore, the CFT also has a low sensitivity using human sera, particularly when detecting past *C. burnetii* infection.

1.5.1.4 Skin test

A skin test has also been developed for *C. burnetii*, largely for use in vaccination screening programs in Australia. The skin test is performed by intradermal injection of diluted human Q fever vaccine (Q-Vax Skin test, CSL Limited), and the reaction to the vaccine is assessed seven days later (Isken et al., 2013). A positive skin test or serological test meets exclusion criteria for vaccination, as it indicates an individual already has a *C. burnetii* specific immune response.

1.5.1.5 Gamma Interferon release assay (IGRA)

IGRA is a blood test used to detect a cell-mediated immune response. The test measures T cell release of interferon-gamma (IFN-gamma) following stimulation by *C. burnetii* antigens (Kersh, et al., 2013). While cellular immunity is thought to be important for pathogen clearance (Andoh et al., 2007), tests for cellular immunity in people are infrequently reported in the *C.
burnetii literature, with the exception of vaccine efficacy research (Marmion et al., 1990; Kersh et al., 2013). Therefore evaluations of immunity against C. burnetii are often solely reliant on the measurement of serum antibodies (Kersh et al., 2013).

In animals, use of IGRA to evaluate cell mediated immunity is uncommon, but has been employed recently in goats inoculated with C. burnetii (Roest et al., 2013). The cell-mediated immune responses in goats however, did not differ enough between Coxiella-infected and non-infected pregnant animals to be used as a screening test (Roest et al., 2013).

1.5.2 Direct C. burnetii tests

The direct methods of diagnosis involve identifying the presence of C. burnetii, indicating colonization of the host. Coxiella burnetii can be directly detected by PCR, immunohistochemistry (IHC), histology and culture, although the latter is rarely performed as C. burnetii is difficult and time-consuming to process, as well as hazardous, requiring biosafety level 3 facilities due to its zoonotic nature (Masala et al., 2004).

1.5.2.6 Immunohistochemistry (IHC)

IHC is a preferred test to support the diagnosis of an abortion in veterinary medicine, as it allows diagnosticians to definitively identify C. burnetii in affected tissues, such as placental lesions (Dilbeck and McElwain, 1994; Anderson et al., 2013a). It makes use of fixed material, and is safe for the operator. The tissue sample best suited for this test is the placental cotyledon, as this region was found to have a high density of C. burnetii antigens in the trophoblast cells (Sánchez et al., 2006). However, a study examining experimental infection in goats identified that IHC was negative in maternal and fetal organs, yet the PCR on these tissues were positive (Sánchez et al., 2006). Authors then speculated that IHC may not have sufficient analytic
sensitivity for use on maternal and fetal organs (Sánchez et al., 2006), as antigens are present in lower concentrations in these tissues than placenta (Hazlett et al., 2013).

1.5.2.7 Polymerase Chain Reaction (PCR)

The PCR is described as being a sensitive method for detecting colonization, as it is more frequently positive among diagnosed cases in humans or animals than when using other direct methods (Turra et al., 2006; Hazlett et al., 2013). As well, a panel of phylogenetically related species of bacteria all tested negative using PCR, confirming good specificity of PCR C. burnetii targets (COM1 and IS1111) (Christensen et al., 2006). Quantitative real-time PCR is now also commonly used in Ontario to support a diagnosis of C. burnetii abortion/stillbirth in animals (Hazlett et al., 2013).

Testing of other abortive agents should always be performed when investigating the cause of small ruminant abortion (Sidi-Boumedine et al., 2010), as C. burnetii can frequently be present when it is not the cause of the abortion (Hazlett et al., 2013). Research has been conducted in sheep and goats using quantitative real-time PCR (qPCR) to quantify the amount of C. burnetii DNA shed, primarily using aborted placental tissue, to address this problem. Pathologists determined the most likely cause of abortion using information from the gross necropsy, clinical history, histology, immunohistochemistry and qPCR (Hazlett et al., 2013). A Receiver Operating Characteristic (ROC) curve of qPCR results using the pathology diagnosis as the reference test was used to determine a cut point of DNA copies at which the qPCR would best predict overall abortion diagnosis (Hazlett et al., 2013). A cut point of $3.78 \times 10^3$ copies/μl for sheep and $6.8 \times 10^4$ copies/μl for goats was established for aborted placental tissue (Hazlett et al., 2013). Therefore, with quantities above these cut points there is a higher degree of certainty
that *C. burnetii* is the definitive cause of the abortion, as opposed to examining merely the presence of *C. burnetii* DNA with the non-quantitative PCR.

PCR has also been evaluated using human sera as a diagnostic tool in the diagnosis of acute (Schneeberger et al., 2010) and chronic Q fever (Fenollar et al., 2004). This technique has proven effective in identifying people who are colonized before antibody responses are detected, as 98% (49/50) of sera from seronegative acute Q fever patients were PCR positive (Schneeberger et al., 2010). This proportion decreases over time, as 90% of patients with IgM-phase II antibodies, the first antibody to appear, were PCR positive (Schneeberger et al., 2010). The use of PCR on sera from chronic Q fever cases has also been investigated and found to have a sensitivity of 64% and specificity of 100%; however, if samples were stored at -20ºC, specificity was decreased to 24% (Fenollar et al., 2004).

1.6 Seroprevalence

1.6.1 Seroprevalence in sheep and goats

The apparent *C. burnetii* seroprevalence reported in the literature at the animal and herd level varies widely (Guatteo et al., 2011). A critical review of seroprevalence estimates in peer reviewed journals in 2010 reported that individual seroprevalence estimates ranged from 0% to 65% in sheep, and 0% to 75% in goats, while herd level estimates ranged from 0% to 89% of sheep farms and 0% to 100% of goats farms (Guatteo et al., 2011). However, of these studies, very few were considered well designed; most (56/69) of the studies used purposive or convenience sampling methods, and none of the studies provided information on the sensitivity and specificity of the tests utilized (Guatteo et al., 2011).

1.6.1.1 Seroprevalence in Canada
Coxiellosis in sheep and goats has been recognized as endemic in Ontario since the 1980s (McLean et al., 1960; Simor et al., 1984). Until the work presented in this thesis was performed, the level of exposure in Ontario sheep or goats had not been systematically evaluated since the 1980s. Researchers found that 21.4% (22/103) of randomly selected Ontario sheep farms had at least one seropositive ewe, and 1.5% (58/3765) of individual ewes were seropositive using a non-commercial ELISA (Lang et al, 1991). In goats, research indicated that 20.0% (4/20) of randomly selected Ontario goat farms had at least one seropositive animal using two different ELISAs (Lang, 1988).

The seroprevalence of sheep and goats has also been investigated in Newfoundland as a result of a goat-associated human Q fever epidemic which occurred in early 1999 (Hatchette et al., 2001). Before this epidemic, 3.1% (9/293) of sheep tested across the province had antibodies to phase II C. burnetii antigen in 1997, as determined by IFA (Hatchette et al., 2002). After the epidemic, samples were collected from a small sample of 34 sheep on farms scattered throughout the province, between May 1999 and January 2000, and 23.5% (8/34) of sampled sheep had antibodies to phase II C. burnetii using the same test (Hatchette et al., 2002). Goats were sampled only in 2000, after the human epidemic. Again using the IFA, 15.6% of sampled goats (10/64) had antibodies to phase II C. burnetii antigens (Hatchette et al., 2002). Goats were purposively sampled, and were from farms that were distant from the location of the 1999 epidemic (Hatchette et al., 2002). Therefore, this estimate of the goat seroprevalence should be interpreted with caution, as it may under-estimate the true proportion of seropositive goats in Newfoundland at that time.
In Quebec, a serological study conducted in 1998 from the lower Saint-Lawrence River region demonstrated a sheep seroprevalence of 41.0% (137/334) using the CFT, and 89.0% (41/46) of sheep farms had at least one seropositive sheep (Dolcé et al., 2003).

1.6.1.2 Seroprevalence in Europe

In the Republic of Ireland, a study to estimate the endemic seroprevalence of C. burnetii in sheep and goats found 0.7% (15/2197) of sheep and 0.3% (2/590) of goats were seropositive using an Indirect ELISA Q fever LSI kit ® (Laboratoire Service International, Lissieu, France) which uses ovine-derived antigens (sensitivity and specificity discussed in section 1.5.1.2) (Ryan et al., 2011). This study used archived serum samples collected from 2005-2007 for other disease surveillance purposes (Brucella melitensis surveillance for sheep and caprine arthritis and encephalitis for goats). However, there may have been additional sampling bias influencing the goat seroprevalence estimate. While the sheep flocks and individual sheep that were tested were a randomly selected subset of the total number of samples collected for Brucella melitensis surveillance, all available goat samples collected for caprine arthritis surveillance were tested. The authors did not explicitly describe the original sampling procedure used for surveillance (Ryan et al., 2011). If the initial surveillance samples were collected non-randomly, then the tested samples may not represent the general population of sheep and goats in the Republic of Ireland.

In northern Spain, sampling of semi-extensive grazing farms in 2007-2008 indicated that the individual seroprevalence in sheep and goats was 12.3% (160/1298) and 8.3% (9/109), respectively (Ruiz-Fons et al., 2010). At the herd level, 74.0% (34/46) of sheep farms and 45.5% (5/11) of goat farms had at least one seropositive animal (Ruiz-Fons et al., 2010).
In Iran, a serological survey using multi-stage random sampling determined that 65.8% (50/76) of individual goats were seropositive, and all farms (100%, 9/9) had at least one positive goat using the IDEXX ELISA test kit ® (Khalili and Sakhaee, 2009).

As noted by Guatteo et al. (2011) in their review of prevalence studies, there is considerable heterogeneity among seroprevalence studies in sheep and goats with respect to the tests, test cut-points, and sampling strategies used, which hinders comparisons between studies or within a country over time. Harmonization of testing procedures is necessary to facilitate such comparisons (EFSA, 2010).

1.6.2 Seroprevalence in people
1.6.2.1 Screening in general populations

Among general populations, the seroprevalence of \textit{C. burnetii} in people has been estimated to be 4.1% (180/4437) in the United States by first screening with an ELISA (Pan Bio Inc., Columbia, MD, United States), and then confirming with a non-commercial IFA (Anderson et al., 2009), 12.8% (778/6080) in Northern Ireland with an ELISA (Vircell, Granada, Spain) (McCaughey et al., 2008), and 7% (171/2438) in the Hunter New England region of New South Wales, Australia using a non-commercial IFA (Islam et al., 2011). These studies performed analyses for \textit{C. burnetii} seropositivity retrospectively, as the samples were collected for larger studies or for different purposes. In addition, there was heterogeneity with respect to test(s) utilized to detect \textit{C. burnetii} antibodies.

Research using samples from Canadian blood donors found a seroprevalence of 11.8% (118/997) in Nova Scotia in 1982 (Marrie et al., 1984), 14.6% (32/219) in Prince Edward Island in 1982 (Marrie et al., 1984), 4.2% (41/966) in New Brunswick in 1986 (Marrie, 1988), and 15.9% (80/503) in Manitoba in 1986 (Marrie, 1988). In the lower Saint-Lawrence River region
of Quebec, 1.2% (1/81) of patients attending a sexually transmitted disease clinic and employees and volunteers of the Centre Hospitalier Régional de Rimouski were seropositive in 1998 (Dolcé et al., 2003). It is important to recognise that these studies may have their own inherent selection bias, as blood donors or hospital staff may not be representative of the populations from which they came. The health screening process preceding blood donation selects for apparently healthy individuals and many diseases and infections lead to exclusion as a blood donor (Nielen et al., 2004). Therefore, the population of blood donors may under-estimate the true seroprevalence among the general population.

1.6.2.2 Estimating seroprevalence in response to epidemics

Seroprevalence studies were also performed in smaller targeted populations as a result of Q fever epidemics. After the large outbreak in the Netherlands from 2007 to 2009, the seroprevalence of blood donors in the area with the highest number of confirmed cases was determined by screening samples using the ELISA; positive or suspicious samples were tested with the IFA for confirmation, with titres ≥ 1:64 classified as seropositive (Hogema et al., 2012). This process resulted in a seroprevalence of 12.2% (66/543) in 2009 (Hogema et al., 2012).

In Newfoundland, an epidemiologic investigation was conducted after workers on goat farms became ill, and found that 44.7% (80/179) of farm workers and their contacts were seropositive against phase II C. burnetii antigens using the IFA cut point of ≥1:8 in 1999 (Hatchette et al., 2001). Similarly, after an abattoir worker in Quebec was diagnosed with Q fever, the remaining workers at the abattoir were subsequently tested and 20% (3/15), including the index case, were seropositive using the CFT (Goyette et al., 1994).

1.6.2.3 Screening in high risk populations (farm workers and veterinarians)
Screening studies have also been conducted in populations that are considered at high risk of Q fever, such as farm workers and veterinarians. All shepherds from registered sheep farms in the lower Saint-Lawrence River region of Quebec were invited to participate in a serological study, with a maximum of three shepherds per farm. Among shepherds that participated, 28.4% (23/81) were seropositive to *C. burnetii* with IFA titres ≥1:32 (Dolcé et al., 2003). In the Kimberley region of north-western Australia, 66% (31/47) of abattoir workers, pastoral industry workers and veterinarians presenting for vaccinations through the National Q fever Management Program, had evidence of exposure as determined by a positive IFA (cut off titre not described) and/or skin test (Mak et al., 2003).

An estimate of *C. burnetii* seropositivity among veterinarians attending a national veterinary convention in the United States in 2006 found 22.2% (113/508) were seropositive (Whitney et al., 2009). Sera were sequentially tested, first with an ELISA (Pan Bio Inc., Columbia, MD, United States), then positive or equivocal samples were re-tested with the more sensitive IFA, and were considered positive when IgG antibody titres were ≥ 1:16 (Whitney et al., 2009). Approximately 65% of Dutch veterinarians and final year veterinary students who attended a veterinary conference in 2009 had both IgG phase I and II titres of ≥1:32 using the IFA and were considered seropositive (Van den Brom et al., 2013). Therefore, among veterinarians which attended professional conferences in the Netherlands and United States exposure to *C. burnetii* was common.

In a more comprehensive approach to sampling, all veterinary students in the Netherlands were invited to provide blood samples for Q fever screening. Approximately 18.7% (126/674) were seropositive, with IFA titres to phase I or phase II *C. burnetii* antigens ≥1:32 (de Rooij et
al., 2012). It is important to note that these samples from veterinary students were collected in 2006, prior to the large human outbreak (de Rooij et al., 2012).

Screening has also been conducted in suspected high risk populations in Nova Scotia; 49% (32/65) of veterinarians and 35% (34/96) of slaughterhouse workers had a titre ≥1:8 to Phase II *C. burnetii* antigens (Marrie and Fraser, 1985). There is considerable evidence that those working in high-risk professions, such as veterinarians or slaughterhouse workers, are commonly exposed to *C. burnetii*.

### 1.6.2.4 Screening in hospitalized patients

In addition to high-risk professions, screening has also been conducted in hospitalized patients in disease categories compatible with Q fever symptoms. In seven Canadian provinces, 13.8% (109/788) of patients with community-acquired pneumonia had antibodies to *C. burnetii* (Marrie and de Carolis, 2002); for Ontario patients specifically, a seroprevalence of 10.8% (21/193) was observed (Marrie and de Carolis, 2002). Seven Canadian patients, one of which was in Ontario, were later diagnosed with acute Q fever, as they exhibited a four-fold increase in their antibody titre six weeks after the initial blood sample (Marrie and de Carolis, 2002).

### 1.7 Case identification of *C. burnetii* associated disease

#### 1.7.1 Case definitions

Case definitions for clinical disease in animals and people include both evidence of infection and the presence of clinical signs. The case definition of coxiellosis or Q fever used in scientific literature may depend on the purpose of testing. The European Food Safety Authority (EFSA) defined a confirmed case of small ruminant coxiellosis at the herd/flock level as one in which multiple abortions/stillbirths have occurred, diagnostic testing confirmed the presence of *C. burnetii* with PCR, and seropositivity among animals that aborted or had a stillbirth (Sidi-
Boumedine et al., 2010). However, this definition does not describe a time frame or magnitude (number or proportions of abortions/stillbirth) required to be considered a positive herd/flock. This case definition is specific with respect to which tests should be used, and this definition would likely be utilized for control strategies where actions taken on positive farms are costly, such as culling. The EFSA also described a probable case at the herd/flock level as one in which multiple abortion/stillbirth has occurred and animals that aborted/ had a stillbirth tested seropositive with the ELISA (Sidi-Boumedine et al., 2010). The probable case only reveals a suspicion of abortions being caused by C. burnetii, and remains only suggestive without direct identification of the agent (Sidi-Boumedine et al., 2010).

1.7.2 Coxiellosis case identification in sheep and goats

Definitive diagnosis of a case of coxiellosis in animals requires the use of diagnostic testing due to the non-specific nature of clinical signs and because multiple agents can cause abortions (Bouvery and Rodolakis, 2005; Georgiev et al., 2013). A case of coxiellosis, defined as an abortion/stillbirth caused by C. burnetii, is immediately reportable in Ontario, as of January 2013 (Animal Health Act, 2013). This regulation requires veterinary laboratories in Ontario, and veterinarians who use a laboratory outside of Ontario, to notify the Ontario Ministry of Agriculture and Food (OMAF) when a case of coxiellosis is diagnosed in an animal from Ontario (Pasma, 2013). There has been a total of 274 cases of coxiellosis in small ruminants (143 in sheep and 131 in goats) diagnosed in Ontario from 2006 to 2012, inclusive (McEwen et al., 2017; McEwen et al., 2008; McEwen et al., 2009; McEwen et al., 2010; McEwen et al., 2011; McEwen et al., 2012; McEwen et al., 2013). However, 202 of those cases were identified in 2010-2011 (McEwen et al., 2013), concurrent with a small ruminant abortion project at the Animal Health Laboratory, in which free diagnostic tests on small ruminant abortion samples
were offered to veterinary practitioners. This project substantially increased the number of submissions received for diagnosis, and subsequently, the number of cases of coxiellosis that were identified (McEwen, personal communication, 2013). Prior to the start of the study (2006-2009), an average of 10 cases were reported per year, while during the study period (2010-2011) an average of 101 cases were reported per year (McEwen et al., 2013; McEwen et al., 2012; McEwen et al., 2011; McEwen et al., 2010; Mcewen et al., 2009; McEwen et al., 2008; McEwen et al., 2007). This demonstrates how the number of cases of coxiellosis identified per year is influenced by the probability of a detailed veterinary investigation, in which the causative agent is identified in the laboratory (Georgiev et al., 2013). Sample submission, particularly following a single abortion, may be uncommon (Georgiev et al., 2013). The disparity between the average number of diagnostic submissions before and during the small ruminant abortion project illustrates under-reporting of coxiellosis in Ontario sheep and goats. Therefore, it is important to consider that prevalence estimates based on diagnosed cases of coxiellosis in sheep and goats may be large under-estimations of the prevalence of coxiellosis in the population, due to cases not being submitted for diagnostic testing. The probability of a full diagnostic investigation occurring when a producer is presented with an abortion or multiple abortions in their animals in Ontario remains unclear.

It is also important to consider that when new tests are introduced into diagnostic laboratory protocols, the laboratory’s ability to diagnose cases can improve. In the fall of 2008, the Animal Health Laboratory implemented the use of a PCR for *C. burnetii* on routine diagnostic small ruminant cases. PCR is a sensitive test for detecting the presence of target *C. burnetii* DNA sequences. However when PCR positive samples were tested using more traditional methods such as histopathology and modified acid fast smears, results often failed to
confirm that *C. burnetii* was the cause of abortion (Hazlett et al., 2013). Therefore, due to the PCR’s conflicting results with the conventional tests and the subsequent acknowledgement of false positives when using PCR to identify *Coxiella*-induced abortions, its inclusion in 2008 may not have led to an increased detection of cases. In 2009, the quantitative real-time PCR was introduced. Now a threshold of target sequence copies needs to be exceeded in order to diagnose an abortion as having been caused by *C. burnetii* (threshold described in Section 1.5.2.6). The advantage of the real-time PCR over the non-quantitative PCR was an increased specificity in detecting abortions caused by *C. burnetii*, and subsequent decrease in false negative cases of coxiellosis from 2009 onwards.

In addition to the number of reported cases, the relative proportion of abortions caused by *C. burnetii* was estimated among sheep and goat placentas submitted for abortive diagnostic testing at the University of Guelph’s Animal Health Laboratory. The majority of placentas were positive for the presence of *C. burnetii* by real-time PCR; 69.0% (113/163) of sheep placentas and 75% (72/96) of goat placentas were positive (Hazlett et al., 2013). However, of those that were PCR positive, the pathologist making the clinical diagnosis by reviewing the history, lesions and microbiology results, considered *C. burnetii* the significant cause of abortion in only 10% (11/113) of sheep cases and 21% (15/72) of goat cases submitted for analysis (Hazlett et al., 2013). Therefore, from the total number of abortion submissions, *C. burnetii* was identified as the cause of the abortion in 6.7% (11/163) of sheep samples, and 15.6% (15/96) of goat samples. However, these estimates are among samples submitted non-randomly for diagnostic testing. As such, the prevalence of coxiellosis among samples should be interpreted with caution, as the study population may not represent the proportion of abortions caused by *C. burnetii* in Ontario sheep and goats due to the non-random sampling procedure used.
1.7.3  *Q* fever case definitions and numbers of human cases

Considerable effort has been made to harmonize the case definitions for probable and confirmed cases of *Q* fever in the European Union. A “probable case” was defined as a person who exhibited at least one clinical sign (fever, pneumonia, hepatitis), and had an epidemiological link of either exposure to a common source (such as raw milk) or animal to human transmission. A “confirmed case” was defined as a person who exhibited at least one clinical sign and was positive on at least one laboratory test (isolation or detection of *C. burnetii* DNA from infected tissues, or *C. burnetii* specific antibody response with IgG or IgM phase II, though a cut point was not noted) (European Parliament Commission decision 2008/426/EC). The same definition is used in Canada for a confirmed case, with the added caveat that when using serological tests, seroconversion or a fourfold increase from a non-negative titre is diagnostic of *Q* fever (OMHLTC, 2010).

*Q* fever is a reportable disease in humans in Ontario under the Health Protection and Promotion Act (*Health Protection and Promotion Act*, 2009). Confirmed and suspected cases must be reported immediately by the attending physician to the local Medical Officer of Health (OMHLTC, 2010). From 2006 to 2011, a total of 47 confirmed cases of *Q* fever were reported in Ontario (PHO, 2012). In 2011, the number of cases increased four-fold from previous years, with 20 cases reported compared to an average of 5 cases reported in the previous 5 years (PHO, 2012). The increase in the number of detected cases during this period must be interpreted carefully because, while an increase in the incidence of disease cannot be ruled out, the rising case numbers may also be a result of the efforts of our research team and collaborators to increase awareness and promote diagnostic testing of sheep and goat farm workers in Ontario (PHO, 2012). Participating farm workers were provided with fact-sheets with information on
symptoms of Q fever, and it was recommended that all farm workers who tested serologically positive visit their physician for further diagnostic testing.

1.7.4 Q fever incidence and seasonality in humans

The incidence risk for Q fever in Ontario is 0.63 cases per million persons per year, based on the number of reported cases between 2006 and 2011 (PHO, 2012), and the average Ontario population from 2006 and 2011 census data as an approximate number at risk (Statistics Canada, 2012a). The Ontario incidence risk is similar to estimates from the province of Alberta, in which the average annual incidence risk was 0.87 cases per million persons between 1998 and 2011 (Snedeker and Sikora, 2013). Reports from the United States from 2000 to 2004, estimated the average annual incidence risk to be 0.28 cases per million persons, with state specific averages ranging from 0 to 2.4 cases per million persons per year (McQuiston et al., 2006). Results from Australia between 1991 and 1994 showed comparable risks, as the average annual incidence was 0.38 cases per million persons, with state and territory specific estimates ranging from 0 to 1.36 cases per million persons per year (Garner et al., 1997). However, differences in incidence risk should be interpreted with caution, as case notification is dependent on the level of local medical and scientific interest in Q fever across locations and time (Maurin and Raoult, 1999), as demonstrated by the increased numbers of cases during our study period (PHO, 2012). As well, there can be significant variability in case ascertainment between surveillance systems, as a variety of tests and case definitions are used to identify cases (EFSA, 2010).

Q fever can vary by geographic distribution, frequency, clinical presentation and time (Georgiev et al., 2013). Cases of Q fever can occur throughout the year, although most cases in Ontario are reported in June and October, and to a lesser extent in November (PHO, 2012). It has been noted that in Alberta (Snedeker and Sikora, 2013), the Netherlands (van der Hoek et al.,
2010), France (Tissot-Dupont et al., 2004), and to a lesser extent the United States (Anderson et al., 2013b), that the timing of clusters of human cases corresponds to the respective lambing and/or kidding seasons observed in each respective area. The timing of case occurrence is similar in the provinces of Alberta and Ontario, as most cases occurred in Alberta between April and June, and a smaller cluster of cases occurring in October to November (Snedeker and Sikora, 2013). During the 2007-2009 epidemic in the Netherlands, the peak of human cases were reported in April-June, approximately one month after peak lambing/kidding season occurring in March-April (van der Hoek et al., 2010; de Valk, 2012). In France, the peak of reported human cases occurred in April-May, approximately one month after the second lambing season, and when the strongest winds blew (Tissot-Dupont et al., 2004). A smaller cluster of cases occurred after the main lambing season in October-November. Infrequent wind during the main lambing season was described as the reason for reduced risk of transmission relative to the second lambing season in France (Tissot-Dupont et al., 2004). The peak of cases in the United States occurred in April-May, but cases were more evenly spread throughout the year (CDC, 2012), compared to Canadian provinces (Snedeker and Sikora, 2013). Some have suggested that this difference may be due to an increased transmission to humans from dairy and beef cattle in the United States, lessening the influence of seasonal lambing or kidding periods on the timing of human cases (Snedeker and Sikora, 2013). Therefore, seasonal patterns in case occurrence are postulated to be driven by the timing of lambing/kidding season, and perhaps to lesser degree, dairy cattle.

In a review of 37 published reports, including case reports and outbreak investigations, 245 symptomatic and 177 asymptomatic cases of acute Q fever in humans were reported in the United States between 1940 and 2001 (McQuiston and Childs, 2002). Among acute Q fever
cases who reported animal contact, sheep were the most frequently implicated species, followed by cattle, then goats (McQuiston and Childs, 2002). Therefore, due to the high number of case reports associated with cattle, and questions pertaining to the different seasonality pattern of cases in the United States, more research is needed to determine the influence of cattle on the seasonality of Q fever case identification. The differences in seasonality between countries and provinces should be interpreted with caution, as these trends were based on a small number of Canadian cases, which can have considerable variability. In addition, the timing lag between disease onset and diagnosis is variable. It can take three to six weeks to make a diagnosis using serological tests, and cases may not be confirmed until years after the infection (Snedeker and Sikora, 2013).

1.8 Risk factors for exposure to C. burnetii

1.8.1 Risk factors for exposure to C. burnetii in sheep and goats

Risk factors for C. burnetii exposure in animals vary by area, yet many factors are linked to contact with local infected animal reservoirs and to animal management practices (Van Der Hoek et al., 2011a; Enright et al., 1971). In addition to sheep and goats, there is evidence that other animals could be potential sources of C. burnetii infection in Canada. Parturient cats and dogs have been implicated in human Q fever cases in the Maritimes (Marrie et al., 1988; Buhariwalla et al., 2010). Since cats and dogs have the potential for transmitting disease to humans, it may also be possible for cats and dogs to transmit C. burnetii to other animals like sheep and goats, although this route of transmission has not been well established. As well, C. burnetii DNA was extracted from genital swabs of rodents in an Ontario National park, most notably from woodland jumping mice and deer mice, which had a significantly higher prevalence than almost all species caught in that study (prevalence – 83.3% and 76.1%, respectively).
C. burnetii infections in humans or livestock species have not been attributed to wildlife, although this has largely remained unexamined. Therefore, the role that rodents play in the transmission of C. burnetii to other species in Canada remains unclear. An investigation in the Netherlands found that the following factors increased the odds of individual goat seropositivity: a cattle density of $\geq 100$ cattle/km$^2$ in farm municipality (OR=4.5, 95% CI=2.0-9.9); controlling nuisance animals, such as wild birds, through covering airspaces (OR=3.7, CI=1.8-7.9); presence of cats in the goat stable (OR=2.6, CI=1.2-5.6); and signs of vermin (rats, mice, birds) in the roughage or litter in the past 12 months (OR=3.3, CI=1.4-7.9) (Schimer et al., 2011). These findings are suggestive of C. burnetii transmission occurring between species, but more research is needed to clarify the conditions and extent of transmission.

In some areas, ticks have been thoroughly investigated as a source of C. burnetii for sheep and goats. In Iran and Cyprus for example, the presence of ticks was significantly associated with sheep and goat seropositivity (Psaroulaki et al., 2006; Asadi et al., 2012), while in the Netherlands and Hungary the role of ticks in animals acquiring C. burnetii infection was deemed to be negligible due to the low prevalence of C. burnetii in ticks (1.1% (20/1891), 0% (0/5402), respectively), as determined by real-time PCR (Gyuranecz et al., 2012; Sprong et al., 2012). As well, the seroprevalence in wildlife in California did not increase during the season of highest tick activity (Enright et al., 1971). The vector competence of ticks has not been well established, as the presence of C. burnetii in ticks does not necessarily mean they are capable of transmitting it to susceptible hosts (Bonnet et al., 2013). Therefore, the role of ticks in the transmission of C. burnetii to sheep and goats remains uncertain.

Considerable increases in small ruminant population numbers (Statistics Canada, 2012b; Statistics Canada, 2012c) and associated livestock management changes over the past decade
may also influence *C. burnetii* seroprevalence by influencing the transmission dynamics and the potential impact of coxiellosis in Ontario sheep and goats. Research from the Netherlands indicated that goats were more likely to be seropositive if they were from herds with more than 800 goats (Schimmer et al., 2011). In addition, goat and cattle studies established that, when a farm was infected, intensive operations with larger herd sizes would result in a larger degree of environmental contamination (Capuano et al., 2001; Hogerwerf et al., 2013). Multiparous sheep (≥3 births) in Iran had 1.63 (CI=1.007-2.66) times the odds of being seropositive compared to primiparous sheep, which may be due to increased cumulative exposure to the bacterium through multiple breeding seasons (Asadi et al., 2012).

A growing body of evidence indicates that weather and geography could play a large role in *C. burnetii* exposure. Dry, windy weather conditions are thought to favour the transmission of *C. burnetii* (Tissot-Dupont et al., 2004). In addition, proximity to infected farms has also been evaluated; an analysis of risk factors among goat farms in the Netherlands identified farm location within 8 kilometers from a bulk tank positive (PCR) farm increased the odds of goat farm seropositivity at the farm (OR=12.9, CI=3.0-54.8) and individual animal level (OR=3.2, CI=1.4-7.3) (Schimmer et al., 2011). A retrospective evaluation of the dairy goat epidemic in the Netherlands suggests that topography may also influence the spread of infection. A study of land use in the Netherlands concluded that sheep and goat husbandry should be avoided in areas of arable land with deep groundwater and little vegetation, because more dust is available for dispersion of *C. burnetii* (Van Der Hoek et al., 2011a). Moreover, *C. burnetii* in soil was found to be persistently infective for 20 days in a range of temperatures (-20, +4, and +20°C), and the survival increased at lower soil temperatures (Evstigneeva et al., 2007).
The animal micro-environment may also play a role in risk of exposure to *C. burnetii*. Animals that were grazed semi-extensively in northern Spain had a low seroprevalence (cattle 6.6% (41/618), goats 8.3% (9/109)), which the authors suspected to be due to reduced contact between individuals when grazing compared to being extensively housed (Ruiz-Fons et al., 2010). The use of windbreak curtains in the Netherlands, alone or in combination with windshields, increased the risk of goat-level seropositivity compared to goats from farms that had none (Schimmer et al., 2011). It was hypothesized that the use of curtains or windshields created a more air-locked stable, which would facilitate accumulation of *C. burnetii* in the barn and within-herd transmission (Schimmer et al., 2011).

1.8.2 *Risk factors for exposure in people*

Contact with, or proximity to, infected livestock have been identified as sources of human infection (Lyytikäinen, et al., 1998, Tissot-Dupont, et al., 1999, Berri, et al., 2003, Bouvery and Rodolakis, 2005, Roest, et al., 2011, Hoek, et al., 2011). Animal or farm contact were reported as possible sources of infection for 58% (21/36) of Q fever cases in Ontario from 2006 to 2011 (PHO, 2012). Among acute Q fever cases who reported animal contact, sheep were the most frequently implicated species, followed by cattle, then goats (McQuiston and Childs, 2002) in the United States. People from rural areas have a higher risk of seropositivity than those from urban areas; those living in rural areas are more likely to be in the presence of livestock and farming activities such as spreading manure (Pascual-Velasco et al., 1998). Human infection has also been occasionally linked to exposure to parturient cats (Marrie et al., 1988), dogs (Buhariwalla et al., 2010), wild rabbits (Marrie et al., 1986), or pigeon feces (Stein and Raoult, 1999). Age has also been identified as a risk factor among general populations, with older people having a higher risk of being seropositive (Pascual-Velasco et al., 1998). This effect is often
credited to cumulative exposure, since IgG antibodies can persist for long periods of time (Pascual-Velasco et al., 1998).

As noted in Section 1.7.2, there appears to be a relationship between the timing of lambing/kidding season(s) and the incidence of Q fever notifications. Studies examining seasonality have found that incidence of clinical disease was higher in spring and early summer than other seasons (Tissot-Dupont et al., 1999; Hellenbrand et al., 2001). Therefore, people may be at higher risk of new exposure to *C. burnetii* during this time. However, seroprevalence studies have not demonstrated a clear relationship between season and seropositivity, as the persistence of *C. burnetii* antibodies through time likely makes the association with seasonality harder to detect.

