Investigating *Coxiella burnetii* at the Livestock - Wildlife Interface

by

Porty, Ariel M.

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science (MSc) in Biology

The Faculty of Graduate Studies
Laurentian University
Sudbury, Ontario, Canada

THESIS DEFENCE COMMITTEE/COMITÉ DE SOUTENANCE DE THÈSE
Laurentian Université/Université Laurentienne
Faculty of Graduate Studies/Faculté des études supérieures

Title of Thesis Titre de la thèse	Investigating Coxiella burnetii at the Livestock - Wildlife Interface
Name of Candidate Nom du candidat	Porty, Ariel Mary
Degree Diplôme	Master of Science
Department/Program Département/Programme	Biology
Date of Defence Date de la soutenance	May 27, 2016

APPROVED/APPROUVÉ

Thesis Examiners/Examinateurs de thèse:

Dr. Albrecht Schulte-Hostedde
(Co-supervisor/Co-directeur(trice) de thèse)

Dr. Claire Jardine
(Co-supervisor/Co-directeur(trice) de thèse)

Dr. Nadia Mykytczuk
(Committee member/Membre du comité)

Dr. Andria Jones-Bitton
(Committee member/Membre du comité)

Dr. Paula Menzies
(Committee member/Membre du comité)

Dr. Fanie Pelletier
(External Examiner/Examinateur externe)

Approved for the Faculty of Graduate Studies
Approuvé pour la Faculté des études supérieures
Dr. Shelley Watson
Madame Shelley Watson
Acting Dean, Faculty of Graduate Studies
Doyenne intérimaire, Faculté des études supérieures

ACCESSIBILITY CLAUSE AND PERMISSION TO USE

General Abstract

The intent of this project was to better understand the role of wildlife in the epidemiology of the zoonotic bacterium, *Coxiella burnetii*, at the livestock-wildlife interface. In Chapter 1, I compared the prevalence of *C. burnetii* DNA in dairy goats, other domestic animals, and wildlife on goat farms and adjacent natural areas. In Chapter 2, I compared the prevalence of *C. burnetii* DNA in different sample types from goats and wildlife, and assessed the level of agreement among the different samples. From April to August 2014, genital, fecal and milk samples were collected from goats on 16 Ontario dairy goat farms. Fecal and genital samples were also collected from other resident animals (cats, chickens, cows, dogs, horses, pigs), and from wildlife (deer mice, house mice, opossums, raccoons, red-backed voles, red squirrels and skunks) live-trapped on farms and from 14 adjacent natural areas. *Coxiella burnetii* was detected by PCR in samples from 89.2% (404/453) of goats, 68.8% (33/48) of other farm animals, 64.7% (44/68) of wild animals sampled on farms, and 58.1% (165/284) of wild animals sampled in natural areas. *Coxiella burnetii* was detected at all study sites and the prevalence in wildlife was similar on farms and adjacent natural areas, independent of site distances. These findings provide evidence to support the hypothesis that wildlife are able to maintain *C. burnetii* independent of livestock and may act as possible maintenance or reservoir hosts of *C. burnetii* at the livestock-wildlife interface. I determined that genital and fecal swab samples, which yielded the highest proportion positives, were optimal sample types to use for the detection of *C. burnetii* DNA in deer mice, eastern chipmunks, and raccoons. Genital swab, fecal swab and fecal material sample types were all suitable for detecting *C. burnetii* DNA in house mice and red squirrels. On the other

hand, genital swab samples were optimal for detecting *C. burnetii* DNA in dairy goats and were significantly more likely to be positive for *C. burnetii* DNA than the other sample types. Additional studies need to be conducted to further elucidate the epidemiology of *C. burnetii* at the livestock-wildlife interface in southern Ontario and to confirm the optimal sample types to use for *C. burnetii* detection in wildlife and dairy goats.

Acknowledgements

This project would not have been possible without the help, support, and guidance of numerous individuals. For this reason, I would like to specifically thank those that were most involved - from the initial origin of the project to sampling the butt end of the goats, and everything in between. My deepest gratitude is intended for my supervisors, Dr. Albrecht Schulte-Hostedde and Dr. Claire Jardine. I will forever be grateful for your endless support and guidance throughout every aspect of this project. You not only provided me with invaluable knowledge about academia and wildlife disease, but also countless life lessons. I would also like to thank my committee members, Dr. Paula Menzies, Dr. Andria Jones-Bitton and Dr. Nadia Mykytczuk for their constant encouragement, edits, and Skype meetings, particularly throughout my topics class.

There were times when it seemed the field methodology for this project was going to be impractical, and in reality, I am still surprised it came together as well as it did. Sampling a total of 30 sites in a short 4-month period is no easy feat, and although I organized the finite details, it was the support of all my witty and wonderful field researchers that made sampling all of these sites possible. A special thank you to Dr. Samantha Allen, Sarah Wilkes, Jared Louw, and Dr. Shannon Meadows. Thank you for your efforts through all the early mornings and countless rainy afternoons. I must also give a special thank you to all the farm producers and managers of the natural areas/conservation authorities that willingly participated in this study. Your generosity and commitment to science is invaluable.

Members of the EBV lab also contributed a plethora of their time and counsel to help me through the tougher components of this project, specifically the statistical

analyses. A special thank you to Dr. Darryl Edwards and Master Colleen Bobbie. I will always admire and appreciate you sharing your stats minds with me. As well, I would like to thank the members of the Keim Lab at Northern Arizona University. In particular, Dr. Talima Pearson, Heidie Hornstra-O'Neill and Emily Kaufman. Your hospitality, laboratory training and dedication to dealing with my precious samples extended beyond what I could have asked for. For this, I thank you.

Last but not least, I would like to thank my family (Porty and Nelson) for always supporting me, even while not always understanding me. My parents are responsible for telling me I could aspire to anything I could dream. Thanks for believing in me! I look forward to your continued love and support while I chase all my other dreams.

Table of Contents

List of Tables

Chapter 1

Chapter 2

List of Figures

Chapter 1

List of Appendices

Chapter 1

Chapter 2

General Introduction

Coxiella burnetii is a zoonotic bacterial pathogen that is known to infect an array of domestic and wildlife species worldwide (Astobiza et al., 2011; reviewed in Mcquiston and Childs, 2002; reviewed in Maurin and Raoult, 1999), excluding New Zealand (Hilbink et al., 1993). The first documentation of *C. burnetii* was in 1937, when feverish illness was reported among abattoir workers in Brisbane, Australia (reviewed in Madariaga et al., 2003; reviewed in Reimer, 1993). Sir MacFarlane Burnet injected blood from infected workers into guinea pigs and observed similar symptoms to that of rickettsial disease (reviewed in Madariaga et al., 2003; Marrie, 1995; reviewed in Oyston & Davies, 2011). Dr. Herald Rae Cox then isolated an unknown infectious agent from ticks that was thought to be an agent of rickettsial disease (reviewed in Madariaga et al., 2003). Together, Cox and Burnet have been honoured with the discovery of *C. burnetii*, which is the causal agent of the disease initially described (reviewed in Madariaga et al., 2003).

Coxiella burnetii is highly virulent and is primarily transmitted through infectious small cell variants (SCV) (Azad and Radulovic, 2003; Tissot-Dupont et al., 2004). It was classified as an agent of bioterrorism in 1942 in the USA (Madariaga et al., 2003), because it consistently causes disability; can be manufactured on a large scale; remains stable under production, storage and transportation conditions; can be efficiently disseminated; and remains viable in the environment for years after dissemination (Azad & Radulovic, 2003). Even though *C. burnetii* is classified as an agent of bioterrorism, the only suspected use of it in the context of bioterrorism was in World War II (Madariaga et al., 2003). However, it is unclear whether troops were infected due to naturally occurring

environmental and airborne SCVs or whether SCVs were distributed for bioterrorism (Spicer, 1978). While *C. burnetii* infection remains reportable for humans in Ontario, in 1978 it was removed from being federally reportable in Canada (Public Health Agency of Canada, 2015). Moreover, it remains an underreported disease due to the similarities of symptoms with the influenza virus (Raoult et al., 2005).

Q fever

Disease in humans is referred to as Q fever and can take the form of an acute or chronic infection (Fenollar et al., 2001; Hartzell et al., 2008). There are two antigenic stages of *C. burnetii*: the virulent phase I and the avirulent phase II (Arricau-Bouvery et al., 2005). Acute Q fever is less serious than the chronic form of the disease, and is attributed to the phase I antigen (Arricau-Bouvery et al., 2005). Approximately half of patients with acute infection will remain asymptomatic (Maurin & Raoult, 1999). Clinical symptoms of acute Q fever are non-specific and include fever, nausea, headache, chest pain, as well as hepatitis, and atypical pneumonia (Reimer, 1993). Symptoms of acute Q fever occur in 60% of cases (Angelakis & Raoult, 2010), and infections usually last 1-2 weeks and symptoms are often self-limiting (Pérez & Rizk, 2004). Chronic Q fever is more serious, in that it is less responsive to antibiotic treatment, and is also attributed to the phase I antigen (Maurin & Raoult, 1999). Not all people with acute Q fever develop chronic Q fever, but those with certain conditions (e.g., pregnancy, immunosuppression, heart valve lesions, and vascular abnormalities) are more susceptible (Carcopino et al., 2009; Fenollar et al., 2001). Chronic Q fever can lead to complications such as meningoencephalitis, myocarditis, chronic endocarditis, and chronic fatigue syndrome (Raoult et al., 2005; Wildman et al., 2002). Although there is no cure for chronic Q fever,

long-term antimicrobial drug therapy, typically of a combination of doxycycline and hydroxychloroquine, can be used to treat the disease (Million et al., 2010).

The main reservoir for human infection has been identified as small ruminants (Maurin & Raoult, 1999; Mcquiston & Childs, 2002). More specifically, goats are known to be the biggest shedders of infectious *C. burnetii*, shedding upwards of 10^9 bacteria per gram of placenta material (Fournier et al., 1998). Humans can become infected primarily when they inhale infectious bacteria shed by these species (Roest et al., 2011). Through occupational exposure, veterinarians and farm workers are at high risk of infection (Thomas et al., 1995); however, because the bacterium remains viable in the environment for extended periods of time (Azad & Radulovic, 2003), humans without occupational exposure are also at risk of infection.

***Coxiella burnetii* Infection in Animals**

Animal disease caused by *C. burnetii* differs from human disease and is referred to as coxiellosis. Small ruminants are most susceptible to clinical disease, and signs include reproductive complications, such as weak or unviable offspring, stillbirths, abortion with marked placentitis, and endometritis (Berri et al., 2001; Bildfell et al., 2000; Guatteo et al., 2012; Moeller, 2001; To et al., 1998a). Small ruminants can shed infectious *C. burnetii* through milk, feces, genital mucus and in birthing materials (Arricau Bouvery et al., 2003; Fournier et al., 1998; Maurin & Raoult, 1999; Rousset et al., 2009). Other farm and domestic animals are known hosts of *C. burnetii*, but clinical symptoms are not common. These animals include cows, horses, chickens, camels, water buffalo, cats and dogs (Buhariwalla et al., 1996; Guatteo et al., 2012; Komiya et al., 2003; Marenzoni et al., 2013; Mohammed et al., 2014; Perugini et al., 2009; Tatsumi et

al., 2006). Sheep and goats are the species that have been attributed to most human Q fever outbreaks worldwide, including several large scale outbreaks (Schimmer et al., 2011; Tilburg et al., 2012), although some outbreaks in North America have been attributed to other species, including parturient cats (Marrie et al., 1988), and pigeons (Stein & Raoult, 1999).

Coxiella burnetii has also been identified from a wide array of wildlife species, including brown and black rats (*Rattus norvegicus* and *Rattus rattus* respectively; Reusken et al., 2011), European hares (*Lepus europaeus*; Astobiza et al., 2011), roe deer (*Capreolus capreolus*; Astobiza et al., 2011), coyotes (*Canis latrans*; Enright et al., 1971; reviewed in Mcquiston and Childs, 2002), red foxes (*Vulpes vulpes*; Meredith et al., 2014), vulture (*Gyps fulvus*), black kite (*Milvus migrans*), wild birds (Astobiza et al., 2011; reviewed in Mcquiston and Childs, 2002), brush rabbits (*Sylvilagus bachmani*; Enright et al., 1971), other rodent species (Meredith et al., 2014), and ticks (Angelakis and Raoult, 2010).

Q fever in the Netherlands

The most recent, and largest Q fever outbreak reported in the literature, took place in the Netherlands from 2007 to 2010 (reviewed in Dijkstra et al., 2012; reviewed in Roest et al., 2011). A European milk quota system was put in place for dairy cattle in 1984 (reviewed in Roest et al., 2011). This system led to a large increase in the number of dairy goat farms in the Netherlands, and this increase in farms is suspected to be an important factor leading to the Netherlands Q fever outbreak (reviewed in Roest et al., 2011). This outbreak led to more than 4,000 reported acute Q fever cases in humans, with as many as 50% of these cases requiring hospitalization (Chmielewski & Tykewska-

Wierzbanowska, 2012; van der Hoek et al., 2010; Schimmer et al., 2011). It is also estimated that this outbreak resulted in 250 chronic Q fever cases and 14 deaths (Roest et al., 2011; Chmielewski & Tykewska-Wierzbanowska, 2012). An increase in reported Q fever cases began in the Netherlands in 2007, and in 2009 a mandatory goat vaccination program was implemented in order to slow the outbreak, and over 100,000 goats were vaccinated (Schimmer et al., 2011; van der Hoek et al., 2010). The vaccination program was ineffective for infected pregnant animals, since they could still shed large amounts of *C. burnetii*, so a mass cull of pregnant animals was also put in place (Roest et al., 2011; Whelan et al., 2011). A total of 50,355 pregnant goats and sheep were killed as a result (Whelan et al., 2011). The outbreak was in decline by 2010 and during this time, researchers were beginning surveillance studies and programs in other geographic locations.

Prevalence of Coxiella burnetii in Ontario

A serological study investigating the seroprevalence of *C. burnetii* in Ontario dairy and meat goats, as well as the people that cared for them, was conducted by the University of Guelph in 2010 and 2011 (Meadows et al., 2015). The Ontario Ministry of Agriculture Food and Rural Affairs reported 230 licensed Ontario dairy goat farms in 2010, and researchers found that 78.6% (33/42) of dairy goat farms surveyed had one or more seropositive animal (Meadows et al., 2015). The same study found that 44.1% (15/34) of meat goat farms were also seropositive (Meadows et al., 2015).

In 2010, researchers at Laurentian University discovered that six of seven wild rodent species were PCR positive for *C. burnetii* from genital swabs collected in Algonquin Provincial Park, Ontario (Thompson et al., 2012). Woodland jumping mice

exhibited the highest prevalence (83.3%, *n* = 30) and no *C. burnetii* DNA was detected from eastern chipmunks (0%, *n* = 12) (Thompson et al., 2012). This was the first study reporting *C. burnetii* in wildlife in an Ontario Provincial Park. These results and those of other studies that have found *C. burnetii* in wildlife species worldwide suggest that infection is common and that it is possible that wildlife species are capable of maintaining *C. burnetii infection* and might be a reservoir.

The Role of Wildlife in Pathogen Transmission

There are several zoonotic diseases that are transmitted amongst and between wildlife and livestock species, including H5N1 avian influenza, bovine tuberculosis, brucellosis, Newcastle disease, and salmonellosis (Gortázar et al., 2007). Each of these diseases has a known source for domestic animal infection. In the example of brucellosis in the Greater Yellowstone Area, the pathogen was first introduced to native wild elk and bison populations from domestic cattle, but has since spilled-back from elk and bison populations to cattle (Cheville et al., 1998). Thus, together these wildlife and livestock animals form components of the reservoir for this disease within this geographic location.

Likewise, *Salmonella* spp. infection has been detected in wild birds and linked to domestic animal and livestock infections (Horton et al., 2013; Refsum et al., 2002; Taylor & Philbey, 2010). Thus, wild bird species are thought to be reservoir and vector species of *Salmonella* spp. infection (Horton et al., 2013).

One important similarity between different zoonotic pathogens at the livestock-wildlife interface is the substantial economic and public health implications. For example, the emergence of highly pathogenic H5N1 avian influenza was estimated to have cost >$10 billion in 2006, including the cost of health care, and export bans for countries with

infected livestock (Kilpatrick et al., 2006). In addition, 340 human cases worldwide at the end of 2007 resulted in public health concerns (reviewed in AbdelGhafar et al., 2008). To avoid the economic and public health impacts of zoonotic pathogens at the livestock-wildlife interface, it is important to understand the epidemiology of diseases and attempt to control them before an emergence opportunity presents. In particular, the transmission complexities of *C. burnetii* need to be better understood in order to prevent future outbreaks.

Identifying Potential Reservoir(s) of Coxiella burnetii

The first step in identifying reservoir(s) of any pathogen is to accumulate epidemiological evidence through prevalence reports and risk factor studies (Haydon et al., 2002). The second step is to identify any and all species that are susceptible to a natural infection (Haydon et al., 2002). Finally, the last step is to understand the transmission between species of natural infection, since not all species of natural infection are involved in the reservoir (Haydon et al., 2002).

In the case of *C. burnetii*, it is known that infection persists globally in both humans and animal species, with the exception of New Zealand (Hilbink et al., 1993; Maurin & Raoult, 1999). The main reservoir for human infection has been identified as small ruminants (Roest et al., 2011); however, wildlife species are known to develop natural infection (Astobiza et al., 2011) and have been attributed to a few human outbreaks (Marrie et al., 1988; Stein & Raoult, 1999). As mentioned earlier, dairy goats and wild rodent species have been identified with natural *C. burnetii* infection in Ontario (Meadows et al., 2015; Thompson et al., 2012); however, the potential transmission

pathway between wildlife-livestock and wildlife-humans have not yet been investigated thoroughly.

Investigating Coxiella burnetii Epidemiology at the Livestock-Wildlife Interface

The primary transmission route of *C. burnetii* for human infection is the inhalation of infectious aerosols containing organisms shed by other animals, particularly small ruminants (Fournier et al., 1998; Maurin & Raoult, 1999; Mcquiston & Childs, 2002; Roest et al., 2011). While this transmission route has been widely studied and supported (reviewed in Marrie & Raoult, 1997; Maurin & Raoult, 1999), there are few studies that have investigated the role of wildlife as maintenance species and potentially forming part of the *C. burnetii* reservoir. *Coxiella burnetii* infection has been reported among an array of wildlife species (Astobiza et al., 2011; Enright et al., 1971; Kazar, 2005; Ho et al., 1995l; Meredith et al., 2014; Mcquiston & Childs, 2002; Reusken et al., 2011; Thompson et al., 2012), however, few studies have investigated the importance of these species in the transmission of *C. burnetii* at the livestock-wildlife interface.

The aim of my study was to investigate the role of wildlife in the epidemiology of *C. burnetii* at the livestock-wildlife interface in Ontario, Canada. The first chapter explores the potential role of wildlife in the transmission of *C. burnetii*. The specific objectives were to: 1) determine the prevalence of *C. burnetii* infection as determined by PCR in wildlife and domestic animals on farms and natural areas, 2) determine the spatial prevalence of *C. burnetii* in southern Ontario, and 3) investigate the role of wildlife in the transmission dynamics of *C. burnetii*. If wildlife are acting as spillover hosts, exposed to *C. burnetii* via infected livestock, but unable to independently maintain the infection, then I predict that *C. burnetii* would occur only in wildlife living in close association with

infected livestock. Alternatively, if wildlife maintain *C. burnetii* independent of livestock, then I predict that *C. burnetii* would also occur in wildlife in natural areas. In addition, if wildlife are able to maintain *C. burnetii* independent of livestock, then I predict that infection prevalence in wildlife would be the same on adjacent farm and natural area sites, regardless of the distance between sites.

In the second chapter, I investigate the prevalence of *C. burnetii* DNA, sensitivity for DNA recovery of different sample types (genital swab, fecal swab, fecal material, and milk) and level of agreement between the sample types collected from dairy goats and five wildlife species, including deer mice (*Peromyscus maniculatus*), eastern chipmunks (*Tamias striatus*), house mice (*Mus musculus*), raccoons (*Procyon lotor*), and red squirrels (*Tamiascurius hudsonicus*), in the absence of a reference sample type.

Literature Cited

AbdelGhafar, A. N., Chotpitayasunondh, T., Gao, Z., Hayden, F. G., Hien, N. D., De Jong, M. D., Naghdaliyev, A., Peiris, J. S. M., Shindo, N., Soeroso, S., & Uyeki, T. M. (2008). Update on avian influenze A (H5N1) virus infection in humans. *New England Journal Of Medicine*, *358*(3), 261–273. http://doi.org/http://dx.doi.org/10.1056/NEJMra0707279

Angelakis, E., & Raoult, D. (2010). Q fever. *Veterinary Microbiology*, *140*(3-4), 297–309. http://doi.org/10.1016/j.vetmic.2009.07.016

Arricau Bouvery, N., Souiriau, A., Lechopier, P., & Rodolakis, A. (2003). Experimental *Coxiella burnetii* infection in pregnant goats: excretion routes. *Veterinary Research*, *34*(4), 423–433. http://doi.org/http://dx.doi.org/10.1051/vetres:2003017

Arricau-Bouvery, N., Souriau, A., Bodier, C., Dufour, P., Rousset, E., & Rodolakis, A. (2005). Effect of vaccination with phase I and phase II *Coxiella burnetii* vaccines in pregnant goats. *Vaccine*, *23*, 4392–4402. http://doi.org/10.1016/j.vaccine.2005.04.010

Astobiza, I., Barral, M., Ruiz-Fons, F., Barandika, J. F., Gerrikagoitia, X., Hurtado, A., & García-Pérez, A. L. (2011). Molecular investigation of the occurrence of *Coxiella burnetii* in wildlife and ticks in an endemic area. *Veterinary Microbiology*, *147*(1-2), 190–194. http://doi.org/10.1016/j.vetmic.2010.05.046

Azad, A. F., & Radulovic, S. (2003). Pathogenic rickettsiae as bioterrorism agents. *Annals of the New York Academy of Sciences*, *990*, 734–738. http://doi.org/10.1086/518147

Berri, M., Souriau, A., Crosby, M., Crochet, D., Lechopier, P., & Rodolakis, A. (2001). Relationships between the shedding of *Coxiella burnetii*, clinical signs and serological responses of 34 sheep. *The Veterinary Record*, *148*(16), 502–505. http://doi.org/10.1136/vr.148.16.502

Bildfell, R. J., Thomson, G. W., Haines, D. M., McEwen, B. J., & Smart, N. (2000). *Coxiella burnetii* infection is associated with placentitis in cases of bovine abortion. *Journal of Veterinary Dagnostic Investigation*, *12*(5), 419–425. http://doi.org/10.1177/104063870001200505

Buhariwalla, F., Cann, B., & Marrie, T. J. (1996). A dog-related outbreak of Q fever. *Clinical Infectious Diseases*, *23*(4), 753–755. http://doi.org/10.1097/00006454-199705000-00034

Carcopino, X., Raoult, D., Bretelle, F., Boubli, L., & Stein, A. (2009). Q fever during pregnancy: a cause of poor fetal and maternal outcome. *Annals of the New York Academy of Sciences, 1166*, 79–89. http://doi.org/10.1111/j.1749-632.2009.04519.x

Cheville, N. F., McCullough, D. R., & Paulson, L. R. (1998). *Brucellosis in the Greater Yellowstone Area*. Washington, DC: National Academy Press.

Chmielewski, T., & Tykewska-Wierzbanowska, T. (2012). Q fever at the turn of the century. *Polish Journal of Microbiology*, *61*(2), 81–93.

Dijkstra, F., van der Hoek, W., Wijers, N., Schimmer, B., Rietveld, A., Wijkmans, C. J., Vellema, P., & Schneeberger, P. M. (2012). The 2007–2010 Q fever epidemic in The Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. *FEMS Immunology and Medical Microbiology*, *64*(1), 3–12. http://doi.org/10.1111/j.1574-695X.2011.00876.x

Enright, J. B., Franti, C. E., Behymer, D. E., Longhurst, W. M., Dutson, V. J., & Wright, M. E. (1971). *Coxiella burnetii* in a wildlife-livestock environment distribution of Q fever in wild mammals. *American Journal of Epidemiology*, *94*(1), 79–90.

Fenollar, F., Fournier, P.-E., Carrieri, M. P., Habib, G., Messana, T., & Raoult, D. (2001). Risks factors and prevention of Q fever endocarditis. *Clinical Infectious Diseases*, *33*(3), 312–316. http://doi.org/10.1086/321889

Fournier, P.-E., Marrie, T. J., & Raoult, D. (1998). Diagnosis of Q Fever. *Journal of Clinical Microbiology*, *36*(7), 1823–1834.

Gortázar, C., Ferroglio, E., Höfle, U., Frölich, K., & Vicente, J. (2007). Diseases shared between wildlife and livestock: a European perspective. *European Journal of Wildlife Research*, *53*(4), 241–256. http://doi.org/10.1007/s10344-007-0098-y

Guatteo, R., Joly, A., & Beaudeau, F. (2012). Shedding and serological patterns of dairy cows following abortions associated with *Coxiella burnetii* DNA detection. *Veterinary Microbiology*, *155*(2-4), 430–433. http://doi.org/10.1016/j.vetmic.2011.09.026

Hartzell, J. D., Wood-Morris, R. N., Martinez, L. J., & Trotta, R. F. (2008). Q fever: Epidemiology, diagnosis, and treatment. *Mayo Clinic Proceedings*, *83*(5), 574–579. http://doi.org/10.1016/S0025-6196(11)60733-7

Haydon, D. T., Cleaveland, S., Taylor, L. H., & Laurenson, M. K. (2002). Identifying reservoirs of infection: A conceptual and practical challenge. *Emerging Infectious Diseases*, *8*(12), 1468–1473. http://doi.org/10.3201/eid0812.010317

Hilbink, F., Penrose, M., Kovacova, E., & Kazar, J. (1993). Q fever is absent from New Zealand. *International Journal of Epidemiology*, *22*(5), 945–949. http://doi.org/10.1093/ije/22.5.945

Ho, T., Htwe, K. K., Yamasaki, N., Zhang, G. Q., Ogawa, M., Yamaguchi, T., Fukushi, H., & Hirai, K. (1995). Isolation of *Coxiella burnetii* from dairy cattle and ticks, and

some characteristics of the isolates in Japan. *Microbiology and Immunology*, *39*(9), 663–671. http://doi.org/10.1111/j.1348-0421.1995.tb03254.x

Horton, R. A., Wu, G., Speed, K., Kidd, S., Davies, R., Coldham, N. G., & Duff, J. P. (2013). Wild birds carry similar *Salmonella enterica* serovar *Typhimurium* strains to those found in domestic animals and livestock. *Research in Veterinary Science*, *95*(1), 45–48. http://doi.org/10.1016/j.rvsc.2013.02.008

Kazar, J. (2005). *Coxiella burnetii* infection. *Annals of the New York Academy of Sciences*, *1063*, 105–14. http://doi.org/10.1196/annals.1355.018

Kilpatrick, A. M., Chmura, A. A., Gibbons, D. W., Fleischer, R. C., Marra, P. P., & Daszak, P. (2006). Predicting the global spread of H5N1 avian influenza. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(51), 19368–19373. http://doi.org/10.1073/pnas.0609227103

Komiya, T., Sadamasu, K., Kang, M.-I., Tsuboshima, S., Fukushi, H., & Hirai, K. (2003). Seroprevalence of *Coxiella burnetii* infections among cats in different living environments. *The Journal of Veterinary Medical Science*, *65*(9), 1047–1048. http://doi.org/10.1292/jvms.65.1047

Madariaga, M. G., Rezai, K., Trenholme, G. M., & Weinstein, R. A. (2003). Q fever: A biological weapon in your backyard. *Lancet Infectious Diseases*, *3*(11), 709–721. http://doi.org/10.1016/S1473-3099(03)00804-1

Marenzoni, M. L., Stefanetti, V., Papa, P., Proietti, P. C., Bietta, A., Coletti, M.,Passamonti, F., & Henning, K. (2013). Is the horse a reservoir or an indicator of *Coxiella burnetii* infection? Systematic review and biomolecular investigation. *Veterinary Microbiology*, *167*(3-4), 662–669. http://doi.org/10.1016/j.vetmic.2013.09.027

Marrie, T. J. (1995). *Coxiella burnetii* (Q fever) pneumonia. *Clinical Infectious Diseases*, *21*(Supplement 3), S253–S264. http://doi.org/10.1093/clind/21.Supplement_3.S253

Marrie, T. J., Durant, H., Williams, J. C., Mintz, E., & Waag, D. M. (1988). Exposure to parturient cats: a risk factor for acquisition of Q fever in Maritime Canada. *The Journal of Infectious Diseases*, *158*(1), 101–108. http://doi.org/10.1093/infdis/158.1.101

Marrie, T. J., & Raoult, D. (1997). Q fever - a review and issues for the next century. *International Journal of Antimicrobial Agents*, *8*(3), 145–161. http://doi.org/10.1016/S0924-8579(96)00369-X

Maurin, M., & Raoult, D. (1999). Q fever. *Clinical Microbiology Reviews*, *12*(4), 518–553.

Mcquiston, J. H., & Childs, J. E. (2002). Q fever in humans and animals in the United States. *Vector Borne and Zoonotic Diseases*, *2*(3), 179–191. http://doi.org/10.1089/15303660260613747

Meadows, S., Jones-Bitton, A., McEwen, S., Jansen, J., & Menzies, P. (2015). *Coxiella burnetii* seropositivity and associated risk factors in goats in Ontario, Canada. *Preventive Veterinary Medicine*, In Press. http://doi.org/10.1016/j.prevetmed.2015.06.014

Meredith, A. L., Cleaveland, S. C., Denwood, M. J., Brown, J. K., & Shaw, D. J. (2014). *Coxiella burnetii* (Q-fever) seroprevalence in prey and predators in the united kingdom: evaluation of infection in wild rodents, foxes and domestic cats using a modified ELISA. *Transboundary and Emerging Diseases*, *62*(6), 639–649. http://doi.org/10.1111/tbed.12211

Million, M., Thuny, F., Richet, H., & Raoult, D. (2010). Long-term outcome of Q fever endocarditis: a 26-year personal survey. *The Lancet Infectious Diseases*, *10*(8), 527–535. http://doi.org/10.1016/S1473-3099(10)70135-3

Moeller, R. B. J. (2001). Causes of caprine abortion: diagnostic assessment of 211 cases (1991-1998). *Journal of Veterinary Diagnostic Investigation*, *13*(3), 265–270. http://doi.org/10.1177/104063870101300317

Mohammed, O. B., Jarelnabi, A. A., Aljumaah, R. S., Alshaikh, M. A., Bakhiet, A. O., Omer, S. A., Alagaili, A. N., & Hussein, M. F. (2014). *Coxiella burnetii*, the causative agent of Q fever in Saudi Arabia: molecular detection from camel and other domestic livestock. *Asian Pacific Journal of Tropical Medicine*, *7*(9), 715–719. http://doi.org/10.1016/S1995-7645(14)60122-X

Oyston, P. C. F., & Davies, C. (2011). Q Fever: the neglected biothreat agent. *Journal of Medical Microbiology*, *60*(1), 9–21. http://doi.org/10.1099/jmm.0.024778-0

Pérez, E. R. F., & Rizk, D. (2004). Acute Q fever. *Hospital Physician*, (September), 22–26.

Perugini, A. G., Capuano, F., Esposito, A., Marianelli, C., Martucciello, A., Iovane, G., & Galiero, G. (2009). Detection of *Coxiella burnetii* in buffaloes aborted fetuses by IS111 DNA amplification: a preliminary report. *Research in Veterinary Science*, *87*(2), 189–191. http://doi.org/10.1016/j.rvsc.2009.01.005

Public Health Agency of Canada. (2015). Notifiable Diseases On-Line. Retrieved from http://dsol-smed.phac-aspc.gc.ca/dsol-smed/ndis/list-eng.php

Raoult, D., Marrie, T. J., & Mege, J. L. (2005). Natural history and pathophysiology of Q fever. *The Lancet of Infectious Diseases*, *5*(4), 219–226. http://doi.org/10.1016/S1473-3099(05)70052-9

Refsum, T., Heir, E., Kapperud, G., Vardund, T., & Holstad, G. (2002). Molecular epidemiology of *Salmonella enterica* serovar Typhimurium isolates determined by pulsed-field gel electrophoresis: comparison of isolates from avian wildlife, domestic animals, and the environment in Norway. *Applied and Environmental Microbiology, 68*(11), 5600–5606. http://doi.org/10.1128/AEM.68.11.5600-5606.2002

Reimer, L. G. (1993). Q fever. *Clinical Microbiology Reviews*, *6*(3), 193–198.

Reusken, C., van der Plaats, R., Opsteegh, M., de Bruin, A., & Swart, A. (2011). *Coxiella burnetii* (Q fever) in *Rattus norvegicus* and *Rattus rattus* at livestock farms and urban locations in the Netherlands; could *Rattus* spp. represent reservoirs for (re)introduction? *Preventive Veterinary Medicine, 101*(1-2), 124–130. http://doi.org/10.1016/j.prevetmed.2011.05.003

Roest, H. I. J., Tilburg, J. J. H. C., van der Hoek, W., Vellema, P., van Zijderveld, F. G., Klaassen, C. H. W., & Raoult, D. (2011). The Q fever epidemic in The Netherlands: history, onset, response and reflection. *Epidemiology Infection*, *139*(1), 1–12. http://doi.org/10.1017/S0950268810002268

Rousset, E., Berri, M., Durand, B., Dufour, P., Prigent, M., Delcroix, T., Touratier, A., & Rodolakis, A. (2009). *Coxiella burnetii* shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds. *Applied and Environmental Microbiology*, *75*(2), 428–433. http://doi.org/10.1128/AEM.00690-08

Schimmer, B., Luttikholt, S., Hautvast, J. L. A., Graat, E. A. M., Vellema, P., & van Duynhoven, Y. T. H. P. (2011). Seroprevalence and risk factors of Q fever in goats on commercial dairy goat farms in the Netherlands, 2009-2010. *BMC Veterinary Research*, *7*, 81–95. http://doi.org/10.1186/1746-6148-7-81

Spicer, A. J. (1978). Military significance of Q fever: a review. *Journal of the Royal Society of Medicine, 71*(10), 762–7. http://doi.org/10.1177/014107687807101011

Stein, A., & Raoult, D. (1999). Pigeon pneumonia in Provence: a bird-borne Q fever outbreak. *Clinical Infectios Disease1*, *29*(3), 617–620. http://doi.org/10.1086/598643

Tatsumi, N., Baumgartner, A., Qiao, Y., Yamamoto, I., & Yamaguchi, K. (2006). Detection of *Coxiella burnetii* in market chicken eggs and mayonnaise. *Annals of the New York Academy of Sciences*, *1078*(1), 502–505. http://doi.org/10.1196/annals.1374.096

Taylor, D. J., & Philbey, A. W. (2010). *Salmonella* infections in garden birds and cats in a domestic environment. *The Veterinary Record*, *167*(1), 26–27. http://doi.org/10.1136/vr.c3156

Thomas, D. R., Treweek, L., Salmon, R. L., Kench, S. M., Coleman, T. J., Meadows, D., Morgan-Capner, P., & Caul, E. O. (1995). The risk of acquiring Q fever on farms: a seroepidemiological study. *Occupational and Environmental Medicine*, *52*(10), 644–647. http://doi.org/10.1136/oem.52.10.644

Thompson, M., Mykytczuk, N., Gooderham, K., & Schulte-Hostedde, A. (2012). Prevalence of the bacterium *Coxiella burnetii* in wild rodents from a Canadian natural environment park. *Zoonoses and Public Health*, *59*(8), 553–560. http://doi.org/10.1111/j.1863-2378.2012.01493.x

Tilburg, J. J. H. C., Roest, H.-J. I. J., Buffet, S., Nabuurs-Franssen, M. H., Horrevorts, A. M., Raoult, D., & Klaassen, C. H. W. (2012). Epidemic genotype of *Coxiella burnetii* among goats, sheep, and humans in the Netherlands. *Emerging Infectious Diseases*, *18*(5), 887–9. http://doi.org/10.3201/eid1805.111907

Tissot-Dupont, H., Amadei, M.-A., Nezri, M., & Raoult, D. (2004). Wind in November, Q fever in December. *Emerging Infectious Diseases*, *10*(7), 1264–9. http://doi.org/10.3201/eid1007.030724

To, H., Htwe, K. K., Kako, N., Kim, H. J., Yamaguchi, T., Fukushi, H., & Hirai, K. (1998). Prevalence of *Coxiella burnetii* infection in dairy cattle with reproductive disorders. *The Journal of Veterinary Medical Science*, *60*(7), 859–861. http://doi.org/10.1292/jvms.60.859

van der Hoek, W., Dijkstra, F., Schimmer, B., Schneeberger, P. M., Vellema, P., Wijkmans, C.,ter Schegget, R., Hackert, V., & van Duynhoven, Y. (2010). Q fever in the Netherlands: an update on the epidemiology and control measures. *Euro Surveillance*, *15*(12), 57–60. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20350500

Whelan, J., Schimmer, B., Schneeberger, P., Meekelenkamp, J., Ijff, A., van der Hoek, W. V, & van Beest Holle, M. R.-D. R. (2011). Q Fever among culling workers, the Netherlands, 2009-2010. *Emerging Infectious Diseases*, *17*(9), 1719–1723. http://doi.org/10.3201/eid1709.110051

Wildman, M. J., Smith, E. G., Groves, J., Beattie, J. M., Caul, E. O., & Ayres, J. G. (2002). Chronic fatigue following infection by *Coxiella burnetii* (Q fever): ten-year follow-up of the 1989 UK outbreak cohort. *Quarterly Journal of Medicine*, *95*(8), 527–538. http://doi.org/http://dx.doi.org/10.1093/qjmed/95.8.527

CHAPTER ONE

Host species and spatial prevalence of *Coxiella burnetii* in Southern Ontario: Wildlife as a reservoir?