By the nature of their occupations, veterinarians, farm workers, and abattoir workers have been identified as having a high risk of *C. burnetii* exposure (Marrie and Fraser, 1985). Within this group of occupations, risk factors for *C. burnetii* exposure among veterinarians included being male, handling sheep placenta (Marrie and Fraser, 1985)\(^1\), more than 30 hours of animal contact per week (OR=16.0, 95% CI=1.8-141.8) relative to < 10 hours/week, more than 22 years since graduation as a veterinarian (OR=58.1, CI=10.3-328.0) relative to < 2 years, living in rural (OR=17.9, 95% CI=3.6-88.1) or semi urban areas (OR=11.9, 95% CI=3.6-68.5) relative to urban areas, being a practicing veterinarian (OR=15.8, 95% CI=2.9-87.2), and occupational contact with swine (OR=3.4, 95% CI=1.1-10.2) (Van den Brom et al., 2013). Occupational contact with swine was also associated with veterinarian seropositivity in the United States, as well as occupational contact with cattle or wildlife, routine contact with pond water, and being ≥46 years of age (Whitney et al., 2009). Swine are not recognized as a reservoir of *C. burnetii* (Marrie, \(^1\) OR estimates not provided by authors
1990), although this has not been thoroughly assessed in the literature. The area of the Netherlands human Q fever epidemic from 2008 to 2010 formerly had a high density of swine farms, although many of these swine farms were converted to goat farms after a classical swine fever epidemic in the 1990s (Enserink, 2010). The C. burnetii seroprevalence in Ontario swine remains unknown, and more research is needed to clarify the role of pigs in the transmission of C. burnetii to other animals and humans. Slaughtering cattle was also a significant risk factor (p=0.004)\(^2\) among abattoir workers in Nova Scotia in 1984 (Marrie and Fraser, 1985).

Similarly, investigation into risk factors in small ruminant farm workers, another high risk profession, has been conducted among different populations. After an epidemic of Q fever among dairy goat farm workers in Newfoundland in 1999, contact with goat placenta, smoking tobacco, and eating cheese made from unpasteurized milk were found to be significantly associated with seropositivity among farm workers and their family in a multivariable analysis (Hatchette et al., 2001). Sheep farmers in the lower Saint-Lawrence region of Quebec had a significantly higher risk of being seropositive (in a univariable analysis) if they spent more than five hours weekly on the sheep farm or if they bought or traded sheep within the previous six months (Dolcé et al., 2003). Finally, multivariable analysis demonstrated several risk factors for seropositivity among Netherlands’ dairy goat farm workers, including: distance to nearest bulk tank milk-positive farm being 8 to 16 km (OR=5.1, 95% CI=1.8-14.1), presence of a cat in the goat stable (OR=2.2, 95% CI=1.01-4.8), distance between residence and goat stable ≤10 meters (OR=2.1, 95% CI=1.3-5.3), and not wearing farm boots among staff and employees (OR=2.5, 95% CI=1.1-5.9) (Schimmer et al., 2012).

\(^2\) OR estimates not provided by authors
Finally, weather has also been identified as a factor contributing to exposure in humans, as dry, windy weather conditions are thought to facilitate the aerosol spread of *C. burnetii* (Georgiev et al., 2013), and have been cited to explain geographical clusters of increased *C. burnetii* exposure in humans (Roest et al., 2011; Asadi et al., 2012). Spatial data demonstrating epidemics of Q fever in humans downwind of sheep rearing areas supports this theory (Tissot-Dupont et al., 1999; Tissot-Dupont et al., 2004). It has also been suggested that wind patterns could account for the appearance of Q fever in urban areas, where patients did not report proximity or contact with animals (Sampere et al., 2003). The potential influence of topography has also been noted in Bulgaria, France and Germany, with human epidemics occurring in towns in valleys close to mountains, or semi-mountainous areas with meadows (Georgiev et al., 2013). Since topography influences airflow (Johansson and Chen, 2003), it follows that the distribution of aerosolized *C. burnetii* can then be influenced by topographical features.

### 1.9 Study rationale

The potential impacts of coxiellosis in sheep and goats are large, due to effects on both reproductive productivity in animals and the potential risk to public health (Sidi-Boumedine et al., 2010). There is a lack of knowledge regarding the current seroprevalence in sheep and goats in Ontario and in the farm workers and veterinarians who care for them. Additionally, risk factors for exposure have not been studied in these populations. This gap in knowledge must be addressed to inform stakeholders of the current risk of exposure and create best practice guidelines based on relevant Ontario data.
1.10 Research objectives

The first objective of this thesis was to describe the individual- and farm-level seroprevalence of *Coxiella burnetii* in sheep and goats in Ontario, in both the meat and dairy sectors. The second was to describe the individual- and farm-level seroprevalence of *C. burnetii* in farm workers caring for the sheep and goats on these farms. The third was to describe the *C. burnetii* seropositivity among small ruminant veterinarians and veterinary students. Lastly, this research set out to identify factors associated with *C. burnetii* seropositivity in the sheep, goats, farm workers, veterinarians and veterinary students in Ontario. To address these objectives, a series of cross sectional-studies was undertaken.
1.11 References


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CHAPTER TWO

Coxiella burnetii seropositivity and associated risk factors in sheep in Ontario, Canada

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Abstract

Coxiella burnetii is a zoonotic pathogen that can cause abortion in sheep in late gestation, as well as the delivery of stillborn, unviable or weak lambs (Rodolakis, 2006). A cross-sectional study was performed to determine the seropositivity and associated risk factors among sheep in Ontario, Canada. Between August 2010 and January 2012, a total of 2363 sheep blood samples from 72 farms across the province were collected and tested for presence of C. burnetii specific antibodies using the CHEKIT Q fever ELISA Test kit (IDEXX Laboratories). Of these farms, 50 were meat sheep farms and 22 were dairy sheep farms. Information on farm demographics and sheep management practices was collected by questionnaire and used to construct a multivariable model with the outcome of sheep seropositivity. Overall, the sheep-level seroprevalence was 14.7% (347/2363, 95% CI=13.3-16.2), and was significantly higher in dairy sheep (24.3%, 181/744) than meat sheep (10.2%, 166/1619) (p<0.0001). At the farm level, 48.6% (35/72, 95% CI=37.2-60.1) of farms had at least one seropositive sheep, and the seroprevalence not significantly different between dairy sheep farms (63.6%, 14/22) and meat sheep farms (42.0%, 21/50) (p=0.09). A mixed logistic model that controlled for farm-level clustering using a random intercept, identified a number of risk factors associated with sheep seropositivity (p<0.05). Increasing the female flock size (logarithmic scale) was associated with increased odds of seropositivity. By way of illustration, sheep on a farm with a female flock size of 200 have 2.26 times (95% CI=1.5-3.5) the odds of seropositivity relative to sheep on a farm with a female flock size of 50.
size of 100. When farms sometimes lambed in an airspace separate from the rest of the flock, sheep had 11.3 times (95%CI= 2.9-43.6) the odds of seropositivity relative to sheep from farms that never lambed in a separate airspace. Loaning sheep that returned to the farm increased the odds of seropositivity by 8.1 times (95%CI: 1.8-33.6). Relative to sheep from farms where all lambing pen hygiene measures were practiced (adding bedding, removing birth materials and disinfection), sheep from farms that only added bedding after lambings had 5.9 times (95%CI= 1.1-32.1) the odds of seropositivity, and those that added bedding and removed birth materials but did not disinfect had 9.0 times (95%CI= 2.2-36.9) the odds of sheep seropositivity. From this research, we can conclude that seropositivity was common among Ontario sheep. Furthermore, the identification of a number of risk factors for sheep seropositivity can be used to inform prevention and control strategies with the aim of reducing *C. burnetii* exposure in sheep.
2.1 Introduction

_Coxiella burnetii_ is a zoonotic bacterium and infection can result in clinical disease in sheep, termed coxiellosis (Maurin and Raoult, 1999). This intracellular pathogen has been recovered from a vast array of other mammals, birds and arthropods worldwide, except in New Zealand (Enright et al., 1971; Maurin and Raoult, 1999; Astobiza et al., 2011; Thompson et al., 2012). Human infection has most frequently been attributed to proximity or contact with infected ruminants, primarily sheep, goats or cattle (Lyytikäinen et al., 1998; Tissot-Dupont et al., 1999; Astobiza et al., 2011; Thompson et al., 2012).

Infection in both humans and animals is considered to be acquired primarily by the inhalation of aerosols contaminated with _C. burnetii_ (Maurin and Raoult, 1999; de Bruin et al., 2012). Sporadic cases attributed to the ingestion of infective materials have also been reported, but this transmission route is considered less efficient than aerosol (Welsh et al., 1945; Benson et al., 1963; Fishbein and Raoult, 1992). In animals, _C. burnetii_ transmission via the oral route is thought to likely occur by ingesting contaminated placentas, pasture, hay, straw and other sources. (Woldehiwet, 2004; Angelakis and Raoult, 2010).

_Coxiella burnetii_ infection in sheep is often subclinical, but it can cause abortion in late gestation, as well as delivery of stillborn, non-viable or weak lambs (Rodolakis, 2006). Large quantities of _C. burnetii_ can be shed by infected ewes in the amniotic fluid, placenta and fetal membranes during an abortion or normal delivery (Berri et al., 2001; Masala et al., 2004; Berri et al., 2005a). In Ontario, a real-time PCR was used to quantify _C. burnetii_ DNA in aborted placental samples, and found an average of $4.82 \times 10^9$ copies of the target DNA sequence (IS1111) per μl of placental tissue (Hazlett et al., 2013). Consequently, during the lambing period, the risk of disease transmission is high due to heavy bacterial loads contaminating the
environment (Maurin and Raoult, 1999; Schulz et al., 2005; McQuiston et al., 2006; Astobiza et al., 2010). However, the risk of transmission extends beyond the lambing period, as intermittent shedding may also occur in the feces, urine and milk for several weeks or months following lambing (Berri et al., 2000; Berri et al., 2001). Contributing to the extended period of transmission is the ability of C. burnetii to remain infective for months in aerosols or contaminated dust that continue to be released as the shed material desiccates (Woldehiwet, 2004).

Q fever in humans and coxiellosis in sheep and goats have been recognized as endemic in Ontario since the 1980s (Palmer et al, 1983; Simor et al., 1984; Simor et al., 1987). While the level of exposure in Ontario sheep has not been evaluated recently, in the 1980s, Lang et al (1991), found that 21.4% (22/103) of randomly-selected Ontario sheep farms had at least one seropositive ewe, and 1.5% (58/3765) of individual ewes were seropositive. The passive surveillance system currently used for coxiellosis in Ontario sheep relies on the reporting of positive diagnostic tests, primarily abortion diagnoses conducted in reference laboratories (McEwen et al., 2012). However, this passive system likely underestimates the true prevalence of C. burnetii infection in sheep due to a high proportion of infections where no clinical signs and no abortions/stillbirths occur (Sidi-Boumedine et al., 2010), and because of under-utilization of available diagnostic services (Georgiev et al., 2013).

Risk factors for C. burnetii exposure in animals include contact with local infected animal reservoirs and specific animal management practices (Enright et al., 1971; Van Der Hoek et al., 2011a). Management practices can influence the farm-level seroprevalence on sheep farms by impacting either the risk of C. burnetii being introduced to farms, or the within-flock seroprevalence by influencing the level of contamination on farm and subsequent degree of...
animal exposure. In Canada, there is evidence that sheep, rodents, cats, cattle, and goats may be a source of *C. burnetii* shedding (Lang, 1988; Thompson et al., 2012; Marrie et al., 1988), but a clearer understanding of the risks of disease transmission between these species is needed.

Contact with infected livestock, or being in the vicinity of infected livestock, have been identified as significant risk factors for human exposure and disease suggesting that it is important to understand infection of sheep in order to develop measures to protect humans (Van Der Hoek et al., 2011b). However, data on risk factors for sheep exposure and disease in Canada is limited. Determination of the seroprevalence and risks for sheep exposure to *C. burnetii* could inform veterinarians, sheep producers and policy makers and aid the development and implementation of practical prevention and control strategies.

The objectives of this study were to: i) determine the flock-level and within flock seroprevalence of *C. burnetii* exposure in Ontario dairy and meat sheep farms, and (ii) identify the demographic and management practices that are risk factors for exposure to *C. burnetii* in Ontario sheep.

### 2.2 Materials and Methods

The University of Guelph Research Ethics Board (Certification of Ethical Acceptability of Research Involving Human Participants – 10JN005) and the University of Guelph Animal Care Committee (Animal Use Protocol – 10R056) approved the study.

#### 2.2.1 Farm selection

This study used a cross-sectional design. Ontario sheep producers were informed of this study through the *Sheep News*, a magazine distributed by the Ontario Sheep Marketing Agency (OSMA) to all producers who market lamb within the province, and *Ontario Farmer*, a newspaper commonly subscribed to by agricultural producers. Meat sheep farms registered in
the OSMA database were selected with a stratified random sampling procedure by OSMA district, using an online random number generator (http://stattrek.com/statistics/random-number-generator.aspx). All solicited producers were asked to provide information regarding their farm’s industry sector and flock numbers, regardless of their intent to participate, to allow for assessment of selection bias. A comprehensive sampling frame for dairy sheep farms in Ontario does not exist; therefore, lists of dairies that milked sheep for human consumption were obtained from sheep milk processors and added to those from an incomplete list from the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). All identified producers in Ontario that met inclusion criteria were solicited for participation. Selected producers from both meat and dairy sheep farms were solicited via letter or telephone. Farm inclusion criteria were: having at least 10 ewes that had given birth in the previous 12 months and being located within 800 km of the University of Guelph. Regions beyond 800 kms of Guelph were excluded due to logistical concerns and low populations of sheep.

2.2.2 Sample size calculation and sheep selection

To estimate the flock-level prevalence using an a priori estimate of 24% with a 95% confidence and 10% allowable error, 72 sheep farms were sampled; 22 dairy sheep farms and 50 meat sheep farms. The number of farms sampled in each sector was proportional to the total estimated number of farms in that sector in Ontario. Farms were enrolled until the target numbers of dairy and meat sheep farms were achieved. To estimate within-farm seroprevalence using an a priori estimate of 10% with a 95% confidence and 10% allowable error, 35 ewes that had lambed in the previous 12 months were sampled per farm. If the farm had less than 35 ewes that lambed in the previous 12 months, samples were taken from all ewes that met this requirement.
Ewes were randomly selected using a random number generator (http://stattrek.com/statistics/random-number-generator.aspx).

2.2.3 Farm-level data collection

Animal management questionnaires were administered on each participating farm by personal interview of the farm manager at the time of animal sampling, or were returned by the farm manager via postal mail after the farm visit was completed. The animal management questionnaire (Appendix I) used a mix of closed and open questions to gather information on farm demographic characteristics, sources of replacement stock, lambing management practices, sheep contact with other animal species (livestock, dogs, cats, rodents, wildlife etc.), proximity to other farms and biosecurity practices.

Measures of reproductive failure, such as average risk of abortions in the preceding three years, and prior reproductive-related diagnostic testing, were also collected for descriptive purposes and to gain insight as to whether current management practices were implemented in response to awareness of prior reproductive challenges in their sheep. These measures of reproductive failure were considered a possible outcome of exposure to C. burnetii, and were therefore not included in the putative risk factor model. Measures of reproductive failure were stratified and compared across risk factor categories in the multivariable model. This was done to allow for comments on potential reverse-causation; if farms in a risk factor category associated with seropositivity in the multivariable model also had a higher risk of reproductive failure, this may indicate that reverse-causation is an issue. Similarly, differences were analysed between farms where farm managers completed the questionnaire with researchers and those that emailed/mailed questionnaires in to assess if method of questionnaire completion may have influenced the multivariable model.
Pre-testing of the animal management questionnaire and consent form was conducted with a total of 10 sheep and goat producers and industry representatives during the spring of 2010. These individuals were purposively selected to represent the different sectors of the sheep and goat industry. Study farms were enrolled beginning in the summer of 2010 until the autumn of 2011. Sampling and questionnaire administration occurred between Aug 2010 and Jan 2012.

2.2.4 Sheep blood collection

Blood samples were collected by jugular venipuncture into 10 ml red top serum BD vacutainer tubes® (Becton, Dickson and Company, Franklin Lakes, New Jersey, USA). Vacutainer tube samples were transported in vertical stopper-up position and chilled on ice at 4-8°C. Sample centrifugation occurred at the University of Guelph if transport time was less than 4 hours or at local veterinary clinics if transport time exceeded 4 hours. Vacutainer tube samples were centrifuged for 10 minutes at 1000 x g and 2ml aliquots of the separated serum were then immediately pipetted into serum micro tubes (Fisher Scientific, Ottawa, CA).

Serum micro tubes that were processed at the University of Guelph during laboratory business hours were submitted immediately to the Animal Health Laboratory (AHL), Laboratory Services Division, University of Guelph. Samples were frozen for up to 48 hours at -20°C prior to submission if either: they were prepared at the University of Guelph after regular business hours, or if they were processed off-site at local veterinary clinics.

2.2.5 Serological analysis

Serology was performed by the AHL using the CHEKIT Q-Fever Antibody ELISA Test Kit (IDEXX Laboratories, Broomfield, CO, USA), which uses the Nine Mile antigen. The results were expressed as the ratio between the Optical Density (OD) of the sample (S) and the OD of the positive control (P) included in the test kit. In accordance with the manufacturer’s
instructions, the ratio of the optical density of the sample(s) to that of the positive control ≥40% was considered seropositive.

2.2.6 Data management and statistical analysis

Questionnaire and serological data were entered into EpiData® v2.2 for file management (EpiData Association®, Odense Denmark) and were manually checked against the original questionnaire for errors. A mixed logistic regression model was constructed in Stata Intercooled Version 10.1 (StataCorp®, 2007) to assess putative risk factor associations with the outcome of sheep seropositivity, while controlling for farm-level clustering using a random intercept.

The initial step in model building was univariable screening of all covariates for association with the outcome at a liberal 20% significance level using the likelihood ratio test (LRT). The linearity of continuous variables was assessed graphically by plotting lowess smoother curves and transforming the variable if necessary to achieve normality (Dohoo et al., 2003). Variables associated with the outcome at a 20% significance level were then screened for pair-wise collinearity using Spearman’s rank correlation; variable pairs were considered collinear when Spearman’s rho was ≥ |0.8| (Mason and Perreault, 2013). Collinear pairs were compared with respect to univariable significance, missing variables, and biological relationship, and the most relevant variable was retained (Dohoo et al., 2003). Variables with more than 25% missing values were excluded from analysis. This cutoff was chosen based on the distribution of missing values, as it minimized the number of putative risk factors excluded from analysis and maximized the number of observations used in the multivariable model. The retained covariates were then used to construct the multivariable model using a manual backward stepwise model-building procedure. Non-significant predictor variables with the highest p-values were removed one at a time from the multivariable model until all variables remaining had a level of
significance of $\alpha < 0.05$. All eliminated variables were then tested for confounding and retained in the model if their inclusion caused a >20% change in the coefficients of one or more of the significant variables. Two-way interaction terms were generated for biologically relevant covariate pairs, and retained in the model if significant ($\alpha < 0.05$). The fit of the model was assessed by examining upper level residuals (by farm) using best linear unbiased predictions (BLUPs) analysis to verify the model assumptions of normality and homoscedasticity (Dohoo et al, 2003). Residuals were visualized in both the fixed and random portions of the model to identify potential outliers that would require further investigation.

2.3 Results

2.3.1 Study Population

Fifty-five percent (22/40) of dairy sheep farms and 30.7% (50/163) of meat sheep farms that were solicited and met the inclusion criteria participated in the study. Nineteen sheep farms mailed back responses indicating they did not wish to participate; of these, four were from meat sheep farms and six were from dairy sheep farms, and nine did not specify sheep farm type. The mean adult female flock size on participating farms was 171 sheep (SD ± 157), and the median was 100 (interquartile range 52-400). Sheep farms were distributed across western, central and eastern Ontario, with a small number of farms in northern Ontario. None of the sheep farms studied reported use of a commercial C. burnetii vaccine (Coxevax®, CEVA Animal Health).

2.3.2 Seroprevalence

Overall, the sheep-level seroprevalence was 14.7% (347/2363, 95% CI=13.3-16.2), and was significantly higher in dairy sheep (24.3%, 181/744) than meat sheep (10.2%, 166/1619) ($p<0.0001$). At the farm level, 48.6% (35/72, 95% CI=37.2-60.1) of farms had at least one seropositive sheep, and the farm level prevalence was not significantly different among dairy
sheep farms (63.6%, 14/22) and meat sheep farms (42.0%, 21/50) (p=0.09). Figures 2.1 and 2.2 demonstrate the distribution of farm level and within-farm seroprevalence in the dairy and meat sectors, respectively.

2.3.3 Risk factor analysis

A descriptive summary of questionnaire data is provided in Appendix II. The factors associated with sheep seropositivity at \(\alpha < 0.20\), are presented in Table 2.1. The following factors were also investigated, but were not significantly associated with sheep seropositivity in the univariable analysis (p\(\geq\)0.20): number of lambing groups; use of artificial insemination; purchase of replacement females; number of replacement animals purchased in the past three years; purchase of animals from show sales or dealer/brokers; taking and returning sheep to/from an agricultural fair; testing for \(C.\ burnetii\); having dogs, cattle, llamas, alpacas, goats, deer, horses, donkeys, pigs or fowl on farm; using cats versus traps for rodent control; having raccoons, opossums or other wildlife other than birds in the barn; lambing outdoors; replacement animals being exposed to adult sheep, their manure, or lambing areas; spreading manure; shearing or crutching ewes prior to lambing; quarantining ewes after aborting; and disposal practices for placentas and aborted fetuses. The variables ‘purchase of male sheep’ and ‘closed flocks’ were negatively correlated with each other (Spearman’s rho= -1.0); ‘closed flocks’ was dropped from the model as it was less significant than ‘purchase of male sheep’ at the univariable level, and had the same number of observations. The final multivariable model is presented in Table 2.2.

Visual assessment of the BLUPs residuals identified three outlier farms. If animals from these three farms were removed, the BLUPs residuals were normally distributed, and the interpretation of the multivariable model did not change. There was no reason for their exclusion, hence; all outliers were retained in the multivariable model. The latent variable technique
(Goldstein et al., 2002) was used to calculate the variance components at the farm and individual levels.

2.3.4 Association of reproductive failure and sheep seropositivity

Measures of reproductive failure were tabulated with significant variables from multivariable model to assess the potential for reverse-causation. Farm-level frequency of reproductive outcomes or testing over the previous three years and the farm-level frequency of non-permanent risk factors in multivariable model are presented in Table 2.3. Based on examination of the raw data in Table 2.3, farms that practiced measures which were associated with seropositivity did not have a higher frequency of C. burnetii abortions, or elevated abortion risk (>5%) over the past three years relative to farms that did not practice those measures.

2.4 Discussion

Exposure to C. burnetii was common among Ontario sheep tested in this study; 48.6% of farms tested had at least one seropositive sheep, and 14.7% of all sheep were seropositive. Dairy sheep were identified as having a higher farm and sheep-level C. burnetii seroprevalence compared to meat sheep. Therefore monitoring C. burnetii infections may be of particular interest in dairy sheep industry in Ontario. The risk factors associated with sheep seropositivity observed here reflect the influence of management practices on transmission dynamics within and between farms. Sheep had higher odds of being seropositive if they were from farms that loaned sheep to other farms and later returned them to the home farm. Loaning sheep to other farms was hypothesized to increase the odds of sheep seropositivity if returning sheep was/were exposed to C. burnetii while away. If the re-introduced seropositive sheep shed C. burnetii, they could expose other sheep on farm, thus further increasing the odds of sheep seropositivity on farm. The risk of introducing the agent to a previously negative flock is the basis of the
restrictions on trade and animal movement during the Q fever epidemic in the Netherlands (EFSA, 2010).

The odds of sheep seropositivity also increased with increasing female flock size on a logarithmic scale. By way of illustration, sheep on a farm with a female flock size of 200 have 2.26 times (95% CI=1.5-3.5) the odds of seropositivity, relative to sheep on a farm with a female flock size of 100. This finding is consistent with goat and cattle studies which established that, when a farm was infected, intensive operations with larger herd sizes would result in a larger degree of environmental contamination (Capuano et al., 2001; Hogerwerf et al., 2013), and subsequently a higher degree of exposure within farms. This is also consistent with research from the Netherlands, which indicated goats were more likely to be seropositive if the herd size was above 800 (Schimmer et al., 2011)

The proportion of animals exposed at a given time may also depend on the distribution of bacteria in the shared environment. Since the lambing area is a likely place for heavy C. burnetii loads, it was anticipated that lambing in a separate airspace from the rest of the flock would serve to minimize the odds of sheep seropositivity (EFSA, 2010). However, on farms where farm managers indicated that ewes sometimes lambed in an airspace separate from the rest of the flock, ewes had significantly higher odds of being seropositive, compared to those from farms that never lambed in a separate airspace. Based on data in Table 2.3, of the 14 farms that sometimes lambed in a separate airspace, one farm had submitted placental or fetal tissues for abortive diagnostic testing, and C. burnetii was not identified as the cause of abortion, nor was it present in submitted tissues. The four farms with elevated abortion risk (above 5%) in the past three years were distributed evenly among all four categories of frequency of separate lambing (always, sometimes, never, not applicable). Therefore, an association between lambing ewes in a
separate airspace and seropositivity was not suspected to be due to reverse causation (i.e. farm managers choosing to lamb in a separate airspace only after they became aware of *Coxiella*-induced reproductive challenges). Research from the Netherlands found the use of windbreak curtains and windshields in barns increased the risk of individual goat seropositivity (Schimmer et al., 2011). The authors reasoned that the restricted air-flow decreased ventilation and facilitated the accumulation of *C. burnetii* in that environment (Schimmer et al., 2011). Therefore, segregating lambing in a separate airspace may have implications for ventilation or air-flow, dependent on the facilities available. If ventilation is more restricted in the lambing area, this could encourage environmental contamination with *C. burnetii* in that space and promote transmission within the segregated group of breeding ewes. However, empirical evidence testing this hypothesis is required.

Sheep seropositivity was also associated with the hygienic practices in place during lambing. Sheep from farms that only added bedding after lambings, and from those that added bedding and removed birth materials, had significantly higher odds of seropositivity compared to sheep from farms that did these things in addition to disinfecting lambing pens. This suggests disinfection may be an effective tool for reducing bacterial burden in the lambing environment. However, the type of disinfectant used was not assessed here, and the efficacy of specific disinfectants for use in lambing pens should be considered for future research. Anecdotally, lambing took place on bedding packs on many farms. Therefore, investigating the efficacy of dry powder disinfectants would be of particular interest, as they would be more practical than liquid disinfectants for use in lambing pens on many Ontario sheep farms. The odds of sheep seropositivity was not significantly different among sheep from farms where none of the lambing pen hygienic practices were in place compared to those where all hygiene measures were taken;
however, the majority of the former farms practiced outdoor lambing. This suggests that that *C. burnetii* shed in outdoor lambings may be subject to other factors, such as increased dispersion through wind (Tissot-Dupont et al., 1999), which can decrease the odds of sheep becoming exposed.

The presence of cats on farm was hypothesized to be a risk of exposure to *C. burnetii* in sheep, as exposure to parturient cats in Maritime Canada was a risk factor for human Q fever (Marrie et al., 1988), and cats and sheep on farms often share the same barn environment. Ninety-two percent (66/72) of farms in this study indicated that they had cats on farm, and this was not significantly associated with *C. burnetii* seropositivity in sheep. Due to the presence of cats being very common among participating sheep farms, it is possible that there was not enough variability in the dataset to detect a statistically significant difference in risk of *C. burnetii* seropositivity between sheep from farms with and without cats.

Wildlife contact was also hypothesized to be a possible risk of exposure to *C. burnetii* for sheep, especially considering the high prevalence of *C. burnetii* found in genital swabs of wild rodents in a national park in Ontario (Thompson et al., 2012). Further, in the Netherlands, rats were found to have active infections outside of the lambing/kidding season and in urban settings (Reusken et al., 2011). Researchers therefore suggested rats may be capable of maintaining *C. burnetii* infection independent of livestock contact. No association between observed wildlife in the barn and sheep seropositivity was found in the present study. However, asking farm managers about wildlife contact may under-estimate its frequency, as it requires some evidence of the presence of wildlife, either from seeing the wild animal itself or seeing droppings or nests, which may not always occur. Also, the presence of wildlife inside the barn may not be required for transmission to sheep to occur, due to the capacity for aerosolized *C. burnetii* to travel in the
wind (Van Der Hoek et al., 2011b). Due to this potential aerosol spread, the degree of contact necessary for transmission to occur from wildlife to livestock remains unclear. Therefore, future research can clarify if wildlife pose a risk of *C. burnetii* exposure in sheep by identifying if wildlife species on or near sheep farms have *C. burnetii* infections, and if so, determining whether the same *C. burnetii* strains are circulating in wildlife and sheep.

Previous research reported that older ewes (>2 years old) were more likely to be seropositive than yearlings (1-2 years old) and replacement lambs (6 months old) (García-Pérez et al., 2009). As such, we attempted to collect individual animal characteristics such as birth date and date of last lambing. While farm managers were able to identify which sheep had lambed in the previous 12 months, it became apparent that many did not keep detailed records with the dates of the last lambing and ewe date of birth, and some did not have adequate animal identification. Additionally, some had purchased adult sheep with no history. As such, we were unable to assess individual sheep risk factors due to high numbers of missing values and likely inaccurate data. It is therefore recommended that the importance of record keeping be emphasized at sheep industry magazines and meetings.

The farm-level seroprevalence obtained in our study (48.6%), differs from that of a previous serosurvey in 1988 of Ontario sheep farms which estimated that 21.4% (22/103) of farms had at least one seropositive sheep (Lang et al., 1991). The apparent increase in seroprevalence in the province over time may signify further spread of *C. burnetii*, but the methodological heterogeneity between these studies also hinders direct comparisons. IDEXX has reported a 100% sensitivity in their ELISA kit (used here), based on comparison with 21 experimentally infected goats, and 100% specificity using 44 goats from known negative herds (IDEXX, 2000). The ideal sensitivity and specificity analysis involving an independent
evaluation of the IDEXX ELISA against a gold standard which uses large sample sizes has yet to be published. The non-commercial ELISA used by Lang et al. (1991) is no longer in use and the degree of formal agreement between these tests is unknown. Therefore, given the dissimilarity in testing used, it remains unclear if there is a true increase in the proportion of Ontario sheep exposed to *C. burnetii* over time, or if the larger proportion of seropositive sheep detected in this study is due to methodological differences. The fluctuations seen in the Canadian sheep population over the past decade (Statistics Canada, 2012) may also affect flock-level and within-flock seroprevalence due to associated changes in sheep loaning, purchasing behaviours and flock sizes.

While long-term persistence of serum antibodies after natural infection has been described in sheep (Berri et al., 2001; Berri et al., 2002), a proportion of sheep in small epidemic scenarios have been shown to shed *C. burnetii* but remain seronegative (Berri et al., 2001; Berri et al., 2001; Berri et al., 2005a). The expected proportion of seronegative shedders in non-epidemic scenarios remains unknown. Therefore, it is possible that both the individual and within-flock seroprevalence reported in this study under-estimate the true prevalence of exposure if seronegative shedders were captured in our sample of sheep.

Given that *C. burnetii* is readily transmitted in aerosols (Stein et al., 2005), we may expect that, on a farm with an active *C. burnetii* infection, most of the sheep will have been exposed to *C. burnetii*. However, since the serological results indicate that the within-flock seroprevalence often did not approach 100%, there may be other mechanisms to explain this effect, such as clearance of *C. burnetii* from cell-mediated immunity (Andoh et al., 2007) and waning *C. burnetii* titres over time (Berri et al., 2001).
If sheep were sampled after a recent infection, but before an immune response had been generated, it is also possible that they could be falsely classified as non-exposed (Berri et al., 2002). Most new infections typically occur during the lambing season (Astobiza et al., 2010), and sero-conversion in sheep typically occurs 3 to 4 weeks after infection (Brooks, 1986). False negatives caused by sampling in the latent period are therefore likely minimized in our study sample, as farm managers had a strong preference to schedule farm visits during non-lambing periods when they had more time available.

There is potential for recall bias when assessing farm manager-reported practices, as managers who know their farms are affected by *C. burnetii* might have an increased sensitivity in recalling practices they deemed to be associated with coxiellosis, when compared to non-affected farms (Dohoo et al., 2003). Efforts were taken to reduce the potential for recall bias by setting a reasonably short recall period, usually assessing either current practices or those conducted in the last year, to minimize misclassification due to recall bias. The questionnaire was completed before the farm manager was informed about their sheep’s serological results, so the majority of farms were blind with respect to their animals’ sero-status, except for the seven farms that had received diagnostic results indicating *C. burnetii* was present in submitted samples prior to participating in this study (Table 2.3). The interpretation of the multivariable model did not change when these farms were excluded (data not shown); hence, we do not believe recall bias had a significant effect.

Farm managers were given the option of completing the questionnaire with a researcher on farm (n=43 farms), or emailing/mailing the questionnaire to researchers (n=29 farms), in order to best facilitate participation. No significant differences were found between those groups with respect to: sheep seropositivity (p=0.98), lambing pen cleaning practices (p=0.86), whether
lambing took place in an airspace separate from the rest of the flock (p=0.85), loaning sheep that return to the farm (p=0.82), or female flock size (logarithmic scale) (p=0.80). Therefore, the method used to complete the questionnaire is not believed to have influenced the risk factor analyses.