Abstract

Q fever is a zoonotic disease caused by *Coxiella burnetii*, a strictly obligate, intracellular bacterial pathogen. The most commonly identified source of human infection is infected parturient small ruminants, including dairy goats. Infected goats shed infectious bacteria in birthing tissues, urine, feces and milk. Recently, *C. burnetii* was detected in six of seven wild rodent species in Algonquin Provincial Park, Ontario; however, the role of wildlife in the maintenance and transmission of *C. burnetii* is not clear. My primary objective was to compare the prevalence of *C. burnetii* DNA in dairy goats, other domestic animals, and wildlife on goat farms and adjacent natural areas. From April to August 2014, genital, fecal and milk samples were collected from goats on 16 Ontario dairy goat farms. Fecal samples and genital swabs were also collected from other resident animals (19 cats, 4 chickens, 6 cows, 13 dogs, 5 horses, 2 pigs), and from wildlife (167 deer mice, 20 house mice, 3 opossums, 86 raccoons, 3 red-backed voles, 14 red squirrels and 2 skunks) live-trapped on-farm and from 14 adjacent natural areas. *Coxiella burnetii* was detected by PCR in samples from 89.2% (404/453) of goats, 68.8% (33/48) of other farm animals, 64.7% (44/68) of wild animals sampled on farms, and 58.1% (165/284) of wild animals sampled in natural areas. *Coxiella burnetii* was detected at all study sites and the prevalence in wildlife was not statistically different between farms and adjacent natural areas, independent of site distances. These findings suggest that wildlife may form part of the *C. burnetii* reservoir in Ontario, Canada.

Keywords: *Coxiella burnetii*, dairy goats, wildlife, DNA, reservoir

Introduction

Coxiella burnetii is a strictly intracellular, gram-negative bacterium (Maurin and Raoult, 1999) that has been reported worldwide, except for New Zealand (Hilbink et al., 1993). The large cell variant (LCV) replicates rapidly and is fragile in the environment, however, the small cell variant (SCV), that develops after several days of infection, remains stable under harsh environmental conditions including high temperatures and low pH (T. J. Marrie, 2003). The main source of human infection is infected and shedding small ruminants (Maurin and Raoult, 1999; Mcquiston and Childs,2002). Humans can become infected when they inhale infectious bacteria shed by these species (Roest et al., 2011). *Coxiella burnetii* is a multi-host pathogen and there is evidence to suggest that humans might also become infected by ingestion of contaminated milk (reviewed in Marrie and Raoult, 1997), and via inhalation of infectious bacteria shed by species other than small ruminants, including cats, dogs, and rabbits (Buhariwalla et al., 1996; Marrie et al., 1986; Marrie and Raoult, 1997).

Q fever and Coxiellosis

Approximately half of human patients infected with *C. burnetii* will remain asymptomatic (Maurin and Raoult, 1999), whereas the remaining half will develop clinical disease, referred to as Q fever. Signs and symptoms can include fever, nausea, headache, chest pain, hepatitis, and atypical pneumonia (Reimer, 1993). Chronic Q fever can occur in infected individuals under certain conditions (e.g., pregnancy, immunosuppression, heart valve lesions, and vascular abnormalities) (Carcopino et al., 2009; Fenollar et al., 2001), and can lead to complications such as meningoencephalitis, myocarditis, chronic endocarditis, and chronic fatigue syndrome (Raoult et al., 2005;

Wildman et al., 2002). In 1999, *C. burnetii* infection in humans became reportable in the United States, and although it became nationally reportable in Canada in 1959 (Mckiel, 1964) surveillance efforts were discontinued in 1978 (Public Health Agency of Canada, 2015). To date, the reporting of human cases in Canada is done by provincial Ministries of Health in most provinces, including Ontario.

Animal infection with *C. burnetii* differs from human infection and is referred to as coxiellosis. Small ruminants are most susceptible to clinical disease, and signs include reproductive complications, such as weak or unviable offspring, stillbirths, abortion with marked placentitis, and endometritis (Berri et al., 2001; Bildfell et al., 2000; Guatteo et al., 2012; Moeller, 2001; To et al., 1998a). While small ruminants are most susceptible to disease and serve as the primary reservoir of human infection, other domestic animals, including cattle (*Bos taurus*; Guatteo et al., 2012), domestic horses (*Equus ferus caballus*; Marenzoni et al., 2013), chickens (*Gallus gallus domesticus*; Tatsumi et al., 2006), camels (*Camelus dromedarius*; Mohammed et al., 2014), water buffalo (*Bubalus bubalis*; Perugini et al., 2009), stray and pet cats (*Felis catus*; Komiya et al., 2003) and dogs (*Canis lupus familiaris*; Buhariwalla et al., 1996) are known sources of *C. burnetii* for human infection. Similarly, numerous wildlife species are known hosts of *C. burnetii* worldwide, including brown and black rats (*Rattus norvegicus* and *Rattus rattus* respectively; Reusken et al., 2011), European hares (*Lepus europaeus*; Astobiza et al., 2011), roe deer (*Capreolus capreolus*; Astobiza et al., 2011), coyotes (*Canis latrans*; Enright et al., 1971; reviewed in Mcquiston and Childs, 2002), red foxes (*Vulpes vulpes*; Meredith et al., 2014), vultures (*Gyps fulvus*), black kite (*Milvus migrans*), wild birds (Astobiza et al., 2011; reviewed in Mcquiston and Childs, 2002), brush rabbits

(*Sylvilagus bachmani*; Enright et al., 1971), other rodent species (Meredith et al., 2014), and ticks (Angelakis and Raoult, 2010). The serological prevalence of *C. burnetii* for wild rodent species, has been documented between 2% for deer mice(*Peromyscus maniculatus*) and pinyon mice (*Peromyscus truii*), and 53% for norway rats (*Rattus norvegicus*; Meerburg & Reusken, 2011). Canada is included in the geographic distribution of *C. burnetii*, and animal disease is annually notifiable in Canada. Recent studies have detected *C. burnetii* in small ruminants and wildlife in Ontario (Meadows et al., 2015; Thompson, et al., 2012).

Coxiellosis in Ontario Wildlife and Domestic Ruminants

In a recent study, 63% (48/76) of Ontario dairy goat farms surveyed had at least one seropositive goat, indicating that exposure to *C. burnetii* is common in goats in Ontario (Meadows et al., 2015). Moreover, in 2010 *C. burnetii* was detected in six of seven wild rodent species sampled in Algonquin Provincial Park, Ontario, with woodland jumping mice (*Napaeozapus insignis*) exhibiting the highest prevalence (83%) and no *C. burnetii* DNA detection from eastern chipmunks (*Tamias striatus*) (0%) (Thompson et al., 2012). While the transmission pathway for human infection of *C. burnetii* has been extensively studied (reviewed in Marrie and Raoult, 1997), the epidemiology of *C. burnetii* at the livestock-wildlife interface is not well understood. In particular, it is unknown whether wildlife serve as a source of *C. burnetii* infection of livestock, or whether *C. burnetii* is spilling over from livestock to wildlife populations.

The objectives of this study were to: 1) determine the prevalence of *C. burnetii* in wildlife and domestic animals on dairy goat farms and natural areas, 2) investigate the spatial prevalence of *C. burnetii* in southern Ontario, and 3) investigate the potential role

of wildlife in the transmission dynamics of *C. burnetii*. If wildlife are acting as spillover hosts exposed to *C. burnetii* via infected livestock but not able to independently maintain the infection, then I predict that *C. burnetii* will occur only in wildlife living in close association with infected livestock. Alternatively, if wildlife are able to maintain *C. burnetii* infection independent of livestock, then I predict that *C. burnetii* will also occur in wildlife in natural areas.

Methods

Study Sites

Sixteen dairy goat farms that produce milk for human consumption, selected from a list of Ontario dairy goat farms licensed to produce milk with the Ontario Ministry of Agriculture, Food and Rural Affairs that had previously participated in a study examining seroprevalence and risk factors for *C. burnetii* exposure (Meadows et al., 2015) were included in this study. Eleven seropositive farms were randomly selected from the 48 seropositive farms identified by Meadows et al. (2015). Five seronegative farms were randomly selected from the 28 seronegative farms identified by Meadows et al. (2015) and were included in this study as control farms. Selected farms were greater than five km apart from one another. A total of 14 natural areas were selected, and were located between two and 26.5 km adjacent to the last 14 randomly selected farm sites. Natural areas were defined as areas used by humans but not agricultural animals and included conservation areas. Each site was sampled for a 1-week period between the end of April and August 2014.

Dairy Goats - Field Methods and Sample Collection

At each farm site, a systematic randomization of up to 30 lactating, recently fresh dairy goats were sampled. A sample size of 30 would allow the estimate of 15% prevalence with 95% confidence and 10.75% allowable error (Sergeant, 2016). The intent was to select goats more likely to be PCR positive on vaginal swabs, feces or milk; research shows that shedding is more prevalent soon after kidding (reviewed in Rodolakis, 2009). However, if fewer than 30 goats had recently given birth on a particular farm, does further in their lactation were included in sampling. Samples

collected from each goat included: two samples of 30 mL of milk (an equal amount of milk was collected from each teat using sterile technique); two genital swabs; five to seven fecal pellets from the rectum using a clean glove, or if no fecal material was present, two fecal swabs. For genital swab samples, individual sterile cotton swabs with wooden handles wrapped in pairs (Covidien Ireland Limited, IDA Business and Technology Park, Tullamore) were introduced into the vagina after parting the vulvar lips, inserted to the cervix and then rotated several times to maximize exposure of the swab surface area. Fecal material was collected with the aid of sterile lubrication and a gloved finger inserted into the rectum to tease out the fecal material. Collected fecal material was placed inside 60 mL sterile urine cups (Starplex Scientific Inc., Etobicoke, ON, Canada) labeled with the site, animal identification number and sample type. If no fecal material was present, individual swabs (as described for genital swabbing) were introduced into the anus and rotated several times in the rectum. The sterile cotton swabs used for genital and fecal swab sampling were immediately placed and, the wooden handles cut, to fit inside 2 mL sterile Cryovials (Simport Scientific, Beloeil, QC, Canada) labelled with the site, animal ID and sample type. Without disinfecting the teats, milk expressed from the teat into 30 mL sterile urine cups (Fisherbrand, Fisher Scientific UK Ltd, Leicestershire, England), and before storage, the outside of each urine cup was wiped with 90% Ethanol solution to remove any residual milk. All samples were then stored in -20^{o}C. To minimize contamination between animals, researchers wore nitrile gloves that were changed between each animal sampled. Approval for dairy goat handling and sampling procedures was granted by the Laurentian University Animal Care Committee (certificate number 2014-01-02).

Other Resident Farm Animals - Field Methods and Sample Collection

When present, other domestic animals residing on the goat farm were sampled. Docile cats and dogs were restrained to collect two genital swabs and two fecal swabs following the same protocol used for goats (approved protocol from Laurentian University Animal Care Committee, certificate number 2014-01-02). Fresh fecal samples were also collected from the environment left by cats, dogs and any other farm animals, including horses, pigs, cows, chickens and ponies, that were too aggressive or too large to restrain. Once collected, all samples were placed in 60 mL sterile urine cups (Starplex Scientific Inc., Etobicoke, ON, Canada), labeled with the site, animal ID and sample type, and stored in -20^{o}C.

Small Mammal Wildlife - Field Methods and Sample Collection

Small mammals were live trapped on 14 natural areas and 16 Ontario dairy goat farms, so 14 farms were partnered with 14 adjacent natural areas. A combination of Sherman (H.B. Sherman Traps, Tallahassee, FL, USA) and Longworth (Rogers Manufacturing Co., Kelowna, BC, Canada) live traps were used to maximize the likelihood of sampling the complete diversity of small mammals at each study site (Anthony et al., 2005). Traps were set at dusk and checked the following morning at dawn, repeated daily for a 4-5 day period at each study site, with the farm site and adjacent natural area site being sampled simultaneously. Traps were set in lines of 10 and each trap was separated by approximately 10 m. GPS waypoints were recorded for the location of the first and last trap of each trap line. At any given site, a total of 30-100 live-traps were set. Each trap was baited with peanut butter and black oil sunflower seeds previously soaked in water. All traps were supplied with polyester bedding to provide

insulation to trapped animals during periods of inclement weather. Traps were not set if temperatures fell below 10^{o}C or if severe weather, such as thunderstorms, were likely to take place. All small mammal wildlife handling and sampling methodology was approved by the Laurentian University Animal Care Committee (certificate number 2014-01-03).

All captured individuals were transferred from the trap into an appropriately sized handling bag made of mesh fabric to facilitate sampling. Weight, sex, and reproductive condition (e.g., pregnant/lactating or not) were recorded and fecal and genital samples were collected. Fresh fecal material, if available, was collected with sterile forceps and placed inside 2 mL sterile Cryovials (Simport Scientific, Beloeil, QC, Canada), labeled with the site, animal ID and sample type. If the individual did not defecate while being handled, and no fresh fecal material available inside the live trap, then two fecal swab samples were collected. Individual swabs were rotated several times on the anus of individuals to maximize exposure of the swab surface area. Two genital swabs were also collected from each individual. Individual swabs were rotated several times on the external genital area of the animal. Sterile cotton swabs with aluminum handles wrapped individually (Puritan Medical, Guilford, Maine, USA) were used to collect all swab samples. Once the swab samples were collected, they were cut to fit inside 2 mL sterile Cryovials labelled with the site, animal ID and sample type. All samples were then stored in -20^{o}C. Lastly, individuals were marked using coloured non-toxic slide staining dye (The Davidson Marking System, Bradley Products Inc., Minnesota, USA) to avoid re-sampling the same individual in the event of a recapture. After all samples were collected and the animal was appropriately marked, animals were released at the same location where they were captured.

Medium-sized Mammal Wildlife - Field Methods and Sample Collection

Medium-sized mammals were live-trapped at all 14 natural areas and 16 Ontario dairy goat farms. Live traps (Tomahawk Live Trap Co., Tomahawk, WI, USA) were set in pairs at each site and strategically placed (e.g., on the floor along interior barn walls, or parallel to a piece of wood on the ground in a wood pile) to maximize the likelihood of capture. At any given site, a total of 20-40 pairs of live-traps were set. Traps were set at dusk, and checked the following morning at dawn for a 4-5 day period. Canned sardines in oil were used as bait, and traps were set in locations where sufficient cover was available to protect trapped animals from inclement weather conditions. All medium-sized mammal wildlife handling and sampling protocols were approved by the Laurentian Animal Care committee (certificate number 2014-01-04).

Prior to sampling the captured animal, towels were used to cover the traps to help keep the individual calm. Weight and sex were recorded and samples were collected while animals were in the trap. Reproductive condition for these species could not be assessed, as they were not manually handled. If the individual defecated inside the trap, then two fecal swabs were collected from the material; otherwise, two fecal swab samples were collected by inserting individual swabs into the rectum of the individual and rotating several times. For males, two genital swabs were collected by rotating the swab on the external genital area. For females, two genital swabs were collected by inserting the swab into the vagina up to the cervix of the individual and rotating several times. All sampled individuals were marked using the same non-toxic slide staining dye used for small mammal wildlife species and released at the same location as their capture site. Sterile cotton swabs with wooden handles wrapped in pairs (Covidien Ireland Limited, IDA

Business and Technology Park, Tullamore) were used for all swab samples. Once individual swab samples were collected, the wooden handles were cut to fit inside 2 mL sterile Cryovials (Simport Scientific, Beloeil, QC, Canada) and labelled with the site, animal ID and sample type. All samples were then stored in -20^{o}C.

When anaesthesia was required for sampling (e.g. skunks and aggressive raccoons), a premixed intramuscular injection of ketamine hydrochloride (Vetalar 100 mg/ml; Bioniche Animal Health, Belleville, ON, Canada; 5 mg/kg bw) and dexmedetomidine hydrochloride (Dexdomitor 0.5 mg/ml; Pfizer Animal Health, Kirkland, Quebec, Canada; 0.025 mg/kg bw) measured in accordance with species and weight was administered. Once anaesthetized, two fecal swabs and two genital swabs were collected, using the same protocol for individuals not anaesthetized. After sample collection, dexmedetomidine was reversed using atipamazole (Antisedan 5 mg/ml; Pfizer Animal Health, Kirkland, Quebec, Canada; 0.25 mg/kg) and were monitored as they recovered from the anesthetic prior to being released at the location of capture.

DNA Extractions

The DNA from all samples was extracted using two separate DNA isolation kits. The DNA from swab samples (fecal and genital) was extracted using the Qiagen DNEasy Blood and Tissue kit (Qiagen Inc, Mississauga, ON, Canada), according to the DNA Purification from Buccal Swabs Spin Protocol according to manufacturer's protocols (2012). Swab type multiples from the same individual were pooled after Step 3 in the Purification Protocol in order to increase the DNA yield.

Milk samples were heat treated in an incubator (Isotemp Standard Lab Incubator, Fisher Scientific, Waltham, MA, USA) at 75^{o}C for 45 min. The DNA from milk samples

was then extracted using the Qiagen DNEasy Blood and Tissue kit, according to the DNA Purification from Blood or Body Fluids Spin Protocol according to manufacturer's protocols (2012).

The DNA from fecal material was extracted using the PowerFecal DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's protocol (version 12192013). All samples were eluted with 50 µL of the final eluent buffer and each tube was vortexed (G-560; scientific Industries, Bohemia, NY, USA) for 15 sec and centrifuged (accuSpin Micro 17; Thermo Fisher Scientific, Nepean, Canada) at 17 000 x*g* for 1 min. This final step was repeated, and thus all samples had a final elution of 100 µL. Samples were labelled with the site, animal ID and sample type and stored in -20^{o}C. The total DNA yield for all samples was determined by spectrophotometer using a NanoDrop 1000 (Thermo Fisher Scientific, Waltham, MA, USA). DNA yields were determined to ensure each sample contained 5-260 µg/µL for PCR processing.

Real-time PCR Detection of Coxiella burnetii

DNA samples were tested in duplicate using real-time PCR. All PCR amplification and data analysis were performed using a 7900HT sequence detection system thermocycler and associated software (ABI PRISM, Applied Biosystems, Foster City, CA). As described in Pearson et al. (2014), a general 16S rRNA assay was first performed to ensure that PCR quality DNA was extracted. Reactions were prepared in 384-well plates with 1.0 µL of template DNA in a 10 µL final reaction volume that contained 1 x SYBR® Green Master Mix (Applied Biosystems by Life Technologies, Foster City, CA, USA). A forward primer 5'-CCTACGGGDGGCWGCA-3', and reverse

primer 5'-GGACTACHVGGGTMTCTAATC-3' targeting the 16S rRNA gene, were used for this assay. Thermal cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min, followed by 50 cycles of 95°C for 15 sec, 60°C for 1 min, and concluding with a dissociation stage of 95°C for 15 sec, 55°C for 15 sec, and 95°C for 15 sec. Positive samples, with cycle thresholds (C_T) of $C_T < 35$ were then tested for the presence of *C. burnetii* (Kersh et al., 2010).

The detection of *C. burnetii* was carried out using an IS*1111* assay, targeting the multicopy IS*1111* transposable element of *C. burnetii*, with a lower limit of detection of one *C. burnetii* organism/1.0 µL of template DNA (Loftis et al., 2006). Similar to the 16S rRNA gene assay, reactions were prepared in 384-well plates with 1.0 µL of template DNA in a 10 µL final reaction volume that contained 1 x TaqMan® Master Mix (Applied Biosystems by Life Technologies, Foster City, CA, USA). The *IS1111* forward primer 5'-CCGATCATTTGGGCGCT-3', reverse primer 5'-CGGCGGTGTTTAGGC-3', and a probe 6FAM-TTAACACGCCAAGAAACGTATCGCTGTG-MGB, were used for this assay. Thermal cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min, followed by 50 cycles of 95°C for 15 s, and 60°C for 1 min. For each PCR, a no template control was included to detect cross contamination during template addition, as well as a synthetic positive control to ensure proper amplification of DNA templates (Pearson et al., 2014). Samples were considered positive if the peak in their thermal dissociation curve occurring between 85-87°C was double that of any background signals (Appendix 1.1; Vogler et al., 2009). If the peak was less than double the size, then the fluorescence curve was investigated. If there was a peak in the fluorescence curve for the SYBR pigment, then the sample was considered positive (Appendix 1.2; Vogler et al., 2009).

Geographical Mapping

The prevalence of *C. burnetii* in natural areas was determined by the prevalence *C. burnetii* in wildlife (small- and medium-mammal species). The collected GPS waypoints from the live trap line placement at each site was used in conjunction with species prevalence, to construct a site prevalence map indicating *C. burnetii* infection prevalence. *Coxiella burnetii* prevalence on farm sites was determined by: (a) dairy goat prevalence and (b) prevalence of wildlife individuals captured on the farm, and two separate maps created. If one or more goat or wildlife individual sampled on the farm tested PCR positive for *C. burnetii,* then the individual was considered positive overall. Similar to individuals sampled on the farm, wildlife sampled in natural areas were considered positive if one or more of their samples tested positive for *C. burnetii*. The maps were constructed using ArcGIS Desktop 10.1 (Environmental Systems Research Institute, Inc., Redlands, CA, USA).

Data Analyses

Infection prevalence of *C. burnetii* in wildlife hosts on farm and natural areas were compared to infection prevalence in goats on farms using a Fisher's exact test ($p < 0.05$). Since there were multiple comparisons, *p*-values were adjusted using a Bonferroni correction (Rice, 1989; reviewed in Cabin and Mitchell, 2000).

Generalized linear mixed-effect models with binomial errors (*GLMEb*) were used to assess the variation of infection prevalence between and within host wildlife species, as well as between and within host resident farm animal species. The specific model is a random effects logistic regression and is a type of *GLMEb*. For the primary analyses, the individual study site the sample came from was entered as a random effect to account for

site-related infection pressures. In addition, individual study sites were nested within the type of study site (farm or natural area) the sample came from for wildlife species and incorporated as a random effect. Age, sex and reproductive condition were included as fixed effects in different species specific univariate *GLMEb* models. For age, individuals were classified as either adult or juvenile, depending on weight and hair colour. For reproductive condition, individuals were considered reproductive or non-reproductive.

To compare the similarity of infection prevalence and the distance between adjacent sites, due to large sample sizes, only deer mice and raccoons were considered. The distance between sites was determined using a "Point-to-Point" distance calculator in ArcGIS Desktop, which is a measure of Euclidean distance. These distances, along with the absolute difference in overall prevalence for each species at adjacent sites, were included in species-specific linear models. For each model, a power analysis was performed using the r-value from the linear model to determine the required number of site comparisons in order to detect a significant difference. The 95% confidence limits for all associated analyses were calculated using the Clopper-Pearson formula. All statistical models were checked for normality using standard residual assessments and analyses were carried out in R (R Core Team, version 3.2.0, 2015).

Results

All study farm sites had at least one dairy goat positive for *C. burnetii*, including farms that had been identified as seronegative in 2010 (Figure 1.1; Meadows et al., 2015). Similarly, the geographic distribution of wildlife species infected with *C. burnetii* was not confined to one cardinal direction, but was widespread throughout the Ontario study sites (Figure 1.2).

Wildlife species included deer mice (*Peromyscus maniculatus*), house mice (*Mus musculus*), red-backed voles (*Microtus pennsylvanicus*), eastern chipmunks (*Tamias striatus*), red squirrels (*Tamiascurius hudsonicus*), raccoons (*Procyon lotor*), skunks (*Mephitis mephitis*), and opossums (*Didelphis virginiana*). On farms, the average prevalence of *C. burnetii* infection in dairy goats was 89.2% (404/453, 95% CI = 86.0-91.1), wildlife sampled on farms was 64.7% (44/68, 95% CI = 52.2-75.9) and wildlife sampled in adjacent natural areas was 58.1% (165/284, 95% CI = 52.3-63.9). Dairy goats had higher infection prevalence than wildlife on farms or in adjacent natural areas (Figure 1.3). No difference in prevalence of *C. burnetii* infection was detected between wildlife sampled on farm sites and those sampled in adjacent natural area sites, including deer mice and raccoon species ($p > 0.3$; Figure 1.3).

The average and range of IS*1111* C_T values varied for each species, and ranged from 13.0 for dairy goats to 43.6 for raccoons (Appendix 1.3). No significant difference was detected in the prevalence of *C. burnetii* infection among species on farms compared to adjacent natural areas ($p > 0.3$) according to the main *GLMEb* (Table 1.1; Appendix 1.4). Similarly, no significant difference was detected for sex ($p > 0.4$) and reproductive condition ($p > 0.5$) of deer mice, eastern chipmunks, house mice, raccoons and red

squirrels (Appendix 1.5). In addition, no significant difference was detected for age ($p > 0.2$) of deer mice, house mice and raccoons (Appendix 1.5). There were insufficient age data for all other wildlife species, so they were not included in the *GLMEb* model.

Other resident farm animals were sampled on 14 of the 16 dairy goat farms included in the study. *Coxiella burnetii* was detected in five of the six species sampled (Table 1.2). Based on the *GLMEb* model, including site as a random effect, there was no significant difference in *C. burnetii* infection prevalence between resident farm animal host species (Appendix 1.6).

The average distance between study farms and adjacent natural areas was 10.9 km (range 2.0-26.5 km). For deer mice, six paired sites (avg. distance = 10.0 km) were investigated to detect whether host species infection prevalence was related to the distance between adjacent study sites. There was no significant difference between infection prevalence and distance between adjacent sites ($F_{(1, 4)} = 1.17$, $p = 0.34$) (Figure 1.4).

In considering the r-value produced from the linear model for deer mice hosts, a power analysis indicated that a sample size of 32 adjacent sites (64 sites in total) would be required to detect a significant association of paired site distance on the difference of infection prevalence. The same analysis was performed for raccoon hosts, where seven adjacent sites (avg. distance = 9 km) were investigated. For raccoons, no significant difference was detected between infection prevalence and distance between adjacent sites ($F_{(1, 5)} = 0.54$, $p = 0.49$) (Figure 1.5). In considering the r-value produced from the raccoon linear model, a power analysis indicates that a sample size of 77 adjacent sites

(154 sites in total) is required to detect a significant effect of adjacent site distance on the difference in infection prevalence of raccoons.

Discussion

Domestic Animal and Wildlife Infection Prevalence

To the best of my knowledge, this is the first study to describe the detection of *C. burnetii* DNA from domestic and wild animals on dairy goat farms and adjacent natural areas. All farm study sites, including sites that were seronegative in 2010, had at least one dairy goat that tested positive for *C. burnetii* (Figure 1.1). The animals on these farms may have been infected and not yet seroconverted or they may have become infected after the 2010 study (Meadows et al., 2015).

The prevalence of *C. burnetii* infection in other domestic animals sampled on farm sites was 64% (Table 1.2). No significant difference in infection prevalence was detected among the sampled farm animals (Appendix 1.5). *C. burnetii* infection was not detected in chickens, in contrast to other studies (Tatsumi et al., 2006; To et al., 1998b). The infection prevalence for all other resident farm animals sampled in this study was consistent with previous literature; greater than 50% infection prevalence for cats, cows, dogs, horses and pigs (Buhariwalla et al., 1996; Guatteo et al., 2012; Komiya et al., 2003; Marenzoni et al., 2013). Since these species exhibited such high infection prevalence, it is possible that they are involved in the transmission and maintenance of *C. burnetii* on farms.

Coxiella burnetii was detected in all wildlife species tested (Table 1.1) and there was no difference in prevalence of infection between natural areas and farms (Appendix 1.4). It is important to acknowledge that while each *GLMEb* model was robust for most species, this is not true for Eastern chipmunks and Red squirrels. The sample size for these species was too low to allow for a robust model, therefore, the *GLMEb* results of

these species should be interpreted with caution. Moreover, the wide 95% confidence intervals indicate that the infection prevalence results for these species also need to be interpreted with caution. Additionally, it is not clear whether wildlife animals sampled in natural areas were a separate population from wildlife on farms. Thus, the prevalence comparisons of animals sampled on farms compared to natural areas may not be independent populations, and results may be misleading. The wildlife prevalence of infection results are consistent with other studies finding *C. burnetii* in a range of wildlife host species (Astobiza et al., 2011; Angelakis and Raoult, 2010; Enright et al., 1971; Meredith et al., 2014; Mcquiston and Childs, 2002; Reusken et al., 2011). In addition, similar to a study conducted by Enright et al. (1971), age, sex and reproductive condition was not significantly associated with the prevalence of *C. burnetii* in wildlife hosts (Appendix 1.5). *Coxiella burnetii* was detected in a variety of wildlife species from both farms and natural areas, indicating that a variety of wildlife hosts may be involved in the maintenance and transmission of *C. burnetii* both on farms and in natural areas.

Spatial Prevalence

Coxiella burnetii was detected on all farms and natural areas, with 14 of 15 study species (wildlife, livestock and other resident farm animals) having one or more animals positive for *C. burnetii*. There was no significant difference in infection prevalence between wildlife on farms and adjacent natural areas, suggesting that wildlife may be able to maintain *C. burnetii* regardless of geographic location (Reusken et al., 2011). In addition, there was no effect of distance between adjacent farm and natural area sites on deer mice and raccoon infection prevalence (Figure 1.4 and 1.5). Although wildlife were sampled in natural areas at a Euclidean distance of 5-25km from adjacent farm sites, it is

possible that wildlife sampled in natural areas were exposed to other farm sites in the area, and potentially exposed to other sources of *C. burnetii* bacteria. More importantly, geographic location was not associated with wildlife infection prevalence, which suggests that wildlife may be able to maintain *C. burnetii* independent of domestic goats. Further *C. burnetii* strain analysis will help us determine the role of wildlife in the epidemiology of *C. burnetii*.

Potential Role of Wildlife in Coxiella burnetii Transmission

If wildlife maintained *C. burnetii* infection independent of livestock, I predicted that infection prevalence of *C. burnetii* would be the same in wildlife sampled on farms, in adjacent natural areas, and in adjacent sites regardless of the distance between sites. My findings support these predictions, in that there was no significant difference detected among wildlife sampled on farms and adjacent natural areas (Figure 1.3). Similarly, in the investigation between deer mice and raccoon infection prevalence and adjacent site distance, no significant relation was detected (Figure 1.4, 1.5). Although there seems to be a potential adjacent site comparison driving the non-significant relation for the raccoon distance model (Figure 1.5), there was no overall significance. Ultimately, more paired sites need to be considered in future distance comparisons to better test the effect of distance between farm and adjacent natural areas on wildlife infection prevalence of *C. burnetii*. Therefore, my overall findings support Ontario wildlife as potential maintenance species of *C. burnetii*.

In conclusion, *C. burnetii* is geographically widespread in wildlife and domestic animals throughout the southern Ontario sites included in this study. Since there is no significant difference in *C. burnetii* prevalence in wildlife species trapped on farms

compared to natural areas (Table 1.1), it is possible that wildlife are involved in the *C. burnetii* reservoir system. However, further studies, comparing *C. burnetii* strain types in wildlife and domestic animals, are required to fully understand the role of wildlife in the epidemiology of *C. burnetii*.

Literature Cited

Angelakis, E., & Raoult, D. (2010). Q fever. *Veterinary Microbiology*, *140*(3-4), 297–309. http://doi.org/10.1016/j.vetmic.2009.07.016

Anthony, N. M., Ribic, C. A., Bautz, R., & Garland, T. J. (2005). Comparative effectiveness of Longworth and Sherman live traps. *Wildlife Society Bulletin*, *33*(3), 1018–1026. http://doi.org/10.2193/0091-7648(2005)33[1018:CEOLAS]2.0.CO;2

Astobiza, I., Barral, M., Ruiz-Fons, F., Barandika, J. F., Gerrikagoitia, X., Hurtado, A., & García-Pérez, A. L. (2011). Molecular investigation of the occurrence of *Coxiella burnetii* in wildlife and ticks in an endemic area. *Veterinary Microbiology*, *147*(1-2), 190–194. http://doi.org/10.1016/j.vetmic.2010.05.046

Berri, M., Souriau, A., Crosby, M., Crochet, D., Lechopier, P., & Rodolakis, A. (2001). Relationships between the shedding of *Coxiella burnetii*, clinical signs and serological responses of 34 sheep. *The Veterinary Record*, *148*(16), 502–505. http://doi.org/10.1136/vr.148.16.502

Bildfell, R. J., Thomson, G. W., Haines, D. M., McEwen, B. J., & Smart, N. (2000). *Coxiella burnetii* infection is associated with placentitis in cases of bovine abortion. *Journal of Veterinary Dagnostic Investigation*, *12*(5), 419–425. http://doi.org/10.1177/104063870001200505

Buhariwalla, F., Cann, B., & Marrie, T. J. (1996). A dog-related outbreak of Q fever. *Clinical Infectious Diseases*, *23*(4), 753–755. http://doi.org/10.1097/00006454-199705000-00034

Cabin, R. J., & Mitchell, R. J. (2000). To Bonferonni or not to Bonferonni: When and how are the questions. *Bulletin of the Ecoloical Society of America*, *81*(3), 246–248. Retrieved from http://www.jstor.org/stable/20168454

Carcopino, X., Raoult, D., Bretelle, F., Boubli, L., & Stein, A. (2009). Q fever during pregnancy: a cause of poor fetal and maternal outcome. *Annals of the New York Academy of Sciences*, *1166*, 79–89. http://doi.org/10.1111/j.1749-6632.2009.04519.x

Enright, J. B., Franti, C. E., Behymer, D. E., Longhurst, W. M., Dutson, V. J., & Wright, M. E. (1971). *Coxiella burnetii* in a wildlife-livestock environment distribution of Q fever in wild mammals. *American Journal of Epidemiology*, *94*(1), 79–90.

Fenollar, F., Fournier, P.-E., Carrieri, M. P., Habib, G., Messana, T., & Raoult, D. (2001). Risks factors and prevention of Q fever endocarditis. *Clinical Infectious Diseases*, *33*(3), 312–316. http://doi.org/10.1086/321889

Guatteo, R., Joly, A., & Beaudeau, F. (2012). Shedding and serological patterns of dairy cows following abortions associated with *Coxiella burnetii* DNA detection. *Veterinary Microbiology*, *155*(2-4), 430–433. http://doi.org/10.1016/j.vetmic.2011.09.026

Hilbink, F., Penrose, M., Kovacova, E., & Kazar, J. (1993). Q fever is absent from New Zealand. *International Journal of Epidemiology*, *22*(5), 945–949.

http://doi.org/10.1093/ije/22.5.945

Kersh, G. J., Wolfe, T. M., Fitzpatrick, K. A., Candee, A. J., Oliver, L. D., Patterson, N. E., Self, J. S., Priestley, R. A., Loftis, A. D., & Massung, R. F. (2010). Presence of *Coxiella burnetii* DNA in the environment of the United States, 2006 to 2008. *Applied and Environmental Microbiology*, *76*(13), 4469–4475. http://doi.org/10.1128/AEM.00042-10

Komiya, T., Sadamasu, K., Kang, M.-I., Tsuboshima, S., Fukushi, H., & Hirai, K. (2003). Seroprevalence of *Coxiella burnetii* infections among cats in different living environments. *The Journal of Veterinary Medical Science*, *65*(9), 1047–1048. http://doi.org/10.1292/jvms.65.1047

Loftis, A. D., Reeves, W. K., Szumlas, D. E., Abbassy, M. M., Helmy, I. M., Moriarity, J. R., & Dasch, G. A. (2006). Surveillance of Egyptian fleas for agents of public health significance: *Anaplasma*, *Bartonella*, *Coxiella*, *Ehrlichia*, *Rickettsia*, and *Yersinia pestis*. *American Journal of Tropical Medicine and Hygiene*, *75*(1), 41–48. http://doi.org/75/1/41 [pii]

Marenzoni, M. L., Stefanetti, V., Papa, P., Proietti, P. C., Bietta, A., Coletti, M., Passamonti, F., & Henning, K. (2013). Is the horse a reservoir or an indicator of *Coxiella burnetii* infection? Systematic review and biomolecular investigation. *Veterinary Microbiology*, *167*(3-4), 662–669. http://doi.org/10.1016/j.vetmic.2013.09.027

Marrie, T. J. (2003). *Coxiella burnetii* pneumonia. *European Respiratory Journal*, *21*(4), 713–719. http://doi.org/10.1183/09031936.03.00099703

Marrie, T. J., & Raoult, D. (1997). Q fever - a review and issues for the next century. *International Journal of Antimicrobial Agents*, *8*(3), 145–161. http://doi.org/10.1016/S0924-8579(96)00369-X

Marrie, T. J., Schlech, W. F., Williams, J. C., & Yates, L. (1986). Q fever pneumonia associated with exposure to wild rabbits. *The Lancet*, *327*(8478), 427–429. http://doi.org/10.1016/S0140-6736(86)92380-9

Maurin, M., & Raoult, D. (1999). Q fever. *Clinical Microbiology Reviews*, *12*(4), 518–553.