When using a cross-sectional study design, it can be difficult to decipher whether the explanatory factors identified truly influence the probability of exposure, or whether they are measures implemented as the result of exposure (Dohoo et al., 2003). While reverse causation may be a possibility, only a small number of farms (7) had *C. burnetii* abortions diagnosed on their farm prior to participating in this study and none had vaccinated their sheep with the *C. burnetii* vaccine (Coxevax®, CEVA Animal Health). Further, a small number of farms had an elevated abortion risk (>5%) over the past three years (4), or had experienced an abortion storm (14). As such, it is unlikely that the management practices associated with *C. burnetii* seropositivity identified in this study were influenced by awareness of previous *C. burnetii* exposure.

The lack of a comprehensive list of dairy sheep producers, and subsequent convenience sampling at the sheep farm-level may hinder the external validity of the dairy sheep results, as it relates to the seroprevalence and the dairy sheep farms influence on the risk factors identified. Convenience recruitment may have caused participating dairy sheep farm workers to be more similar with respect to some factors (e.g. milk processors, geography and social circles) than non-participating dairy sheep farm workers. We exhausted all known sources to identify sheep dairy farms in Ontario. To the best of our knowledge, a high percentage (55%, 22/40) of the total number of estimated dairy sheep farms participated in our study, but this high response does not guarantee the lack of response bias (Bjertnaes et al., 2008).
An animal vaccine (Coxevac, Ceva Animal Health) is available for use in sheep in Ontario. Veterinarians need to apply to the Canadian Centre for Veterinary Biologics for access to the vaccine, providing details regarding where and why the Coxevac vaccine will be used, as the vaccine remains unlicensed in Canada. A recent meta-analysis of available literature concluded that vaccination did not have a significant effect on the risk of *C. burnetii* shedding in sheep (O’Neill et al., 2013); however, analysis was is based only on three studies. At this time, there appears to be limited utility of vaccinating sheep with the Coxevac vaccine, although this should be re-evaluated if new evidence is established.

Since persistent contamination of the environment with *C. burnetii* is a large concern, future research demonstrating how to effectively mitigate the bacterial burden on farm is needed, including; reducing animal shedding; limiting introduction of *C. burnetii* from wildlife and other domestic animals; proper composting of placenta/aborted fetuses and manure; and effective disinfection practices. While this study identified a protective effect of disinfecting the lambing pens and removing placental material on seropositivity, further experimental research should be conducted to provide a set of evidence-based guidelines for best-practice protocols for disinfecting lambing pens.

This study evaluated management practices associated with seropositivity. Moving forward, a better understanding of how *C. burnetii* spreads within and between sheep flocks, as well as from other host species, would assist to improve the ability to implement effective management interventions.

### 2.5 Conclusion

Results from this study indicate that exposure to *C. burnetii* was common among Ontario sheep tested in this study; 48.6% of farms tested had at least one seropositive ewe, and 14.7% of
all ewes were seropositive. This research has also provided a better understanding of the
demographic and management practices associated with *C. burnetii* seropositivity in Ontario
sheep. Lambing area hygiene was noted as an important factor protecting against exposure.
Sheep farm workers that disinfect lambing pens, in addition to removing birthing materials and
adding bedding should continue to do so; however, more research is needed to identify which
disinfectants are effective against *C. burnetii* in lambing pens so that specific recommendations
can be made. Since loaning sheep to other farms that then return was associated with a
significant risk of exposure, animal movement should be voluntarily restricted (“closed” flock
policy), where possible, to mitigate the risk of *C. burnetii* introduction. Producers from larger
flocks should be mindful that there is an increasing risk of exposure as the female flock size
increases. Therefore, more stringent hygiene practices and biosecurity may be required on large
farms to counterbalance this effect. The findings from this work can also be used to direct future
research and provide evidence to support the development and validation of control strategies
and policies based on preventing or mitigating exposure of sheep to *C. burnetii*.

2.6 Acknowledgements

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2.7 References


EFSA, 2010: Scientific opinion on Q fever. EFSA J. 8, 1–114.


Figure 2.1 Percentage of *C. burnetii* seropositive sheep per farm on 22 dairy sheep farms in Ontario, Canada (Aug 2010-Jan 2012)*

* Farms have been sorted from lowest to highest within-herd seroprevalence
Figure 2.2 Percentage of *C. burnetii* seropositive sheep per farm on 50 randomly selected meat sheep farms in Ontario, Canada (Aug 2010-Jan 2012)*

* Farms have been sorted from lowest to highest within-herd seroprevalence
Table 2.1 Farm management variables associated (p<0.2) with sheep seropositivity for *C. burnetii*, as observed through univariable mixed-effects logistic regression of data collected from 72 sheep farms in Ontario, Canada (Aug 2010-Jan 2012)

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>OR</th>
<th>OR 95% CI</th>
<th>P-value</th>
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</thead>
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<tr>
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<td>Meat</td>
<td>Ref</td>
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<tr>
<td>Add bedding, remove birthing materials and disinfecting pen</td>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Lambing ewes in separate area from flock (LRT ( \chi^2 = 362.96 ), P-value = 0.1310)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Not Applicable (do not lamb indoors)</td>
<td>0.18</td>
<td>-4.85-4.075</td>
<td>0.281</td>
</tr>
<tr>
<td>Sometimes</td>
<td>4.93</td>
<td>0.86-28.20</td>
<td>0.073</td>
</tr>
<tr>
<td>Always</td>
<td>2.87</td>
<td>0.34-24.44</td>
<td>0.336</td>
</tr>
<tr>
<td>Days replacement lambs spend with dam before weaning (Log_{10} scale)</td>
<td>0.50</td>
<td>0.23-1.08</td>
<td>0.0760</td>
</tr>
<tr>
<td>Other sheep or goat farms within 5km (LRT ( \chi^2 = 411.79 ), P-value = 0.1774)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>5.59</td>
<td>0.86-36.13</td>
<td>0.071</td>
</tr>
<tr>
<td>Unknown</td>
<td>8.12</td>
<td>0.23-289.00</td>
<td>0.251</td>
</tr>
</tbody>
</table>

Ref= Referent category

LRT = Likelihood ratio test for categorized variable
Table 2.2 Farm management variables associated (p<0.05) with *C. burnetii* seropositivity in sheep, as observed through multivariable mixed-effects logistic regression of data collected from 72 sheep farms in Ontario, Canada (Aug 2010-Jan 2012)

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>OR</th>
<th>OR 95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lambing pen cleaning practices</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(LRT $\chi^2 = 11.71, p = 0.0084$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Add bedding, remove birthing materials and disinfect</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Add bedding and remove birthing materials</td>
<td>8.96</td>
<td>2.17-36.90</td>
<td>0.002</td>
</tr>
<tr>
<td>Add bedding only</td>
<td>5.94</td>
<td>1.10-32.12</td>
<td>0.038</td>
</tr>
<tr>
<td>Do nothing</td>
<td>0.97</td>
<td>0.04-23.29</td>
<td>0.986</td>
</tr>
<tr>
<td><strong>Lambing ewes housed in separate airspace from flock</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(LRT $\chi^2 = 11.77, p = 0.0082$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>2.08</td>
<td>0.43-10.10</td>
<td>0.362</td>
</tr>
<tr>
<td>Sometimes</td>
<td>11.26</td>
<td>2.91-43.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Never</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Not Applicable (do not lamb indoors)</td>
<td>0.44</td>
<td>0.02-11.01</td>
<td>0.614</td>
</tr>
<tr>
<td><strong>Loanng sheep that return to farm</strong></td>
<td>8.14</td>
<td>1.81-36.61</td>
<td>0.006</td>
</tr>
<tr>
<td>Female flock size (Log$_{10}$ scale)</td>
<td>3.24</td>
<td>1.71-6.15</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

| **Random Intercept Parameter**                                               |      |           |         |
| Farm                                                                        | 1.59 | 1.11-2.26 | <0.00001|

Ref= Referent category, SD= Standard deviation

*a* LRT = Likelihood ratio test for categorized variable

*b* Partitioning the variance, 43.3% occurred at the farm-level and 56.7% occurred at the sheep-level
Table 2.3 Farm-level frequency of reproductive testing and outcomes over the previous 3 years and frequency of non-permanent risk factors in multivariable model, on 72 sheep farms in Ontario, Canada (Aug 2010- Jan 2012)

<table>
<thead>
<tr>
<th>Reproductive outcomes</th>
<th>C. burnetii identified as cause of abortion</th>
<th>C. burnetii identified in placental/fetal samples but not cause of abortion</th>
<th>Elevated abortion risk (&gt;5%) over previous 3 years</th>
<th>Experienced abortion storm</th>
<th>Submitted abortive diagnostic samples to AHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambing pen hygiene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nothing</td>
<td>1/7</td>
<td>2/7</td>
<td>1/7</td>
<td>3/7</td>
<td>3/7</td>
</tr>
<tr>
<td>Add bedding only</td>
<td>1/11</td>
<td>1/11</td>
<td>2/11</td>
<td>2/11</td>
<td>4/11</td>
</tr>
<tr>
<td>Add bedding and remove birth materials</td>
<td>1/23</td>
<td>0/23</td>
<td>0/23</td>
<td>4/23</td>
<td>5/23</td>
</tr>
<tr>
<td>Disinfect, add bedding and remove birth materials</td>
<td>1/22</td>
<td>0/22</td>
<td>1/22</td>
<td>5/22</td>
<td>6/22</td>
</tr>
<tr>
<td>Separating lambing ewes to separate airspace</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not applicable</td>
<td>2/5</td>
<td>2/5</td>
<td>1/5</td>
<td>3/5</td>
<td>4/5</td>
</tr>
<tr>
<td>Never</td>
<td>2/36</td>
<td>1/36</td>
<td>1/36</td>
<td>6/36</td>
<td>10/36</td>
</tr>
<tr>
<td>Sometimes</td>
<td>0/14</td>
<td>0/14</td>
<td>1/14</td>
<td>3/14</td>
<td>1/14</td>
</tr>
<tr>
<td>Always</td>
<td>0/8</td>
<td>0/8</td>
<td>1/8</td>
<td>2/8</td>
<td>3/8</td>
</tr>
<tr>
<td>Loaned sheep return to farm</td>
<td>0/10</td>
<td>0/8</td>
<td>1/8</td>
<td>2/8</td>
<td>3/8</td>
</tr>
</tbody>
</table>

In bold are the risk factor categories which have a significant positive association with seropositivity, which are of particular interest for reverse causation.

a Abortion risk defined as percent of ewes that aborted/ number of ewes bred.
b Abortion storm defined as a cluster of abortions, usually greater than 5% of pregnancies and occurring within a three-week period.
CHAPTER THREE

Coxiella burnetii seropositivity and associated risk factors in goats in Ontario, Canada

Prepared for: Journal of Zoonoses and Public Health

Abstract

Coxiella burnetii is a zoonotic bacterium that can cause coxiellosis in goats, which can result in abortion/stillbirth or birth of weak unviable kids. A cross-sectional study was conducted to identify the seroprevalence and risk factors for C. burnetii exposure in Ontario goats. A total of 2195 goat serum samples were collected from does on 76 goat farms (42 dairy goat and 34 meat goat) across the Ontario, Canada, between August 2010 and February 2012. Sera were then tested for C. burnetii specific antibodies using an enzyme-linked immunosorbent assay (IDEXX). Data on farm demographics and goat management practices were collected via questionnaire and used to construct a mixed multivariable model with goat seropositivity as the outcome, while controlling for farm-level clustering using a random intercept.

Overall, 63.2% (48/76, 95% CI=51.9-73.4) of farms had at least one seropositive goat. A higher farm-level seroprevalence of 78.6% (33/42) was found on dairy goat farms, compared to 44.1% (15/34) on meat goat farms (p<0.01). At the overall individual-animal level, 32.5% (714/2195, 95% CI=30.6-34.5) of goats were seropositive. Similarly, a higher individual-level seroprevalence was identified for dairy goats (43.7%, 633/1447) and compared to meat goats (10.8%, 81/748) (p<0.0001).
Increasing the female herd size (logarithmic scale) was associated with increased odds of seropositivity. By way of illustration, goats on a farm with 200 females would have 6.3 times (95% CI= 3.04-13.01) the odds of seropositivity, compared to goats on farms with 100 females. In contrast, increases in male herd size had a negative association with seropositivity. For example, goats on farms with a four males had 0.3 times (95% CI=0.1-0.6) the odds of seropositivity compared to goats with two males. If does kidded in a separate airspace from the rest of the herd, goats had 14.3 times (95% CI=1.9-110.1) the odds of seropositivity relative to those that never kid in a separate airspace. If other sheep or goat farms were located in a 5 km radius, goats had 5.6 times (95% CI=1.01-5.6) times the odds of seropositivity compared to those without sheep or goat farms within 5 km. Relative to goats from farms where all lambing pen hygiene was practiced (adding bedding, removing birth materials and disinfection after kidding), goats from farms which only added bedding and removed birth materials had a higher odds of seropositivity (OR=19.3, 95% CI= 1.1-330.4), as did goats from farms which practiced none of these measures (OR=161.0, 95% CI= 2.4-10822.2). An interaction term revealed kidding outdoors when there were no swine on farm had a protective effect on seropositivity compared to kidding indoors. Among goats from farms that kidded outdoors, goats from farms which had swine had 83.3 times the odds of seropositivity compared to goats without swine (95% CI: 4.95-1428.6, p=0.02). Therefore, we conclude that *C. burnetii* exposure was common among goats in Ontario. As well, identified management practices associated with goat seropositivity provide a better understanding of how to prevent or mitigate exposure to *C. burnetii* in Ontario.
3.1 Introduction

Coxiella burnetii (C. burnetii) is a zoonotic intracellular bacterium that can infect a wide variety of mammals, birds and arthropods (Enright et al., 1971; Maurin and Raoult, 1999; Astobiza et al., 2011; Thompson et al., 2012). In goats, C. burnetii infection can result in clinical disease, called coxiellosis (Angerholm et al., 2013). Human C. burnetii infection, or Q fever, has most frequently been attributed to indirect or direct contact with infected ruminants, primarily sheep, goats or cattle (Lyytikäinen et al., 1998; Tissot-Dupont et al., 1999; Astobiza et al., 2011; Thompson et al., 2012).

A large human Q fever epidemic occurred in the Netherlands between 2007-2009, and was attributed to transmission of C. burnetii from infective dairy goats, and to a lesser extent, dairy sheep (Roest et al., 2011). This epidemic demonstrated the potential impacts of coxiellosis on goat production and subsequently, public health (Roest et al., 2011). Important gaps in knowledge were also identified, including the impact of C. burnetii infection in humans and animal reservoirs, risk factors for infection in these populations, and the effectiveness of various prevention and control strategies (de Valk, 2012).

Infection in both humans and animals is considered to be acquired primarily by the inhalation of aerosols contaminated with C. burnetii (Maurin and Raoult, 1999; de Bruin et al., 2012). Sporadic human cases attributed to the ingestion of contaminated materials have also been reported, but this transmission route is considered less efficient than aerosol (Welsh et al., 1945; Benson et al., 1963; Fishbein and Raoult, 1992). In animals, C. burnetii transmission via the oral route is thought to occur by ingesting contaminated placentas, pasture, hay, straw and other sources (Woldehiwet, 2004; Angelakis and Raoult, 2010). Q fever in humans and coxiellosis in goats have been recognized as endemic in Ontario since the 1980s (Palmer et al, 1983; Simor et
al., 1984; Simor et al., 1987). In 1984, research indicated that 20.0% (4/20) of goat farms had at least one seropositive animal using two different ELISAs (Lang, 1988). Other than a case report of goat associated infections which occurred at an agricultural fair (Sanford et al., 1994), little to no research on the infection of goats in Canada has been published since.

*Coxiella burnetii* infection in goats is often subclinical, but it can cause abortion in late gestation, as well as delivery of stillborn, or nonviable kids (Rousset et al., 2009). In 2013, coxiellosis became an immediately notifiable disease in Ontario. The passive surveillance system currently used for coxiellosis in Ontario goats relies on the reporting of positive diagnostic tests, primarily abortion diagnoses conducted at reference laboratories, to the Ontario Ministry of Agriculture and Food (McEwen et al., 2012). However, this passive system likely underestimates the true prevalence of *C. burnetii* infection in goats due to a high proportion of asymptomatic infections where no abortions/stillbirths occur (Sidi-Boumedine et al., 2010). If abortions or stillbirth do occur, the likelihood of identifying a case of coxiellosis is still influenced by the probability of a detailed veterinary investigation occurring, in which the causative agent is identified in the laboratory (Georgiev et al., 2013), and by the sensitivity of the diagnostic tested used. Diagnostic sample submission, particularly following a single abortion in a flock or herd may be uncommon (Georgiev et al., 2013).

During abortion and normal deliveries, large quantities of *C. burnetii* bacteria can be shed by does in the amniotic fluid, placenta and fetal membranes (Berri et al., 2001; Masala et al., 2004; Berri et al., 2005). In aborted goat placentas submitted in Ontario for an abortion diagnostics project, the average number of *C. burnetii* DNA copies per μl of aborted placenta was $3.20 \times 10^{10}$ (Hazlett et al., 2013). Therefore, during kidding season, the risk of disease transmission is high due to heavy bacterial loads contaminating the kidding environment (Maurin
and Raoult, 1999; Schulz et al., 2005; McQuiston et al., 2006; Astobiza et al., 2010). However, intermittent shedding may continue to occur in the feces, vaginal discharge, urine and milk for several weeks or months following kidding (Berri et al., 2000; Berri et al., 2001; Rodolakis et al., 2007). *C. burnetii* can also remain infective for months in aerosols or contaminated dust that continue to be released as the shed material desiccates (Woldehiwet, 2004).

Risk factors identified for *C. burnetii* exposure in animals have included animal management practices and contact with local infected animal reservoirs (Enright et al., 1971; Schimmer et al., 2011). In Canada, there is evidence that sheep, rodents, cats, cattle, and goats shed *C. burnetii* (Lang, 1988; Marrie et al., 1988; Thompson et al., 2012), but a clearer understanding of the risks of disease transmission between these species is needed. Contact with infected livestock or being in the vicinity of infected livestock have been identified as significant risk factors for human exposure and disease (Van Der Hoek et al., 2011). However, data on prevalence and risk factors for Ontario livestock exposure and disease are limited. Further investigation of seroprevalence and risks for goat exposure to *C. burnetii* could inform veterinarians, goat producers and policy makers, and aid in the development and implementation of practical prevention and control strategies.

The objectives of this study were to: (i) determine the herd-level and within-herd seroprevalence of *C. burnetii* exposure in Ontario goat farms, and (ii) identify demographic and management practices that are risk factors for exposure to *C. burnetii* in Ontario goats.

### 3.2 Materials and Methods

#### 3.2.1 Raising awareness of study

Prior to solicitation, Ontario goat producers were informed of this study through articles in: (i) the *Canadian Meat Goat Journal*, the official publication of the Canadian Meat Goat
Association distributed to over 300 members and subscribers, (ii) the *Ontario Goat newsletter*, distributed to producers who raise goats in Ontario, as well as subscribers, and (iii) the *Ontario Farmer*, a subscription weekly newspaper targeted to farmers in the agricultural community in Ontario.

### 3.2.2 Farm and animal selection

This study used a cross-sectional design and multi-stage random sampling. Three goat producer databases were used to construct the farm-level sampling frame: Ontario Goat, Canadian Meat Goat Association, and a list of dairy goat producers provided by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), the Ontario provincial ministry responsible for licensing goat milk production. To estimate the herd-level prevalence using an *a priori* estimate of 24% with a 95% confidence and 10% allowable error, 76 goat farms were sampled; 42 dairy goat farms and 34 meat goat farms. The number of farms sampled in each sector was proportional to the total estimated number of farms in that sector in Ontario. Goat producers were randomly selected from the sampling frame using an online random number generator (http://stattrek.com/statistics/random-number-generator.aspx), and were solicited for enrolment via letter or telephone. All solicited producers were asked to complete information regarding their farm’s industry sector and herd numbers, regardless of their intention to participate, to allow for assessment of selection bias. Farm inclusion criteria were: having at least 10 does that gave birth in the previous 12 months, and being located in Ontario within 800-km of the University of Guelph. Farms beyond this distance were excluded due to logistical concerns and small goat populations. Farms were enrolled until the target numbers for dairy and meat goat farms were achieved. Enrollment occurred between the summer of 2010 and the autumn of 2011.
To estimate within-farm seroprevalence using an *a priori* estimate of 10% with a 95% confidence and 10% allowable error, 35 does that had kidded in the previous 12 months were randomly sampled per farm using an online random number generator (http://stattrek.com/statistics/random-number-generator.aspx). If the farm had less than 35 does that kidded in the previous 12 months, samples were taken from all does that met this requirement.

The University of Guelph Research Ethics Board (Certification of Ethical Acceptability of Research Involving Human Participants – 10JN005) and the University of Guelph Animal Care Committee (Animal Use Protocol – 10R056) approved the study.

### 3.2.3 Farm-level data collection

Detailed descriptions of the farm-level data collection methodologies were described in Section 2.2.3. In brief, an animal management questionnaire (Appendix I) was administered between August 2010 and February 2012, to the producer considered to be the farm manager, on each farm in order to gather information on farm demographics (e.g. male herd size, female herd size), sources of replacement stock, kidding management practices, goat contact with other animal species (livestock, dogs, cats, rodents, wildlife etc.), proximity to other farms, and biosecurity practices.

Measures of reproductive failure, such as average risk of abortions in the preceding three years, and prior reproductive-related diagnostic testing, were collected for descriptive purposes and to gain insight as to whether current management practices were likely to have been implemented in response to awareness of prior reproductive challenges in their goats. These measures of reproductive failure were considered a possible outcome of exposure to *C. burnetii*, and therefore were not included in the putative risk factor model, so as to avoid problems with
reverse-causation. Descriptive statistics of measures of reproductive failure and non-permanent risk factors associated with sheep seropositivity were assessed separately to allow for comments on possible reverse-causation.

3.2.4 Goat blood collection

Blood samples were collected via jugular venipuncture into 10ml red top serum BD vacutainer tubes® (Becton, Dickson and Company, Franklin Lakes, New Jersey, USA) via jugular venipuncture. Samples were then transported on ice at 4-8°C. Blood centrifugation occurred at the University of Guelph if transport time was less than 4 hours or at a nearby veterinary clinic if transport time exceeded 4 hours. Vacutainer tube samples were centrifuged for 10 minutes at 1000 x g and 2ml aliquots of the separated serum were pipetted into serum micro tubes (Fisher Scientific, Ottawa, CA). Serum micro tubes that were processed during laboratory business hours were submitted immediately to the Animal Health Laboratory (AHL), Laboratory Services Division, University of Guelph. Samples were frozen for up to 48 hours at -20°C prior to submission if either: they were prepared at the University of Guelph after regular business hours, or if they were processed off-site at a nearby veterinary clinic.

3.2.5 Serological analysis

Serology was performed by the AHL using the IDEXX CHEKIT Q-Fever Antibody ELISA Test Kit (IDEXX Laboratories, Broomfield, CO, USA). In accordance with the manufacturer’s instructions, the ratio of the optical density of the sample(s) to that of the positive control ≥ 40% was considered seropositive.
3.2.6 Data management and statistical analysis

Questionnaire and serological data were entered into EpiData® v2.2 for file management (EpiData Association®, Odense Denmark) and were manually checked against the original questionnaires and laboratory reports for errors. A mixed logistic regression model of seropositivity was constructed in Stata Intercooled Version 10.1 (StataCorp®, 2007), using manual backwards elimination and controlling for farm-level clustering by including farm as a random intercept. The initial step in model building was a univariable screening of all covariates for association with goat seropositivity, and were retained if the level of significance of $\alpha < 0.20$ using the likelihood ratio test (LRT) for categorical variables with more than two categories and Wald’s test for dichotomous variables. Linearity of continuous variables was assessed graphically by plotting lowess smoother curves and transforming the variable, if necessary, to achieve normality (Dohoo et al., 2003). Explanatory variables associated with the outcome at $p<0.20$ were then screened for pair-wise collinearity using Spearman’s rank correlation; variable pairs were considered collinear if Spearman’s rho was $\geq |0.8|$ (Mason and Perreault, 2013). If collinear, the covariates were compared with respect to univariable significance, missing data, and biological relationship, and the most relevant variable was retained (Dohoo et al., 2003). Retained variables were then used to construct the multivariable model with significance set at $\alpha <0.05$. Variables with more than 25% missing values were excluded from analysis. After examination of the distribution of missing values, this cut point of 25% was chosen as it maximized both the number of variables examined for inclusion in multivariable model building and the number of observations used in the multivariable model. Eliminated variables were tested for confounding and retained if their inclusion caused a $>20\%$ change in the coefficient of one or more of the significant variables. Biologically plausible two-way interaction terms were generated and retained if significant in the multivariable model. The fit of the model was
assessed by examining upper level residuals (by farm) using best linear unbiased predictions (BLUPs) analysis to verify the model assumptions of normality and homoscedasticity (Dohoo et al., 2003). Residual analysis was conducted in both the fixed and random portions of the model to identify potential outliers. The latent variable technique (Goldstein et al., 2002) was used to calculate the variance components at the farm and individual levels.

Measures of reproductive failure on farm were assessed separately in a mixed univariable analysis against the serological outcome to allow for comments on the relationship between doe seropositivity and the occurrence of adverse parturient events. Variables significant at p<0.20 were not included in the risk factor model of doe seropositivity, as measures of reproductive failure were deemed a consequence of exposure rather than a risk factor.

Measures of reproductive failure were described among categories of non-permanent risk factors associated with goat seropositivity. This was done to allow for comments on the potential for reverse-causation.

### 3.3 Results

#### 3.3.1 Study population

Forty percent (42/105) of dairy goat farms and 36.6% (34/93) of meat goat farms that were solicited and met inclusion criteria participated in the study. Twenty-nine goat farms mailed back responses indicating they did not wish to participate, of those twenty-nine, 11 were from meat goat farms, 15 were from dairy goat farms, and three did not specify goat farm type. The mean herd size on participating farms was 256 goats (SD ± 281), and the median was 144 goats (interquartile range 60-420). Goat farms were distributed across western, central and eastern Ontario, with a few farms in northern Ontario. None of the goat farms studied reported use of a C. burnetii vaccine (Coxevax®, CEVA Animal Health).
3.3.2 Seroprevalence

Overall, 63.2% (48/76) of goat farms had at least one seropositive goat. Significantly more dairy goat farms (78.6%, 33/42) than meat goat farms (44.1%, 15/34) were seropositive by this definition (p=0.001). The overall animal-level seroprevalence was 32.5% (714/2195), and was significantly higher in dairy goats (43.7%, 633/1447) than meat goats (10.8%, 81/748) (p<0.0001). Figures 3.1 and 3.2 demonstrate the distribution of within-farm seroprevalence in the meat and dairy sectors, respectively.

3.3.3 Risk factor analysis

A descriptive summary of questionnaire data is provided in Appendix III. The variables associated with goat seropositivity at p<0.20 in the univariable models are presented in Table 3.1. The following variables were also investigated, but were not significantly associated with doe seropositivity in the univariable analysis (p≥0.20): kidding in the fall, winter or spring; annual kidding; accelerated kidding; use of artificial insemination; having a closed herd; purchase of replacement females under one year of age; purchase of replacement females over one year of age; purchase of pregnant females; purchase of replacement males under one year of age; ordinal category for number of replacement animals purchased in the past three years; purchase of animals from show sales or dealer/brokers; taking and returning goats to/from an agricultural fair; loaning goats to other farms; having other livestock or non-livestock domestic animals on farm; method of rodent control; having non-avian wildlife in the barn; replacement animals being exposed to adult goats, or their manure; method of spreading manure; quarantining does after aborting; and method of disposal of placentas or aborted fetuses.

Two variables that were initially multilevel were dichotomized: kidding outdoors (farms that always and frequently kidded outdoors were collapsed and compared with farms that never kidded outdoors) and quarantining new animals (farms that always quarantined new animals
were compared to farms that sometimes or never quarantined new animals). This was done to account for small sample sizes and lack of variability with respect to seropositivity among farms that always kidded outdoors and farms that sometimes quarantined new animals.

The final model is presented in Table 3.2. The following variables were identified as risk factors: log (base 10) of female flock size, sheep or goat farms within 5km, kidding in an airspace separate from the rest of the herd, and only adding bedding or only removing birthing materials and adding bedding for kidding pen hygiene measures compared to disinfecting pens, removing birth materials and adding bedding. In addition, log (base 10) of male herd size had protective effect. An interaction term between having swine on farm and kidding outdoors was also significant in the final multivariable model. Figure 3.3 shows the relationship between the predicted outcome, seropositivity, and the four categories of the interaction term, while all other variables in the multivariable model were held at median values. Kidding outdoors when there were no swine on farm had a protective effect on seropositivity compared to kidding indoors. Further, among farms that kidded outdoors, goats from farms which had swine had 83.3 times the odds of seropositivity compared to goats without swine (95% CI: 4.95-1428.6, p=0.02).

Purchasing males over one year of age was just beyond the cut-point of significance (OR=3.21, 95% CI 1.00-10.26, p=0.05), but met criteria for retention in the model as a confounding variable.

Visual assessment of the BLUPs residuals did not identify any major departures from normality, nor was there evidence of heteroscedasticity. Visualization of the residuals at the lower individual level showed high Pearson residuals for three farms. The interpretation of the multivariable model did not change if these outliers were excluded from the analysis, and there was no reason for their exclusion, hence; all outliers were retained in the multivariable model.
3.3.4 Association of reproductive failure and doe seropositivity

Measures of reproductive failure were considered a possible result of exposure to *C. burnetii* rather than a potential risk factor, and were therefore assessed separately to allow for discussion on reverse causation, and were not included in the putative risk factor multivariable model. These variables were hypothesized to possibly affect change in animal management practices if a producer suspected or was aware of their herd’s *C. burnetii* infection status. Farm-level frequency of reproductive testing or outcomes over the previous three years and the farm-level frequency of non-permanent risk factors in multivariable model are presented in Table 3.3. Based on examination of these data, farms that practiced measures that were associated with seropositivity did not have a significantly higher frequency (one-sided Fisher’s exact P<0.05) of *C. burnetii* abortions, or elevated abortion risk (>5%) over the past three years, compared to the farms that practiced measures which were not associated with seropositivity. We therefore do not believe reverse causation bias to be likely in the risk factors analyses.

3.4 Discussion:

Overall, *C. burnetii* exposure in Ontario goats was common, and a number of risk factors for doe seropositivity were identified that highlight the importance of hygiene and biosecurity practices. Poor kidding hygiene practices were strongly associated with doe seropositivity, and this parallels what has been described in sheep (Section 2.3.3). Relative to goats from farms where all lambing pen hygiene was practiced (adding bedding, removing birth materials and disinfection after kidding), goats from farms which added bedding and removed birth materials and goats from farms which practiced none of these practices, had significantly higher odds of seropositivity. This evidence indicates that hygiene may be an effective tool for reducing bacterial burden in the kidding environment. The type of disinfectants used in kidding pens by
these farm workers was not assessed, and while the effectiveness of disinfectant use has been described in controlled settings (Scott and Williams, 1990) but has not been evaluated on farm conditions. Further research to investigate the effectiveness of various disinfectants to reduce C. burnetii burden in kidding pens would be useful to inform control measures.

We hypothesized that kidding in a separate airspace would decrease the odds of goat seropositivity, as it would decrease the opportunity for transmission of C. burnetii between those animals kidding and the rest of the herd (Reichel et al., 2012); however, the opposite association was observed. The possibility of reverse causation was investigated (i.e. when farm workers came to know or suspect C. burnetii infections in their animals they implemented control measures, like kidding in a separate airspace), which could make a control measure appear as a risk factor for seropositivity. Of the 22 farms on which goats kidded in a separate airspace, three had experienced an elevated abortion risk (>5%) over the past three years, and one farm had C. burnetii identified as the cause of an abortion. If these four farms were removed from the model, kidding in an airspace separate from the flock is still significantly associated with seropositivity, although the odds of seropositivity when goats kidded in an airspace separate from the flock was lower (OR=6.7) with these four farms removed than it was in the multivariable model (OR=14.3). Therefore, reverse causation may have had only limited impact on the association between kidding in a separate airspace and goat seropositivity. In the Netherlands, the use of windbreak curtains and windshields (to protect against sun, and rain) increased the risk of goat seropositivity in a multivariable analysis (Schimmer et al., 2011). The authors suggested that using windbreak curtains and windshields decreased air-flow, and may have facilitated accumulation of C. burnetii in the barn (Schimmer et al., 2011). Therefore, dependent upon the kidding facilities available, segregating kidding of does in a separate space may have
implications to ventilation and accumulation of _C. burnetii_ in the segregated environment. If the segregated kidding area does have decreased ventilation, this may increase the transmission within the kidding group. At this stage it is premature to make recommendations based on this finding, as empirical evidence is needed to support this hypothesis that ventilation may be reduced in segregated kidding areas. Further research in this area would be useful.

The presence of other sheep or goat farms within a 5km radius had a significant positive association with doe seropositivity. An effective range of airborne _C. burnetii_ spread of <5 km has been reported (Schimmer et al., 2010), as has effective airborne transmission of _C. burnetii_ within clusters (Reusken et al., 2011). Similar transmission patterns may be responsible for the positive association with nearby farms observed here.

Female herd size had a strong positive association with doe seropositivity in the multivariable model. This finding is consistent with other literature that indicates that animals in large herds have more opportunities for transmission between individual animals, or increased chance of introduction of _C. burnetii_ on larger farms (Hogerwerf et al., 2013), especially from breeding females who are the primary shedders of _C. burnetii_ (Rodolakis, 2009). In the univariable analysis, both the log of female and male herd sizes were significantly and positively associated with doe seropositivity and were moderately correlated (Spearman’s rho = 0.461), but not enough to meet exclusion criteria for collinearity. In contrast to females, male herd size (log scale) had a negative association with goat seropositivity in the multivariable model. After controlling for female herd size, for every 1 unit increase in the log (base 10) of male herd size, the odds of doe seropositivity decreased by 6.25 times. It is possible that farms with higher number of males were more likely to be farms which sold replacement bucks, and may have had more protective measures in place (e.g. more strict biosecurity), that were not captured in the
questionnaire. If this is true, it could account for a reduced risk of *C. burnetii* introduction on breeding stock farms.

In contrast, purchasing male goats may increase the odds of goat seropositivity. The level of significance of purchasing male goats over one year of age was marginally significant (OR=3.21, 95% CI 1.00-10.26, p=0.05). While it did not meet the *a priori* inclusion criteria for statistical significance (p<0.05), purchasing male goats may still contribute a biologically significant risk for *C. burnetii* exposure in goat herds. This increase in risk could be due to direct effects from purchasing infected males, although males are less likely to transmit *C. burnetii* than females (Guatteo et al., 2011). Alternatively, risk could be due to indirect effects; purchase of male goats may be a proxy measure for another factor, such as more risky animal purchasing behaviour, that increased the odds of *C. burnetii* seropositivity.

The influence of birthing outdoors compared to indoors on seropositivity has not been evaluated thoroughly in goats or other species. There is however, more general data comparing the seroprevalence in cattle kept indoors and outdoors in Italy. Non-housed cattle were found to have a significantly lower *C. burnetii* seroprevalence than housed cattle (Capuano et al., 2001). The significant interaction term between the presence of swine on farm and kidding outdoors in the multivariable model here showed a protective effect of kidding outdoors in the absence of swine on seropositivity. Therefore it is possible that the presence of swine negated any benefit from kidding outdoors. However, recommending outdoor kidding as a result of this information remains questionable, as kidding outdoors may have a protective effect for goat seropositivity, but human epidemics of Q fever have been attributed to outdoor animal births in goats, sheep and cattle, due to the increased opportunity for aerosol transmission after outdoor birthing (Hawker et al., 1998; Lyytikäinen et al., 1998; Tissot-Dupont et al., 2004; Georgiev et al., 2013).
As well, there are a number of other considerations when considering kidding outdoors such as predators and parasites. Swine are not recognized as a reservoir of *C. burnetii* (Marrie, 1990), although this has not been thoroughly assessed in the literature. There is reference to the area of the Netherlands Q fever outbreak from 2008 to 2009 formerly having a high density of swine farms, although many of these swine farms were converted to goat farms after a classical swine fever epidemic in the 1990s (Enserink, 2010). Occupational contact with swine has been associated with veterinarian seropositivity in the Netherlands and United States (Whitney et al., 2009; Van den Brom et al., 2013). Researchers in the United States suggested that swine were potential *C. burnetii* reservoirs (Whitney et al., 2009), while authors in the Netherlands hypothesized that the apparent association with swine contact was confounded by the location of swine farms corresponding with the high-incidence area of the human Q fever epidemic related to dairy goats (Van den Brom et al., 2013). Alternatively, rooting behaviour in swine has been hypothesized to produce infectious aerosols from pathogens found in the earth or bedding (Cornick and Vukhac, 2008). If rooting behaviour among swine does indeed promote aerosolization of *C. burnetii* that has contaminated that environment, this could explain the increased odds of exposure in goats on the same farm, as observed here. The *C. burnetii* seroprevalence in Ontario swine has not been investigated and more research is needed to clarify the role of pigs, if any, in the transmission of *C. burnetii* to goats and other animals. Furthermore, investigation is also required to identify how the presence of pigs on farm may influence management factors important in goat *C. burnetii* seropositivity.

Cats have been suspected as a reservoir of *C. burnetii* infection in Canada (Marrie et al., 1988), thus the presence of cats on farm was hypothesized to increase the odds of seropositivity in does. The presence of cats on farm was not significant in the univariable analysis here.
(p=0.9491), regardless of whether cats were kept in the barn or house. However, the majority of goat farms kept cats (70/76), so we may not have had sufficient power to detect an effect if one existed.

The presence of wildlife in the barn was also hypothesized to increase the odds of seropositivity, as recent evidence demonstrated a high prevalence of *C. burnetii* among mice, voles and squirrels in a National park in Ontario (Thompson et al., 2012). Additionally, researchers in the Netherlands found actively infected rats outside the lambing/kidding season and in urban areas. They then suggested that rats may not be spill-over hosts, and may be capable of maintaining *C. burnetii* infection cycles independent of livestock contact (Reusken et al., 2011). The presence of wildlife, including raccoons, opossums and other non-avian wildlife, in the barn was not uncommon in the present study (36.8% (28/76) farms had witnessed wildlife or their droppings in the barn), but it was not significantly associated with seropositivity. Future research to elucidate the risk of transmission between wildlife and livestock would be useful in understanding the epidemiology of this disease.

We attempted to collect individual goat data, such as birth date and date of last kidding, since characteristics such as parity and age have been shown to influence seropositivity in other ruminant studies (García-Pérez et al, 2009; Asadi et al., 2012). However, it became apparent during data collection that many farm managers did not keep detailed records with this information, and some did not have adequate animal identification. Additionally, some had purchased goats with no history. As such, we were unable to assess individual goat-level risk factors due to high numbers of missing dates and likely inaccurate data. Therefore, we recommended that the importance of record keeping at the individual-level be emphasized through goat industry meetings and goat producer directed magazines.
To best facilitate participation, producers could fill out the questionnaire on the farm visit (n=54 farms), or they could email/mail the questionnaire (n=22 farms). These two groups of farms (in person or email/mail completion) were not significantly different with respect to: seropositive goats (p=0.964), female herd size (logarithmic scale) (p=0.93), male herd size (logarithmic scale) (p=0.86), kidding pen hygiene practices (p=0.83), kidding outdoors (p=0.90), having swine on farm (p=0.90), being within 5km of other sheep or goat farms (p=0.86) or kidding in an airspace separate from the rest of the herd (p=0.90). Therefore, the method of questionnaire completion is not believed to have influenced the multivariable risk factor model.

This research has identified a number of management factors that can be altered with the goal of decreasing the risk of *C. burnetii* seropositivity among Ontario goats. For factors that are not easily modified, such as female herd size or proximity to other sheep and goat farms, more stringent biosecurity and hygiene measures may be required to help offset this effect. Animal vaccination (Coxevac, Ceva Animal Health) has been proven effective in reducing shedding of *C. burnetii* in dairy goats (O’Neill et al., 2013), and could thus be used on Ontario goat farms to reduce the risk of human exposure and subsequent infection (Isken et al., 2013).

### 3.5 Conclusion:

This study found *C. burnetii* exposure in Ontario goats to be common, with 63.2% (48/76) of goat farms and 32.5% (714/2195) of individual goats being seropositive. Kidding area hygiene was highlighted as an important factor protecting against exposure. Best practices during kidding season (e.g. disinfecting kidding pens, as well as removing birthing materials and adding new bedding) can be promoted to goat producers. As well, large female flock size and having other sheep or goat farms in close proximity was associated with increased odds of goat
seropositivity. This study identified practices associated with goat exposure to \textit{C. burnetii}, which can be used to direct future research and provide evidence to support the development and validation of control strategies and policies to prevent or mitigate exposure of goats to \textit{C. burnetii}.

3.6 Acknowledgements

The authors would like to thank: the Ontario Ministry of Agriculture, Food and Rural Affairs - University of Guelph Agreement through the Animal Health Strategic Investment fund (AHSI) managed by the Animal Health Laboratory of the University of Guelph; Ontario Ministry of Health and Long-Term Care; National Sciences and Engineering Research Council of Canada; Public Health Ontario; and Ontario Goat. The authors would also like to acknowledge the cooperation of all goat producers that participated in the study.
3.7 References:


system to identify a dairy goat farm as the most likely source of an urban Q fever outbreak. 
BMC Infect. Dis. 10, 69.


Figure 3.1 Percentage of *C. burnetii* seropositive goats per farm on 34 randomly selected meat goat farms in Ontario, Canada (Aug 2010-Feb 2012) *

*Farms have been sorted from lowest to highest within-herd seroprevalence*
Figure 3.2 Percentage of *C. burnetii* seropositive goats per farm on 42 randomly selected dairy goat farms in Ontario, Canada (Aug 2010-Feb 2012) *

* Farms have been sorted from lowest to highest within-herd seroprevalence
Figure 3.3 Predicted values for *C. burnetii* seropositivity in goats as determined by a mixed multivariable model with an interaction term between the variables of presence of pigs on farm and kidding outdoors.

* Signifies significant different category (P-value = 0.02)

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3 All other fixed effects not included in interaction are set to median values
Table 3.1 Farm management variables associated (p<0.2) with doe seropositivity for *C. burnetii*, in univariable mixed-effects logistic regressions of data collected from 76 goat farms in Ontario, Canada (Aug 2010-Feb 2012).

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat industry sector</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dairy</td>
<td>14.27</td>
<td>3.72-54.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female herd size (<em>Log₁₀ scale</em>)</td>
<td>3.30</td>
<td>1.96-5.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male herd size (<em>Log₁₀ scale</em>)</td>
<td>3.35</td>
<td>1.27-8.84</td>
<td>0.014</td>
</tr>
<tr>
<td>Summer breeding season</td>
<td>12.07</td>
<td>2.38-61.21</td>
<td>0.003</td>
</tr>
<tr>
<td>Purchasing males goats over 1 year of age</td>
<td>3.61</td>
<td>0.82-15.89</td>
<td>0.090</td>
</tr>
<tr>
<td>Purchasing goats from sales barns</td>
<td>11.29</td>
<td>0.74-173.02</td>
<td>0.082</td>
</tr>
<tr>
<td>Always quarantining new goats</td>
<td>0.15</td>
<td>0.03-0.708</td>
<td>0.017</td>
</tr>
<tr>
<td>Pigs on farm</td>
<td>0.127</td>
<td>0.02-1.03</td>
<td>0.053</td>
</tr>
<tr>
<td>Kidding in separate area from rest of herd</td>
<td>0.20</td>
<td>0.04-1.09</td>
<td>0.062</td>
</tr>
<tr>
<td>Kidding outdoors</td>
<td>0.09</td>
<td>0.02-0.55</td>
<td>0.009</td>
</tr>
<tr>
<td>Replacement animals have access to kidding area after weaning</td>
<td>0.13</td>
<td>0.03-0.57</td>
<td>0.007</td>
</tr>
<tr>
<td>Disposed placenta in manure pile</td>
<td>17.42</td>
<td>2.50-121.26</td>
<td>0.004</td>
</tr>
<tr>
<td>Kidding pen hygiene practices (LRT $\chi^2$=7.93, P-value=0.0475)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do nothing</td>
<td>15.88</td>
<td>0.38-672.07</td>
<td>0.148</td>
</tr>
<tr>
<td>Add bedding only</td>
<td>20.64</td>
<td>1.94-219.34</td>
<td>0.012</td>
</tr>
<tr>
<td>Add bedding and remove birthing materials</td>
<td>13.15</td>
<td>1.80-95.81</td>
<td>0.011</td>
</tr>
<tr>
<td>Add bedding, remove birthing materials and disinfect</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Other sheep or goat farms within 5km from farm</td>
<td>4.36</td>
<td>0.71-26.83</td>
<td>0.112</td>
</tr>
</tbody>
</table>

Ref= referent category, OR= odds ratio, CI= Confidence Interval.

- LRT = Likelihood ratio test for categorized variable
- Referent category is the absence of this practice

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4 “Always” quarantining new animals is compared to “sometimes” and “never” quarantining new animals
5 “Always” and “sometimes” kidding outdoors collapsed and compared to never kidding outdoors
Table 3.2 Farm management variables associated (p<0.05) with *C. burnetii* seropositivity in goats, as observed through multivariable mixed-effects logistic regression of data collected from 76 goat farms in Ontario, Canada (Aug 2010-Feb 2012).

<table>
<thead>
<tr>
<th>Significant Risk Factors</th>
<th>OR</th>
<th>OR 95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female herd size (Log_{10} scale)</td>
<td>13.66</td>
<td>4.89-38.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male herd size (Log_{10} scale)</td>
<td>0.16</td>
<td>0.06-0.46</td>
<td>0.001</td>
</tr>
<tr>
<td>Kidding pen hygiene practice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(LRT $\chi^2$=11.50, P-value =0.0093) $^a$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do nothing</td>
<td>160.97</td>
<td>2.39-10822.20</td>
<td>0.018</td>
</tr>
<tr>
<td>Add bedding only</td>
<td>4.65</td>
<td>0.26-84.11</td>
<td>0.298</td>
</tr>
<tr>
<td>Add bedding and remove birthing materials</td>
<td>19.30</td>
<td>1.13-330.35</td>
<td>0.041</td>
</tr>
<tr>
<td>Add bedding, remove birthing materials and disinfect</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kidding outdoors</td>
<td>0.01</td>
<td>0.001-0.17</td>
<td>0.001</td>
</tr>
<tr>
<td>Pigs on farm</td>
<td>3.42</td>
<td>0.56-20.68</td>
<td>0.181</td>
</tr>
<tr>
<td>Interaction of kidding outdoors and pigs on farm</td>
<td>29.41</td>
<td>1.26-685.49</td>
<td>0.035</td>
</tr>
<tr>
<td>Other sheep or goat farms within 5km $^b$</td>
<td>5.59</td>
<td>1.01-30.85</td>
<td>0.048</td>
</tr>
<tr>
<td>Kidding in separate airspace from rest of herd $^7$</td>
<td>14.33</td>
<td>1.86-110.14</td>
<td>0.011</td>
</tr>
<tr>
<td>Forced into model as confounding variable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quarantining new animals</td>
<td>0.23</td>
<td>0.05-1.08</td>
<td>0.062</td>
</tr>
<tr>
<td>Kidding in summer season</td>
<td>0.962</td>
<td>0.19-4.86</td>
<td>0.963</td>
</tr>
<tr>
<td>Purchasing males over 1 year of age</td>
<td>3.21</td>
<td>1.00-10.26</td>
<td>0.050</td>
</tr>
<tr>
<td>Purchasing animals from sales barn</td>
<td>1.62</td>
<td>0.23-11.19</td>
<td>0.625</td>
</tr>
<tr>
<td>Disposing of placenta in manure pile</td>
<td>0.22</td>
<td>0.03-1.85</td>
<td>0.165</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Random Intercept Parameter</th>
<th>SD</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm $^b$</td>
<td>1.13</td>
<td>0.79-1.61</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

Ref= referent category, OR= odds ratio, CI= Confidence Interval SD= Standard deviation

$^a$ LRT= Likelihood ratio test for categorical variable

$^b$ Partitioning the variance, 27.9% occurred at the farm-level and 72.1% occurred at the goat-level

$^6$ Other sheep and goat farms with in 5km, “yes” is compared to “no” and “not sure”

$^7$ “Always” or “sometimes” kidding in a separate airspace was collapsed and compared with “never”
Table 3.3 Farm-level frequency of reproductive testing and outcomes over the previous 3 years and frequency of non-permanent risk factors in multivariable model, on 76 goat farms in Ontario, Canada (Aug 2010- Feb 2012)

<table>
<thead>
<tr>
<th>Reproductive outcomes</th>
<th>C. burnetii identified as cause of abortion</th>
<th>C. burnetii identified in placental/fetal samples but not cause of abortion</th>
<th>Elevated abortion risk (&gt;5%) over previous 3 years</th>
<th>Experienced abortion storm</th>
<th>Submitted abortive diagnostic samples to AHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambing pen hygiene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nothing</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>1/3</td>
</tr>
<tr>
<td>Add bedding only</td>
<td>1/13</td>
<td>0/13</td>
<td>3/13</td>
<td>5/13</td>
<td>4/13</td>
</tr>
<tr>
<td>Add bedding and remove birth materials</td>
<td>5/36</td>
<td>0/36</td>
<td>6/36</td>
<td>10/36</td>
<td>13/36</td>
</tr>
<tr>
<td>Disinfect add bedding and remove birth materials</td>
<td>1/18</td>
<td>1/18</td>
<td>2/18</td>
<td>3/18</td>
<td>5/18</td>
</tr>
<tr>
<td>Kid outdoors</td>
<td>1/17</td>
<td>0/17</td>
<td>1/17</td>
<td>3/17</td>
<td>3/17</td>
</tr>
<tr>
<td>Pigs on farm</td>
<td>0/14</td>
<td>1/14</td>
<td>3/14</td>
<td>4/14</td>
<td>2/14</td>
</tr>
<tr>
<td>Other sheep or goat farms within 5km</td>
<td>6/49</td>
<td>0/49</td>
<td>10/49</td>
<td>5/49</td>
<td>18/49</td>
</tr>
<tr>
<td>Kidding in a separate airspace</td>
<td>1/22</td>
<td>0/22</td>
<td>3/22</td>
<td>6/22</td>
<td>4/22</td>
</tr>
</tbody>
</table>

In bold are the risk factor categories which have a significant positive association with seropositivity, which are of particular interest for reverse causation

a Abortion risk defined as percent of does that aborted/ number of does bred.
b Abortion storm defined as a cluster of abortions, usually greater than 5% of pregnancies and occurring within a three-week period.
CHAPTER FOUR

Coxiella burnetii seropositivity and associated risk factors in sheep and goat farm workers in Ontario, Canada

Prepared for: Canadian Medical Association Journal

Abstract

Coxiella burnetii is a zoonotic bacterium that causes Q fever in humans. The objective of this study was to determine the seroprevalence and risk factors for C. burnetii exposure in workers on sheep and goat farms in Ontario, Canada. Between August 2010 and March 2012, 172 people (≥ 14 years of age) from 78 farms provided a blood sample and completed a questionnaire to collect data on demographics, lifestyle factors, farm practices, and medical history. Sera were analysed at the Public Health Ontario Laboratory with an immunofluorescence assay (Focus Diagnostics); phase I or II IgG titres ≥ 1:16 indicated seropositivity. A mixed multivariable logistic regression model was constructed to identify risk factors for farm worker seropositivity, while accounting for clustering by farm using a random intercept.

Individual-level and farm-level seroprevalence (where ≥1 person tested positive) for C. burnetii were 64.5% (111/172, 95% CI=57.2-71.4) and 76.3% (58/76, 95% CI=65.8-84.6), respectively. An increasing percentage of seropositive sheep/goats on-farm was positively associated with farm worker seropositivity (OR=1.04; 95% CI 1.02-1.07). By way of illustration, a 20% rise in the proportion of seropositive sheep/goats in the flock/herd would increase the odds of farm worker seropositivity by 2.4 times (95% CI 1.3-4.2). A higher odds of seropositivity was also observed for people working on dairy goat farms compared to dairy sheep.
OR=0.04; 95% CI 0.003-0.53) or meat goat (OR=0.09; 95% CI 0.01-0.67) farms. A history of ever having smoked tobacco was also marginally associated with seropositivity (OR=4.03; 95% CI 0.88-18.45, p=0.072), and was retained in the model as it was determined to be a confounding variable between industry sector and farm worker seropositivity. C. burnetii seropositivity was common in workers on sheep and goat farms in Ontario. The risk factors identified provide insight into the relationship of disease transmission between sheep and goats and their farm workers in Ontario.

4.1 Introduction

Coxiella burnetii is an intracellular zoonotic bacterium (Maurin and Raoult, 1999) that causes Q fever in humans and is a common infection of a large number of animal species (Marrie, 1995). Q fever has been reported sporadically in humans in Ontario with small outbreaks occurring since the 1980s (McLean et al., 1960; Simor et al., 1984; Simor, 1987; PHO, 2012). Sheep, goats and cattle are considered the main reservoirs of C. burnetii, and are also the main species implicated as sources for human Q fever cases in the United States (McQuiston and Childs, 2002) and Canada (Lang, 1989). Due to the nature of their occupations, farm workers, particularly those working with sheep, goats and cattle, are at high risk of C. burnetii exposure (Marrie and Fraser, 1985).

Coxiella burnetii infection can be asymptomatic, or present as acute or chronic disease. In general, approximately 60% of cases are asymptomatic, 38% experience mild symptoms without the need for hospitalization, 1.8% are hospitalized with acute Q fever, and 0.2% develop chronic Q fever (Maurin and Raoult, 1999). Symptomatic acute Q fever manifests primarily as a self-limiting febrile illness associated with severe headaches, atypical pneumonia, or granulomatous hepatitis; endocarditis and a form of chronic fatigue syndrome are common
presentations of chronic Q fever (Maurin and Raoult, 1999; Limonard et al., 2010). The non-specific nature of signs and symptoms associated with Q fever may contribute to under-diagnosis by clinicians, and thus, under-reporting (Marrie and de Carolis, 2002; PHO, 2012; de Valk, 2012). The case definition of acute Q fever in humans includes exhibiting at least one clinical sign (fever, pneumonia, hepatitis) and demonstrated evidence of *C. burnetii* infection with at least one positive diagnostic test (isolation or detection of *C. burnetii* DNA from infected tissues, or demonstrating seroconversion or a fourfold increase from a non-negative titre) (European Parliament Commission decision 2008/426/EC; OMHLTC, 2010). Chronic Q fever is typically characterized by an infection lasting longer than six months (Maurin and Raoult, 1999).

In Ontario, confirmed and suspected cases of Q fever are reportable to the local Medical Officer of Health under the Health Protection and Promotion Act (OMHLTC, 2010). From 2006 to 2011, 47 confirmed cases of Q fever were reported in Ontario (PHO, 2012). It is likely, however, that *C. burnetii* infection is under-reported.

The presence of *C. burnetii*-specific antibodies in serum is used to indicate past exposure to the bacterium. The immunofluorescence assay (IFA) is the reference serological test used in human diagnostics (Tissot Dupont et al., 1994; Maurin and Raoult, 1999; Sidi-Boumedine et al., 2010). The IFA detects the immunoglobulin G (IgG) antibody response to phase I and phase II *C. burnetii* antigens (Focus Diagnostics, 2011). The IFA allows for the differentiation between suspected acute or chronic infections in humans, based on the ratio of phase I and phase II antibodies (Wielders et al., 2012). If the phase I titre is ≥ phase II, the sample is indicative of a chronic exposure, and if the phase II titre is > the phase I titre, the sample is indicative of an acute exposure. There is evidence of long-term persistence of *C. burnetii* in human hosts, in both acute and chronic cases (Harris et al., 2000). This makes serological testing useful for identifying
the prevalence of exposure to *C. burnetii* in human populations. IgG half times have been estimated to be up to several years among acute Q fever patients (Teunis et al., 2013), although the decay rate of IgG among chronic Q fever patients or asymptomatic individuals has not been established.

Ontario has the highest number of both sheep and goat farms of all Canadian provinces (Statistics Canada, 2012a; Statistics Canada, 2012b), but prior to the research presented here, no recent *C. burnetii* research has been conducted in sheep and goats in Ontario, and the *C. burnetii* seroprevalence among Ontario farm workers has not been investigated. Additionally, limited *C. burnetii* research has been conducted examining farm workers in other Canadian provinces (Marrie and Pollak, 1995; Marrie and de Carolis, 2002; Dolcé et al., 2003). Higher seroprevalence was observed among veterinarians and slaughterhouse workers in Nova Scotia (Marrie and Fraser, 1985) and sheep farm workers in Quebec (Dolcé et al., 2003) compared to general populations in the 1980s (Marrie et al., 1984; Dolcé et al., 2003). A recent human Q fever epidemic in the Netherlands attributed to infected dairy goats and to a lesser extent dairy sheep highlighted the public health importance of this disease (De Valk, 2012). As such, it is important to investigate seropositivity and factors influencing seropositivity in these high risk populations in Ontario.

The first objective of this study was to assess the seroprevalence to *C. burnetii* among farm workers that care for sheep and goats in Ontario, Canada. The second objective was to investigate farm management practices, demographic characteristics and lifestyle measures for association with exposure to *C. burnetii* in this population.

**4.2 Materials and Methods**

*4.2.1 Farm and farm worker selection*
This research was part of a larger cross-sectional study to determine the *C. burnetii* seroprevalence of sheep and goats in Ontario, Canada (Chapter 2 & Chapter 3). Briefly, dairy goat and meat goat farms were randomly selected from the Ontario Goat, Ontario Meat Goat Association and Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) databases, as described in Section 3.2.2. Meat sheep farms were randomly selected from the *Ontario Sheep Marketing Agency* database. As a comprehensive database for dairy sheep farms in Ontario does not exist, all dairy sheep farms identified by sheep milk processors and OMAFRA were solicited for participation (Section 2.2.3). A total of 148 farms were enrolled (50 meat sheep farms, 22 dairy sheep farms, 34 meat goat farms, and 42 dairy goat farms). Workers 14 years and older and who cared for the sheep and goats (e.g. owners, family members, employees) on these farms were asked to participate in the research presented here. Those aged 14 to 17 years required consent of a guardian to participate. Hence, a cluster sampling procedure was used for farm workers (i.e. selection first occurred at the farm level for animal sampling, and on participating farms, all farm workers which met eligibility criteria were asked to participate in human sampling). Study farms were enrolled beginning in the summer of 2010 until the autumn of 2011. Sampling and questionnaire administration occurred between August 2010 and March 2012.

The University of Guelph Research Ethics Board (Certification of Ethical Acceptability of Research Involving Human Participants – 10JN005) and the University of Guelph Animal Care Committee (Animal Use Protocol – 10R056) approved the study.

4.2.2 Questionnaire development and administration

A questionnaire was developed to collect information from farm workers (Appendix IV). It was pre-tested with 10 sheep and goat producers and industry representatives during the spring
of 2010. These individuals were purposively selected to represent the different sectors of the sheep and goat industries. Questions deemed lengthy or confusing were re-worded.

The questionnaires were administered on each participating farm by personal interview, or were given to participants on the farm and were subsequently completed and mailed/emailed back to the researcher. The questionnaire used a mix of closed- and open-ended questions to gather information on: personal demographic characteristics; medical history, including illnesses that were possibly Q fever sequelae; prior Q fever diagnostic testing; practices for assisting with animal births; individual biosecurity, hygiene, animal management practices; contact with non-livestock animals; and lifestyle habits including smoking, alcohol and raw milk product consumption. Data obtained from sheep and goat serological testing (Chapters 2 and 3), including the percentage of animals that tested seropositive at the farm-level, were also used and tested for association with farm worker seropositivity.

4.2.3 Blood collection

All blood was collected by a health care professional licensed to do so in the province of Ontario. Participants were asked to visit local participating human phlebotomy laboratories (CML Health Care or Gamma Dynacare) for blood collection. A mobile phlebotomist was sent to the farm worker’s residence to collect blood samples where possible, preferentially for those without laboratories within 50 kilometers of their residence or for old-order Amish or Mennonite participants without access to an automobile.

Blood samples were collected by venipuncture into 10ml red top serum BD vacutainer tubes® (Becton, Dickson and Company, Franklin Lakes, New Jersey, USA). The mobile phlebotomist transported vacutainer tube samples in the stopper-up position and chilled on ice at 4-8°C to the University of Guelph for centrifugation. Samples collected at the phlebotomy
laboratories were centrifuged on-site. Vacutainer tube samples were centrifuged for 10 minutes at 1000 x g and 2ml aliquots of the separated serum were then immediately pipetted into serum micro tubes (Fisher Scientific, Ottawa, CA). Serum micro tubes that were processed at the University of Guelph were frozen at -80°C for a maximum of 6 weeks before serological analysis, while the sera collected at phlebotomy laboratories were frozen at -20°C and submitted for serological analysis the next business day.

4.2.4 Serological analysis

Serological analysis was performed at the Public Health Ontario Laboratory using Focus Diagnostic’s © Indirect Immunofluorescence Assay (IFA) (Cypress, California, United States), according to manufacturer instructions. Serum samples were stored at 4°C if tested within 48 hours of collection, or stored at -20°C if tested beyond 48 hours. Participants were considered seropositive when either the phase I or phase II IgG titre was ≥1:16, as per (Focus Diagnostics, 2011).

4.2.5 Data management and statistical analysis

Questionnaire and serological data were entered into EpiData® v2.2 for file management (EpiData Association®, Odense Denmark) and were manually checked against the original questionnaires and laboratory reports for errors. A mixed logistic regression model was constructed in Stata Intercooled Version 10.1 (StataCorp®, 2007) to assess putative risk factor associations with the outcome of farm worker seropositivity, while controlling for clustering by using farm as a random intercept. The initial step in model building utilized univariable screening of all covariates for association with farm worker seropositivity: a liberal level of significance of 20% using the likelihood ratio test (LRT) for categorical variables with more than two categories and Wald’s test for dichotomous variables was used. The linearity of continuous
variables was assessed graphically by plotting lowess smoother curves and transforming the 
variable, if necessary, to achieve normality (Dohoo et al., 2003). Covariates were then screened 
for pair-wise collinearity using Spearman’s rank correlation; variable pairs were considered 
collinear when Spearman’s rho was $\geq 0.8$ (Mason and Perreault, 2013), in which case, the 
covariates were compared with respect to univariable significance, missing variables, and 
biological relationship, and the most relevant variable was retained (Dohoo et al., 2003). The 
retained covariates were then used to construct the multivariable model using a manual backward 
stepwise model-building procedure. Non-significant predictor variables with the highest p-values 
were removed one at a time from the multivariable model until all variables had a significance 
level of $\alpha < 0.05$. All eliminated variables were then tested for confounding and retained in the 
model if their inclusion caused a $>20\%$ change in the coefficients of one or more of the 
significant variables. Two-way interaction terms were generated for biologically relevant 
covariate pairs, and retained in the model if significant ($\alpha < 0.05$). The fit of the model was 
assessed by examining upper level residuals (by farm) using best linear unbiased predictions 
(BLUPs) analysis to verify the model assumptions of normality and homoscedasticity (Dohoo et 
al, 2003). Residuals were visualized in both the fixed and random portions of the model to 
identify potential outliers that would require further investigation. The latent variable technique 
(Goldstein et al., 2002) was used to calculate the variance components at the farm and individual 
levels.
4.3 Results

4.3.1 Study population and seroprevalence

Of the 148 farms on which sheep or goats were tested, 52.7% (78/148) of farms had at least one person participate in human sampling (average 2.2). The farm-level participation in human sampling varied by sector; 73.8% (31/42) of dairy goat farms, 55.9% (19/34) of meat goat farms, 42.0% (21/50) of meat sheep farms and 22.7 % (5/22) of dairy sheep farms participated in the human component of the study. Additionally, two farms had both sheep and goats; one with both meat sheep and meat goats, and the other had meat sheep and dairy goats. In those workers, the variable that was used in the analysis to indicate the percentage of seropositive sheep/goats used all animals (sheep and goat) sampled on that farm.

Five of 172 participants (2.9%) that provided blood samples failed to submit their questionnaire. Therefore, 167 individuals had submitted both the questionnaire and a blood sample. In addition to the 172 individuals who provided blood samples, 43 people completed the questionnaire but failed to have a blood sample drawn. The data from these 43 individuals were used to assess the potential for selection bias. Age had a significant association with the odds of submitting a blood sample (LRT p=0.0033). Relative to 40-49 year olds, the odds of providing a blood sample were 6.2 times higher (95%CI=2.0-18.7, p=0.001) among 18-29 year olds, 4.8 times higher (95% CI=1.8-13.2, p=0.02) among 50-59 year olds, and 12.3 times higher (95%CI=1.5-104.9, p=0.018) among 60-69 year olds. The odds of providing a sample were not significantly different (p≥0.05) among 14-17 year olds, 30-39 year olds and 40-49 year olds. Relative to those who worked on meat sheep farms, the odds of submitting a sample was 5.2 times higher (95%CI= 1.8-15.2, p=0.003) among those that worked on meat goat farms, and 4.9 times higher (95%CI=2.1-11.6, p<0.0001) among those that worked dairy goat farms. Relative to
meat goats, there was no significant difference in the odds of submitting a sample compared to dairy sheep farm workers (OR=4.0, 95%CI=0.8-19.6, p=0.08), or farm workers with both sheep and goats (OR=0.4, 95% CI=0.1-1.7, p=0.20). Those that provided samples were not significantly different than those that did not provide samples with respect to the percentage of seropositive animals on their farm (OR=0.9, 95%CI=0.9-1.0, p=0.14), or smoking history (OR=0.7, 95% CI=0.3-1.4 p=0.25).

In total, complete questionnaires and serological results were available from 167 participants. Participation was similar among genders; 49.4% (85/172) of participants were female and 50.6% (87/172) were male. The age distribution was as follows: 9.0% (15/167) were 14-17 years old, 24.6% (41/167) were 18-29 years old, 13.2% (22/167) were 30-39 years old, 14.4% (24/167) were 40-49 years old, 27.0% (45/167) were 50-59 years old, 10.2% (17/167) were 60-69 years old, and 1.8% (3/167) were 70 years old or more. On average, participants had been working with sheep or goats for 10.8 years (±9.3), and any livestock for 25.1 years (±16.6) and spent an average of 4.1 (±3.2) hours per day in contact with sheep or goats. The majority (91.0%, 152/167) of participants lived on the farm on which sheep and/or goats were sampled.