Mckiel, J. A. (1964). Q Fever in Canada. *The Canadian Medical Association Journal*, *91*(11), 573–577.

Mcquiston, J. H., & Childs, J. E. (2002). Q fever in humans and animals in the United States. *Vector Borne and Zoonotic Diseases*, *2*(3), 179–191. http://doi.org/10.1089/15303660260613747

Meadows, S., Jones-Bitton, A., McEwen, S., Jansen, J., & Menzies, P. (2015). *Coxiella burnetii* seropositivity and associated risk factors in goats in Ontario, Canada. *Preventive Veterinary Medicine*, In Press. http://doi.org/10.1016/j.prevetmed.2015.06.014

Meerburg, B. G., & Reusken, C. B. E. M. (2011). The role of wild rodents in spread and

transmission of *Coxiella burnetii* needs further elucidation. *Wildlife Research*, *38*, 617–625. http://doi.org/10.1071/WR10129

Meredith, A. L., Cleaveland, S. C., Denwood, M. J., Brown, J. K., & Shaw, D. J. (2014). *Coxiella burnetii* (Q-fever) seroprevalence in prey and predators in the united kingdom: evaluation of infection in wild rodents, foxes and domestic cats using a modified ELISA. *Transboundary and Emerging Diseases*, *62*(6), 639–649. http://doi.org/10.1111/tbed.12211

Moeller, R. B. J. (2001). Causes of caprine abortion: diagnostic assessment of 211 cases (1991-1998). *Journal of Veterinary Diagnostic Investigation*, *13*(3), 265–270. http://doi.org/10.1177/104063870101300317

Mohammed, O. B., Jarelnabi, A. A., Aljumaah, R. S., Alshaikh, M. A., Bakhiet, A. O., Omer, S. A., Alagaili, A. N., & Hussein, M. F. (2014). *Coxiella burnetii*, the causative agent of Q fever in Saudi Arabia: molecular detection from camel and other domestic livestock. *Asian Pacific Journal of Tropical Medicine*, *7*(9), 715–719. http://doi.org/10.1016/S1995-7645(14)60122-X

Pearson, T., Hornstra, H. M., Hilsabeck, R., Gates, L. T., Olivas, S. M., Birdsell, D. M., Hall, C. M., German, S., Cook, J. M., Seymour, M. L., Priestley, R. A., Kondas, A. V., Clark Friedman, C. L. Price, E. P., Schupp, J. M. Liu, C. M., Pricem L. B., Massung, R. F., Kersh, G. J., & Keim, P. (2014). High prevalence and two dominant host-specific genotypes of *Coxiella burnetii* in U.S. milk. *BMC Microbiology*, *14*(1), 41–50. http://doi.org/10.1186/1471-2180-14-41

Perugini, A. G., Capuano, F., Esposito, A., Marianelli, C., Martucciello, A., Iovane, G., & Galiero, G. (2009). Detection of *Coxiella burnetii* in buffaloes aborted fetuses by IS111 DNA amplification: a preliminary report. *Research in Veterinary Science*, *87*(2), 189–191. http://doi.org/10.1016/j.rvsc.2009.01.005

Public Health Agency of Canada. (2015). Notifiable Diseases On-Line. Retrieved from http://dsol-smed.phac-aspc.gc.ca/dsol-smed/ndis/list-eng.php

Raoult, D., Marrie, T. J., & Mege, J. L. (2005). Natural history and pathophysiology of Q fever. *The Lancet of Infectious Diseases*, *5*(4), 219–226. http://doi.org/10.1016/S1473-3099(05)70052-9

Reimer, L. G. (1993). Q fever. *Clinical Microbiology Reviews*, *6*(3), 193–198.

Reusken, C., van der Plaats, R., Opsteegh, M., de Bruin, A., & Swart, A. (2011). Coxiella burnetii (Q fever) in *Rattus norvegicus* and *Rattus rattus* at livestock farms and urban locations in the Netherlands; could *Rattus* spp. represent reservoirs for (re)introduction? *Preventive Veterinary Medicine*, *101*(1-2), 124–130. http://doi.org/10.1016/j.prevetmed.2011.05.003

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution*, *43*(1), 223–225. Retrieved from http://www.jstor.org/stable/2409177

Rodolakis, A. (2009). Q fever in dairy animals. *Annals of the New York Academy of Sciences*, *1166*, 90–93. http://doi.org/10.1111/j.1749-6632.2009.04511.x

Roest, H. I. J., Tilburg, J. J. H. C., van der Hoek, W., Vellema, P., van Zijderveld, F. G., Klaassen, C. H. W., & Raoult, D. (2011). The Q fever epidemic in The Netherlands: history, onset, response and reflection. *Epidemiology Infection*, *139*(1), 1–12. http://doi.org/10.1017/S0950268810002268

Sergeant, E. S. G. (2016). No Title. Retrieved from http://epitools.ausvet.com.au

Tatsumi, N., Baumgartner, A., Qiao, Y., Yamamoto, I., & Yamaguchi, K. (2006). Detection of *Coxiella burnetii* in market chicken eggs and mayonnaise. *Annals of the New York Academy of Sciences*, *1078*(1), 502–505. http://doi.org/10.1196/annals.1374.096

Thompson, M., Mykytczuk, N., Gooderham, K., & Schulte-Hostedde, A. (2012). Prevalence of the bacterium *Coxiella burnetii* in wild rodents from a canadian natural environment park. *Zoonoses and Public Health*, *59*(8), 553–560. http://doi.org/10.1111/j.1863-2378.2012.01493.x

To, H., Htwe, K. K., Kako, N., Kim, H. J., Yamaguchi, T., Fukushi, H., & Hirai, K. (1998). Prevalence of *Coxiella burnetii* infection in dairy cattle with reproductive disorders. *The Journal of Veterinary Medical Science*, *60*(7), 859–861. http://doi.org/10.1292/jvms.60.859

To, H., Sakai, R., Shirota, K., Kano, C., Abe, S., Sugimoto, T., Takehara, K., Morita, C., Takashima, I., Maruyama, T., Yamaguchi, T., Fukushi, H., & Hirai, K. (1998). Coxiellosis in domestic and wild birds from Japan. *Journal of Wildlife Diseases*, *34*(2), 310–316. http://doi.org/10.1292/jvms.60.859

Vogler, A. J., Birdsell, D., Price, L. B., Bowers, J. R., Beckstrom-Sternberg, S. M., Auerbach, R. K., Beckstrom-Sternberg, J. S., Johansson, An., Clare, A., Buchhagen, J. L., Peterson, J. M., Pearson, T., Vaissaire, J., Dempsey, M. P., Foxall, P., Engelthaler, D. M., Wagner, D. M., & Keim, P. (2009). Phylogeography of *Francisella tularensis*: global expansion of a highly fit clone. *Journal of Bacteriology*, *191*(8), 2474–2484. http://doi.org/10.1128/JB.01786-08

Wildman, M. J., Smith, E. G., Groves, J., Beattie, J. M., Caul, E. O., & Ayres, J. G. (2002). Chronic fatigue following infection by *Coxiella burnetii* (Q fever): ten-year follow-up of the 1989 UK outbreak cohort. *Quarterly Journal of Medicine*, *95*(8), 527–538. http://doi.org/http://dx.doi.org/10.1093/qjmed/95.8.527

Tables and Figures

Table 1.1. Prevalence of *Coxiella burnetii* infection in wildlife species sampled in 2014 on 16 Ontario dairy goat farms, and 14 adjacent natural areas in Ontario, as determined by PCR testing of genital and fecal samples.

Species	Farm		Natural Area	
	Sample Size	% Positive (95% CI)	Sample Size	% Positive (95% CI)
Deer mouse	30	70 (51-85)	137	59 (50-67)
Eastern chipmunk	-	-	57	80 (68-90)
House mouse	20	50 (27-73)	-	-
Opossum	2	100 (16-100)	1	0 (0-98)
Raccoon	12	58 (28-85)	74	43 (32-55)
Red-backed vole	1	100 (3-100)	2	0 (0-84)
Red squirrel	2	100 (16-100)	12	50 (21-79)
Skunk	1	100 (3-100)	1	0 (0-98)

Table 1.2. Prevalence of *Coxiella burnetii* infection in resident farm animals on 16 goat farms in Ontario. Average IS*1111* threshold cycle (C_T) values are indicated. Individual infection prevalence was determined by individual analysis of the dissociation and fluorescence curve for each sample tested for IS*1111*.

Species	**Sample Size**	**% Positive (95% CI)**	**Average IS*1111* C_T (range)**
Cat	18	83 (58.6-96.4)	32 (16.4-37.8)
Chicken	4	0 (0-60.2)	-
Cow	6	50 (11.8-88.2)	38 (37.1-38.8)
Dog	13	69 (38.6-90.9)	27 (19.9-31.2)
Horse	5	80 (28.4-99.5)	38 (35.4-39.7)
Pig	2	100 (15.8-100)	38 (37.1-38.3)

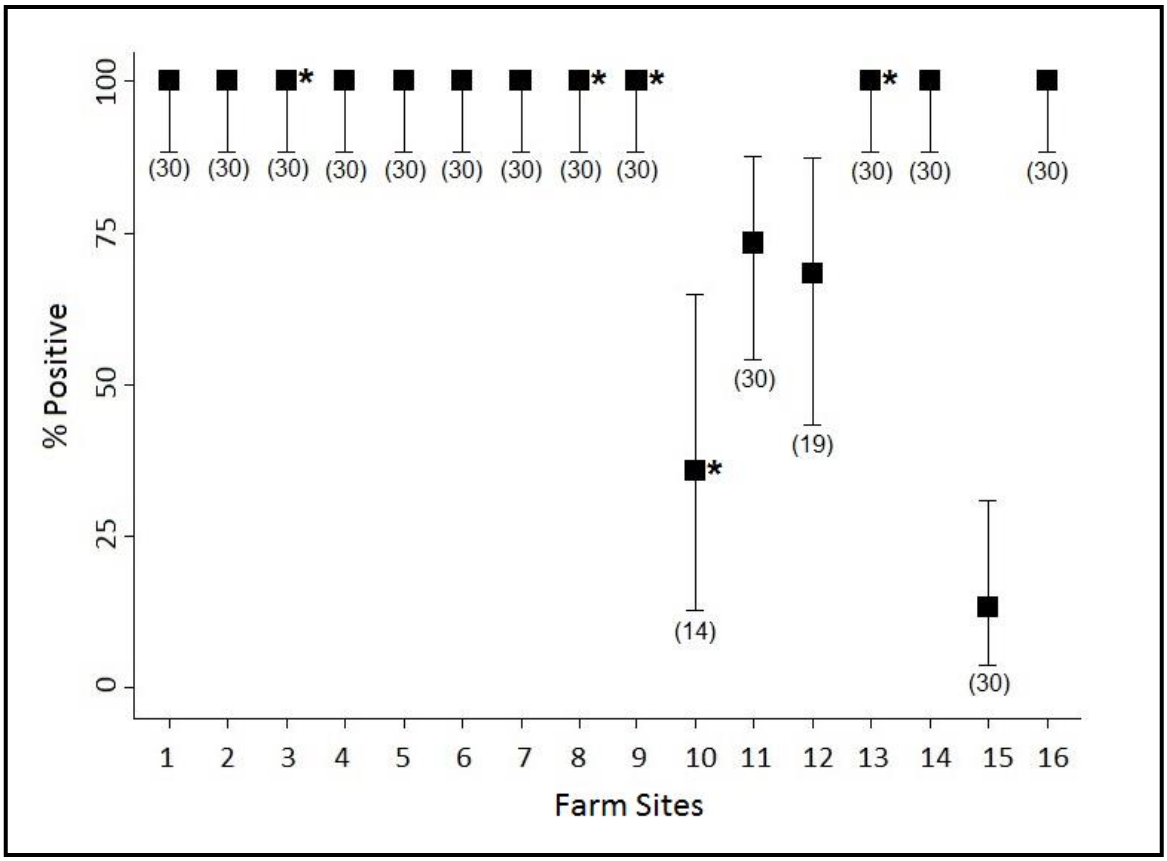


Figure 1.1. Prevalence of *Coxiella burnetii* infection in recently kidded goats on 16 Ontario dairy goat farms. The sample size of each site is indicated in parentheses underneath the lower confidence limit. Error bars represent 95% confidence limits calculated using the Clopper-Pearson formula. Farm sites with an asterisk beside their point are sites that were included in the study as control farms (i.e. were ELISA seronegative in 2010; Meadows et al., 2015).

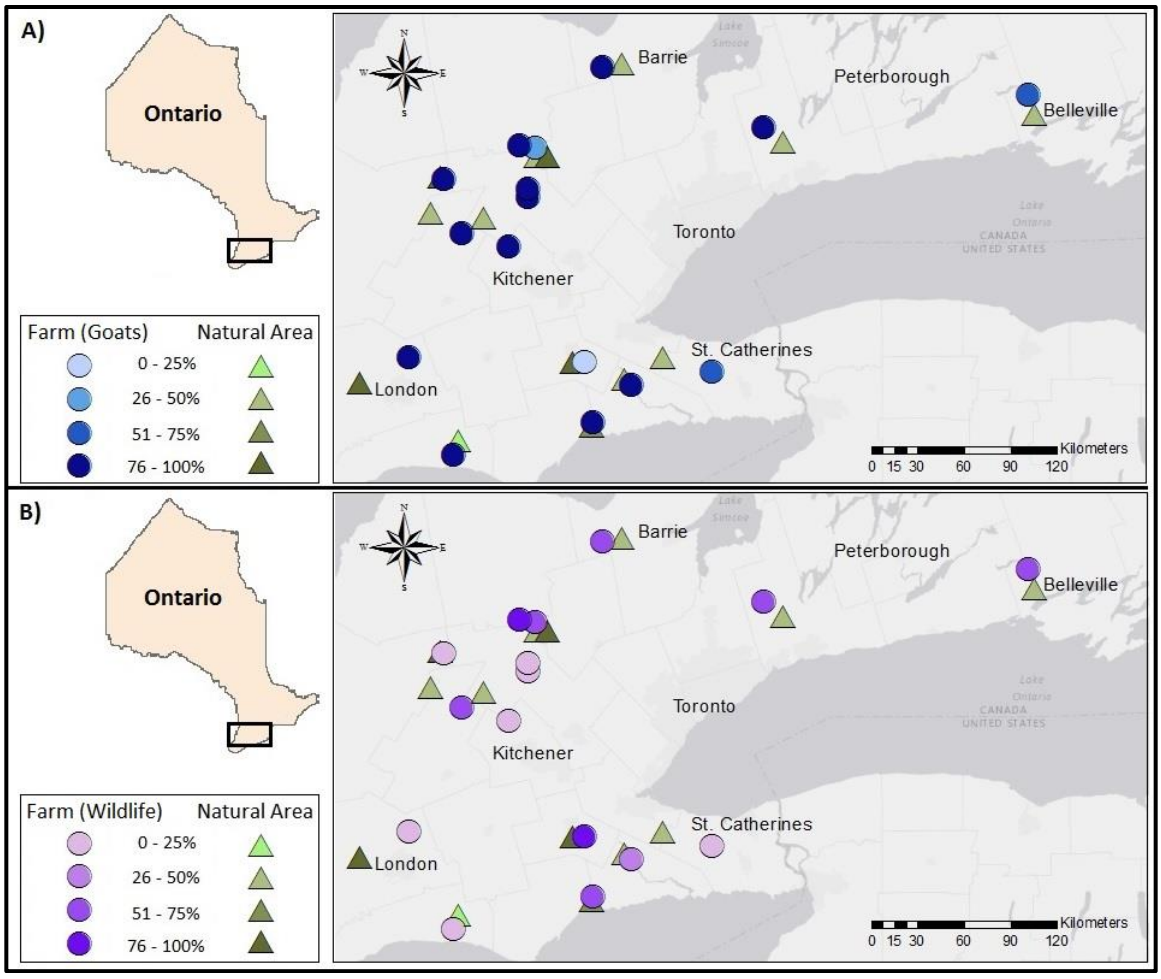


Figure 1.2. Prevalence of *Coxiella burnetii* infection on 30 sites (16 dairy goat farms and 14 adjacent natural areas) sampled across southern Ontario, summer 2014. Darker shades of each colour represent higher prevalence of *C. burnetii* infection. (A) Farm prevalence is representative of recently kidded dairy goats as hosts of current *C. burnetii* infection, and natural area prevalence is representative of wildlife species (deer mice, eastern chipmunks, raccoons, red-backed voles, skunks and opossums) as host species of current *C. burnetii* infection. (B) Farm prevalence is representative of wildlife species (deer mice, house mice, raccoons, red-backed voles, skunks and opossums), and natural area prevalence is representative of wildlife species as in (A).