Overall, one or more farm workers tested positive on 76.3% (58/76, 95% CI=65.8-84.6) of farms (farm-level prevalence); 96.8% (30/31, 95% CI=83.3-99.9) on dairy goat farms, 60.0% (3/5, 95% CI=14.7-94.7) on dairy sheep farms, 71.4% (15/21, 95% CI=47.8-88.7) on meat sheep farms, 47.4% (9/19, 95% CI=24.5-71.4) on meat goat farms, and 50.0% (1/2, 95% CI=1.3-98.7) on farms with animals from multiple sectors. Therefore, the farm-level seroprevalence was significantly higher on dairy goat farms compared to meat goat farms, but were not significantly different from those that worked with dairy or meat sheep, or those that worked with both sheep and goats.
Overall, 64.5% (111/172, 95% CI=57.2-71.4) of sheep and goat farm workers (individual-level prevalence) were seropositive for C. burnetii (i.e. had phase I or phase II ≥ 1:16). By sector, the individual farm worker seroprevalence was significantly higher on dairy goat farms (68/80, 85.0%, 95% CI=75.9-91.6) compared to farm workers on dairy sheep farms (4/13, 30.8%, 95% CI=10.63-58.7), meat goat farms (19/42, 45.2%, 95% CI=30.8-60.4) and meat sheep farms (19/34, 55.9%, 95% CI=39.1-71.8), but was not statistically different from farm workers that worked with both sheep and goats (33.3%, 1/3, 95% CI=0.8-90.6). Table 4.1 demonstrates the distribution of phase I and phase II IgG titres to C. burnetii antigens among the study population, as determined by the IFA, and the classifications as suspected acutely or chronically exposed.

4.3.2 Prior diagnostic testing and history of illness possibly related to Q fever

Twenty participants reported that they suspected they had previously been ill with Q fever. The reported symptoms in decreasing frequency were: fatigue (14/20), muscle aches (13/20), cough, headache and fever (10/20), vomiting/nausea (6/20), and rash (3/20). Included in those 20 people are three individuals from one farm who indicated they suspected they had had Q fever (because their animals had a high rate of abortion on their farm), even though they had reported having no signs or symptoms of disease. Reported signs and symptoms compatible with Q fever in seven of the 20 people occurred two to eight weeks after contact with aborting ewes/does, and in five of the 20 people after lambing ewes or kidding does with no history of abortions. A total of nine of the 20 people sought medical attention for their symptoms, seven of whom requested a test for Q fever. Six of these reported that a Q fever test was performed, five of which tested positive. Of these five people with a positive blood test, three were contacted by the local public health unit as per protocols for routine follow up with reportable diseases.
With respect to general medical history, a total of 46 of 167 people (27.5%) indicated they had been physician-diagnosed with one or more of: pneumonia (34), asthma (22), heart disease (9), cancer (7), low immune function (5), and emphysema (1). Of the 47 (56.6%, 47/83) female participants of childbearing ages (14–49 years of age), 14 had been pregnant within the previous two years, and five of these women were pregnant at the time of sampling. Almost all of these women (13/14) indicated they had been in the barn during lambing or kidding when pregnant, and nine assisted with lambing or kidding while they were pregnant. Three of the 14 women (21.4%) who had been pregnant within the previous two years reported an adverse pregnancy outcome (miscarriage/stillbirth).

4.3.3 Risk factor analysis

A descriptive summary of the questionnaire data is provided in Appendix V. The variables unconditionally associated (p < 0.20) with farm worker seropositivity are presented in Table 4.2. The following variables were also investigated, but were not significantly associated with seropositivity in the univariable analysis (p > 0.20): age category; years working with current herd/flock, sheep/goats, or any livestock; average hours spent in contact with sheep/goats per day; living on the sampled farm; pregnancy history in the past two years; presence in the barn or assisting with births while pregnant; miscarriage of a pregnancy after 12 weeks of gestation; children present on farm or in the barn; personal protective equipment practices when assisting with deliveries such as wearing gloves, wearing a N95 respirator mask, washing hands with a disinfectant soap, changing coveralls, changing or disinfecting footwear; regular personal protective equipment practices including hand washing routines, practices for changing coveralls or barn clothing, washing hands with a disinfectant soap immediately after handling placenta or stillborn/aborted lambs/kids; showering after handling goats before entering the home; having
biosecurity protocols for visitors; having cats on farm, kittens being born on the farm, contact with pregnant cats or newborn kittens; having dogs on the farm, puppies born on the farm, contact with pregnant dogs or newborn puppies born on the farm; contact with vermin, wildlife or their feces; consumption of alcohol; frequency of alcohol consumption; frequency of tobacco use; and frequency of raw milk consumption.

The final mixed-effect multivariable model, which excluded variables possibly related to Q fever sequelae, is shown in Table 4.3. Farm workers from dairy goat farms had the highest odds of being seropositive among all industry sectors. Dairy goat farm workers had 11 times higher odds of being seropositive, compared to meat goat farm workers (95% CI 1.5-83.3), and 25 times higher odds of being seropositive compared to dairy sheep farm workers (95% CI 1.88-333.3), but were not significantly different from meat sheep (p=0.18) or farm workers with multiple species (p=0.54). The odds of farm worker seropositivity also increased with the proportion of tested sheep and goats that were seropositive on their farm. Interpreting this change over a reasonable biological range, an increase of 20% in the percentage of sampled sheep and/or goats that tested positive on the farm (e.g. from 30% to 50%) increased the odds of a farm worker being seropositive by 2.4 times (95% CI 1.3-4.2).

Of the farms that participated in human sampling, dairy goat farms had a significantly higher percent of farms with an individual animal seroprevalence >50% (61.3%, 19/31, 95% CI=42.2-78.2), compared to meat sheep farms (9.5%, 2/21, 95% CI=1.2-31.7), and meat goat farms (5.3%, 1/19, 95% CI=0.1-26.0), but were not significantly different from dairy sheep farms (20.0%, 1/5 95%, CI=0.5-71.6).

Any history of having smoked tobacco (including current smokers and those who previously smoked at any point in their lives but had quit) was marginally associated with
seropositivity (p=0.072). This variable was also determined to confound the relationship between industry sector and seropositivity. It should be noted that the likelihood ratio test statistic, comparing the multivariable model with and without the smoking history variable, was significant (LRT p=0.038); however, the significance of the Wald’s test statistic was decided 

*apriori* as the test to determine inclusion of dichotomous variables in the multivariable model. As such, ever having smoked tobacco remained in the model only as a confounder.

A visual assessment of the model residuals identified three outliers with large Pearson and Anscombe residuals. These three individuals were seronegative and were from dairy goat farms where: all other farm workers from their farm were seropositive, and more than 57% of tested goats on their farm were seropositive. The interpretation of the multivariable model did not change if these outliers were excluded from the analysis, and there was no reason for their exclusion, hence; all outliers were retained in the final model.

**4.4 Discussion**

This is the first comprehensive study to investigate exposure to *C. burnetii* among people working on sheep and goat farms in Ontario, Canada. *Coxiella burnetii* exposure was common; 64% of individuals overall were seropositive, and 76% of farms had one or more seropositive farm workers. It is also important to note that testing here was conducted with the aim of identifying previous exposure to *C. burnetii* using seropositivity; this is not a measure of clinical disease, particularly as the majority of Q fever cases tend to be asymptomatic or mild self-limiting infections (Maurin and Raoult, 1999). The seroprevalence observed here was higher than the 28.4% (23/81) prevalence observed among sheep farm workers in the lower Saint-Lawrence River Region of Quebec in 1998 (Dolcé et al., 2003); however, the latter study used a higher IFA titre cut-off of ≥1:32 to *C. burnetii* phase I or phase II antigens to indicate seropositivity (Dolcé
et al., 2003). We used an IFA titre cut-off of ≥1:16, as this is the cut point recommended by the manufacturers and it maximizes case capture of those who have been previously exposed to *C. burnetii* (Dohoo et al., 2003; Focus Diagnostics, 2011). Although an IFA titre cut point analysis using past *C. burnetii* exposure as the outcome has not been published, manufacturers reported a high specificity (100%) using the cut-cut off of ≥1:16, so false positives are not anticipated (Focus Diagnostics, 2011). Using a lower titre cut-off also contributes to minimizing false negatives caused by waning titres over time (Teunis et al., 2013). As well, different sampling procedures were used between studies, as a convenience sample of all registered sheep producers within the lower Saint-Lawrence Region were eligible to participate in the Quebec study, which may limit external validity (Dohoo et al., 2003). The lack of homogeneity with respect to test cut-offs, and differences in study time periods and sampling procedures makes direct comparisons challenging.

After an epidemic of Q fever among dairy goat farm workers in Newfoundland in 1999, contact with goat placenta, smoking tobacco and eating cheese made from unpasteurized milk were found to be independently associated with seropositivity among farm workers and their families (Hatchette et al., 2001). Sheep farmers in the lower Saint-Lawrence region of Quebec also had a significantly higher risk of being seropositive if they spent more than five hours weekly on the sheep farm or if they bought or traded sheep within the previous six months (Dolcé et al., 2003). However, bias by confounding may be a concern, as the study in Quebec estimated only univariable associations. Multivariable analysis demonstrated several risk factors for seropositivity among Netherlands’ dairy goat farm workers, including: less than 16 km distance to nearest bulk tank milk-positive farm (relative to those ≥16 km distance, OR=5.1, 95% CI=1.8-14.1 for 8 to <16km distance, OR=4.1, 95% CI=1.5-11.1 for 4 to <8 km distance and
OR=4.2, 95% CI=1.6-11.1 for 0 to <4 km distance), presence of a cat in the goat stable (OR=2.2, 95% CI=1.01-4.8), distance between residence and goat stable is ≤10 meters (OR=2.1, 95% CI=1.3-5.3), and staff and employees not wearing farm boots (OR=2.5, 95% CI=1.1-5.9) (Schimmer et al., 2012). Age has also been identified as a risk factor among general populations, with older people having a higher risk of being seropositive (Pascual-Velasco et al., 1998)\(^8\); this effect is often credited to multiple exposures to \textit{C. burnetii} over time boosting immunity, since IgG antibodies can persist for several months or years (Pascual-Velasco et al., 1998).

There was a strong positive association between the percentage of seropositive animals and farm worker seropositivity, which is likely reflective of the highly transmissible nature of \textit{C. burnetii}. Infected sheep and goats may shed \textit{C. burnetii} in the placenta, vaginal secretions, manure, milk, and urine (Berri et al., 2001; Guattéo et al., 2006) and \textit{C. burnetii} can remain infective for months in aerosols or contaminated dust which continue to be released as the placental/fecal material desiccates (Woldehiwet, 2004). Inhalation of contaminated aerosols is the major mechanism whereby \textit{C. burnetii} is transmitted to humans (Gonder et al., 1979; Marrie et al., 1989; Williams et al., 1991; Stein et al., 2005). Secondarily, cases have also been attributed to ingesting \textit{C. burnetii} by consuming unpasteurized milk from an infected farm (Welsh et al., 1945; Benson et al., 1963; Fishbein and Raoult, 1992). Consequently, people can become infected from either direct contact to shedding animals (Fournier et al., 1998), contaminated materials such as bedding or manure, or through sharing a \textit{C. burnetii} contaminated environment for a proportion of the day (Rodolakis, 2009). \textit{Coxiella burnetii} exposure among farm workers’ sheep and goats was common (Section 2.3.2 & Section 3.3.2), therefore, a proportion of exposed animals were presumably shedding \textit{C. burnetii} at some point

\(^8\)No odds ratio provided by authors
on positive farms, which may have resulted in transmission to humans on the farms. Since C. burnetii shedding was not directly measured for this research, we do not know if any sampled animals were shedding at the time of sampling; however, one of the criteria that is predictive of an active infection at the herd-level is a within-herd seroprevalence above 50% (Sidi-Boumedine et al., 2010). Using this cut-point, there may have been an active infection in 22.3% (33/148) of the sheep and goat farms in Ontario. However, this criterion only reveals suspicion that an active C. burnetii infection is present without other positive diagnostic tests such as a quantitative PCR on aborted placental tissue (Sidi-Boumedine et al., 2010). As shown in Appendix VI, farms above and below the 50% seroprevalence cut-point were not different with respect to having C. burnetii identified as the cause of abortion, or experiencing an elevated abortion risk (>5%) or abortion storm in sheep/goats in the previous three years. Therefore, this finding suggests that the 50% seroprevalence cut-point may not be a good measure of active infections at the herd-level in our population, although power was likely an issue. It is also possible that the persistence of C. burnetii titres may affect the relationship between seroprevalence and the presence of active infections, though further research is needed to confirm this hypothesis.

Due to the relationship between animal and human exposure, implementing measures to prevent or control the infection in animals, such as keeping a closed herd/flock, limiting loaning animals, or sheep/goat vaccination against C. burnetii, could reduce the likelihood of infection among the people in contact with them. Animal vaccination has been used in the Netherlands to reduce the risk of human Q fever (Isken et al., 2013). A meta-analysis suggested that vaccination of naïve goats reduced the risk of shedding in uterine and vaginal secretions, milk and feces (O’Neill et al., 2013). In addition, the levels of bacteria shed from placental and vaginal routes were reduced in vaccinated goats compared to naive controls. The data in sheep are limited; the
meta-analysis concluded that vaccination did not have a significant effect on the risk of shedding in sheep (O’Neill et al., 2013).

There were higher odds of seropositivity amongst workers on dairy goat farms compared to meat goat and dairy sheep farms. This association was significant even after accounting for the percentage of animals per farm that tested positive. This suggests that other characteristics of dairy goat farms may influence the risk of human exposure. Prior research indicated that the degree of human-animal contact was associated with human seropositivity (Van den Brom et al., 2013). It stands to reason that there may be more intensive animal contact on dairy animal farms compared to meat animals farms, particularly during milking where farm workers handle animals daily and are close to the animal’s escutcheon and udder where C. burnetii may be shed via feces, milk or urine (Porter et al., 2011). We identified that significantly more dairy goat farms had a within-farm seroprevalence >50%, compared to meat sheep and meat goat farms. This may indicate that dairy goat farms had more active C. burnetii infections at the herd-level than meat sheep or meat goat farms, making dairy goat farm workers at higher risk of exposure. Although, as noted previously, a within-farm seroprevalence > 50% only reveals suspicion of an active C. burnetii infection without other diagnostic tests. Appendix VII shows the distribution of sheep/goat reproductive testing and outcomes in each small ruminant sector. Due to the small number of farms with C. burnetii abortions and elevated abortion risk, a relationship with industry sector is not evident. The shedding routes between sheep and goats may also differ, as research suggests that sheep shed C. burnetii mainly in feces and vaginal mucous, while goats excreted C. burnetii mostly in milk (Rodolakis et al., 2007).

Other research has identified a positive association between smoking tobacco and evidence of C. burnetii exposure (Hatchette et al., 2001; Karagiannis et al., 2009). An outbreak
investigation in Newfoundland in 1999 concluded that smokers had 3.27 times the odds of being seropositive to *C. burnetii* compared to non-smokers (Hatchette et al., 2001). Smoking may increase the likelihood of ingestion of *C. burnetii* via hand-to-mouth contact among smokers compared to non-smokers (Hatchette et al., 2001), and there may also exist reduced antibacterial host defences among smokers (Herr et al., 2009). Though the Wald’s p-value indicated the variable for the smoking history was marginally associated with seropositivity, an examination of the Wald’s test and LRT by Hanuck and Donner (1977), suggested the LRT p-value may be a better indication of significance, particularly when data are sparse. Therefore, the association between smoking history and seropositivity may be considered both statistically and biologically significant. This evidence could be used for the justification of promoting smoking prevention programs among those working with sheep or goats. This may be particularly important among sheep and goat workers with pre-existing health conditions who are more susceptible to the development of chronic Q fever (Kampschreur et al., 2012).

Age was hypothesized to have a positive association with farm worker seropositivity as previously described (Marrie and Pollak, 1995; Psaroulaki et al., 2006; Whitney et al., 2009; Anderson et al., 2009), because of the potential for cumulative exposure to *C. burnetii* with increasing age (Whitney et al., 2009). However, age was not significantly associated with seropositivity in our study. Many of the participating farm workers were relatively new to working with sheep and goats (median= 8 years, mean=11 years); therefore, farm workers who are new to working with sheep and goats may not have equivalent cumulative *C. burnetii* compared to long-standing sheep and goat producers, regardless of age.

The presence of barn cats on farm had been identified as a risk factor for seropositivity among Netherland’s dairy goat farm workers (Schimmer et al., 2012), but we did not observe
this association in our study. This could be due to a lack of variability of this exposure, as only 16% (26/162) of participating farm workers did not have cats on the farm. Eating cheese made from unpasteurized milk was found to be independently associated with seropositivity among farm workers and their family after an epidemic of Q fever among dairy goat farm workers in Newfoundland (Hatchette et al., 2001). In our study, the consumption of unpasteurized dairy products was not significantly associated with seropositivity. In the multivariable model, much of the variability for consuming unpasteurized dairy products was likely explained by farm sector. As expected, dairy sheep and dairy goat farms had higher proportions of producers consuming unpasteurized dairy products than meat sheep and meat goat farm workers. In addition, dairy farm workers may have been exposed via other routes, such as aerosolized milk, manure and vaginal secretions during the milking procedure.

In this research, seropositivity was used to indicate past exposure. However, it has been noted in other studies that some infected animals do seroconvert (Rousset et al., 2009). It is also possible then, that people do not always seroconvert when infected with C. burnetii, although this has not been thoroughly examined as diagnosing C. burnetii infection is often solely reliant on a positive serological test, providing an individual has compatible symptoms (Fournier and Raoult, 2003). Therefore, a proportion of exposed individuals may be misclassified as non-exposed due to their lack of C. burnetii specific antibodies. While slow decay rates of C. burnetii titres have been demonstrated in humans, there is also considerable heterogeneity with respect to how long these specific antibodies persist in the human blood, particularly between acute and chronic cases (Teunis et al., 2013). The titre decay rates are the slowest for IgG phase I (Teunis et al., 2013); therefore, the duration of seropositivity is longer with chronic cases compared to acute cases. As a result, there may be an increased chance of identifying seropositive chronic
cases compared to seropositive acute cases in seroprevalence studies of humans. If a factor, such as smoking, increased the likelihood of leading to chronic Q fever compared to acute Q fever, this factor would be more likely to be associated with seropositivity in this study, due to the slow decay rate of IgG phase I relative to IgG phase II. Research has demonstrated smoking suppresses the innate and adaptive immune systems, making smokers more susceptible to chronic infections (Sopori, 2002; Herr et al., 2009). This research was not specific to C. burnetii infection, therefore more work is needed to confirm whether smoking increases the likelihood of chronic Q fever compared to acute Q fever.

Selection bias was assessed to the extent possible, by comparing risk factor, demographic, and animal serological data among 167 participants with questionnaire and serological data, and 43 individuals who filled out questionnaires but failed to provide blood samples. Participation among this group was not significantly associated with the proportion of seropositive animals on their farm (p=0.14), nor whether there was at least one or more positive animals on their farm (p=0.93). The low participation among dairy sheep farms in the human testing may be due to a high proportion of Old-Order Amish and Mennonite participants in this group. These individuals may have had different attitudes regarding blood donation, or may have found it more difficult to travel to a phlebotomy laboratory. The latter was addressed by taking efforts to maximize human participation by utilizing a mobile phlebotomist where possible, and working with two different phlebotomy companies with laboratories across Ontario, which minimized the distance participants would need to travel to have samples collected. Unfortunately, some rural areas were under-serviced by these phlebotomy laboratories, which made sample collection less convenient. Due to the low participation and non-random farm
selection among dairy sheep farms, there is more uncertainty surrounding the representativeness of the seroprevalence estimate in that population.

To maximize participation, farm workers had the option to complete the questionnaire at the farm visit with a researcher (n=111), or email/mail the questionnaire to the research team (n=61). Completing the questionnaire in person or via email/mail was not associated with farm worker seropositivity (p=0.99), small ruminant sector (p=0.72), a history of smoking tobacco (p=0.63), or the proportion of seropositive animals on their farm (p=0.96).

Given the high proportion of farm workers with evidence of exposure, there is a need to more closely examine the prevalence of acute and chronic cases through active surveillance efforts, and subsequently, examine the burden of illness in farm workers exposed to *C. burnetii*. Physicians, particularly those with patients living in rural areas, should be cognisant of the high risk of *C. burnetii* exposure among those working with sheep and goats. As well, farm workers with signs and symptoms compatible with Q fever should be encouraged to discuss diagnostic testing with their physicians. In addition to increased surveillance efforts, vaccinating sheep or goat farm workers, particularly those at risk of developing chronic Q fever, merits future consideration. At the moment, a licensed human vaccine is only commercially available in Australia (Q Vax, Commonwealth Serum Laboratories, Melbourne, Australia), and is used for abattoir workers, farm workers and veterinarians (Gidding et al., 2009). During the Netherlands Q fever epidemic, the Dutch government also offered the unlicensed Q fever vaccine to patient groups at high risk of Q fever complications (Isken et al., 2013). Alternatively, animal vaccination (Coxevac, CEVA Animal Health) can be used on sheep and goat farms affected by coxiellosis to reduce the risk of human Q fever via reduction in animal shedding (Isken et al., 2013). Animal vaccination could be particularly beneficial on dairy goat farms, as *C. burnetii*
exposure in both dairy goats and their farm workers was common, and the vaccine has been proven effective in reducing shedding in dairy goats (O’Neill et al., 2013).

4.5 Conclusion

_C. burnetii_ exposure was common among sheep and goat farm workers in Ontario; 76% of farms and 64.5% of individuals were seropositive. Two risk factors were identified which increased the odds of seropositivity among farm workers: increases in the proportion of seropositive animals on their farm, and working on a dairy goat farm. As well, smoking may also increase the odds of seropositivity.

4.6 Acknowledgements

We thank the Ontario Ministry of Agriculture, Food and Rural Affairs - University of Guelph Agreement through the Animal Health Strategic Investment fund (AHSI) managed by the Animal Health Laboratory of the University of Guelph, the Ontario Ministry of Health and Long-Term Care, the National Sciences and Engineering Research Council of Canada, Public Health Ontario, the Ontario Sheep Marketing Agency, and Ontario Goat. We gratefully acknowledge the cooperation of all goat producers that participated in the study.
4.7 References


Table 4.1 Number of individual sheep and goat farm workers (percentage of total samples) with specific immunoglobulin G serum titres to Phase I and Phase II *Coxiella burnetii* antigens (n = 172) Ontario, Canada, as determined by the Immunofluorescence assay (Focus Diagnostics) (August 2010-March 2012)

<table>
<thead>
<tr>
<th>Phase II</th>
<th>NR</th>
<th>1:16</th>
<th>1:32</th>
<th>1:64</th>
<th>1:128</th>
<th>≥1:256</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR</td>
<td>61 (35.5)</td>
<td>7 (4.0)</td>
<td>3 (1.7)</td>
<td>1 (0.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>72 (41.9)</td>
</tr>
<tr>
<td>1:16</td>
<td>5 (2.9)</td>
<td>4 (2.3)</td>
<td>5 (2.9)</td>
<td>1 (0.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>15 (8.7)</td>
</tr>
<tr>
<td>1:32</td>
<td>1 (0.6)</td>
<td>10 (5.8)</td>
<td>10 (5.8)</td>
<td>6 (3.5)</td>
<td>2 (1.2)</td>
<td>1 (0.6)</td>
<td>30 (17.4)</td>
</tr>
<tr>
<td>1:64</td>
<td>0 (0)</td>
<td>1 (0.6)</td>
<td>3 (1.7)</td>
<td>11 (6.4)</td>
<td>5 (2.9)</td>
<td>1 (0.6)</td>
<td>21 (12.2)</td>
</tr>
<tr>
<td>1:128</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.6)</td>
<td>12 (7.0)</td>
<td>5 (2.9)</td>
<td>18 (10.5)</td>
</tr>
<tr>
<td>≥1:256</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (2.3)</td>
<td>0 (0)</td>
<td>12 (7.0)</td>
<td>16 (9.3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>67 (39.0)</td>
<td>22 (12.8)</td>
<td>21 (12.2)</td>
<td>24 (14.0)</td>
<td>19 (11.0)</td>
<td>19 (11.0)</td>
<td>172 (100.0)</td>
</tr>
</tbody>
</table>

NR- Not reactive

- 35.5% (61/172) Unexposed (Phase I and Phase II IgG not reactive)
- 14.5% (25/172) Titres suggestive of an acute exposure (Phase II titre > Phase I titre)
- 50.0% (86/172) Titres suggestive of a chronic exposure (Phase I titre ≥ Phase II titre)
Table 4.2. Covariates associated (p<0.2) with farm worker seropositivity for *C. burnetii*, as observed through univariable mixed-effects logistic regression of data collected from sheep and goat farm workers in Ontario, Canada (August 2010-March 2012)

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Industry Sector (LRT $\chi^2 = 15.13$, p=0.0044)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy goats</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dairy sheep</td>
<td>0.030</td>
<td>0.003-0.342</td>
<td>0.005</td>
</tr>
<tr>
<td>Meat goats</td>
<td>0.039</td>
<td>0.006-0.242</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Meat sheep</td>
<td>0.113</td>
<td>0.023-0.555</td>
<td>0.007</td>
</tr>
<tr>
<td>Multiple$^9$</td>
<td>0.042</td>
<td>0.001-2.106</td>
<td>0.112</td>
</tr>
<tr>
<td>Percentage sheep and/or goats seropositive</td>
<td>1.051</td>
<td>1.026-1.077</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ever smoked tobacco$^{10}$</td>
<td>3.968</td>
<td>0.800-19.686</td>
<td>0.092</td>
</tr>
<tr>
<td>Consumed raw milk in the past two years</td>
<td>2.564</td>
<td>0.683-9.619</td>
<td>0.163</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2.815</td>
<td>1.033-7.669</td>
<td>0.043</td>
</tr>
<tr>
<td>Female</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wear gloves while assisting with presumed abortion (LRT $\chi^2 = 6.20$, p=0.185)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NA$^b$</td>
<td>1.605</td>
<td>0.294-8.765</td>
<td>0.585</td>
</tr>
<tr>
<td>Infrequently</td>
<td>0.281</td>
<td>0.016-4.837</td>
<td>0.382</td>
</tr>
<tr>
<td>Frequently</td>
<td>2.13</td>
<td>0.286-15.869</td>
<td>0.460</td>
</tr>
<tr>
<td>Always</td>
<td>0.372</td>
<td>0.072-1.920</td>
<td>0.0238</td>
</tr>
</tbody>
</table>

$^9$ Both sheep and goats (multiple industry sectors) on farm

$^{10}$ Current smokers and those who had previously smoked in their lives but had quit were collapsed and represented by variable ‘ever smoked tobacco’
<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash hands after assisting with normal births</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(LRT $\chi^2 = 6.89$, p=0.142)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NA$^c$</td>
<td>3.368</td>
<td>0.187-60.523</td>
<td>0.041</td>
</tr>
<tr>
<td>Infrequently</td>
<td>18.181</td>
<td>0.239-1383.412</td>
<td>0.189</td>
</tr>
<tr>
<td>Frequently</td>
<td>16.475</td>
<td>0.844-321.656</td>
<td>0.065</td>
</tr>
<tr>
<td>Always</td>
<td>1.41</td>
<td>0.138-14.468</td>
<td>0.772</td>
</tr>
<tr>
<td>Change coveralls or barn clothing after assisting with normal births (LRT $\chi^2 = 8.79$, p=0.0667)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NA$^c$</td>
<td>1.334</td>
<td>0.147-12.120</td>
<td>0.798</td>
</tr>
<tr>
<td>Infrequently</td>
<td>1.054</td>
<td>0.262-4.243</td>
<td>0.941</td>
</tr>
<tr>
<td>Frequently</td>
<td>4.730</td>
<td>0.513-43.600</td>
<td>0.017</td>
</tr>
<tr>
<td>Always</td>
<td>0.141</td>
<td>0.025-0.804</td>
<td>0.027</td>
</tr>
<tr>
<td>Wash hands with soap after handling sheep/goats (LRT $\chi^2 = 6.61$, p=0.0855)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Infrequently</td>
<td>4.322</td>
<td>0.362-51.646</td>
<td>0.248</td>
</tr>
<tr>
<td>Frequently</td>
<td>0.990</td>
<td>0.147-6.678</td>
<td>0.992</td>
</tr>
<tr>
<td>Always</td>
<td>0.300</td>
<td>0.050-1.802</td>
<td>0.188</td>
</tr>
<tr>
<td>Wash hands with soap before entering the house</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(LRT $\chi^2 = 5.97$, p=0.113)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Infrequently</td>
<td>3.004</td>
<td>0.344-26.241</td>
<td>0.320</td>
</tr>
<tr>
<td>Frequently</td>
<td>2.401</td>
<td>0.406-14.191</td>
<td>0.334</td>
</tr>
<tr>
<td>Always</td>
<td>0.368</td>
<td>0.082-1.656</td>
<td>0.193</td>
</tr>
<tr>
<td>Visitors wash hands</td>
<td>0.249</td>
<td>0.035-1.756</td>
<td>0.163</td>
</tr>
</tbody>
</table>

LRT= Likelihood ratio test

$^a$ Farms had both sheep and goats

$^b$ NA= Not applicable - individual either does not assist with births or no abortions have occurred

$^c$ NA= Not applicable - individual does not assist with births
Table 4.3. Final multivariable mixed-effects logistic regression model of farm worker seropositivity for *C. burnetii* based on serological and questionnaire data collected from sheep and goat farm workers in Ontario, Canada (August 2010-March 2012) (n=167)

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Industry Sector (LRT $\chi^2 = 11.48$, p=0.022) $^a$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy goats</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dairy sheep</td>
<td>0.040</td>
<td>0.003-0.531</td>
<td>0.015</td>
</tr>
<tr>
<td>Meat goats</td>
<td>0.091</td>
<td>0.012-0.669</td>
<td>0.018</td>
</tr>
<tr>
<td>Meat sheep</td>
<td>0.303</td>
<td>0.053-1.734</td>
<td>0.180</td>
</tr>
<tr>
<td>Multiple $^b$</td>
<td>0.290</td>
<td>0.006-15.200</td>
<td>0.540</td>
</tr>
<tr>
<td>Percentage sheep and or goats seropositive</td>
<td>1.044</td>
<td>1.015-1.074</td>
<td>0.003</td>
</tr>
<tr>
<td>Ever smoked tobacco</td>
<td>4.032</td>
<td>0.881-18.452</td>
<td>0.072</td>
</tr>
</tbody>
</table>

**Random Intercept Parameter**

<table>
<thead>
<tr>
<th>Farm $^c$</th>
<th>SD</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.56</td>
<td>0.76-3.21</td>
<td>0.0019</td>
</tr>
</tbody>
</table>

Ref= referent category, OR= odds ratio, CI= Confidence Interval, SD= Standard Deviation

$^a$ LRT= Likelihood ratio test for categorical variable

$^b$ Farms had animals in more than one category

$^c$ Partitioning the variance, 42.7% of variation in multivariable model occurred at the farm-level and 57.3% at the farm worker-level
Prevalence and risk factors for *Coxiella burnetii* seropositivity in small ruminant veterinarians and veterinary students in Ontario, Canada

Abstract:

*Coxiella burnetii* is a zoonotic pathogen that causes Q fever in humans. A serological survey was conducted and a questionnaire administered to veterinarians and veterinary students who were members of the Small Ruminant Veterinarians of Ontario and who attended a professional education meeting in February, 2012. Serum was tested for *C. burnetii* specific antibodies using the immunofluorescence assay (IFA, Focus Diagnostics), and those with phase I or phase II IgG titres of ≥1:16 were classified as seropositive. Fifty-nine percent (19/32, 95% CI=41.9-75.2) of participants were seropositive. In univariable exact logistic regression models, being a veterinary student had a sparing effect, as students had 0.06 times (95% CI: 0.0011-0.65) the odds of seropositivity compared to practicing veterinarians (p=0.014). In addition, participants aged 30-39 and 40-49 years had 15.46 times (95% CI: 1.0088-1059.64) and 12.96 times (95% CI: 1.26- +Infinity) the odds of seropositivity, respectively, compared to those aged 18-29.

Overall, seropositivity was common among this population, presumably due to their contact with, or proximity to, infectious animals. Veterinarians and veterinary students should be mindful that exposure to *C. burnetii* was common, and ask their physicians about diagnostic testing should they present with signs of Q fever. The use of a human Q fever vaccine (Q Vax, Commonwealth Serum Laboratories, Melbourne, Australia) for seronegative veterinarians and veterinary students also merits future consideration.
5.1 Introduction

*Coxiella burnetii* (*C. burnetii*) is a zoonotic bacterium, which causes Q fever in humans (Maurin and Raoult, 1999). Approximately 60% of cases are asymptomatic, 38% experience mild symptoms without the need for hospitalization, 1.8% are hospitalized with acute Q fever, and 0.2% develop chronic Q fever (Maurin and Raoult, 1999). Patients with Q fever may present with acute or chronic clinical manifestations (Maurin and Raoult, 1999). Symptomatic acute Q fever manifests primarily as a febrile illness associated with severe headaches, atypical pneumonia, or granulomatous hepatitis; while endocarditis is a common presentation of chronic Q fever (Maurin and Raoult, 1999; Limonard et al., 2010). Additionally, a form of chronic fatigue syndrome can also occur in acute and chronic Q fever cases (Ayres et al., 1998; Wildman et al., 2002). The non-specific nature of symptoms associated with Q fever may contribute to under-diagnosis and subsequent under-reporting (Marrie and de Carolis, 2002; PHO, 2012; de Valk, 2012). Primarily, infection is considered to be acquired from inhalation of aerosols contaminated with *C. burnetii* organisms (Maurin and Raoult, 1999). Human infection has most frequently been attributed to indirect or direct contact with infected ruminants, primarily sheep, goats and cattle (Lyytikäinen et al., 1998; Tissot-Dupont et al., 1999; Astobiza et al., 2011; Thompson et al., 2012). Therefore, by the nature of their occupations, veterinarians have been identified as having a higher risk of *C. burnetii* exposure than the general population (Marrie and Fraser, 1985; Whitney et al., 2009). Identified risk factors for *C. burnetii* exposure among Nova Scotia veterinarians included being male and handling sheep placenta (Marrie and Fraser, 1985). In the Netherlands, identified risk factors for veterinarians’ exposure to *C. burnetii* included: hours of animal contact per week, increasing number of years graduated as a veterinarian, living
in rural or semi-urban areas, being a practicing livestock veterinarian, and occupational contact with swine (Van den Brom et al., 2013).