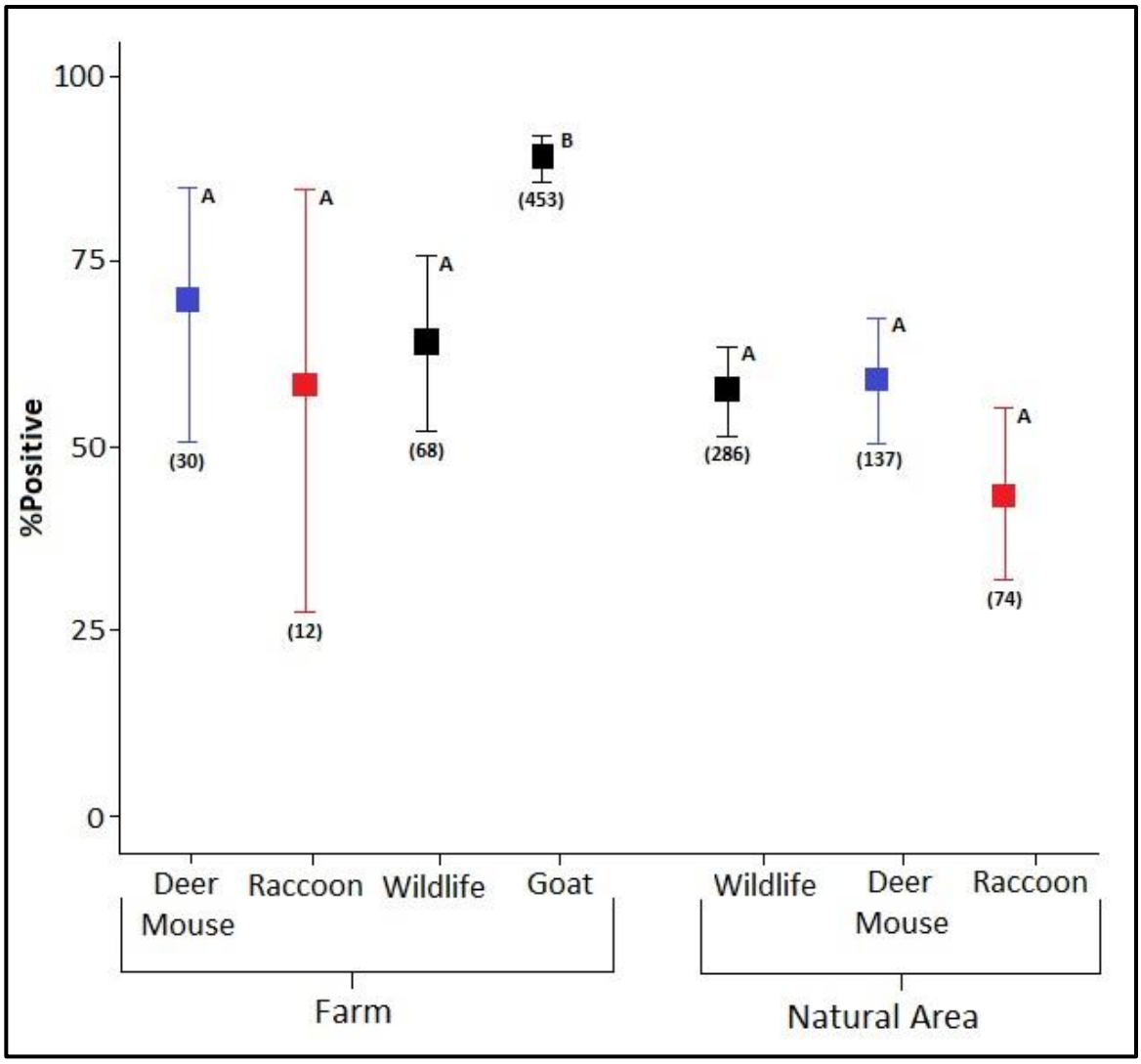


Figure 1.3. Coxiella burnetii infection prevalence detected in dairy goats and wildlife sampled on 16 Ontario dairy goat farms and 14 adjacent natural areas in 2014. The sample size of animals sampled at each site is indicated in parentheses underneath the lower confidence limit. Animal groups with the same letter beside their whiskers are not significantly different based on a Fisher's exact test ($p > 0.05$). Error bars represent 95% confidence limits calculated using the Clopper-Pearson formula.

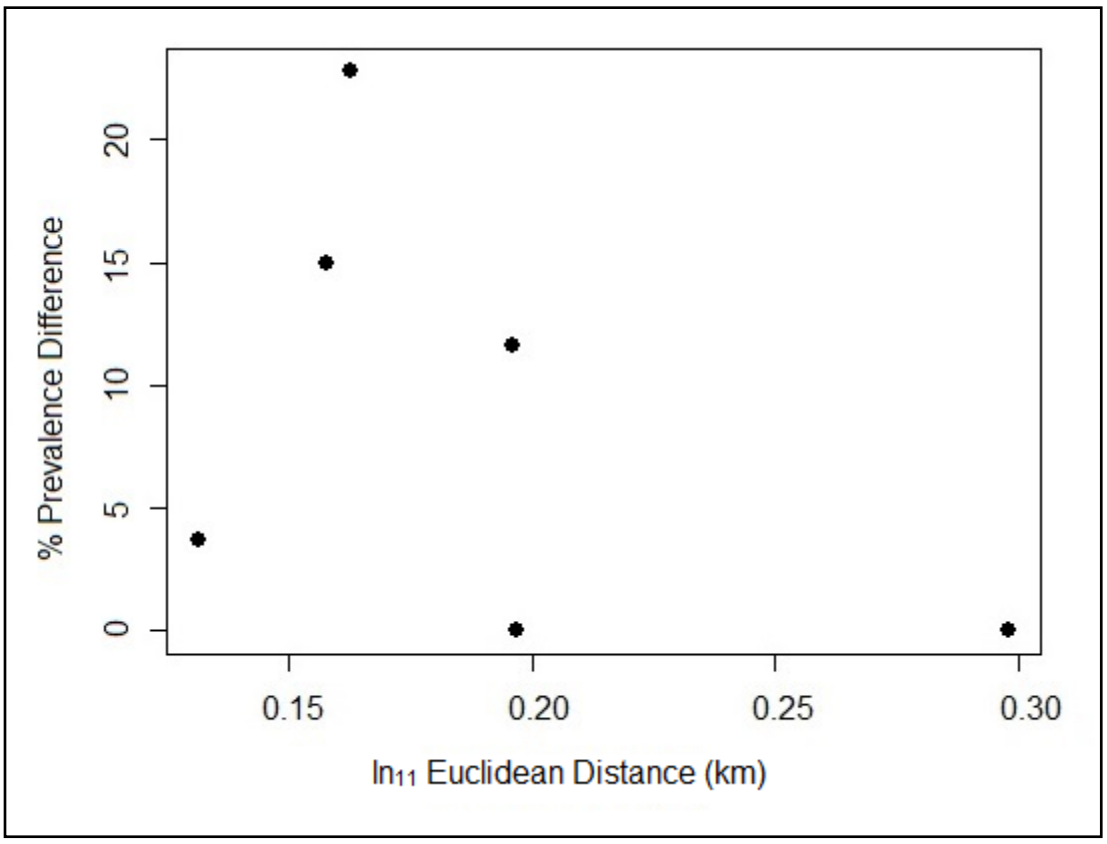


Figure 1.4. Absolute difference of prevalence of *Coxiella burnetii* infection in wild deer mice hosts on farm and adjacent natural area sites, whereby the Euclidean distance between the two sites with a $\ln_{11}$ transformation is considered. No difference was detected between prevalence differences when comparing sites close together and those farther apart ($F_{(1, 4)} = 1.17$, $p = 0.34$).

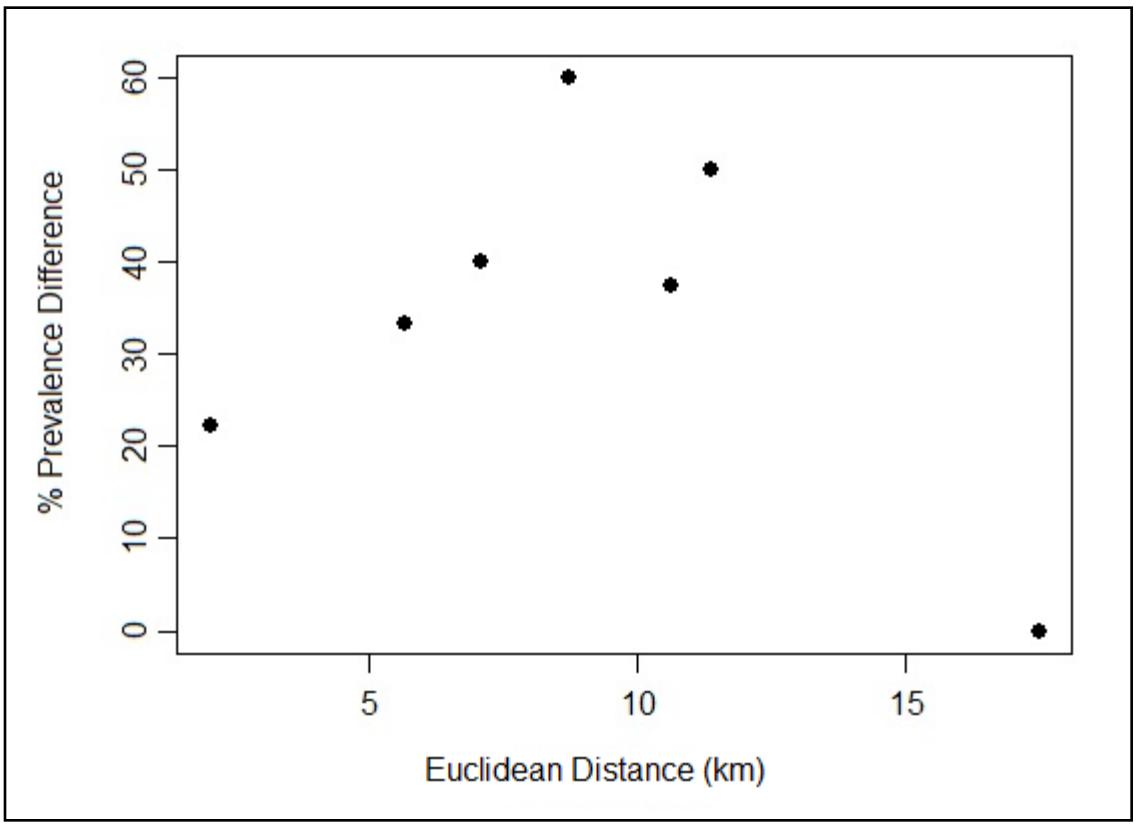
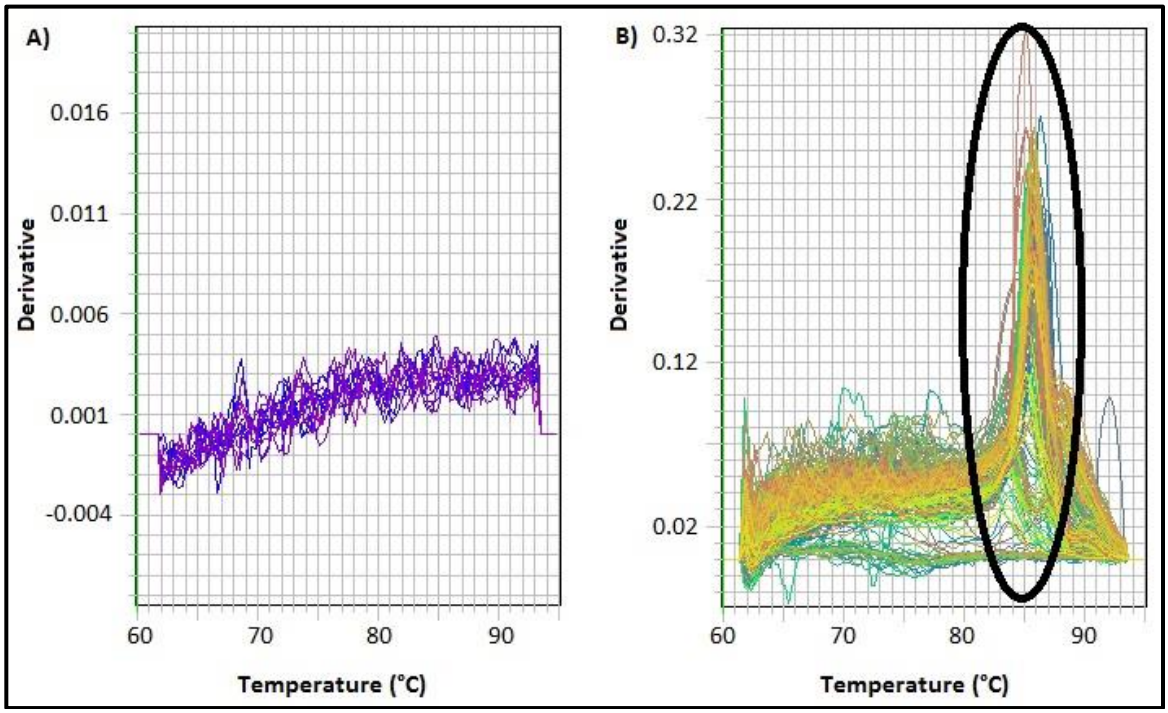
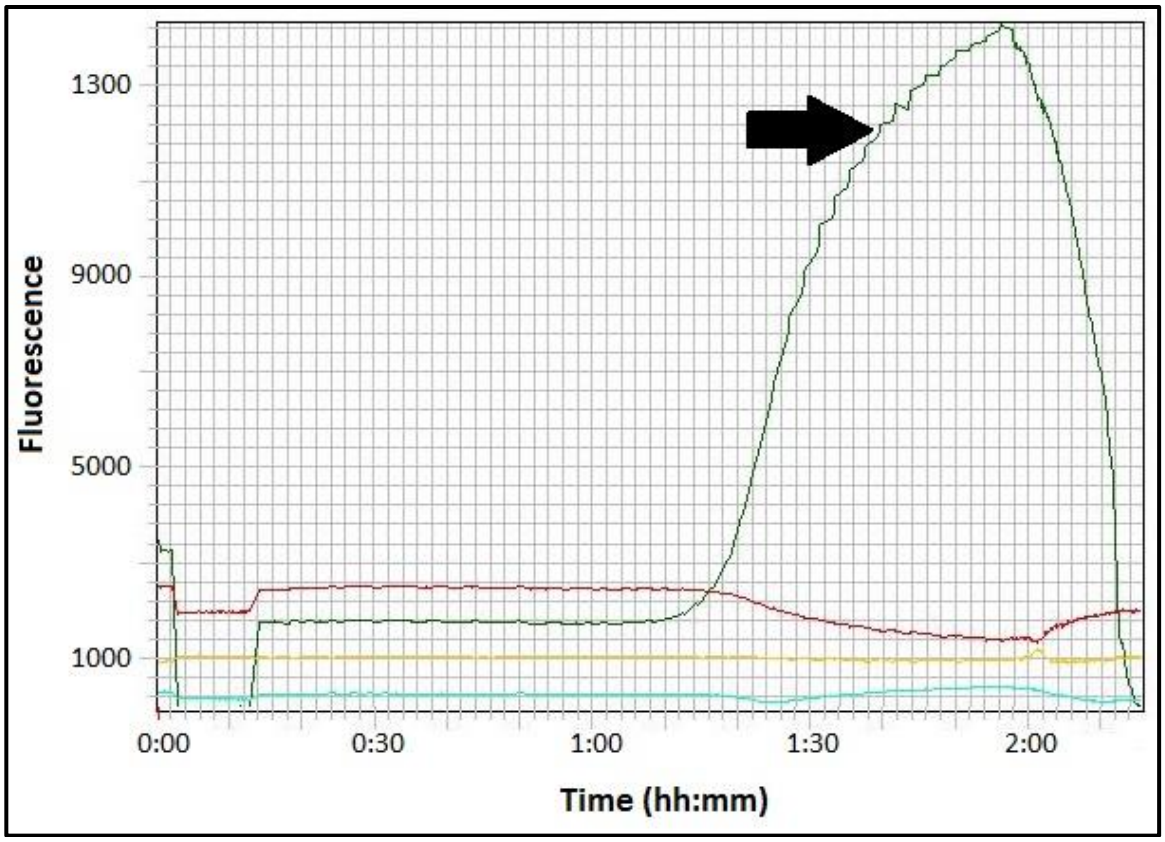


Figure 1.5. Absolute difference of prevalence of *Coxiella burnetii* infection in wild raccoon hosts on farm and adjacent natural area sites, whereby Euclidean distance between the two sites is considered. No difference was detected between prevalence differences when comparing sites close together and those farther apart ($F_{(1, 5)} = 0.54$, $p = 0.49$).

Appendix



Appendix 1.1. (A) Dissociation curves for no template control samples. (B) Dissociation curves for one full plate of DNA samples run for the detection of IS*1111* from *Coxiella burnetii*. Each line indicates a single sample. Samples with a peak double the size or greater than the rest of the curve (indicated within the black oval), are considered *C. burnetii* positive. When the peak is not double in size, then the fluorescent curve is investigated (see Appendix 1.2).



Appendix 1.2. Fluorescence curve for a single sample tested for IS*1111* as representative of *Coxiella burnetii* presence. If a sample fluoresced the SYBR pigment, then a peak in the curve would be apparent, indicating a positive sample. The fluorescence peak for this sample is indicated by the black arrow. If no fluorescent peak was apparent, then the sample was considered negative.

Appendix 1.3. The average and range of IS*1111* critical threshold (C_T) values for the different study species. Wildlife on farms included all wildlife species sampled on registered Ontario dairy goat farms, and wildlife in natural areas included all wildlife species sampled in natural areas. IS*1111* C_T values were determined from all sample types collected from each individual (genital swab, fecal swab, fecal material and milk).

Species	**Avg. IS*1111* C_T**	**Range IS*1111* C_T**
Goats	33.2	13.0-39.5
Wildlife on Farms	36.5	34.6-38.9
Wildlife in Natural Areas	36.6	35.8-37.6
Deer mouse	36.1	32.0-39.3
Eastern chipmunk	35.8	32.5-39.7
House mouse	34.6	20.7-38.9
Opossum	38.9	36.2-41.5
Raccoon	37.4	33.2-43.6
Red-backed vole	35.9	35.9
Red squirrel	36.5	34.1-39.8
Skunk	37.7	37.7

Appendix 1.4. Comparative prevalence of *Coxiella burnetii* infection in selected wildlife sampled on farm sites versus adjacent natural area sites. In the *GLMEb* model, the individual site the species were sampled at was included as a random effect and the type of site (farm or natural area) was nested within the type of site as a random effect. No significant difference was detected for the listed wildlife species, with respect to *C. burnetii* infection prevalence on farms compared to adjacent natural areas. Only the species listed contained enough data to be included within the *GLMEb*.

Species	**Estimate**	**Std. Error**	**Z value**	***p*-value**
Deer mouse	-0.03	0.47	-0.05	0.96
Raccoon	-0.97	0.96	-1.02	0.31
Red squirrel	-37.5	2048	-0.02	0.99

Appendix 1.5. The potential effect of age, sex, and reproductive condition as a variable that influences the infection prevalence of different wildlife host species of *Coxiella burnetii*. In species specific models, the sampling site was nested within the type of site (farm or natural area) and included as a random effect, while age, sex and reproductive condition were included as fixed effects.

Species	**Variable**	**Estimate**	**Std. Error**	**Z value**	***p*-value**
Deer mouse	Age	0.34	0.54	0.63	0.53
	Sex	0.11	0.4	0.27	0.79
	Reproductive Condition	0.26	0.46	0.56	0.58
Eastern chipmunk	Sex	-25.2	603	-0.04	0.97
	Reproductive Condition	24.2	603	0.04	0.97
House mouse	Age	-2.1	2.54	-0.83	0.41
	Sex	0.82	1.78	0.46	0.65
	Reproductive Condition	-2.72	3.58	-0.76	0.45
Raccoon	Age	1.03	0.87	1.18	0.24
	Sex	0.05	0.69	0.07	0.94
	Reproductive Condition	-0.55	0.85	-0.65	0.52
Red squirrel	Sex	-18.6	11496	-0.002	0.99
	Reproductive Condition	19.2	11496	0.002	0.99

CHAPTER TWO

A Comparison of *Coxiella burnetii* DNA Detection in Fecal, Milk and Genital Samples from Dairy Goats and Wildlife in Ontario

Abstract

Q fever is a zoonotic disease caused by *Coxiella burnetii*, a gram-negative, intracellular, zoonotic bacterium. The most commonly identified source of human infection is parturient small ruminants, including dairy goats; however, this bacterium is known to infect other domestic and wild animal species worldwide. Infected animals shed infectious bacterial spores in birthing tissues, urine, feces and milk. To date, there is no suggested sample type for the detection of *C. burnetii* DNA. The objectives of this study were to: 1) compare the prevalence of *C. burnetii* in different sample types (i.e., milk, genital, and fecal samples) from dairy goats and wildlife; and 2) assess the level of agreement among these sample types. Genital, fecal and milk samples were collected from 368 goats on 16 Ontario dairy goat farms, and fecal and genital samples were collected from 248 animals representing five wildlife species that were live-trapped on farms and 14 adjacent natural areas. It was determined that genital and fecal swab samples were the optimal sample types to use for the detection of *C. burnetii* DNA in deer mice, eastern chipmunks and raccoons, yielding the highest proportion positives. Genital swab, fecal swab and fecal material sample types were not significantly different from one another in detecting *C. burnetii* DNA in house mice and red squirrels. Of fecal, milk and genital swab samples, the latter sample type yielded significantly higher proportion positives and thus, were determined the optimal sample type for detecting *C. burnetii* DNA in recently kidded dairy goats. Additional studies, including larger sample sizes from wildlife and goats in different stages of reproduction, are needed to assess the generalizability of the results of this study.

Keywords: *Coxiella burnetii*, DNA, dairy goats, wildlife

Introduction

Coxiella burnetii is a gram-negative, intracellular, zoonotic bacterium that is primarily transmitted via airborne droplets and is known to infect domestic and wild animal species (Maurin and Raoult, 1999; Marrie, 2003; Mcquiston and Childs, 2002; Astobiza et al., 2011). Studies have identified small ruminants, including dairy goats, as the primary human reservoir of *C. burnetii* bacteria (Maurin and Raoult, 1999; Roest et al., 2011). Infection with *C. burnetii* can lead to acute and chronic Q fever in humans (Fenollar et al., 2001; Hartzell et al., 2008) and coxiellosis in other animal species (reviewed in Guatteo et al., 2011; Astobiza et al., 2011; Meredith et al., 2014). Due to the intracellular nature of *C. burnetii*, conventional diagnostic confirmation of infection has proven to be extremely challenging and diagnostic confirmation is often limited to antibody detection from serology samples, including enzyme-linked immunosorbent assays (ELISA), indirect fluorescent antibody tests (IFAT) and complement fixation tests, which indicate exposure rather than infection (CFT) (Field et al., 2000; Field et al., 2002; Villumsen et al., 2009).

Although serology is a good indicator of prior exposure to *C. burnetii*, the detection of *C. burnetii* antibodies during the early onset of acute Q fever infection is often inaccurate due in part to the latent development of antibodies after infection (Wegdam-Blans et al., 2012; Schneeberger et al., 2010). Thus, most studies suggest a combination of serology and PCR tests for accurate detection of infection (Fournier and Raoult, 2003; Schneeberger et al., 2010). Several studies investigating dairy goat infection used serological tests rather than DNA detection (reviewed in Guatteo et al., 2011; Schimmer et al., 2011; Rousset et al., 2007), however, there are studies that have

investigated the DNA detection in different goat sample types, including birthing tissues (Masala et al., 2004; Roest et al., 2011), milk (Berri et al., 2007; Rousset et al., 2009), vaginal mucus (Berri et al., 2007; Rousset et al., 2009) and feces (Rousset et al., 2009). Similarly, *C. burnetii* DNA detection has been investigated in different wildlife species using tissues (e.g., spleen, lung, bone marrow, liver and kidney; Astobiza et al., 2011; Rijks et al., 2011; Reusken et al., 2011), genital swabs (Minor et al., 2013), and feces (Davoust et al., 2014). However, there have been no published studies comparing the detection of *C. burnetii* DNA among these different sample types, in either goats or wildlife.

Determining an Optimal Sampling Procedure for Detecting Coxiella burnetii in Wildlife

Most studies aim to impute, adjust or construct an optimal reference detection method in order to determine detection or accuracy of screening and diagnostic tests (Rutjes et al., 2007; Reitsma et al., 2009). For example, historically, a combination of immunoassay and culture was considered to be the optimal detection method for detecting *Chlamydophila abortus* infection; however, more recently a combination of immunoassay, culture detection, and PCR results was found to be superior (reviewed in Alonzo and Pepe, 1999). When there is not an optimal detection method available, it is important to compare the effectiveness of different detection methods when considering infection status.

Often, when an optimal detection method is available, newly developed methods are compared to the optimal method using Cohen's kappa statistic (Viera and Garrett, 2005). Moreover, Cohen's kappa statistic can also be used to compare different detection

methods when there is not an optimal method available (Allen et al., 2013). Cohen's kappa statistic becomes unstable in situations of very high or low prevalence, as estimated by at least one of the two detection methods (Byrt et al., 1993). This statistic is also affected by the bias of one detection method assigning more positive results than the other (Byrt et al., 1993). Although bias is not normally a problem, it can substantially reduce the kappa score and create misleading results (Mak et al., 2004). With this said, a prevalence-adjusted and bias-adjusted kappa (PABAK) can replace the standard Cohen's kappa and eliminate the two sources of bias (Byrt et al., 1993; Mak et al., 2004). The kappa and PABAK values can be used to interpret the level of agreement between optimal and other sample types (Sim and Wright, 2005). In addition, McNemar's χ^2 test is a widely used method for comparing differences between tests run on paired samples (Lachenbruch and Lynch, 1998). Similarly, a first-order agreement coefficient (AC1) can also be used to assess the level of agreement between detection methods (Gwet, 2008).

The goals of this chapter are to compare the prevalence of *C. burnetii* DNA detection, as well as the level of agreement, among different sample types collected from dairy goats (i.e., genital, fecal and milk samples) and five wildlife species (deer mice (*Peromyscus maniculatus*), eastern chipmunks (*Tamias striatus*), house mice (*Mus musculus*), raccoons (*Procyon lotor*), and red squirrels (*Tamiascurius hudsonicus*) (i.e. genital and fecal samples) in the absence of an optimal reference sample.

Methods

Study Sites and Species

All field methods, including live-trapping, handling of all animal species and sample collection (genital swabs, fecal swabs, fecal material and milk), were conducted as described in Chapter 1, Methods and were approved by the Laurentian University Animal Care Committee (2014-01-02, 2014-01-03, 2014-01-04). A total of 368 dairy goats, and wildlife species including 113 deer mice, 12 house mice, 29 eastern chipmunks, 85 raccoons, and 9 red squirrels were included in this study. DNA extractions and PCR detection of *C. burnetii* in each sample were also conducted as described in Chapter 1, Methods. The DNA from fecal material was extracted using the PowerFecal DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA), which includes standard protocol to remove inhibitors using ceramic beads. The removal of inhibitors for these sample types, allows for a robust comparison of *C. burnetii* DNA detection prevalence to other sample types. All directions were followed according to the manufacturer's protocol (version 12192013).

Data Analyses

To date, there is no consensus as to an optimal sample type(s) to use for *C. burnetii* DNA detection for dairy goats and wildlife species. Therefore, different sample types were collected and compared for each wildlife species (Appendix 2.1) and dairy goats (Appendix 2.2). Either fecal swabs or fecal material were collected from wildlife, not both. Thus, for wildlife species there are two separate sample comparison tests (genital swab/fecal swab and genital swab/fecal material). Fecal material, milk and

genital swab samples were collected from dairy goats; hence, there is one parallel sample comparison test inclusive of all three sample types (genital swab/fecal material/milk).

DNA detection prevalence for individual sample types for each species were calculated by dividing the number of animals positive for each sample by the total number of each sample type tested. The sensitivity of individual sample types for *C. burnetii* DNA detection for each species was calculated by dividing the number of animals positive for each sample type by the number of animals positive for all samples tested in parallel.

A McNemar's χ^2 test was used to determine if *C. burnetii* DNA detection differed between paired sample types. In addition, the levels of agreement beyond chance between sample types were assessed using PABAK and AC1. For each agreement test, the strength of agreement was classified using the criteria by Landis and Koch (1977). All statistical models were checked for normality using standard residual assessments and analyses were carried out in R (R Core Team, version 3.2.0, 2015) using the 'epiR' package for McNemar's χ^2 and PABAK tests. Statistical significance was regarded as $p < 0.5$ and exact *p*-values were calculated for tables with discordant cells <10 using the "exact2x2" package in R.

Results

Wildlife Genital Swab and Fecal Swab Sample Comparison

The prevalence of *C. burnetii* DNA by sample type and species are listed in Table 2.1. The prevalence of *C. burnetii* DNA positive samples ranged from 26-86% and the sensitivity of *C. burnetii* DNA detection ranged from 56-100 (Table 2.1). There was no significant difference between genital swab and fecal swab samples in the detection of *C. burnetii* DNA for any of the wildlife species (p =0.6-0.18; Table 2.2). The level of agreement beyond chance between sample types ranged from 0.17 (slight agreement) for deer mice, according to PABAK and AC1, to 0.68 (substantial agreement) for eastern chipmunks, according to AC1 (Table 2.2).

Wildlife Genital Swab and Fecal Material Sample Type Comparison

The prevalence of *C. burnetii* DNA by sample type and species are listed in Table 2.3. The prevalence of *C. burnetii* DNA positive samples ranged from 0-100% and the sensitivity of *C. burnetii* DNA detection ranged from 0-100 (Table 2.3). For deer mice and eastern chipmunks, genital swabs were significantly more likely to test positive for *C. burnetii* DNA than fecal material ($p < 0.005$ and $p = 0.01$ respectively; Table 2.2). There were no significant differences between genital swab and fecal material sample types for the remaining wildlife species ($p > 0.5$). The level of agreement beyond chance between the sample types ranged from 0.2 (slight agreement) for red squirrels according to PABAK and 0.68 (substantial agreement) for house mice according to AC1 (Table 2.2).

Dairy Goat Sample Type Comparisons

The prevalence of *C. burnetii* DNA positive sample types ranged from 25-89% and the sensitivity of *C. burnetii* DNA detection ranged from 28-99 (Table 2.4). Genital

swabs were significantly more likely to test positive than fecal material or milk samples ($p < 0.0005$; Table 2.5). On the contrary, there was no significant difference in *C. burnetii* detection between fecal material and milk samples. The agreement beyond chance between fecal material and milk sample types ranged from 0.22-0.36 (fair agreement) according to PABAK and AC1 (Table 2.5).

Discussion

This is the first study to compare the effectiveness of different samples types in the detection of *C. burnetii* DNA from an array of wildlife and livestock species. To date, the majority of studies have investigated seroprevalence of *C. burnetii* infection (Villumsen et al., 2009; Marrie, 2003; Berri et al., 2001). The detection of *C. burnetii* DNA shed from infected animals is becoming a more common way to determine infection (Masala et al., 2004; Rousset et al., 2009; Astobiza et al., 2011; Davoust et al., 2014); however, there is currently no recommendation on which sample types are more likely to be positive for wildlife species and dairy goats. By comparing the prevalence, sensitivity of each sample type (genital swab, fecal swab, fecal material and milk sample types) for *C. burnetii* DNA detection, and level of agreement between the sample types, this study is the first to offer a suggested sample type(s) for optimal *C. burnetii* DNA detection for wildlife species and dairy goats.

Coxiella burnetii DNA Detection from Wildlife Samples

There was no significant difference between genital swab and fecal swab sample types in detection of *C. burnetii* DNA for any of the wildlife species in this study. There was substantial agreement between these sample types for eastern chipmunks, indicating that both sample types are equally as effective at detecting *C. burnetii* DNA for this species, and moderate agreement when used in deer mice, house mice and red squirrels. A plausible explanation for the similarity is the difficultly in assuring no cross-contamination between swabbing the genital or anal region in these mammals. The swabs used in the study were the smallest that were commercially available; however, they were still large in relation to the genital area of the wildlife species, particularly the small-

mammals. Thus, when collecting genital swab samples, there was likely overlap onto the anal area, and vice versa for fecal swab samples. So it is possible that genital swab and fecal swab sample types were not region specific, therefore explaining the similarity and level of agreement between them in the detection of *C. burnetii* DNA.

There were significant differences in prevalence of *C. burnetii* DNA between genital swab and fecal material sample types for deer mice and eastern chipmunks, where genital swabs exhibited higher prevalence and sensitivity for DNA detection than fecal material sample types. However, for house mice and red squirrels there was no significant difference and the level of agreement was moderate to substantial, suggesting that genital swab and fecal material sample types are equal in detecting *C. burnetii* DNA. In this study, fecal swabs and genital swabs were equally effective in the detection of *C. burnetii* DNA for wildlife species. In general, the ease of collecting genital swab, fecal swab and fecal material sample types is relatively equal. Collecting fecal material samples is less invasive in that fresh fecal material was collected in the live-traps left by the captured individual. However, the animal still needed to be trapped to ensure its identity. On the other hand, once the individual was comfortably restrained, genital swab and fecal swab sample types were easy to collect.

Optimal Sample Types for Dairy Goats

There was no significant difference in *C. burnetii* DNA prevalence between fecal material and milk samples, and there was fair to moderate agreement between these sample types. On the contrary, I was more likely to detect *C. burnetii* DNA in genital swab samples compared to either milk and fecal material samples. Genital swabs also exhibited the highest sensitivity for DNA detection. Consequently, genital swab samples

are the best sample type to detect *C. burnetii* DNA in dairy goats reported by the owner to have recently kidded. These findings agree with studies that have suggested that infected dairy goats shed highest amounts of infectious *C. burnetii* DNA in birthing tissue (Fournier et al., 1998).

Although this study provides valuable information about the effectiveness of different sample types for detecting *C. burnetii* DNA for dairy goats and an array of wildlife species, some limitations need to be addressed. First, the sample size of individual red squirrels ($n < 10$) and house mice ($n < 15$) were low, which likely reduced statistical power. As such, we may not have had a sufficient sample size to detect a difference if one was present. Second, the inability to compare fecal swab/fecal material sample types among wildlife species reduced my ability to recommend optimal sample types with assurance. A more inclusive study needs to be conducted that compares all sample types within one parallel analysis, similar to Khalesi et al. (2005) investigating the accuracy of multiple diagnostic methods in the detection of beak and feather disease virus in psittacine birds. Third, the sensitivity calculations and scoring system in this study are traditionally used for subjective studies in the presence of an optimal sample type or detection method and not for objective measures as put forth in this study. Thus, the results from this study need to be interpreted with caution. Lastly, the results from this study indicate that genital swab samples are optimal samples for the detection of *C. burnetii* DNA for dairy goats that have recently kidded; however, these results might not be true for dairy goats in other stages of production. Therefore, additional studies need to be conducted to confidently determine optimal sample types in the detection of *C. burnetii* DNA for the wildlife and livestock species included in this study.

In conclusion, I found that genital swabs are the optimal sample for detecting *C. burnetii* DNA in recently kidded dairy goats. Optimal sample types for wildlife varied among species. While genital swab samples were more sensitive and more likely to be positive compared to fecal material samples, there was no significant difference detected between genital swab and fecal swab samples for deer mice and eastern chipmunks. Thus, genital swab and fecal swab sample types may both be suitable for the detection of *C. burnetii* DNA for deer mice and eastern chipmunks, as well as raccoons. Similarly, there was no significance detected between genital swab/fecal swab and genital swab/fecal material sample types for house mice and red squirrels. Thus, all three sample types may be suitable for detecting *C. burnetii* DNA in these species; however further studies with larger sample sizes are required to determine if there is an optimal sample to use for *C. burnetii* DNA detection in wildlife.

Literature Cited

Allen, S. E., Janecko, N., Pearl, D. L., Boerlin, P., Reid-Smith, R. J., & Jardine, C. M. (2013). Comparison of *Escherichia coli* recovery and antimicrobial resistance in cecal, colon, and fecal samples collected from wild house mice (*Mus musculus*). *Journal of Wildlife Diseases*, *49*(2), 432–6. http://doi.org/10.7589/2012-05-142

Alonzo, T. A., & Pepe, M. S. (1999). Using a combination of reference tests to assess the accuracy of a new diagnostic test. *Statistics in Medicine*, *18*(22), 2987–3003. http://doi.org/10.1002/(SICI)1097-0258(19991130)18:22<2987::AID-SIM205>3.0.CO;2-B

Astobiza, I., Barral, M., Ruiz-Fons, F., Barandika, J. F., Gerrikagoitia, X., Hurtado, A., & García-Pérez, A. L. (2011). Molecular investigation of the occurrence of *Coxiella burnetii* in wildlife and ticks in an endemic area. *Veterinary Microbiology*, *147*(1-2), 190–194. http://doi.org/10.1016/j.vetmic.2010.05.046

Berri, M., Rousset, E., Champion, J. L., Russo, P., & Rodolakis, A. (2007). Goats may experience reproductive failures and shed *Coxiella burnetii* at two successive parturitions after a Q fever infection. *Research in Veterinary Science*, *83*(1), 47–52. http://doi.org/10.1016/j.rvsc.2006.11.001

Berri, M., Souriau, A., Crosby, M., Crochet, D., Lechopier, P., & Rodolakis, A. (2001). Relationships between the shedding of *Coxiella burnetii*, clinical signs and serological responses of 34 sheep. *The Veterinary Record*, *148*(16), 502–505. http://doi.org/10.1136/vr.148.16.502

Byrt, T., Bishop, J., & Carlin, J. B. (1993). Bias, prevalence and kappa. *Journal of Clinical Epidemiology*, *46*(5), 423–429. http://doi.org/10.1016/0895-4356(93)90018-V

Davoust, B., Marié, J.-L., de Santi, V. P., Berenger, J.-M., Edouard, S., & Raoult, D. (2014). Three-toed sloth as putative reservoir of *Coxiella burnetii*, Cayenne, French Guiana. *Emerging Infectious Diseases*, *20*(10), 1760–1761. http://doi.org/10.3201/eid2010.140694

Fenollar, F., Fournier, P.-E., Carrieri, M. P., Habib, G., Messana, T., & Raoult, D. (2001). Risks factors and prevention of Q fever endocarditis. *Clinical Infectious Diseases*, *33*(3), 312–316. http://doi.org/10.1086/321889

Field, P. R., Mitchell, J. L., Santiago, A., Dickeson, D. J., Chan, S., Ho, D. W. T., Murphy, A. M., Cuzzubbo, A. J., & Devine, P. L. (2000). Comparison of a commercial Enzyme-Linked Immunosorbent Assay with Immunofluorescence and Complement Fixation Tests for detection of *Coxiella burnetii* (Q Fever) Immunoglobulin M. *Journal of Clinical Microbiology*, *38*(4), 1645–1647.