The presence of \textit{C. burnetii} specific antibodies in serum is used to indicate past exposure to the bacterium. The immunofluorescence assay (IFA) is the reference serological test used to diagnose human Q fever (Sidi-Boumedine et al., 2010). The IFA detects the immunoglobulin G (IgG) antibody response to phase I and phase II \textit{C. burnetii} antigens (Focus Diagnostics, 2011). A phase II titre > phase I is suggestive of acute exposure, while phase I ≥ phase II is suggestive of chronic exposure (Focus Diagnostics, 2011). There is evidence of long-term persistence of \textit{C. burnetii} in human hosts, in both acute and chronic cases (Harris et al., 2000). This persistence of \textit{C. burnetii} antibodies, and the potential boosts in immunity due to re-exposure, makes serological testing useful for identifying the prevalence of past exposure to \textit{C. burnetii} (Marrie and Pollak, 1995).

In 2007-2009, a human Q fever epidemic occurred in the Netherlands in which 3523 cases were diagnosed (Roest et al., 2011). This epidemic was later attributed to transmission to humans from infective dairy goats, and to a lesser extent, dairy sheep (Roest et al., 2011). This outbreak raised awareness of Q fever and its public health implications. Since then, efforts have been made to screen high risk populations, such as veterinarians in the United States and the Netherlands, and to learn more about how disease can be prevented. In Canada, sero-surveys were conducted in the 1980s in Nova Scotia veterinarians and slaughterhouse workers, and in sheep farmers in Quebec (Marrie and Fraser, 1985; Dolcé et al., 2003); however, this is the first examination of \textit{C. burnetii} seropositivity among veterinarians in Ontario. The objectives of this study were to: (i) determine the prevalence of \textit{C. burnetii} seropositivity in Ontario small
ruminant veterinarians and veterinary students, and (ii) identify demographic, hygiene, biosecurity and lifestyle risk factors associated with seropositivity.

5.2 Materials and Methods

5.2.1 Selection of participants

A convenience sampling procedure was used; all attendees at the Small Ruminant Veterinarians of Ontario (SRVO) Annual General Meeting (AGM), which took place in Orangeville, Ontario, on February 24, 2012, were invited to participate. SRVO is a voluntary professional organization of veterinarians and veterinary students with an interest in the health of ovine, caprine, cervid or camelid species. Informed written consent was obtained from all participants. The University of Toronto Research Ethics Board (Certification of Ethical Acceptability of Research Involving Human Participants: Reference 27340) approved the study.

5.2.2 Questionnaire description and administration

The questionnaire (Appendix VIII) was self-administered at the AGM and used a mix of closed- and open-ended questions to gather information on: age, gender, years working in veterinary practice, percent of clinical practice time devoted to different animal species, contact with different animal species, number of hours in contact with sheep/goats, personal protective equipment practices when assisting births of lambs/kids, prior Q fever testing and diagnoses, general medical history related to Q fever sequelae, pregnancy and adverse pregnancy events (women), lifestyle questions regarding smoking habits, alcohol and raw milk product consumption, and C. burnetii vaccination history. On average, the questionnaire took approximately 10 minutes to complete.
5.2.3 Blood collection and serological analyses

Blood samples were collected on-site by a certified phlebotomist via venipuncture into 10ml red top serum BD vacutainer tubes® (Becton, Dickson and Company, Franklin Lakes, New Jersey, USA). Vacutainer tube samples were then transported in the stopper-up position on ice at 4-8°C to the Public Health Ontario Laboratory for centrifugation and serological analysis. Samples were centrifuged for 10 minutes at 1000 x g and 2ml aliquots of the separated serum were then immediately pipetted into serum micro tubes (Fisher Scientific, Ottawa, CA).

Serological analysis was performed at the Public Health Ontario Laboratory in Toronto using the Focus Diagnostic © IFA (Cypress, California, United States), according to the manufacturer instructions. Serum samples were stored at 4°C if tested within 48 hours, or stored at -20°C if tested beyond 48 hours. Samples were considered seropositive when either the phase I or phase II IgG titre was ≥1:16, as per (Focus Diagnostics, 2011).

5.2.4 Data management and statistical analysis

Univariable exact logistic models were constructed in Stata Intercooled Version 10.1 (StataCorp®, 2007) to assess putative risk factor associations with the outcome of seropositivity. Exact logistic regression modelling was used in place of standard asymptotic logistic regression, as the former is ideal for analyzing small, skewed or sparse datasets (Dohoo et al., 2003). Associations were considered significant if the confidence level of α <0.05. If a covariate predicted seropositivity perfectly, an estimate of the coefficient was calculated using a median unbiased estimates procedure to give a reasonable estimate of the covariate of interest (Dohoo et al., 2003). The dataset was considered too small for the development of a multivariable model.
5.3 Results

5.3.1 Study population

There were 36 attendees at the SRVO AGM; 88.9% (32/36) participated by providing blood samples and completing the questionnaire. One attendee declined and three agreed to participate, but the phlebotomist was unable to collect a sufficient blood sample for analysis. Participants represented 33.3% (32/96) of the source population of SRVO members at the time of sampling. To estimate the target population size, an estimate of the total number of veterinarians in Ontario working with small ruminants was obtained from the College of Veterinarians of Ontario (http://www.cvo.org/imis15/CVO/); 562 veterinarians were listed as working with small ruminants in the spring of 2013. However, misclassification of individuals in this list was evident, specifically with respect to the presence of false positives (i.e. individuals classified as small ruminant veterinarians, but were not) (Dr. Jocelyn Jansen, personal communication, 2013). Hence, this estimate is conservative and likely over-estimates the number of small ruminant veterinarians in the target population. The target population for veterinary students included 96 students at the Ontario Veterinary College who considered small ruminants to be an important part of their caseload or anticipated caseload (Dr. Paula Menzies, personal communication, 2013). Using the sum of these veterinarians and veterinary students as a conservative estimate of our target population, participants in the study represented 4.9% (32/658) of all Ontario veterinarians and veterinary students working with small ruminants.

5.3.2 Prior diagnostic testing

At the time of sampling, five participants indicated in the questionnaire that they suspected they had had Q fever at some point in the past. Two of these individuals sought medical attention; however, serological tests for *C. burnetii* were negative for both individuals.
The reported symptoms attributed to Q fever by the five individuals were: fever (5), headache and muscle ache (3), fatigue (2), cough (1) and sore throat (1). All five of these individuals were seropositive for *C. burnetii* at the time of sampling. None of the 14 female participants were pregnant at the time of sampling. Of the four women that reported having been pregnant in the previous two years, all tested seropositive; none reported an adverse pregnancy outcome.

### 5.3.3 Seropositivity

Serosurvey results indicated that 59.4% (19/32, 95% CI: 40.6%-76.3%) of participating veterinarians and veterinary students were seropositive to *C. burnetii*. Practicing veterinarians had a seroprevalence of 76.2% (16/21, 95% CI: 52.8%-91.8%) compared to a seroprevalence of 50.0% (2/4, 95% CI: 6.8%-93.2%) in non-practicing veterinarians, and 14.3% (1/7, 95% CI: 0.3%-57.9%) in veterinary students. Table 5.1 demonstrates the distribution of phase I and phase II antibodies to *C. burnetii* antigens among the study population; 9.4% (3/32) and 50.0% (16/32) were considered to have acute and chronic exposures, respectively.

### 5.3.4 Risk factors

A descriptive summary of selected questionnaire data and their association with seropositivity is presented in Table 5.2. The exact logistic univariable analysis identified two covariates associated with seropositivity at α<0.05. Being a veterinary student had a sparing effect, as students had 0.06 times (95% CI: 0.0011-0.65) the odds of seropositivity compared to practicing veterinarians (p=0.014); the odds of seropositivity among non-practicing veterinarians were not significantly different from either practicing veterinarians or veterinary students. In addition, participants aged 30-39 and 40-49 years had 15.46 times (95% CI: 1.0088-1059.64) and 12.96 times (95% CI: 1.26- +Infinity) the odds of seropositivity, respectively, compared to those aged 18-29.
While not found to be significantly associated with seropositivity in this study, the questionnaire data highlighted some potentially hazardous practices among the study group. For example, thirty-two percent (9/28) of participants indicated they never or only infrequently wore gloves while assisting with normal births of lambs or kids. As well, 57% (16/28) indicated that they never or only infrequently take off their coveralls before entering the vehicle after assisting with births.

5.4 Discussion

This is the first investigation examining *C. burnetii* seropositivity among veterinarians and veterinary students in the province of Ontario, and the second in Canada (Marrie and Fraser, 1985). The seroprevalence (59.4%, 19/32) indicates that exposure to *C. burnetii* was common. The seropositivity among the general population in Ontario has not been evaluated recently. A random sample of blood donors from the urban centre of Toronto, Ontario, in 1982 indicated 0.6% (2/360) were seropositive using the Compliment Fixation Test (CFT) (Simor et al., 1984); however, the CFT has demonstrated a low sensitivity (Sn=71%), compared to 99%-100% in the IFA (Field et al., 2000). This, and the age of the data, therefore makes it a poor basis for comparison.

*C. burnetii* was identified as an occupational hazard for veterinarians and veterinary students elsewhere. For example, a serosurvey conducted in Nova Scotia that occurred in the summer of 1984 and used two non-commercial *C. burnetii* antibody tests. Researchers concluded that 17% (11/65) of veterinarians had compliment fixing antibody titres ≥1:8 for phase II *C. burnetii* antigens in 1984 (Marrie and Fraser, 1985), compared to 4.1% (41/997) in Nova Scotia blood donors in 1982. The analysis was repeated with IFA, which resulted in 49% (32/65) of Nova Scotia veterinarians (Marrie and Fraser, 1985) and 11.8% (118/997) (Marrie et al., 1984)
of blood donors having an antibody titre ≥1:8 to phase II C. burnetii antigens (Marrie et al., 1984). The sampling strategy used was not explicitly described (Marrie and Fraser, 1985), and the agreement between the non-commercial tests used in the Nova Scotia study with the commercial IFA used in our study is unknown. For the present research, an IFA titre cut-off of ≥1:16 was used, as this is the cut point recommended by manufacturers (Focus Diagnostics, 2011), and the low cut-point maximizes case capture of those who have been previously exposed to C. burnetii (Dohoo et al., 2003). Though an IFA titre cut point analysis using past C. burnetii exposure as the outcome has not been published, manufacturers reported a high specificity (100%) using the cut-off of ≥1:16, so false positives are not anticipated (Focus Diagnostics, 2011). Using a lower titre cut-off also contributes to minimizing false negatives caused by waning titres over time (Teunis et al., 2013).

Seropositivity was examined among PCR positive blood donors in 2009-2010 in the area of the Netherlands with the highest Q fever notifications during the 2007-2009 epidemic was 12.2% (66/543) using an ELISA and IFA interpreted in series (Hogema et al., 2012). Also, in 2009, seropositivity among attendees a veterinary conference in the Netherlands concluded that 69.2% (117/169) of Dutch livestock veterinarians, and 30.0% (6/20) of livestock veterinary students in their final year of studies, were seropositive to C. burnetii (Van den Brom et al., 2013). These authors considered a sample seropositive if both IgG phase I and phase II titres were ≥1:32, if IgG phase II titre alone was ≥1:512 (IFA, Focus Diagnostics), or if the IgM titre for both phase I and phase II were ≥1:32 (ELISA, Focus Diagnostics) (Van den Brom et al., 2013). A direct comparison of these results with the present study is therefore difficult due to the use of different titre cut-points to denote positive samples, and different sampling procedures.
Approximately 22.2% (113/508) of companion, equine and food animal veterinarians attending a national veterinary convention in the United States in 2006 were seropositive to *C. burnetii* (Whitney et al., 2009). Of those that participated, 25.6% (130/508) practiced in either a food animal or mixed animal practice, with 69.1% (351/508) of participants practicing small/companion animal medicine and the remaining 5.3% (27/508) practicing in equine, exotics or other species (Whitney et al., 2009). If only those veterinarians who practiced mixed or food animal medicine in the United States are considered, the estimate of seroprevalence is 45.4% (59/130) (Whitney et al., 2009), which more closely resembles the seroprevalence identified here among small ruminant veterinarians in Ontario. Sera were sequentially tested, first with an ELISA (PanBio) (sensitivity = 71%) (Field et al., 2002), then positive or equivocal samples were re-tested with the more sensitive IFA (sensitivity=100%) (Focus Diagnostics, 2011), and were considered positive if IgG antibody titres were ≥ 1:16 (Whitney et al., 2009). As the PanBio ELISA is less sensitive than the IFA, and a series interpretation was used (whereby only samples positive to the first test were subjected to IFA testing), there may have been a decreased sensitivity for detecting seropositive individuals in the US study compared to the present study.

Within our study group, there was an increased risk of *C. burnetii* seropositivity among practicing veterinarians compared to veterinary students. This could be explained by more opportunity for exposure to infected animals among veterinarians. While veterinary students perform similar activities as veterinarians, particularly in upper years of study (de Rooij et al., 2012), they typically do not have as much exposure to potentially infected animals as veterinarians. Other studies have also identified significant risk factors for seropositivity that are consistent with dose-response relationships between degree of animal exposure and human seropositivity, including age, number of hours with animal contact per week, number of years
graduated as a veterinarian, and number of years lived on a farm (Whitney et al., 2009; de Rooij et al., 2012; Van den Brom et al., 2013). Young participants (aged 18-29) here had a decreased odds of seropositivity compared to those who were 30-39 and 40-49; however, all seven of the participating veterinary students, and three of the practicing veterinarians, were between 18-29 years of age. When students were excluded from the analysis, age was no longer associated with seropositivity. Age may therefore be an explanatory antecedent of position (practicing veterinarian/student) (Dohoo et al., 2003), since age can largely explain what stage of career participants were in. Due to the small sample size of our dataset, we were unable to determine whether age confounded the relationship between stage of career and seropositivity.

Researchers in Nova Scotia identified male veterinarians as being at higher risk of seropositivity than female veterinarians, but it was not evident to the authors why this association was observed (Marrie and Fraser, 1985). The authors did not stratify based on practice type, but they did note that the highest relative risk of seropositivity occurred among males exposed to sheep placentas (Marrie and Fraser, 1985). If male veterinarians in Nova Scotia in the 1980s were more likely to be exposed to sheep placenta than female veterinarians, this may explain why male veterinarians had a higher risk of seropositivity, as sheep placenta can contain high concentrations of C. burnetii (Hazlett et al., 2013). In our study, gender did not have a significant association with seropositivity. The majority of our participants (28/32) had assisted with delivery of sheep or goats, thereby having had contact with sheep or goat placenta, and both genders were represented (18 females, 10 males). In the study from Nova Scotia, 45 males and 20 females participated, but the ratio of gender among those that had contact with sheep placenta was not described.
We hypothesized that the percent of a veterinarian’s practice dedicated to sheep, goats, or to a lesser extent cattle, would have had an association with seropositivity, as these species have been demonstrated to shed up to $1 \times 10^9$ organisms in the sheep/goat placenta (Sánchez et al., 2006) and have been linked to human cases of Q fever (Maurin and Raoult, 1999); however, this study failed to demonstrate any relationship. There may have been insufficient variation within the study group since almost all veterinarians were food animal practitioners. While one-third of all SRVO members were sampled for this study, the sample size was nevertheless quite small. Given our sample size, and an expected proportion $C.\ burnetii$ exposure of 59%, the power of this study is calculated at 16.5%. Larger sample sizes may be required to further elucidate the relationship between species-specific contact and veterinarian seropositivity in Ontario. The exact logistic models served to limit the bias of the coefficients and p-values obtained from these small sample sizes (Dohoo et al., 2003). The potential animal source of $C.\ burnetii$ among seropositive veterinarians and veterinary students remains unclear. While sheep and goats have demonstrated high seroprevalences in Ontario (Chapter 2 and 3), $C.\ burnetii$ could have been transmitted to participants from cows, cats, or other animals.

This study, which utilized convenience sampling as have others (Whitney et al., 2009; Van den Brom et al., 2013), and may have been subject to selection bias. Our sampling frame of SRVO members is not an exhaustive list of all veterinarians and veterinary students who work with small ruminants in Ontario. As previously noted, SRVO is a voluntary organization and is involved in continuing education the health and welfare of small ruminants. SRVO members who attended the AGM may therefore be more engaged in learning about small ruminants and consider small ruminants as a more important part of their caseload or anticipated caseload, than non-attending SRVO members and non-members. In addition, research has identified a number
of other barriers to participation in continuing education events among veterinarians including: timing of events, distance from the venue, money, ownership of a solo practice, stage of career and family demands (Moore et al., 1998). These factors may have influenced attendance to the AGM, and thus, subsequent participation. Most participants had not been tested for *C. burnetii* exposure prior to participating in our study and most did not suspect that they have had Q fever. However, this may not be true for non-participants. If a livestock veterinarian had a serious illness due to chronic Q fever, they may be less likely to continue with clinical practice due to the physical demands involved with this occupation. Since a small proportion of seropositive humans are anticipated to develop serious clinical disease to the point they may be removed from the study population (Rousset et al., 2009; Angelakis and Raoult, 2010), survival bias may have limited influence on the estimates of seropositivity in and risk factors identified in this population.

By the nature of their occupations, small ruminant veterinarians and veterinary students may be exposed to *C. burnetii* frequently throughout their career. The animal Q fever vaccine is now licensed for use in Ontario in sheep and goats. However, since veterinarians potentially have contact with animals on many farms, use of the vaccine in sheep and goats may not infer veterinarian protection unless use was widespread. Therefore, vaccinating unexposed veterinary students and veterinarians, particularly those at high risk of developing chronic Q fever (e.g. those with pre-existing heart disease), with a human *C. burnetii* vaccine (Q Vax, Commonwealth Serum Laboratories, Melbourne, Australia), merits future consideration. The Q Vax vaccine is currently used in Australia and the Netherlands, and research has demonstrated it to establish a long-lived immune response to *C. burnetii* (Kersh et al., 2013).

### 5.5 Conclusion
Coxiella burnetii seropositivity was common among SRVO members, particularly among veterinarians, presumably due to their contact with infectious animals. Veterinarians should be alert to the signs and symptoms of Q fever, and advocate for clinical testing should these signs and symptoms appear. Hygiene and biosecurity practices, while not statistically associated with seropositivity here, are encouraged for their utility in preventing occupational exposure to not only C. burnetii, but also several other zoonotic diseases (Van den Brom et al., 2013).

5.6 Acknowledgements

The authors would like to thank: the Ontario Ministry of Agriculture, Food and Rural Affairs -University of Guelph Agreement through the Animal Health Strategic Investment fund (AHSI) managed by the Animal Health Laboratory of the University of Guelph; the Ontario Ministry of Health and Long-Term Care; the National Sciences and Engineering Research Council of Canada; and Public Health Ontario. The authors would also like to acknowledge the cooperation of SRVO and all of the small ruminant veterinarians and veterinary student members that participated in this study.
5.7 References


Table 5.1 Number of individuals (percentage of total samples) with specific serum titres for immunoglobulin G to anti Phase I and Phase II *Coxiella burnetii* antigens, among 32 small ruminant veterinarians and veterinary students in Ontario, Canada, as determined by the Immunofluorescence assay (Focus Diagnostics), February 24, 2012.

<table>
<thead>
<tr>
<th>Phase II</th>
<th>NR</th>
<th>1:16</th>
<th>1:32</th>
<th>1:64</th>
<th>1:128</th>
<th>≥1:256</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR</td>
<td>13 (40.6)</td>
<td>2 (6.3)</td>
<td>1 (3.1)</td>
<td>1 (3.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>17 (53.1)</td>
</tr>
<tr>
<td>1:16</td>
<td>1 (3.1)</td>
<td>0 (0)</td>
<td>1 (3.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (6.2)</td>
</tr>
<tr>
<td>1:32</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (6.3)</td>
<td>3 (9.4)</td>
<td>2 (6.3)</td>
<td>0 (0)</td>
<td>7 (22.0)</td>
</tr>
<tr>
<td>1:64</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (6.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (6.3)</td>
</tr>
<tr>
<td>1:128</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (6.3)</td>
<td>0 (0)</td>
<td>2 (6.3)</td>
<td>0 (0)</td>
<td>4 (12.6)</td>
</tr>
<tr>
<td>≥1:256</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>14 (43.7)</td>
<td>2 (6.3)</td>
<td>6 (18.8)</td>
<td>6 (18.8)</td>
<td>4 (12.6)</td>
<td>0 (0)</td>
<td>32 (100.0)</td>
</tr>
</tbody>
</table>

NR= not reactive

- **40.6% (13/32)** Non-exposed (Phase I and Phase II IgG not reactive)
- **9.4% (3/32)** Titres suggestive of an acute exposure (Phase II titre > Phase I titre)
- **50.0% (16/32)** Titres suggestive of a chronic exposure (Phase I titre ≥ Phase II titre)
Table 5.2 Selected descriptive statistics and univariable exact logistic analysis of associations between putative risk factors and *Coxiella burnetii* seropositivity (as determined by immunofluorescence assay (Focus Diagnostics) among 32 small ruminant veterinarians and veterinary students in Ontario, Canada, February 24, 2012.

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Percent (Frequency)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Position</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Practicing veterinarian</td>
<td>65.6 (21/32)</td>
<td>Ref</td>
<td>-</td>
</tr>
<tr>
<td>Non-practicing veterinarian</td>
<td>12.5 (4/32)</td>
<td>0.33 (0.019-5.64)</td>
<td>0.61</td>
</tr>
<tr>
<td>Veterinary student</td>
<td>21.9 (7/32)</td>
<td>0.06 (0.0011-0.65)</td>
<td>0.014</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-29</td>
<td>31.3 (10/32)</td>
<td>Ref</td>
<td>-</td>
</tr>
<tr>
<td>30-39</td>
<td>18.8 (6/32)</td>
<td>15.46 (1.0088-1059.64)</td>
<td>0.049</td>
</tr>
<tr>
<td>40-49</td>
<td>12.5 (4/32)</td>
<td>12.96 (1.26-+Inf)</td>
<td>0.030</td>
</tr>
<tr>
<td>50-59</td>
<td>31.3 (10/32)</td>
<td>8.15 (0.88-127.0090)</td>
<td>0.070</td>
</tr>
<tr>
<td>60+</td>
<td>6.3 (2/32)</td>
<td>3.46 (0.034-352.33)</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>40.6 (13/32)</td>
<td>Ref</td>
<td>-</td>
</tr>
<tr>
<td>Male</td>
<td>59.4 (19/32)</td>
<td>1.16 (0.22-6.37)</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>78.1 (25/32)</td>
<td>Ref</td>
<td>-</td>
</tr>
<tr>
<td>Quit</td>
<td>21.9 (7/32)</td>
<td>0.89 (0.12-7.44)</td>
<td>1.00</td>
</tr>
<tr>
<td>Current smokers</td>
<td>0 (0/32)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Frequency of consuming raw milk products in the past 2 years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>78.1 (25/32)</td>
<td>Ref</td>
<td>-</td>
</tr>
<tr>
<td>Less than 12 times per year</td>
<td>12.5 (4/32)</td>
<td>0.20 (0.0034-2.90)</td>
<td>0.36</td>
</tr>
<tr>
<td>More than 12 times per year</td>
<td>9.4 (3/32)</td>
<td>1.12 (0.051-73.65)</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Any contact with any of following species:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy Cattle</td>
<td>53.1 (15/32)</td>
<td>0.57 (0.10-2.91)</td>
<td>0.67</td>
</tr>
<tr>
<td>Beef cattle (cow-calf)</td>
<td>50.0 (16/32)</td>
<td>0.78 (0.15-3.93)</td>
<td>1.00</td>
</tr>
<tr>
<td>Beef cattle (feedlot)</td>
<td>34.4 (11/32)</td>
<td>0.75 (0.13-4.22)</td>
<td>0.98</td>
</tr>
<tr>
<td>Swine</td>
<td>34.4 (11/32)</td>
<td>0.43 (0.073-2.36)</td>
<td>0.43</td>
</tr>
<tr>
<td>Poultry</td>
<td>34.4 (11/32)</td>
<td>1.30 (0.24-8.05)</td>
<td>1.00</td>
</tr>
<tr>
<td>Sheep</td>
<td>56.3 (18/32)</td>
<td>0.42 (0.067-2.16)</td>
<td>0.39</td>
</tr>
<tr>
<td>Goat</td>
<td>43.8 (14/32)</td>
<td>1.42 (0.28-7.80)</td>
<td>0.89</td>
</tr>
<tr>
<td>Camelid</td>
<td>25.0 (8/32)</td>
<td>1.18 (0.18-9.43)</td>
<td>1.00</td>
</tr>
<tr>
<td>Farmed deer</td>
<td>6.3 (2/32)</td>
<td>0.68 (0.0081-56.65)</td>
<td>1.00</td>
</tr>
<tr>
<td>Horses</td>
<td>62.5 (20/32)</td>
<td>1.07 (0.19-5.71)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

11 Significant variables at p<0.05 shown in bold
<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Percent (Frequency)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
<td>78.1 (25/32)</td>
<td>0.52 (0.042-3.97)</td>
<td>0.78</td>
</tr>
<tr>
<td>Cats</td>
<td>84.4 (27/32)</td>
<td>0.97 (0.070-10.019)</td>
<td>1.00</td>
</tr>
<tr>
<td>“Pocket pets” (e.g. mice)</td>
<td>21.9 (7/32)</td>
<td>0.20 (0.015-1.54)</td>
<td>0.15</td>
</tr>
<tr>
<td>Pet birds</td>
<td>12.5 (4/32)</td>
<td>0.66 (0.042-10.30)</td>
<td>1.00</td>
</tr>
<tr>
<td>Wildlife</td>
<td>12.5 (4/32)</td>
<td>0.66 (0.042-10.30)</td>
<td>1.00</td>
</tr>
<tr>
<td>Currently live on sheep/goat farm</td>
<td>9.4 (3/32)</td>
<td>1.40 (0.066-90.13)</td>
<td>1.00</td>
</tr>
<tr>
<td>Pregnant in the previous 2 years</td>
<td>28.6 (4/14)</td>
<td>5.89 (0.58-+Inf)</td>
<td>0.14</td>
</tr>
<tr>
<td>Wear gloves while assisting normal births (exact p=0.19)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>17.9 (5/28)</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Infrequently</td>
<td>14.3 (4/28)</td>
<td>0.54 (0.0064-16.50)</td>
<td>1.00</td>
</tr>
<tr>
<td>Frequently</td>
<td>7.1 (2/28)</td>
<td>2.22 (0.14-+Inf)</td>
<td>0.57</td>
</tr>
<tr>
<td>Always</td>
<td>60.7 (17/28)</td>
<td>3.37 (0.29-52.59)</td>
<td>0.47</td>
</tr>
<tr>
<td>Wash hands with disinfectant soap after assisting normal births (exact p=0.47)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>0.0 (0/27)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Infrequently</td>
<td>7.4 (2/27)</td>
<td>Ref</td>
<td>-</td>
</tr>
<tr>
<td>Frequently</td>
<td>11.1 (3/27)</td>
<td>1.5 (0.038-+Inf)</td>
<td>0.80</td>
</tr>
<tr>
<td>Always</td>
<td>81.5 (22/27)</td>
<td>1.42 (0.017-121.98)</td>
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</tr>
<tr>
<td>Changing coveralls after assisting normal births (exact p=0.66)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>11.1 (3/27)</td>
<td>Ref</td>
<td>-</td>
</tr>
<tr>
<td>Infrequently</td>
<td>18.5 (5/27)</td>
<td>2.60 (0.079-235.00)</td>
<td>1.00</td>
</tr>
<tr>
<td>Frequently</td>
<td>33.3 (9/27)</td>
<td>5.73 (0.20-470.32)</td>
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</tr>
<tr>
<td>Always</td>
<td>37.0 (10/27)</td>
<td>2.8 (0.11-205.94)</td>
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</tr>
<tr>
<td>Changing footwear or disinfecting boots after assisting with normal births (exact p=0.12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>11.1 (3/27)</td>
<td>Ref</td>
<td>-</td>
</tr>
<tr>
<td>Infrequently</td>
<td>11.1 (3/27)</td>
<td>0.30 (0-4.92)</td>
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</tr>
<tr>
<td>Frequently</td>
<td>7.4 (2/27)</td>
<td>0.58 (0.0043-78.17)</td>
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</tr>
<tr>
<td>Always</td>
<td>70.4 (19/27)</td>
<td>1.38 (0.020-32.39)</td>
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<tr>
<td>Taking off coveralls before entering vehicle after assisting births (exact p=0.84)</td>
<td></td>
<td></td>
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<tr>
<td>Never</td>
<td>34.6 (9/26)</td>
<td>Ref</td>
<td>-</td>
</tr>
<tr>
<td>Infrequently</td>
<td>26.9 (7/26)</td>
<td>1.23 (0.095-20.53)</td>
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</tr>
<tr>
<td>Frequently</td>
<td>23.1 (6/26)</td>
<td>0.52 (0.038-6.57)</td>
<td>0.91</td>
</tr>
<tr>
<td>Always</td>
<td>15.4 (4/26)</td>
<td>1.46 (0.070-104.64)</td>
<td>1.00</td>
</tr>
<tr>
<td>Risk Factors</td>
<td>Percent (Frequency)</td>
<td>OR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>------------------------------------------------------------------------------</td>
<td>---------------------</td>
<td>----------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Frequency of alcohol consumption in previous 2 years (exact p=0.26)</td>
<td></td>
<td>0.38 (0-3.791)</td>
<td>0.265</td>
</tr>
<tr>
<td>None</td>
<td>9.7 (3/31)</td>
<td>Ref</td>
<td>-</td>
</tr>
<tr>
<td>Less than once/week and not more than 1-2 drinks at a time</td>
<td>38.7 (12/31)</td>
<td>0.32 (0-3.51)</td>
<td>0.37</td>
</tr>
<tr>
<td>More than once/week and not more than 1-2 drinks at a time</td>
<td>45.2 (14/31)</td>
<td>0.55 (0-6.04)</td>
<td>0.65</td>
</tr>
<tr>
<td>More than once/week and more than 1-2 drinks at a time</td>
<td>6.5 (2/31)</td>
<td>0.67 (0-26.00)</td>
<td>0.80</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Mean (95%CI)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of practice devoted to:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy Cattle</td>
<td>30.31 (28.06-32.71)</td>
<td>1.031 (0.99-1.10)</td>
<td>0.24</td>
</tr>
<tr>
<td>Beef cattle (cow-calf)</td>
<td>10.68 (9.36-12.14)</td>
<td>0.94 (0.83-1.063)</td>
<td>0.34</td>
</tr>
<tr>
<td>Beef cattle (feedlot)</td>
<td>2.91 (2.24-3.71)</td>
<td>1.07 (0.82-1.57)</td>
<td>0.83</td>
</tr>
<tr>
<td>Swine</td>
<td>4.68 (3.82-5.68)</td>
<td>1.18 (0.94-49.056)</td>
<td>0.75</td>
</tr>
<tr>
<td>Poultry</td>
<td>0.95 (0.59-1.46)</td>
<td>0.97 (0.80-+Inf)</td>
<td>1.00</td>
</tr>
<tr>
<td>Sheep</td>
<td>8.45 (7.28-9.76)</td>
<td>0.91 (0.77-1.0068)</td>
<td>0.07</td>
</tr>
<tr>
<td>Goat</td>
<td>5.09 (4.19-6.13)</td>
<td>1.24 (0.90-2.00)</td>
<td>0.26</td>
</tr>
<tr>
<td>Camelid</td>
<td>1.045 (0.66-1.57)</td>
<td>1.033 (0.43-2.78)</td>
<td>1.00</td>
</tr>
<tr>
<td>Farmed deer</td>
<td>0.14 (0.028-0.40)</td>
<td>2.65 (0.097-+Inf)</td>
<td>0.65</td>
</tr>
<tr>
<td>Horses</td>
<td>21.00 (19.13-23.0051)</td>
<td>0.98 (0.94-1.029)</td>
<td>0.47</td>
</tr>
<tr>
<td>Dogs</td>
<td>8.82 (7.62-10.15)</td>
<td>1.0063 (0.93-1.11)</td>
<td>0.95</td>
</tr>
<tr>
<td>Cats</td>
<td>6.23 (5.23-7.36)</td>
<td>0.99 (0.90-1.11)</td>
<td>0.83</td>
</tr>
<tr>
<td>Pocket pets</td>
<td>0.18 (0.050-0.47)</td>
<td>0.91 (0.11-26.62)</td>
<td>1.00</td>
</tr>
<tr>
<td>Pet birds</td>
<td>0.045 (0.0011-0.25)</td>
<td>0.29 (0.00-11.47)</td>
<td>0.45</td>
</tr>
<tr>
<td>Total years working with current practice</td>
<td>12.98 (8.54-17.42)</td>
<td>1.00 (0.91-1.10)</td>
<td>1.00</td>
</tr>
<tr>
<td>Total years working in veterinary practice</td>
<td>18.56 (13.62-23.51)</td>
<td>1.00 (0.92-1.07)</td>
<td>0.91</td>
</tr>
<tr>
<td>Hours per week in contact with sheep/goats</td>
<td>5.0 (0.96-9.094)</td>
<td>0.89 (0.68-1.020)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

\(^a\) Median Unbiased estimate (MUE)

\(^b\) Infinity

\(^c\) Exact P-value based on conditional scores for categorical variables
CHAPTER SIX

Discussion

Coxiellosis in animals and Q fever in humans, both caused by *Coxiella burnetii*, can have significant impacts on livestock health and public health (Roest et al., 2011). The largest Q fever epidemic on record occurred between 2007-2009 in the Netherlands and was later attributed to transmission from infective dairy goats, and to a lesser extent, dairy sheep (Roest et al., 2011). The consequences of this outbreak were severe, both in terms of animal health and welfare, human health, and economics. The cost of outbreak control measures was estimated to be €50 million (approximately $75 million CAD) (Bruschke and Emeka, 2011). The total cost of the outbreak, including societal (lost quality of life, productivity, etc.), human health and veterinary costs was estimated to be €250-600 million (approximately $378-900 million CAD) (Morroy et al., 2012). This epidemic revealed important gaps in knowledge concerning the impact of *C. burnetii* infection in different populations at risk, risk factors for infection, and the effectiveness of various prevention and control strategies, that are applicable globally (De Valk, 2012).

Human infection has most frequently been attributed to either direct or indirect contact with infected ruminants, primarily sheep, goats and cattle (Lyytikäinen et al., 1998; Tissot-Dupont et al., 1999; Astobiza et al., 2011; Thompson et al., 2012). Hence, people who spend significant amounts of time around sheep or goats, such as farm workers and veterinarians, have been reported to be at higher risk for *C. burnetii* exposure than the general population (Marrie and Fraser, 1985; Lang, 1989; McQuiston et al., 2006; Whitney et al., 2009). In recent decades, few studies have been conducted in Canada, and particularly, Ontario, to understand the epidemiology of Q fever and coxiellosis, including estimation of prevalence and the
investigation of risk factors. Therefore, the intent of this thesis was to estimate the prevalence of exposure to *C. burnetii* in sheep, goats, and small ruminant farm workers, veterinarians and veterinary students in Ontario, as well as to identify risk factors associated with *C. burnetii* exposure in these populations.

### 6.1 Study strengths

Effective management of zoonoses necessitates multi-sectoral approaches (Aenishaenslin et al., 2013), which relate animal and public health. Therefore, a One Health framework was utilized for this research to assess connections between animal and human health. A number of studies have examined *C. burnetii* in sheep, goat and human populations in isolation (McCaughey et al., 2008; Islam et al., 2011; Schimmer et al., 2012; De Lange et al., 2013). One of the major strengths of this research was the ability to examine the association between sheep/goat seropositivity and farm worker seropositivity on the same farm. Few studies have examined this relationship (Dolcé et al., 2003; Nahed and Khaled, 2012); use of a harmonized sampling scheme for animals and humans in the *C. burnetii* literature is relatively novel.

This is the first comprehensive examination of *C. burnetii* seropositivity among farm workers and veterinarians in Ontario, and the first examination of putative risk factors associated with seropositivity in these populations. As such, the results of this research provide much needed estimates of baseline exposure in these high risk groups.

Similarly, seroprevalence estimates for sheep and goats in Ontario, or other parts of Canada have not been investigated in recent decades. Current estimates are important as the seroprevalence in these populations can change through time. The seroprevalence estimates for sheep and goats here were based on large sample sizes, particularly relative to other estimates in the literature (Hatchette et al., 2002; Nahed and Khaled, 2012; De Lange et al., 2013), and with
the exception of dairy sheep, were solicited using a randomized process. The large sample sizes provided more precise estimates of the true population seroprevalence, while the randomized process for solicitation and animal selection aided in maintaining external and internal validity, respectively.

6.2 Major contributions of research

6.2.1 Seroprevalence of sheep, goats, farm workers and veterinarians

Animal seropositivity was common; the individual seroprevalence was 32.5% (714/2195) in goats and 14.7% (347/2363) in sheep. At the farm-level, 63.2% (48/76) of goat farms and 48.6% (34/72) of sheep farms had at least one seropositive animal. Given the high seroprevalence identified among sheep and goats in Ontario, it was not surprising that seropositivity among people working with these animals was also common. Overall, the individual seroprevalence among sheep and goat farm workers was 64.5% (111/172), and 76.3% (58/76) of farms had at least one seropositive person. Similarly, 59.4% (19/32) of small ruminant veterinarians and veterinary students attending the Small Ruminant Veterinarians of Ontario annual general meeting in 2012 were seropositive for *C. burnetii*.

6.2.2 Factors associated with seropositivity in sheep and goats

Some covariates were associated with seropositivity in both the sheep and goat mixed logistic regression models. This consistency between models strengthens our confidence in making inferences about the risk factors for *C. burnetii* exposure. In both sheep and goat models, female herd size had a strong positive association with seropositivity. Others have also identified this relationship and have hypothesized that this effect is caused by increased opportunities for transmission between individual animals within a herd (Hogerwerf et al., 2013), especially from breeding females that are the primary shedders of *C. burnetii* (Rodolakis, 2009). Another
possibility is the increased chance of introduction of *C. burnetii* onto larger farms through purchased animals (Schimmer et al., 2011).

Lambing/kidding pen hygiene practices were also significantly associated with seropositivity in both sheep and goat models. In addition to adding bedding and removing birthing material, if lambing/kidding pens were also disinfected, then sheep and goats were less likely to be seropositive, compared to sheep and goats from farms where disinfection did not occur. This indicates that having good lambing/kidding pen hygiene, including disinfection, removing birthing materials and adding bedding, may be an effective tool for reducing bacterial burden in the birthing environment.

The practice of kidding or lambing in an airspace separate from the rest of the herd/flock increased the odds of seropositivity in the sheep and goat models, which contradicts what was hypothesized *a priori*. We hypothesized that giving birth in a separate airspace would decrease the odds of ewes/does being seropositive, as it could decrease the opportunity for transmission between the lambing/kidding animals and the rest of the herd during this high risk period (Reichel et al., 2012). Research from the Netherlands found the use of windbreak curtains and windshields in barns also increased the risk of individual goat seropositivity (Schimmer et al., 2011). The authors reasoned that the restricted air-flow decreased ventilation and facilitated the accumulation of *C. burnetii* in that environment (Schimmer et al., 2011). With the restriction of lambing/kidding to an airspace separate from the rest of the herd, there may be implications to air-flow into the segregated lambing/kidding area, depending on the facilities used. If the airspace used for segregated lambing/kidding group is poorly ventilated compared to those lambing/kidding in the same airspace as the rest of the flock/herd, this could promote transmission of *C. burnetii* within the segregated group, and increase the odds of seropositivity.
among breeding ewes or does on farm. However, empirical evidence testing this hypothesis is required before recommendations based on this idea are made. It is also possible that farm workers decided to have lambing/kidding in a separate airspace after abortions/stillbirths occurred on their farm. The possibility of this “reverse causation” was assessed, but since few farms lambing in separate airspaces reported *C. burnetii* abortions or elevated abortion risk, there was not good evidence that reverse causation existed.

Ewes had higher odds of being seropositive if they were from farms that loaned sheep to other farms. We postulated that loaning seronegative sheep to other farms would increase the flock-level seroprevalence if the sheep were exposed to *C. burnetii* prior to return to the home farm. If the re-introduced seropositive sheep then shed *C. burnetii*, they could expose other sheep on the home farm, thus further increasing the within-flock seroprevalence. The risk of introducing the agent to previously negative flocks/herds is the basis of the restrictions on trade and animal movement in place during the Q fever epidemic in the Netherlands (EFSA, 2010). This finding supports the recommendation that animal movement should be voluntarily restricted (“closed” flock policy), where possible, to mitigate the risk of *C. burnetii* introduction.

In the goat model, the presence of other sheep or goat farms within a 5km radius had a positive association with doe seropositivity. An effective range of airborne *C. burnetii* spread of up to 5 km has been reported (Schimmer et al., 2010), as has effective airborne transmission of *C. burnetii* within geographical clusters (Reusken et al., 2011). Therefore, airborne transmission between farms within 5km may be responsible for this association.

The goat model also demonstrated a strong protective effect of outdoor kidding on goat seropositivity, but only when swine were not present on farm. The influence of birthing outdoors (compared to indoors) on seropositivity has not been evaluated thoroughly in goats or other
species. There is however, more general data comparing the seroprevalence in cattle kept indoors and outdoors in Italy. Non-housed cattle were found to have a significantly lower *C. burnetii* seroprevalence than housed cattle (Capuano et al., 2001). The protective effect of giving birth outdoors may be partially explained due to lower stocking densities and/or less environmental accumulation of the pathogen outdoors due to air-flow and dispersion of *C. burnetii* (Schimmer et al., 2011). It is premature, however, to recommend outdoor kidding on the basis of this finding. While kidding outdoors may have a protective effect for goat seropositivity, human epidemics of Q fever have been attributed to outdoor animal births in goats, sheep and cattle, presumably due to the increased opportunity for aerosol transmission after outdoor birthing (Hawker et al., 1998; Lyytikäinen et al., 1998; Tissot-Dupont et al., 2004; Georgiev et al., 2013).

Swine have not been recognized as a source of *C. burnetii* infection in other animals (Marrie, 1990), although this has not been thoroughly assessed in the literature. Swine seropositivity data are scarce; however, research from Uruguay indicated a 21.2 % (83/391) seroprevalence among a survey of swine going to slaughter in 1984 and 0% (0/88) in 1985 using the microagglutination test (Somma-Moreira et al., 2013). However, the sampling methodology used in those studies, was not described and sample sizes were small (391 and 88), particularly in 1985 (88). Occupational contact with swine was associated with veterinarian seropositivity in the Netherlands and United States (Whitney et al., 2009; Van den Brom et al., 2013). Researchers suggested that swine were potential reservoirs of *C. burnetii* infection in the United States (Whitney et al., 2009). Alternatively, authors in the Netherlands hypothesized that the apparent association with swine contact was confounded by the location of swine farms, as swine farms were located in areas with a high density dairy goat farms in the Netherlands (Van den Brom et al., 2013). The *C. burnetii* seroprevalence in Ontario swine has not been investigated and more
research is needed to clarify the role of pigs, if any, in the direct transmission of \( C. \ burnetii \) to goats and other animals. Furthermore, investigation is also required to identify how the presence of pigs on farm influences goat management on the same farm, such as disposal of placentas or spreading manure, that are important in contributing to exposure/seropositivity in goats.

6.2.3 \textit{Factors associated with seropositivity in humans working with sheep and goats}

There was a strong positive association between the percentage of seropositive animals on farms and farm worker seropositivity. This likely reflects the highly transmissible nature of \( C. \ burnetii \), through either direct contact with shedding animals (Fournier et al., 1998), contaminated materials such as bedding or manure, or through sharing a \( C. \ burnetii \) contaminated environment for a proportion of the day (Rodolakis, 2009). However, seropositivity is not a perfect predictor of shedding at the animal-level, as not all seropositive animals shed \( C. \ burnetii \), and seronegative animals can shed \( C. \ burnetii \) (Rousset et al., 2009). Since \( C. \ burnetii \) shedding was not directly measured for the present research, we do not know if any sampled animals were shedding at the time of sampling. However, one of the criteria that is suggestive of an active \( C. \ burnetii \) infection (i.e. shedding) at the herd-level is a within-herd seroprevalence beyond 50% (Sidi-Boumedine et al., 2010). Of the 83 sheep and goat farms that had at least one seropositive animal, the average within-herd seroprevalence was 39.0\% (± 27.0 SD), with 39.8\% (33/83) of these farms above the threshold of 50\% seroprevalence. Using this cut-point, 22.3\% (33/148) of farms had a within-farm seroprevalence greater than 50\% at the time of sampling. \textit{Coxiella. burnetii} shedding was not measured in this study, therefore we can only determine the proportion of farms that met this one of the criteria (>50\% seroprevalence) that is suggestive of an active \( C. \ burnetii \) infection at the herd-level.
Due to the relationship between animal and human exposure, measures to prevent or control the infection in animals, such as keeping a ‘closed flock/herd,’ disinfecting birthing pens in addition to adding bedding and removing birthing materials, and use of animal vaccination against *C. burnetii*, could reduce the likelihood of infection among susceptible people. Additionally, use of the human *C. burnetii* vaccine (Q-Vax) among those that are unexposed (negative serology and skin test) can also reduce the likelihood of infection (Kersh et al., 2013). Vaccination may be particularly beneficial early in an individual’s veterinary or farming career, when they may be more likely to be unexposed.

Working on dairy goat farms increased the odds of farm workers being seropositive compared to working on meat goat and dairy sheep farms, even after accounting for the percentage of test-positive animals per farm. This suggests that other characteristics of dairy goat farms may influence the risk of human exposure. The degree of human-animal contact has been associated with human seropositivity in other studies (Van den Brom et al., 2013). It stands to reason that there may be more intensive animal contact on dairy farms compared to meat farms, particularly due to milking where farm workers regularly handle animals and are close to the animal’s escutcheon where *C. burnetii* may be shed via feces, or urine (Porter et al., 2011). Data from a small ruminant abortion study found that aborted sheep placentas contained an average of $4.82 \times 10^9$ *C. burnetii* DNA copies and aborted goat placentas contained $3.00 \times 10^{10}$ copies (Hazlett et al., 2013). Therefore, if infected goats shed more *C. burnetii* on average than infected sheep, this may explain the increased risk of exposure among people working with goats. It is also possible that dairy goats in our study were more likely to be undergoing active infection and shedding of *C. burnetii* at the time of sampling, compared to meat goats or meat sheep. Of all farms that participated in human sampling, 9.5% (2/21) of meat sheep farms, 5.3% (1/19) of
meat goat farms, 20.0% (1/5) of dairy sheep farms, and 61.3% (19/31), of individual dairy goat farms had an individual animal seroprevalence >50%. This suggests that dairy goat farms may have had more active \textit{C. burnetii} infections at the herd-level, contributing to the higher observed risk of exposure of workers on these farms compared to other industry groups. However, since information on \textit{C. burnetii} shedding was not measured, this hypothesis remains unconfirmed.

Smoking has been identified as a risk factor for seropositivity in other studies (Hatchette et al., 2001; Karagiannis et al., 2009); however, the covariate indicating a history of smoking tobacco (including current smokers and those who had quit) was only marginally associated with farm worker seropositivity here. This marginal association may be sufficient for the justification of promoting smoking prevention programs among those working with sheep or goats. This may be particularly important among sheep and goat workers with pre-existing health conditions who are more susceptible to the development of chronic Q fever (Kampschreur et al., 2012).

Finally, practicing veterinarians had an increased risk of \textit{C. burnetii} seropositivity compared to veterinary students. This is likely reflective of an increased risk of human \textit{C. burnetii} exposure when an individual spends more time with potentially infected animals. While exposure to potentially infectious animals for those in the veterinary profession is a challenge to mitigate, use of the human vaccine (Q Vax, Commonwealth Serum Laboratories, Melbourne, Australia), could be a potential method to decrease the risk of \textit{C. burnetii} infection among veterinarians and veterinary students in Ontario.

\textbf{6.3 Study Limitations}

Many of the covariates investigated, including lifestyle and animal management practices, are non-permanent and may change over time. A cross-sectional study design limits the ability to establish a temporal sequence for such factors (i.e. ability to know that the
covariates of interest preceded seroconversion). For example, farm workers or veterinarians may have made a change after a *C. burnetii*-related problem occurred. In this research, prior awareness of Q fever or coxiellosis status was gauged by collecting information on prior diagnostic testing, potential disease outcomes of coxiellosis such as abortion history, and signs/symptoms and potential health outcomes of Q fever. Overall however, clinical testing, prior diagnoses and associated outcomes of coxiellosis and Q fever were rare among animal and human participants. Therefore, we do not suspect reverse causation to have had a major influence on the significant covariates identified among the statistical models.

There may be a risk of survival bias among sheep and goats sampled, as animals that experienced abortion or stillbirth may have been at higher risk of being culled or removed from the breeding population. Similarly, if a farm worker or veterinarian had a serious illness due to chronic Q fever, they may be less likely to continue with farming and clinical practice due to the physical demands involved with these occupations. However, the relationship between clinical disease and seropositivity should also be taken into consideration in the assessment of survival bias. In this scenario, the seropositive animals or people without serious clinical disease (abortions/stillbirth in animals and chronic Q fever in humans) would remain in the population. Since a small proportion of seropositive humans are anticipated to develop serious clinical disease to the point they may be removed from the study population (Rousset et al., 2009; Angelakis and Raoult, 2010), survival bias is likely limited. The animal questionnaire results indicated 5.6 % (4/72) of sheep farms and 14.5% (11/76) of goat farms reported an elevated abortion risk (>5%) over the past three years, indicating selection bias may have also been limited in animal study populations.
As with most observational studies, there also exists the potential for non-response bias, as those who chose to participate may have been systematically different from those who chose not to participate. Few cases of coxiellosis and Q fever are reported annually in Ontario; therefore, few producers, if any, on our study farms were aware of their disease status when invited to participate. All solicited producers were asked to complete a form regarding their farm’s industry sector and herd/flock size, regardless of their intent to participate, to allow for assessment of non-response bias. This information was often poorly filled out by non-participants. Missing information for industry sector and herd/flock size made comparisons difficult between responders and non-responders. Dairy sheep farms were solicited through conversation with researchers. Contacts were made via other dairy sheep producers, milk truck drivers, and contacting those in the OMAFRA database, as a complete list of dairy sheep producers in Ontario does not exist. Therefore, participating dairy sheep farms was a non-random convenience sample of this industry. Therefore, the response rate may be artificially higher in this population due to this recruitment practice, as farm workers may have been more likely to participate after having a conversation with researchers, compared to reading a letter from researchers. As well, the timing of our study was at a stage of heightened awareness of Q fever and coxiellosis due to the large human epidemic in the Netherlands. Therefore, news of the culling of pregnant animals taking place on positive farms, and the restrictions in place to prevent further human disease in the Netherlands (Dijkstra et al., 2012), may have decreased participation rates in our study, if producers were concerned about the ramifications of testing positive in Ontario.

The potential for non-response bias may have also influenced human sampling among farm workers. Factors such as distance from the farm to the closest phlebotomy laboratory, and
whether the mobile phlebotomist was able to visit the farm, may have influenced their participation. Two different phlebotomy companies with laboratories across Ontario were used to minimize the distance participants would need to travel to have samples collected. Unfortunately, some rural areas were under-serviced, which made sample collection less convenient. As well, the mobile phlebotomist was only able to collect from farms near central Ontario. Therefore, non-response may have been higher among farm workers who did not drive, particularly if they lived outside of the area which could be visited by the mobile phlebotomist, and/or among the Mennonite/Amish participants. Where the budget allows, it would be ideal in the future for blood collection to occur on farm to reduce potential for non-response bias.

The lack of a comprehensive list of dairy sheep producers and subsequent non-random sampling of dairy sheep farms may have also hindered the external validity of our study findings. Convenience recruitment may mean that participating producers were more similar with respect to milk processors, geography and social circles than non-participating producers. To the best of our knowledge, a high proportion of the total number of estimated dairy sheep farms participated in our study (22/40), but this high response does not guarantee the lack of response bias (Bjertnaes et al., 2008).

We attempted to collect individual animal characteristics such as birth date and date of last lambing/kidding, as they were hypothesized to be associated with seropositivity. However, it became apparent that many producers did not keep detailed records of birthing dates and ages of individuals, and some did not have adequate animal identification. Additionally, some had purchased adult animals with no history. As such, we were unable to assess individual animal risk factors due to missing values and likely inaccurate data. It is therefore recommended that the importance of record keeping be emphasized at sheep and goat industry meetings.
In this research, seropositivity was used to indicate past exposure. However, it has been noted in other studies that some infected animals do not demonstrate seroconversion (Rousset et al., 2009). Therefore, a proportion of exposed individuals may be misclassified as non-exposed due to their lack of *C. burnetii* specific antibodies. While slow decay rates of *C. burnetii* titres have been demonstrated in humans, there is also considerable heterogeneity with respect to how long these specific antibodies persist in the human blood, particularly between acute and chronic cases (Teunis et al., 2013). The titre decay rates are the slowest for IgG phase I (Teunis et al., 2013); therefore, the duration of seropositivity is longer with chronic cases compared to acute cases. As a result, there may be an increased chance of identifying seropositive chronic cases compared to seropositive acute cases in seroprevalence studies of humans. If a factor, such as smoking, increased the likelihood of leading to chronic Q fever compared to acute Q fever, this factor would be more likely to be associated with seropositivity in this study, due to the slow decay rate of Phase I IgG relative to Phase II IgG. Research has demonstrated smoking suppresses the innate and adaptive immune systems, making smokers more susceptible to chronic infections (Sopori, 2002; Herr et al., 2009). This research was not specific to *C. burnetii* infection, therefore more work is needed to confirm whether smoking increases the likelihood of chronic Q fever compared to acute Q fever.

### 6.4 Suggestions for Future Research

#### 6.4.1 Experimental evidence evaluating efficacy of birthing pen disinfectants

Since disinfecting the lambing/kidding area was protective against *C. burnetii* seropositivity in both sheep and goat models, further research is needed to identify which specific disinfectant(s) are effective against *C. burnetii* and are appropriate for use in lambing/kidding pens. Anecdotally, some producers that disinfected the lambing/kidding pens...
mentioned that they used Agricultural Lime (calcium carbonate). Dry powder disinfectants are of particular interest, as many sheep and goat producers used bedding packs where the use of liquid disinfectants is not practical. Firstly, dry powder disinfectants need to be shown to be efficacious in disinfecting *C. burnetii* in laboratory conditions, and then be tested in field conditions (i.e. lambing/kidding pens). If successful, then a randomized control trial can be conducted, with sheep/goats randomly allocated to disinfected or control lambing/kidding pens. *Coxiella burnetii* contamination in lambing pens can then be quantified to examine if disinfectants had a significant influence on bacterial load.

6.4.2 *Investigate efficacy of prevention and control programs*

Our risk factor research was intended to be a starting point to better understand factors that influence exposure to *C. burnetii* in high-risk animal and human populations in Ontario. Our study showed evidence that biosecurity and hygiene practices, particularly around lambing or kidding, were important factors influencing seropositivity. Ideally, future research will build upon this evidence and prevention and control programs be established to reduce the risk of *C. burnetii* exposure, and subsequently, infection. Additionally, examination of risk factors for seroconversion, infection, shedding, and clinical disease is also important for prevention and control strategies. The effectiveness of implementing prevention and control measures against infection and environmental contamination with *C. burnetii* has not been formally examined (EFSA, 2010) and needs to be scrutinized.

Cohort studies could help understand how biosecurity and hygiene practices influence the odds of seroconversion. As well, understanding factors that influence the odds of clinical disease is important. This may be investigated with a case control study, using animals having abortions or stillbirths due to coxiellosis as cases, and seropositive animals without clinical disease as
controls. Secondly, the population attributable fraction, approximated from such a case control study, could be used to develop a cost-benefit analysis. Costs associated with interventions, and benefits accrued in the form of decreased risk of clinical disease, in both animals and people, should be examined. This analysis may be useful in farm workers adopting heightened biosecurity and hygiene measures to prevent or control infection of *C. burnetii*, as well as other zoonotic diseases.

While it is important to investigate the effectiveness of prevention or control measures independent of one another in a controlled manner, it is also important that multiple prevention strategies or multiple control strategies be evaluated together due to the multitude of pathways in which *C. burnetii* transmission may occur. There may be synergistic effects when strategies are combined (e.g. prevention programs including rodent control and strict biosecurity protocols).

6.4.3 *Update seroprevalence estimate in general population*

Evidence indicates that people in Ontario working with sheep or goats are at a high risk of *C. burnetii* exposure. That being said, the seroprevalence among the general Ontario population was not investigated; therefore, it remains unclear how the seroprevalence among people working with sheep and goats compares to the general population. Therefore, updating the baseline *C. burnetii* seroprevalence in the general population is recommended to allow for comments on whether *C. burnetii* exposure is truly an occupational hazard for sheep and goat farm workers, veterinarians and veterinary students. Baseline seroprevalence in the general population would also be useful to inform policy with respect to the risk of *C. burnetii* exposure in general.

6.4.4 *Mathematical modelling to understand the influence of seropositivity on the occurrence of epidemics*
There are two schools of thought on how a high seroprevalence influences the probability of an epidemic occurring in a population. The first is, a high seroprevalence is suggestive that the agent, *C. burnetii* is common. Since the agent is common, seronegative individuals may have an increased likelihood of being exposed to *C. burnetii* and becoming infected, compared to if the agent was uncommon. This would serve to increase the probability of an epidemic. On the other hand, a high seroprevalence suggests that a high proportion of the population have immunity against re-infection with *C. burnetii*, which may decrease the probability of an epidemic occurring. Therefore, to elucidate the relationship between seroprevalence and the probability of epidemics occurring, compartmental SIR models can be constructed, with a stochastic process for the occurrence of infection to determine which scenario (high/low seroprevalence) favours the occurrence of an epidemic.
6.5 References:


EFSA, 2010: Scientific opinion on Q fever. EFSA J. 8, 1–114.


Schimmer, B., R. Ter Schegget, M. Wegdam, L. Zächner, A. de Bruin, P. M. Schneeberger, T. Veenstra, P. Vellema, and W. van der Hoek, 2010: The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q fever outbreak. BMC Infect. Dis. 10, 69.


APPENDIX I

Animal Management Questionnaire

1. CONTACT INFORMATION

Owner’s Name: Date:

Email: ___________________________________ Farm name:__________________________________________

Mailing Address: ________________________________________________________________

Postal Code: ____________________________

Flock / Herd Veterinarian (Optional) ________________________________________________

Clinic Name:_______________________________________________________________

Email:________________________ Fax: ________________________________

Clinic Mailing Address: __________________________________________________________

2. GENERAL HERD/FLOCK INFORMATION

This section refers to how the herd/flock was managed in the previous 12 months, i.e. ________ to today.

a. What is your farm’s primary production type? (Check one):

☐ Meat Sheep ☐ Dairy Sheep

☐ Meat Goats ☐ Dairy Goats

b. What management system did you use in this time period? (Check one):

☐ Annual lambing/kidding (females give birth once / year)

If annual, how many lambing / kidding groups? ________

☐ Accelerated lambing/ kidding (females give birth more than once/year)

If accelerated, how many lambing / kidding groups? ________

c. Size of Adult Herd/Flock Today: # of breeding age females ________

# of breeding age males ________
d. Which months are your lambing/kidding season?

3. REPLACEMENT STOCK – MALES AND FEMALES INTENDED FOR BREEDING USE

Over the last 3 years, i.e. since __________ to ______________

a. Indicate all sources of replacement stock (both males and females) to your farm:
(Check all that apply)

□ Closed flock (no sheep or goats have entered flock or herd)

OR

□ Artificial Insemination (AI) or Embryo Transfer (ET)

□ Purchased males (rams or bucks)

□ Youngstock (less than 1 year of age)

□ One or more adults (greater than 1 year of age)

Purchased females (ewes or does)

□ Youngstock < 1 year of age and were not pregnant at the time of purchase

□ One or more females ≥ 1 year of age and were not pregnant at the time of purchase

□ One or more females (any age) and were pregnant at the time of purchase

If you have not brought in any live animals, please skip to question # 4

In the last 3 years...

b. How many replacement animals have you purchased? (Check one):

□ 6 or fewer

□ 7 to 20

□ 21 to 50

□ 51 or more
c. From where have you purchased replacement animals? (Check all that apply):

- Private sale direct from producer
- Private sale from dealer or broker (middleman)
- Sales Barn
- Show Sale
- Other – please explain _________________________

i. If you only checked “Private sale direct from producer,” from how many farms have you purchased replacement animals? (Check one):

- 1
- 2 to 5
- 6 or more

d. Before you agree to purchase an animal, have you asked about any of the following health issues of the flock / herd of origin (not the animal purchased)? (Circle Yes or No)

<table>
<thead>
<tr>
<th>Health Issue</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of abortions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Status of Q Fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Status of Johne’s Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Status of Caseous lymphadenitis (CLA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Status of Caprine Arthritis-Encephalitis (CAE) (goat) or Maedi Visna (MV) (sheep)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridial (tetanus / pulpy kidney) vaccination history?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abortion (chlamydia / campylobacter) vaccination history?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other: specify _____________________________</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
e. If you answered yes to any of the above, have you ever rejected animals based on the information you received? (Circle Yes or No):

- History of abortions
- Q Fever
- Johne’s Disease
- CLA
- CAE/MVV
- Vaccination history (clostridial, e.g. pulpy kidney)
- Vaccination history (abortion)
- Other: specify____________________

4. ANIMAL MOVEMENT ON AND OFF FARM

Within the last 3 years, i.e. since _______ _____ to ______________

   a. Have any of your sheep /goats: (Check all that apply)
      
      □ Been loaned to another flock / herd and returned (e.g. 4H animals, breeding rams/bucks)
      
      □ Travelled to an agricultural show / fair / display and returned
      
   b. Have any sheep/goats been loaned into your flock / herd from another farm, and then returned (e.g. breeding ram / buck or 4H animal)? (circle one)
      
      Yes               No

   c. If yes, did any of those animals from b) give birth on your farm? (circle one)
      
      Yes               No

In the last 3 years, if you have not brought in any live animals or had animals leave and return, please skip to question # 6
5. **ANIMAL ENTRY PROTOCOLS**

Are purchased or returning animals (sheep / goats):

a. Quarantined from your existing sheep / goats? (Check one):

   - □ Always
   - □ Sometimes
   - □ Never

   i. For how long? _______ days / weeks (circle one)

b. Tested for Johne’s?

   - □ Always
   - □ Sometimes
   - □ Never

c. Tested for Q-Fever?

   - □ Always
   - □ Sometimes
   - □ Never
6. CONTACT WITH OTHER ANIMALS

Within the last 3 years, i.e. since _____ ______ to ________________

a. With regards to animal contact: (Y = Yes; N = No; DK = Don’t Know)

<table>
<thead>
<tr>
<th>Have you had these animals on your farm?</th>
<th>Have they given birth?</th>
<th>Have they experienced an abnormal birthing event (such as an abortion or stillbirth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cats:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barn / Feral cats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>House cats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogs:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guard dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herding dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pet Dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Llamas or Alpacas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep or Goats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmed deer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horses or Donkeys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fowl: e.g. chickens, ducks</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

b. Please check any of the following measures you have used to control rodents?

- Poison baits
- Traps (live or dead)
- Cats
- Other: ________________________________

c. Have raccoons, opossums or other wildlife other than birds been found in the barn?

- Yes
- No
7. COLOSTRUM & MILK FEEDING

Please answer the following questions on colostrum and milk feeding as it applies to potential replacement animals. In the last 12 months, have you used the following practices?

<table>
<thead>
<tr>
<th>Always</th>
<th>Frequently &gt;50% of the time</th>
<th>Infrequently &lt; 50% of the time</th>
<th>Never</th>
</tr>
</thead>
<tbody>
<tr>
<td>COLOSTRUM FEEDING</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed colostrum from the dam their own kid(s)/lamb(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed colostrum from 1 dam to kid(s)/lamb(s) not their own</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pool colostrum from more than 1 dam to feed to a kid(s)/lamb(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed heat treated colostrum (56 to 60 °C for 1 hr).</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Feed a commercial colostrum replacement product.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed colostrum sourced from off-farm from the same species.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed colostrum from another species (e.g. cattle).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MILK FEEDING</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do kids/lambs nurse their dam? If yes, how long?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>______(hrs/dy/wk)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed pooled milk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed milk replacer</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Feed pasteurized milk</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Feed acidified milk</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Feed milk from other species</td>
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</tbody>
</table>
8. **HOUSING**

In the last 12 months ______________ to ________________

a. When lambing / kidding indoors, were the ewes/does housed in a separate airspace from the rest of the flock/herd? (Check one)

   - Always
   - Sometimes
   - Never
   - Don’t lamb/kid indoors

b. Have ewes/does lambed/kidded outdoors (e.g. on pasture, paddock or dry lot)? (check one)

   - Always
   - Sometimes
   - Never

c. On average, when are your potential replacement lambs / kids removed from their dam? ___________ (hrs / days / weeks ) (circle one)

d. Are replacement ewe lambs / doelings exposed to adult sheep / goats or their manure?

   - Yes
   - No

e. Are replacement ewe lambs / doelings exposed to areas where adult sheep / goats have lamb/kidded in the previous 12 months?

   - Yes
   - No

9. **MANURE MANAGEMENT**

Within the last 3 years, i.e. since ____________ to ________________

a. Has manure from sheep, goats or cattle been spread on grazing pastures or hayfields?

   - Yes
   - No

i. If yes, how long before it was spread, was fresh manure last added to the storage pile? (“zero” if manure was spread right out of the barn)

   ________________ (days/weeks/months/years) (circle one)
ii. if yes, which months was manure spread on your farm in the last 12 months? (Circle all that apply)

<table>
<thead>
<tr>
<th>Month</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
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</tr>
</tbody>
</table>

b. Do you avoid spreading manure under the following conditions? (circle yes or no)

- Rainy weather: Yes  No
- Windy weather: Yes  No
- Hot temperatures: Yes  No
- Freezing temperatures: Yes  No
10. ANIMAL MANAGEMENT PROTOCOLS

a. Typically are ewes crutchted or shorn prior to lambing? (Check one):
   - [ ] Always
   - [ ] Sometimes
   - [ ] Never
   - [ ] Not applicable (goats or hair sheep)

b. Are ewes/ does that have aborted quarantined immediately after aborting?
   - [ ] Always
   - [ ] Sometimes
   - [ ] Never

b. How frequently do you do the following practices with the placenta and stillborn/aborted lambs/kids?

   *Always = “A”*;  *Frequently (>50%) = “F”*;  *Infrequently (<50%) = “INF”*;  *Never = “N”*

<table>
<thead>
<tr>
<th></th>
<th>Normal Lambing / Kidding</th>
<th>Abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temporarily stored in a closed container?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temporarily stored in an open container?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buried in the ground?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buried in the manure pile?</td>
<td></td>
<td></td>
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<tr>
<td>Composted?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placed in disposal vessel?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incinerated?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shipped off-farm for disposal?</td>
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</tr>
</tbody>
</table>
c. How frequently are the following practices done with lambing/kidding pens?

*Always = “A”; Frequently (>50%) = “F”; Infrequently (<50%) = “INF”; Never = “N”; Not Applicable = “NA”*

<table>
<thead>
<tr>
<th>Is bedding added (and no other measure taken)?</th>
<th>Normal Lambing / Kidding</th>
<th>Abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>After individual lambing / kidding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After group has finished lambing / kidding</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Are birthing materials removed and bedding added?</th>
<th>Normal Lambing / Kidding</th>
<th>Abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>After individual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After group</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Are pens cleaned out, disinfected and bedded?</th>
<th>Normal Lambing / Kidding</th>
<th>Abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>After individual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After group</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Other (Specify):</th>
<th>Normal Lambing / Kidding</th>
<th>Abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>After individual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After group</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
12. Q FEVER BACKGROUND INFORMATION

Has Q Fever (*Coxiella burnetii*) been diagnosed by a veterinarian on your farm in the last 10 years? (Including all species: e.g. cattle, sheep, goats, cats, dogs or other):

☐ Yes    ☐ No

*If yes:* How was the diagnosis made __________________________

What year? ________

What species of animal(s)? ___________________

Was the animal: (check one)

☐ Home raised    ☐ Purchased    ☐ Unknown

Have you ever had your sheep / goats tested for Q Fever?

☐ Yes    ☐ No

Have you ever had your sheep / goats vaccinated with a Q Fever vaccine (Coxevax, CEVA Sante Animal)?

☐ Yes    ☐ No

Have you ever submitted an aborted foetus to the Animal Health Laboratory/ University of Guelph for an abortion work-up?

☐ Yes    ☐ No

*If no, go to question v)*

Was *Coxiella burnetii* (agent of Q-fever) identified as the cause of abortion?

☐ Yes    ☐ No    ☐ Unknown

Was *Coxiella burnetii* (agent of Q-fever) identified in the aborted tissues but was not considered the cause of abortion?

☐ Yes    ☐ No    ☐ Unknown
What was the average abortion rate on your farm for the last 3 years [(# of ewes /does that abort in a year divided by # bred in a year) X 100%]? (Check one):

- □ < 2%
- □ 2 to 5%
- □ > 5% but < 30%
- □ 30% and higher

Have your sheep/goats had an abortion “storm” (i.e. a cluster of abortions occurring in a 3 week period within a group of pregnant females) within the last 3 years?

- □ Yes  □ No

   *If yes, please estimate the proportion of the group that aborted during that “storm” _____%*

Are there other livestock farms within 5 Kms of your farm (approximately 2 concession lines each way)?

- □ Yes  □ No  □ Unknown

   *If yes, are any of them sheep or goat farms? (Check one):*

- □ Yes  □ No  □ Unknown*
**APPENDIX II**

**Sheep Questionnaire Results**

Table 8.1 Descriptive statistics for covariate describing frequency of lambing ewes in an airspace separate from rest of flock as per animal management questionnaire responses in Ontario sheep farms (Aug 2010-Jan 2012)

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Percent (Frequency)</th>
<th>Mean seroprevalence (95% CI)</th>
<th>Average female flock size (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambing ewes housed in separate airspace from flock</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>13.8 (260/1883)</td>
<td>0.12 (0.08-0.16)</td>
<td>260 (247-300)</td>
</tr>
<tr>
<td>Sometimes</td>
<td>21.6 (406/1883)</td>
<td>0.22 (0.18-0.26)</td>
<td>111 (101-122)</td>
</tr>
<tr>
<td>Never</td>
<td>55.3 (1042/1883)</td>
<td>0.09 (0.07-0.11)</td>
<td>153 (145-161)</td>
</tr>
<tr>
<td>Not Applicable (do not lamb indoors)</td>
<td>9.3 (175/1883)</td>
<td>0.01 (0.0-0.03)</td>
<td>298 (269-327)</td>
</tr>
</tbody>
</table>
Table 8.2 Animal management questionnaire answers for sheep study including variables p<0.20 in univariable screening

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Mean (95% CI)</th>
<th>Percent (Frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female flock size</td>
<td>171.3 (164.4-178.2)</td>
<td></td>
</tr>
<tr>
<td>Number of days replacement lambs spend with dam before weaning</td>
<td>13.2 (12.3-14.1)</td>
<td></td>
</tr>
<tr>
<td>Risk Factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sector</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td>68.5 (1619/2363)</td>
<td></td>
</tr>
<tr>
<td>Dairy</td>
<td>31.5 (744/2363)</td>
<td></td>
</tr>
<tr>
<td>Lambing pen cleaning practices</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Add bedding, remove birthing materials and disinfecting pen</td>
<td>33.3 (695/2087)</td>
<td></td>
</tr>
<tr>
<td>Add bedding and remove birthing materials</td>
<td>36.9 (771/2087)</td>
<td></td>
</tr>
<tr>
<td>Add bedding only</td>
<td>18.2 (379/2087)</td>
<td></td>
</tr>
<tr>
<td>Do nothing</td>
<td>11.6 (242/2087)</td>
<td></td>
</tr>
<tr>
<td>Return of loaned sheep</td>
<td>16.3 (341/2087)</td>
<td></td>
</tr>
<tr>
<td>Closed Flock</td>
<td>10.0 (213/2122)</td>
<td></td>
</tr>
<tr>
<td>Purchasing sheep from sales barns</td>
<td>7.5 (140/1874)</td>
<td></td>
</tr>
<tr>
<td>Purchasing replacement males</td>
<td>85.0 (1804/2122)</td>
<td></td>
</tr>
<tr>
<td>Poison baits for rodent control</td>
<td>70.2 (1464/2087)</td>
<td></td>
</tr>
<tr>
<td>Barn cats on farm</td>
<td>87.5 (1825/2087)</td>
<td></td>
</tr>
<tr>
<td>Lambing ewes in separate area from flock</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Applicable (do not lamb indoors)</td>
<td>8.4 (175/2087)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>58.0 (1211/2087)</td>
<td></td>
</tr>
<tr>
<td>Sometimes</td>
<td>21.1 (441/2087)</td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>12.5 (260/2087)</td>
<td></td>
</tr>
<tr>
<td>Other sheep or goat farms within 5km</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>25.1 (497/1982)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>69.6 (1381/1982)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>5.3 (105/1982)</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX III

Goat Questionnaire Results

Table 9.1 Animal management questionnaire answers for goat study including variables p<0.20 in univariable screening

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female herd size</td>
<td>225.8 (213.5-238.1)</td>
</tr>
<tr>
<td>Male herd size</td>
<td>5.8 (5.5-6.1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Percent (Frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat industry sector</td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td>34.1 (748/2195)</td>
</tr>
<tr>
<td>Dairy</td>
<td>65.9 (1447/2195)</td>
</tr>
<tr>
<td>Summer breeding season</td>
<td>71.2 (1221/1715)</td>
</tr>
<tr>
<td>Purchasing males over 1 year of age</td>
<td>50.4 (962/1910)</td>
</tr>
<tr>
<td>Purchasing animals from sales barns</td>
<td>8.0 (150/1875)</td>
</tr>
<tr>
<td>Quarantining new animals</td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>46.0 (892/1941)</td>
</tr>
<tr>
<td>Sometimes</td>
<td>18.2 (354/1941)</td>
</tr>
<tr>
<td>Never</td>
<td>35.7 (695/1941)</td>
</tr>
<tr>
<td>Pigs on farm</td>
<td>15.7 (317/2015)</td>
</tr>
<tr>
<td>Kidding in separate area from rest of herd</td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>7.64 (151/1976)</td>
</tr>
<tr>
<td>Sometimes</td>
<td>24.1 (447/1976)</td>
</tr>
<tr>
<td>Never</td>
<td>68.2 (1348/1976)</td>
</tr>
<tr>
<td>Kidding outdoors</td>
<td>1.44 (29/2011)</td>
</tr>
<tr>
<td>Always</td>
<td>19.64 (395/2011)</td>
</tr>
<tr>
<td>Sometimes</td>
<td>78.9 (1587/2011)</td>
</tr>
<tr>
<td>Replacement animals have access to kidding area</td>
<td>61.1 (1228/2011)</td>
</tr>
<tr>
<td>after weaning</td>
<td></td>
</tr>
<tr>
<td>Disposed placenta in manure pile</td>
<td>48.3 (970/2008)</td>
</tr>
<tr>
<td>Always</td>
<td>16.4 (329/2008)</td>
</tr>
<tr>
<td>Frequently</td>
<td>13.4 (268/2008)</td>
</tr>
<tr>
<td>Never</td>
<td>21.9 (440/2008)</td>
</tr>
<tr>
<td>Risk Factors</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>------------------------------------------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Kidding pen hygiene practices</td>
<td></td>
</tr>
<tr>
<td>Do nothing</td>
<td>4.0 (81/2015)</td>
</tr>
<tr>
<td>Add bedding only</td>
<td>21.3 (429/2015)</td>
</tr>
<tr>
<td>Add bedding and remove birthing materials</td>
<td>52.4 (1056/2015)</td>
</tr>
<tr>
<td>Add bedding, remove birthing materials and disinfect</td>
<td>22.3 (449/2015)</td>
</tr>
<tr>
<td>Other sheep or goat farms within 5km from farm</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>75.6 (1433/1895)</td>
</tr>
<tr>
<td>No</td>
<td>21.9 (415/1895)</td>
</tr>
<tr>
<td>Not sure</td>
<td>2.5 (47/1895)</td>
</tr>
</tbody>
</table>
APPENDIX IV

Human Questionnaire

CONTACT INFORMATION

Name:__________________________________________________________

Date: __________________________________________________________

Farm Name: ____________________________________________________

Mailing Address: _______________________________________________

Postal Code: ________________

For purposes of this questionnaire, “barn” refers to that part of the farm where sheep or goats are housed and may include pastures, dry lots and paddocks.

1. DEMOGRAPHIC INFORMATION:

   Age (years) (check one):

   □ 14-17                          □ 50-59
   □ 18-29                          □ 60-69
   □ 30-39                          □ 70 and above
   □ 40-49

b. Gender: ____ (M/F)

c. Years working with this current sheep/goat flock/herd: ____
d. Total years working with sheep/goats on any flock / herd: ____

e. Years worked with any livestock: _________

f. On average, how many hours per day do you spend in contact with sheep / goats?
   ____ hours

g. Do you currently live on the sheep / goat farm being sampled? (Check one):

   ☐ Yes        ☐ No

2. Q FEVER HISTORY:

   a. Have you ever suspected that you have had Q-Fever? (Check one):

      ☐ Yes        ☐ No

      If yes, please answer the questions below, otherwise proceed to Section 3.
i. What signs / symptoms did you have that made you suspect Q-Fever? (check all that apply)

- [ ] Fever
- [ ] Head ache
- [ ] Fatigue
- [ ] Vomiting / Nausea
- [ ] Muscle ache
- [ ] Cough
- [ ] Rash
- [ ] Other: ________________________________

ii. When did this occur (nearest month and year)? ________________

iii. Did the symptoms occur 2 to 8 weeks after contact with? (Check one)

- [ ] Aborting ewes / does
- [ ] Lambing ewes / kidding does with no abortions
- [ ] Not within 2 to 8 weeks after either of these events

iv. Did you seek medical attention for these signs (Check one)?

- [ ] Yes
- [ ] No

*If yes to iv.,

(1) Did you request a test for Q-fever (Check one)?

- [ ] Yes
- [ ] No
If yes to (1)

(a) Did the doctor have a test for Q-fever performed (i.e. blood test) (Check one)?

☐ Yes  ☐ No

If yes to (a)

(i) Was the result of the blood test positive (Check one)?

☐ Yes  ☐ No  ☐ Don’t know

If yes to (i)

I. Were you contacted by the local health unit (Medical Health Officer or designate) (Check one)?

☐ Yes  ☐ No

If no to (a)

(i) Why did the doctor decline to perform the test? (Check all that apply)

☐ Doctor did not believe that the signs were compatible with Q-fever.

☐ Doctor was not familiar with the disease Q-fever.

☐ Doctor was familiar with the disease, but did not know how to test for Q-fever.

☐ Other: ___________________________________________
3. MEDICAL HISTORY

Have you ever been diagnosed by a physician with any of the following conditions?

☐ Asthma  ☐ Cancer

☐ Pneumonia  ☐ Heart Disease

☐ Emphysema  ☐ Low Immune Function

4. WOMEN OF CHILD BEARING AGES ONLY

a. Are you currently pregnant? (Check one):

☐ Yes  ☐ No

b. Have you been pregnant within the last 2 years i.e. __________ to __________?

(Check one):

☐ Yes  ☐ No

If yes to either a or b:

i. Have you been in the barn during lambing or kidding while pregnant?

☐ Yes  ☐ No

ii. Have you assisted with lambing or kidding while pregnant?

☐ Yes  ☐ No

iii. Have you miscarried (lost) a pregnancy after 12 weeks of gestation in the last 2 years?

☐ Yes  ☐ No
5. REGARDING CHILDREN ON THE FARM

a. Do you have care of children less than 18 years old that live or visit this farm?

☐ Yes       ☐ No

If yes, please answer the following. If no, proceed to Section 6.

i. Do children enter the barn where sheep / goats are housed?

☐ Yes       ☐ No

If yes, (1) Indicate how often the following ages of children are present in the barn during lambing / kidding:

Always (A); Sometimes (S); Never (N); or no children that age (NA)

(a) _____ Infants less than 1 year of age

(b) _____ Toddlers 1 year to 5 years of age

(c) _____ Young children 6 to 12 years of age

(d) _____ Teenagers 13 to 17 years of age
6. PROTOCOLS FOR ASSISTING BIRTHS OF LAMBS / KIDS

Within the last 2 years i.e. ________________ to ________________

a. Please indicate how frequently you take the following measures when assisting with lambing/ kidding: Always (A); Frequently (F); Infrequently (INF); Never (N); Not Applicable (NA):

<table>
<thead>
<tr>
<th>Measure</th>
<th>Normal Birth</th>
<th>Unexpected abortion</th>
<th>Presumed abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wearing protective sleeves/gloves while assisting births</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wearing a N95 (^{12}) respirator mask while assisting births</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washing hands with disinfectant soap after assisting births</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changing coveralls or barn clothes after assisting births</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changing footwear or disinfecting boots after assisting births</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taking off coveralls before entering home after assisting births</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

b. If any other precautions are taken, please describe:

________________________________________________________________________

7. PROTOCOLS FOR BARN CLOTHING WORN WHILE TAKING CARE OF LIVESTOCK

This includes any of the following: protective coveralls/overalls, pants & shirts (if no protective coveralls worn), coats, vests, hats, gloves, outer footwear (e.g. rubber boots, work shoes).

\(^{12}\) N95 Respirator mask is a specially fitted mask designed to prevent inhalation of infectious organisms. It is different from masks for renovation work, or from masks worn by surgeons.
a. How frequently do you take the following measures?

*Always (A); Frequently (>50%) (F); Infrequently (<50%) (INF); Never (N)*

i. Remove all barn clothing when leaving the barn

ii. Remove all barn clothing either in the barn or in the “mud” room\(^\text{13}\) prior to entering the house.

iii. Wear article(s) of barn clothing into the house.

(1) Indicate which articles are worn into the house:

_____________________________________________________________________

b. How frequently do you take the following measures?

*Always (A); Frequently (>50%) (F); Infrequently (<50%) (INF); Never (N); Not Applicable (NA)*

i. Wash hands with disinfectant soap immediately after handling sheep / goats.

ii. Wash hands with disinfectant soap immediately after handling placenta or stillborn / aborted lambs/kids.

iii. Wash hands with disinfectant soap before entering the house.

iv. Shower after handling sheep/goats and before entering house.

---

\(^{13}\) Mud Room = Separate hall or room usually connected to the house with direct outside access, or drive-shed / garage that is connected to the house.
8. PROTOCOLS FOR VISITORS

a. What biosecurity protocols and policies are currently used on your farm for all visitors? (Check all that apply):

☐ No visitors are allowed on-farm.

☐ Visitors are recorded in a log

☐ Visitors must first wash their hands with a disinfectant soap before entering the animal housing area.

☐ Visitors are not allowed to handle animals.

☐ Visitors must wear coveralls provided to them.

☐ Visitors must first use a foot bath to disinfect outer footwear.

☐ Visitors must wear boots provided to them.

☐ Visitors must wear provided disposable boot covers over their footwear.

☐ Other- Please describe:

________________________________________________________

9. NON-LIVESTOCK ANIMALS:

Within the last 2 years, i.e. _________________ to _________________

a. Do you have cats on the farm?

☐ Yes ☐ No

i. If yes, have any kittens been born on the farm?

☐ Yes ☐ No
(1)  If yes, have you had contact with pregnant cats or newborn kittens born on this farm?

☐ Yes    ☐ No

b.  Do you have any dogs on the farm?

☐ Yes    ☐ No

i.  If yes, have any puppies been born on this farm?

☐ Yes    ☐ No

(1)  If yes, have you had contact with pregnant dogs or newborn puppies born on this farm?

☐ Yes    ☐ No

c.  Have you had direct contact with vermin, wildlife or their feces in last 2 years?

☐ Yes    ☐ No

i.  If yes, please describe


10.  LIFESTYLE

Within the last 2 years, i.e. _________________ to _________________

a.  Have you consumed alcohol?

☐ Yes    ☐ No
i. If yes, how would you describe your consumption? (Check one)

- Less than once / week and not more than 1-2 drinks at a time.
- More than once / week and not more than 1-2 drinks at a time.
- More than once / week and more than 1-2 drinks at a time.

b. Have you smoked tobacco? (Including cigarettes, cigars or pipes)

- Yes
- No

i. If yes, how frequently do you smoke? (Check one)

- I quit. How long ago? ______________ (weeks / months / years) (circle one)
- I smoke less than once/day.
- I smoke daily.

11. RAW MILK CONSUMPTION:

Within the last 2 years, i.e. _______________ to _______________

a. Have you consumed raw milk, or raw milk products?

- Yes
- No

i. If yes, how frequently do you consume these products? (Check one)

- At least once / week
- At least once / month
- < 12 times per year
- Only once or twice in last 2 years
## APPENDIX V

### Human Questionnaire Results

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of seropositive sheep and or goats sampled</td>
<td>31.9 (27.4-36.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Percent (Frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Industry Sector</strong></td>
<td></td>
</tr>
<tr>
<td>Dairy goats</td>
<td>46.5 (80/172)</td>
</tr>
<tr>
<td>Dairy sheep</td>
<td>7.6 (13/172)</td>
</tr>
<tr>
<td>Meat goats</td>
<td>24.4 (42/172)</td>
</tr>
<tr>
<td>Meat sheep</td>
<td>19.8 (34/172)</td>
</tr>
<tr>
<td>Multiple&lt;sup&gt;14&lt;/sup&gt;</td>
<td>1.7 (3/172)</td>
</tr>
<tr>
<td><strong>Tobacco smoking history</strong></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>75.4 (126/167)</td>
</tr>
<tr>
<td>Quit</td>
<td>14.4 (26/167)</td>
</tr>
<tr>
<td>Less than once per day</td>
<td>3.6 (3/167)</td>
</tr>
<tr>
<td>Smoke daily</td>
<td>5.4 (9/167)</td>
</tr>
<tr>
<td><strong>Consumed raw milk or raw milk products in the past 2 years</strong></td>
<td>51.2 (85/166)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>50.6 (87/172)</td>
</tr>
<tr>
<td>Female</td>
<td>49.4 (85/172)</td>
</tr>
</tbody>
</table>

<sup>14</sup> Farm worker worked on farm with sheep and goats in multiple industry sectors
<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Percent (Frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wearing gloves while assisting with presumed abortion</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>21.0 (35/167)</td>
</tr>
<tr>
<td>NA&lt;sup&gt;15&lt;/sup&gt;</td>
<td>29.9 (50/167)</td>
</tr>
<tr>
<td>Infrequently</td>
<td>5.4 (9/167)</td>
</tr>
<tr>
<td>Frequently</td>
<td>14.4 (24/167)</td>
</tr>
<tr>
<td>Always</td>
<td>29.3 (49/167)</td>
</tr>
<tr>
<td>Washing hands after assisting with normal births</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>7.2 (12/167)</td>
</tr>
<tr>
<td>NA&lt;sup&gt;16&lt;/sup&gt;</td>
<td>9.0 (15/167)</td>
</tr>
<tr>
<td>Infrequently</td>
<td>6.0 (10/167)</td>
</tr>
<tr>
<td>Frequently</td>
<td>15.6 (26/167)</td>
</tr>
<tr>
<td>Always</td>
<td>62.3 (104/167)</td>
</tr>
<tr>
<td>Changing coveralls or barn clothing after assisting with normal births</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>38.2 (64/167)</td>
</tr>
<tr>
<td>NA&lt;sup&gt;13&lt;/sup&gt;</td>
<td>9.0 (15/167)</td>
</tr>
<tr>
<td>Infrequently</td>
<td>26.4 (44/167)</td>
</tr>
<tr>
<td>Frequently</td>
<td>11.4 (19/167)</td>
</tr>
<tr>
<td>Always</td>
<td>15.0 (25/167)</td>
</tr>
<tr>
<td>Wash hands with soap after handling sheep/goats</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>16.6 (27/163)</td>
</tr>
<tr>
<td>Infrequently</td>
<td>14.7 (24/163)</td>
</tr>
<tr>
<td>Frequently</td>
<td>25.2 (41/163)</td>
</tr>
<tr>
<td>Always</td>
<td>43.6 (71/163)</td>
</tr>
</tbody>
</table>

<sup>15</sup> NA= Not applicable - individual either does not assist with births or no abortions have occurred  
<sup>16</sup> NA= Not applicable - individual does not assist with births
<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Percent (Frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash hands with soap before entering the house</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>30.4 (49/161)</td>
</tr>
<tr>
<td>Infrequently</td>
<td>13.7 (22/161)</td>
</tr>
<tr>
<td>Frequently</td>
<td>17.4 (28/161)</td>
</tr>
<tr>
<td>Always</td>
<td>38.5 (62/161)</td>
</tr>
<tr>
<td>Visitors wash hands</td>
<td>23.8 (39/164)</td>
</tr>
</tbody>
</table>
# APPENDIX VI

**Sheep/Goat seropositivity data and frequency of reproductive testing and outcomes**

Table 12.1 Farm-level frequency of reproductive testing and outcomes over the previous 3 years and percentage of seropositive sheep/goats on farm on 148 sheep and goat farms in Ontario, Canada (Aug 2010- Feb 2012)

<table>
<thead>
<tr>
<th>Reproductive outcomes</th>
<th>C. burnetii identified as cause of abortion</th>
<th>C. burnetii identified in placental/fetal samples but not cause of abortion</th>
<th>Elevated abortion risk (&gt;5%) over previous 3 years</th>
<th>Experienced abortion storm (^b)</th>
<th>Submit abortive diagnostic samples to AHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of C. burnetii seropositive sheep/goats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 50%</td>
<td>4/33</td>
<td>0/33</td>
<td>4/33</td>
<td>8/33</td>
<td>12/33</td>
</tr>
<tr>
<td>&gt;50%</td>
<td>7/115</td>
<td>4/115</td>
<td>11/115</td>
<td>24/115</td>
<td>29/115</td>
</tr>
</tbody>
</table>

\(^a\) Abortion risk defined as percent of ewes/does that aborted/ number of ewes/does bred.

\(^b\) Abortion storm defined as a cluster of abortions, usually greater than 5% of pregnancies and occurring within a three-week period.
### APPENDIX VII

Sheep/Goat seropositivity data and frequency of reproductive testing and outcomes

Table 13.1 Farm-level frequency of reproductive testing and outcomes over the previous 3 years and sheep and goat industry sector on farm on 148 sheep and goat farms in Ontario, Canada (Aug 2010- Feb 2012)

<table>
<thead>
<tr>
<th>Reproductive outcomes</th>
<th>C. burnetii identified as cause of abortion</th>
<th>C. burnetii identified in placental/fetal samples but not cause of abortion</th>
<th>Elevated abortion risk (&gt;5%) over previous 3 years</th>
<th>Experienced abortion storm</th>
<th>Submit abortive diagnostic samples to AHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Industry sector</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat sheep</td>
<td>3/50</td>
<td>2/50</td>
<td>2/50</td>
<td>9/50</td>
<td>12/50</td>
</tr>
<tr>
<td>Meat goat</td>
<td>2/34</td>
<td>0/34</td>
<td>4/34</td>
<td>6/34</td>
<td>5/34</td>
</tr>
<tr>
<td>Dairy sheep</td>
<td>1/22</td>
<td>1/22</td>
<td>2/22</td>
<td>5/22</td>
<td>6/22</td>
</tr>
<tr>
<td>Dairy goat</td>
<td>5/42</td>
<td>1/42</td>
<td>7/42</td>
<td>12/42</td>
<td>18/42</td>
</tr>
</tbody>
</table>

*Abortion risk defined as percent of ewes/does that aborted/ number of ewes/does bred.*

*b Abortion storm defined as a cluster of abortions, usually greater than 5% of pregnancies and occurring within a three-week period.*
APPENDIX VIII
Veterinary Questionnaire

CONTACT INFORMATION

Name: ____________________________ Date: ________________________

Practice Name or Class Year (Undergraduate DVM): ____________________________

Mailing Address: __________________________________________________________

Postal Code: ______________ Email: _________________________________

*The purpose of this questionnaire is to determine important risk factors or protective behaviours for Q fever infection status in veterinarians and veterinary students. Please answer all questions below. For most questions, the time frame is for the last two years, i.e. February 2010 to February 2012.*

1) DEMOGRAPHIC INFORMATION:

a) Age (years):

☐ 18-29 ☐ 30-39 ☐ 40-49 ☐ 50-59 ☐ 60-69 ☐ 70 and above

b) Gender: ____ (M/F)

c) Indicate the species that you work with routinely in practice (i.e. over the last 2 years), and estimate percentage of your professional practice time devoted to veterinary care of that species (should add to 100%):

☐ Dairy cattle ______% ☐ Sheep ______% ☐ Dogs ______%

☐ Beef (cow-calf) ______% ☐ Goat ______% ☐ Cats ______%

☐ Beef (feedlot) ______% ☐ Camelid ______% ☐ Pocket pets ______%

☐ Swine ______% ☐ Farmed deer ______% ☐ Pet birds ______%

☐ Poultry ______% ☐ Horses ______% ☐ Other (specify) ______%
☐ No Applicable  If not applicable, what is the reason?

☐ Retired  ☐ DVM Student  ☐ Do not practice clinical medicine

d) Years working with this veterinary practice: _____(yrs)  ☐ Not applicable

e) Total years working in any veterinary practice: _____(yrs)  ☐ Not applicable

f) Please indicate if you have contact with any of the following species outside of practice, e.g. as livestock raised by you, household pets, wildlife as a hunter, as a DVM student getting experience, etc. (check all that apply).

If checked, please indicate approximately how frequently over the last 12 months:

D=daily; W=weekly; M=monthly; S=2 to 10 times per year; Y=once/year or less frequently.

☐ Dairy cattle  _____  ☐ Sheep  _____  ☐ Dogs  _____

☐ Beef (cow-calf)  _____  ☐ Goat  _____  ☐ Cats  _____

☐ Beef (feedlot)  _____  ☐ Camelid  _____  ☐ Pocket pets  _____

☐ Swine  _____  ☐ Farmed deer  _____  ☐ Pet birds  _____

☐ Poultry  _____  ☐ Horses  _____  ☐ Wildlife  _____

☐ Other (Specify) _____________________________  ☐ Not applicable

g) On average, how many hours per week do you spend in contact with sheep / goats?

_____ hours

h) Do you currently live on a sheep / goat farm? (Check one):

☐ Yes  ☐ No
2) **Q FEVER HISTORY:**

a) Have you ever suspected or known that you have had Q-Fever? (Check one):

- Yes  
- No

b) *If yes, please answer the questions below, otherwise proceed to Section 3.*

i) What signs / symptoms did you have that made you suspect Q-Fever? (check all that apply)

- Fever  
- Head ache  
- Muscle Ache  
- Cough  
- Fatigue  
- Vomiting / Nausea  
- Rash  
- Other:_____________________

ii) When did this occur (nearest month and year)? ____________________

iii) Did the symptoms occur 2 to 8 weeks after contact with? (Check one)

- Aborting ewes / does  
- Lambing ewes / kidding does with no abortions  
- Another suspected exposure (specify) _________________________________  
- Not within 2 to 8 weeks after any specific event

iv) Did you seek medical attention for these signs (Check one)?  

- Yes  
- No

*If yes to iv.,*

(1) Did you request a test for Q-fever (Check one)?  

- Yes  
- No

*If yes to (1)*

(a) Did the doctor have a test for Q-fever performed (i.e. blood test) (Check one)?  

- Yes  
- No
If yes to (a)

(i) Was the result of the blood test positive (Check one)?

☐ Yes  ☐ No  ☐ Don’t know

If yes to (i)

1. Were you contacted by the local health unit (Medical Health Officer or designate) regarding your test result (Check one)?

☐ Yes  ☐ No

If no to (a)

(ii) Why did the doctor decline to perform the test? (Check all that apply)

☐ Doctor did not believe that the signs were compatible with Q-fever.

☐ Doctor was not familiar with the disease Q-fever.

☐ Doctor was familiar with the disease, but did not know how to test for Q-fever.

☐ Other: __________________________________________________________

3) MEDICAL HISTORY

a) Have you ever been diagnosed by a physician with any of the following conditions (check all that apply)?

☐ Asthma  ☐ Cancer  ☐ Pneumonia  ☐ Heart Disease

☐ Emphysema  ☐ Low Immune Function
4) **WOMEN OF CHILD BEARING AGES ONLY**

a) Are you currently pregnant? (Check one):

- Yes
- No

b) Have you been pregnant within the last 2 years i.e. February 2010 to February 2012?

(Check one):

- Yes
- No

*If yes to a) or b)*:

i) Have you been present in a barn during lambing or kidding while pregnant?

- Yes
- No

ii) Have you assisted with lambing or kidding while pregnant?

- Yes
- No

iii) Have you miscarried (lost) a pregnancy after 12 weeks of gestation in the last 2 years?

- Yes
- No
5) PROTOCOLS FOR ASSISTING BIRTHS OF LAMBS / KIDS

Within the last 2 years i.e. February 2010 to February 2012

a) Please indicate how frequently you take the following measures when assisting with lambing/ kidding:

Always (A); Frequently (F); Infrequently (INF); Never (N); Not Applicable (NA):

<table>
<thead>
<tr>
<th>Measure</th>
<th>Normal birth</th>
<th>Unexpected abortion</th>
<th>Presumed abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wearing protective sleeves/gloves while assisting births</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wearing a N95 17 respirator mask while assisting births</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washing hands with disinfectant soap after assisting births</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changing coveralls or protective wear after assisting births</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changing footwear or disinfecting boots after assisting births</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taking off coveralls before entering the vehicle after assisting births</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Unexpected abortion = abortion problems were not suspected in the flock when assisting with deliveries

Presumed abortion = Ongoing issues in flock with abortion

b) If any other precautions are taken, please describe:

__________________________________________________________________________________________________

__________________________________________________________________________________________________

__________________________________________________________________________________________________

17 N95 Respirator mask is a specially fitted mask designed to prevent inhalation of infectious organisms. It is different from masks for renovation work, or from masks worn by surgeons.
6) LIFESTYLE

Within the last 2 years, i.e. February 2010 to February 2012

a) Have you consumed alcohol? □ Yes □ No

If yes, how would you describe your consumption? (Check one)

□ Less than once / week and not more than 1-2 drinks at a time.

□ More than once / week and not more than 1-2 drinks at a time.

□ More than once / week and more than 1-2 drinks at a time.

b) Have you smoked tobacco? (Including cigarettes, cigars or pipes) □ Yes □ No

If yes, how frequently do you smoke? (Check one)

□ I quit. How long ago? ______________(weeks / months / years) (circle one)

□ I smoke less than once/day.

□ I smoke daily.

7) RAW MILK CONSUMPTION:

Within the last 2 years, February 2010 to February 2012

a) Have you consumed raw milk, or raw milk products? □ Yes □ No

If yes, how frequently do you consume these products? (Check one)

□ At least once / week

□ At least once / month

□ < 12 times per year

□ Only once or twice in last 2 years
8) Q FEVER VACCINATION

a) Animal Vaccination

i) If *Coxiella burnetii* infection is diagnosed in one of your herds or flocks, as the herd veterinarian would you advise vaccination with a killed commercial vaccine if it were available (Coxevac, Ceva Animal Health\(^{18}\))

☐ Yes   ☐ No

Comments:

b) Human Vaccination

i) If a human vaccine were available which would protect humans against Q fever, would you:

(1) Request vaccination of yourself  ☐ Yes   ☐ No

(2) Recommend vaccination of your co-workers (i.e. veterinarians and assistants)

☐ Yes   ☐ No

(3) Recommend vaccination to your small ruminant clients and families. ☐ Yes   ☐ No

*Thank you for your time in completing this questionnaire. If there is anything else you would like to add, please feel free to write comments below:*

---

\(^{18}\) Coxevac has been shown to be effective in preventing abortion due to *C. burnetii* and to prevent shedding of the bacteria in birth fluids, feces and milk of domestic ruminants. It is most effective when given as a primary series prior to infection and boostered annually. Licensing of this vaccine may be possible in Canada but the company is trying to determine market capacity.