Field, P. R., Santiago, A., Chan, S., Dhara, B. P., Dickeson, D., Mitchell, J. L., Devine, P. L., & Murphy, A. M. (2002). Evaluation of a novel commercial Enzyme-Linked Immunosorbent Assay detecting *Coxiella burnetii* -Specific Immunoglobulin G for Q fever prevaccination screening and diagnosis. *Journal of Clinical Microbiology*, *40*(9), 3526–3529. http://doi.org/10.1128/JCM.40.9.3526

Fournier, P.-E., Marrie, T. J., & Raoult, D. (1998). Diagnosis of Q Fever. *Journal of Clinical Microbiology*, *36*(7), 1823–1834.

Fournier, P.-E., & Raoult, D. (2003). Comparison of PCR and serology assays for early diagnosis of acute Q fever. *Journal of Clinical Microbiology*, *41*(11), 5094–5098. http://doi.org/10.1128/JCM.41.11.5094

Guatteo, R., Seegers, H., Taurel, A.-F., Joly, A., & Beaudeau, F. (2011). Prevalence of *Coxiella burnetii* infection in domestic ruminants: A critical review. *Veterinary Microbiology*, *149*(1-2), 1–16. http://doi.org/10.1016/j.vetmic.2010.10.007

Gwet, K. L. (2008). Computing inter-rater reliability and its variance in the presence of high agreement. *The British Journal of Mathematical and Statistical Psychology*, *61*(1), 29–48. http://doi.org/10.1348/000711006X126600

Hartzell, J. D., Wood-Morris, R. N., Martinez, L. J., & Trotta, R. F. (2008). Q fever: Epidemiology, diagnosis, and treatment. *Mayo Clinic Proceedings*, *83*(5), 574–579. http://doi.org/10.1016/S0025-6196(11)60733-7

Khalesi, B., Bonne, N., Stewart, M., Sharp, M., & Raidal, S. (2005). A comparison of haemagglutination, haemagglutination inhibition and PCR for the detection of psittacine beak and feather disease virus infection and a comparison of isolates obtained from loriids. *The Journal of General Virology*, *86*, 3039–46. http://doi.org/10.1099/vir.0.81275-0

Lachenbruch, P. A., & Lynch, C. J. (1998). Assessing screening tests: Extensions of McNemar's test. *Statistics in Medicine*, *17*(19), 2207–2217. http://doi.org/10.1002/(SICI)1097-0258(19981015)17:19<2207::AID-SIM920>3.0.CO;2-Y

Landis, J. R., & Koch, G. G. (1977). The measurement of observer agreement for categorical data. *Biometrics*, *33*(1), 159–174. http://doi.org/10.2307/2529310

Mak, H. K. F., Yau, K. K. W., & Chan, B. P. L. (2004). Prevalence-adjusted bias-adjusted k values as additional indicators to measure observer agreement. *Radiology*, *232*(1), 302–303. http://doi.org/10.1148/radiol.2321031974

Marrie, T. J. (2003). *Coxiella burnetii* pneumonia. *European Respiratory Journal*, *21*(4), 713–719. http://doi.org/10.1183/09031936.03.00099703

Masala, G., Porcu, R., Sanna, G., Chessa, G., Cillara, G., Chisu, V., & Tola, S. (2004). Occurrence, distribution, and role in abortion of *Coxiella burnetii* in sheep and goats in Sardinia, Italy. *Veterinary Microbiology*, *99*(3-4), 301–305. http://doi.org/10.1016/j.vetmic.2004.01.006

Maurin, M., & Raoult, D. (1999). Q fever. *Clinical Microbiology Reviews*, *12*(4), 518–553.

Mcquiston, J. H., & Childs, J. E. (2002). Q fever in humans and animals in the United States. *Vector Borne and Zoonotic Diseases*, *2*(3), 179–191. http://doi.org/10.1089/15303660260613747

Meredith, A. L., Cleaveland, S. C., Denwood, M. J., Brown, J. K., & Shaw, D. J. (2014). *Coxiella burnetii* (Q-fever) seroprevalence in prey and predators in the united kingdom: evaluation of infection in wild rodents, foxes and domestic cats using a modified ELISA. *Transboundary and Emerging Diseases*, *62*(6), 639–649. http://doi.org/10.1111/tbed.12211

Minor, C., Kersh, G. J., Gelatt, T., Kondas, A. V., Pabilonia, K. L., Weller, C. B., Dickerson, B. R., & Duncan, C. G. (2013). *Coxiella burnetii* in Northern fur seals and Steller sea lions of Alaska. *Journal of Wildlife Diseases*, *49*(2), 441–446. http://doi.org/10.7589/2012-09-226

Reitsma, J. B., Rutjes, A. W. S., Khan, K. S., Coomarasamy, A., & Bossuyt, P. M. (2009). A review of solutions for diagnostic accuracy studies with an imperfect or missing reference standard. *Journal of Clinical Epidemiology*, *62*(8), 797–806. http://doi.org/10.1016/j.jclinepi.2009.02.005

Reusken, C., van der Plaats, R., Opsteegh, M., de Bruin, A., & Swart, A. (2011). *Coxiella burnetii* (Q fever) in *Rattus norvegicus* and *Rattus rattus* at livestock farms and urban locations in the Netherlands; could *Rattus* spp. represent reservoirs for (re)introduction? *Preventive Veterinary Medicine*, *101*(1-2), 124–130. http://doi.org/10.1016/j.prevetmed.2011.05.003

Rijks, J. M., Roest, H. I. J., van Tulden, P. W., Kik, M. J. L., IJzer, J., & Gröne, A. (2011). *Coxiella burnetii* infection in Roe deer during Q fever epidemic, the Netherlands. *Emerging Infectiour Diseases*, *17*(12), 2369–2371. http://doi.org/10.3201/eid1712.110580

Roest, H. I. J., Tilburg, J. J. H. C., van der Hoek, W., Vellema, P., van Zijderveld, F. G., Klaassen, C. H. W., & Raoult, D. (2011). The Q fever epidemic in The Netherlands: history, onset, response and reflection. *Epidemiology Infection*, *139*(1), 1–12. http://doi.org/10.1017/S0950268810002268

Rousset, E., Berri, M., Durand, B., Dufour, P., Prigent, M., Delcroix, T., Touratier, A., & Rodolakis, A. (2009). *Coxiella burnetii* shedding routes and antibody response after

outbreaks of Q fever-induced abortion in dairy goat herds. *Applied and Environmental Microbiology*, *75*(2), 428–433. http://doi.org/10.1128/AEM.00690-08

Rousset, E., Durand, B., Berri, M., Dufour, P., Prigent, M., Russo, P., Delcroix, T., Touratier, A., Rodolakis, A., & Aubert, M. (2007). Comparative diagnostic potential of three serological tests for abortive Q fever in goat herds. *Veterinary Microbiology*, *124*(3-4), 286–297. http://doi.org/10.1016/j.vetmic.2007.04.033

Rutjes, A. W. S., Reitsma, J. B., Coomarasamy, A., Khan, K. S., & Bossuyt, P. M. M. (2007). Evaluation of diagnostic tests when there is no gold standard. A review of methods. *Health Technology Assessment*, *11*(50), iii, ix–51. http://doi.org/10.3310/hta11500

Schimmer, B., Luttikholt, S., Hautvast, J. L. A., Graat, E. A. M., Vellema, P., & van Duynhoven, Y. T. H. P. (2011). Seroprevalence and risk factors of Q fever in goats on commercial dairy goat farms in the Netherlands, 2009-2010. *BMC Veterinary Research*, *7*, 81–95. http://doi.org/10.1186/1746-6148-7-81

Schneeberger, P. M., Hermans, M. H. A., van Hannen, E. J., Schellekens, J. J. A., Leenders, A. C. A. P., & Wever, P. C. (2010). Real-time PCR with serum samples is indispensable for early diagnosis of acute Q fever. *Clinical and Vaccine Immunology*, *17*(2), 286–290. http://doi.org/10.1128/CVI.00454-09

Sim, J., & Wright, C. C. (2005). The kappa statistic in reliability studies: Use, interpretation, and sample size requirements. *Physical Therapy*, *85*(3), 257–268. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15733050

Viera, A. J., & Garrett, J. M. (2005). Understanding interobserver agreement: The kappa statistic. *Family Medicine*, *37*(5), 360–363.

Villumsen, S., Jørgensen, C. S., Smith, B., Uldum, S., Schiellerup, P., & Krogfelt, K. A. (2009). Determination of new cutoff values for indirect immunofluorescence antibody test for Q fever diagnosis in Denmark. *Diagnostic Microbiology and Infectious Disease*, *65*(2), 93–98. http://doi.org/10.1016/j.diagmicrobio.2009.06.004

Wegdam-Blans, M. C. A., Wielders, C. C. H., Meekelenkamp, J., Korbeeck, J. M., Herremans, T., Tjhie, H. T., Bijlmer, H. A., Koopmans, M. P. G., & Schneeberger, P. M. (2012). Evaluation of commonly used serological tests for detection of *Coxiella burnetii* antibodies in well-defined acute and follow-up sera. *Clinical and Vaccine Immunology*, *19*(7), 1110–1115. http://doi.org/10.1128/CVI.05581-11

Tables and Figures

Table 2.1. Prevalence of *Coxiella burnetii* DNA detected from genital and fecal swab sample types collected from 5 different wildlife species on Ontario dairy goat farms and anearby natural areas in 2014. The sensitivity of *C. burnetii* DNA detection from the sample types was determined by dividing the number of animals positive for each sample type by the number of animals positive when considering all sample types interpreted in parallel.

Species	Sample Type	% Positive (95% CI)	Sensitivity (95% CI)
	(*n* = 48)		**(*n* = 33)**
Deer mouse	Genital Swab	42 (27.6-56.8)	61 (42.1-77.1)
	Fecal Swab	54 (39.2-68.6)	79 (61.1-91)
	(*n* = 29)		**(*n* = 26)**
Eastern chipmunk	Genital Swab	86 (68.3-96.1)	96 (80.4-99.9)
	Fecal Swab	69 (49.2-84.7)	77 (56.4-91)
	(*n* = 9)		**(*n* = 7)**
House mouse	Genital Swab	44 (13.7-78.8)	57 (18.4-90.1)
	Fecal Swab	78 (40-97.2)	100 (59-100)
	(*n* = 85)		**(*n* = 39)**
Raccoon	Genital Swab	26 (17.0-36.5)	56 (39.6-72.2)
	Fecal Swab	35 (25.2-46.4)	77 (60.7-88.9)
	(*n* = 9)		**(*n* = 6)**
Red squirrel	Genital Swab	44 (13.7-78.8)	67 (22.3-95.7)
	Fecal Swab	67 (29.9-92.5)	100 (54.1-100)

Table 2.2. Comparison of sample agreement in the detection of *Coxiella burnetii* DNA collected in 2014 from wildlife species using McNemar's χ^2, PABAK (prevalence-adjusted and bias-adjusted kappa test) and AC1 (first-order agreement coefficient) tests.

Species	Test	McNemar's χ^2 test *P* value	PABAK (95% CI)	AC1 (95% CI)
Deer mouse	Genital Swab/ Fecal Swab	0.18	0.17 (-0.14-0.45)	0.17 (0.07-0.29)
	Genital Swab/ Fecal Material	**<0.0001**	0.06 (-0.13-0.25)	0.15 (0.07-0.27)
Eastern chipmunk	Genital Swab/ Fecal Swab	0.13	0.52 (0.13-0.79)	0.63 (0.50-0.74)
	Genital Swab/ Fecal Material	**0.01**	0.28 (-0.15-0.64)	0.38 (0.26-0.52)
House mouse	Genital Swab/ Fecal Swab	0.25	0.33 (-0.40-0.85)	0.36 (0.24-0.51)
	Genital Swab/ Fecal Material	1.00	0.50 (-0.14-0.89)	0.68 (0.56-0.78)
Raccoon	Genital Swab/ Fecal Swab	0.12	0.39 (0.17-0.58)	0.47 (0.33-0.60)
Red squirrel	Genital Swab/ Fecal Swab	0.50	0.56 (-0.20-0.90)	0.56 (0.40-0.69)
	Genital Swab/ Fecal Material	0.25	0.20 (-0.71-0.89)	0.41 (0.29-0.54)

For significant McNemar's, the sample type with the highest DNA detection prevalence and sensitivity is bolded.

Table 2.3. Prevalence of *Coxiella burnetii* DNA detected from genital swabs and fecal material sample types collected from 4 different wildlife species sampled on Ontario dairy goat farms and nearby natural areas in 2014. The sensitivity of *C. burnetii* DNA detection from the sample types was determined by dividing the number of animals positive for each sample type by the number of animals positive when considering all sample types interpreted in parallel.

Species	Sample Type	% Positive (95% CI)	Sensitivity (95% CI)
	(*n* = 113)		**(*n* = 65)**
Deer mouse	Genital Swab	49 (39.2-58.3)	85 (73.5-92.4)
	Fecal Material	20 (12.6-28)	34 (22.6-46.6)
	(*n* = 25)		**(*n* = 19)**
Eastern chipmunk	Genital Swab	64 (42.5-82)	84 (60.4-96.6)
	Fecal Material	24 (9.4-45.1)	32 (12.6-56.6)
	(*n* = 12)		**(*n* = 4)**
House mouse	Genital Swab	17 (2.1-48.4)	50 (6.8-93.2)
	Fecal Material	8 (0.2-38.5)	25 (0.6-80.6)
	(*n* = 3)		**(*n* = 3)**
Red squirrel	Genital Swab	100 (29.2-100)	100 (29.2-100)
	Fecal Material	0 (0-70.8)	0 (0-70.8)

Table 2.4. Prevalence of *Coxiella burnetii* DNA detected from genital swabs, fecal material and milk samples collected from dairy goats on registered Ontario dairy goat farms in 2014. The sensitivity of *C. burnetii* DNA detection from the sample types was determined by dividing the number of animals positive for each sample type by the number of animals positive when considering all sample types interpreted in parallel.

Sample Type	**% Positive (95% CI)**	**Sensitivity (95% CI)**
	***n* = 368**	***n* = 331**
Genital Swab	89 (85.8-92.4)	99 (97.8-99.9)
Fecal Material	29 (24-33.4)	32 (26.7-37)
Milk	25 (20.4-29.5)	28 (23-32.5)

Table 2.5. Comparison of sample agreement in the detection of *Coxiella burnetii* DNA collected in 2014 from Ontario dairy goats using McNemar's χ^2, PABAK (prevalence-adjusted and bias-adjusted kappa test) and AC1 (first-order agreement coefficient) tests. For significant McNemar's, the sample type with highest DNA detection prevalence and sensitivity is bolded. Sample types with an asteric indicate the sample with the highest sensitivity.

Test	McNemar's χ^2 test *P* value	PABAK (95% CI)	AC1 (95% CI)
Genital Swab/ Fecal Material	**<0.0005**	0.22 (0.11-0.32)	0.43 (0.30-0.55)
Genital Swab/ Milk	**<0.0001**	0.32 (0.21-0.41)	0.52 (0.40-0.64)
Fecal Material/ Milk	0.24	0.22 (0.11-0.32)	0.36 (0.24-0.49)

For significant McNemar's, the sample type with the highest DNA detection prevalence and sensitivity is bolded.

Appendix

Appendix 2.1. The 2 x 2 table used to assess the level of agreement in the detection of *Coxiella burnetii* DNA from three different sample types (genital swabs, fecal swabs and fecal material) collected from five wildlife species. The tables were used for McNemar's χ^2, PABAK and AC1 test comparisons.

Species	2 x2 Tables for Sample Comparison		
Deer mouse		Positive (Fecal Swab)	Negative (Fecal Swab)
	Positive (Genital Swab)	13	7
	Negative (Genital Swab)	13	15
		Positive (Fecal Material)	Negative (Fecal Material)
	Positive (Genital Swab)	12	43
	Negative (Genital Swab)	10	48
Eastern chipmunk		Positive (Fecal Swab)	Negative (Fecal Swab)
	Positive (Genital Swab)	19	6
	Negative (Genital Swab)	1	3
		Positive (Fecal Material)	Negative (Fecal Material)
	Positive (Genital Swab)	3	13
	Negative (Genital Swab)	3	6
House mouse		Positive (Fecal Swab)	Negative (Fecal Swab)
	Positive (Genital Swab)	4	0
	Negative (Genital Swab)	3	2
		Positive (Fecal Material)	Negative (Fecal Material)
	Positive (Genital Swab)	0	2
	Negative (Genital Swab)	1	9
Raccoon		Positive (Fecal Swab)	Negative (Fecal Swab)
	Positive (Genital Swab)	13	9
	Negative (Genital Swab)	17	46
Red squirrel		Positive (Fecal Swab)	Negative (Fecal Swab)
	Positive (Genital Swab)	4	0
	Negative (Genital Swab)	2	3
		Positive (Fecal Material)	Negative (Fecal Material)
	Positive (Genital Swab)	0	3
	Negative (Genital Swab)	0	2

Appendix 2.2. The 2 x 2 table used to assess the level of agreement in the detection of *Coxiella burnetii* DNA from three different sample types (genital swabs, fecal material, and milk) collected from recently kidded dairy goats. The tables were used for McNemar's, PABAK and AC1 test comparisons.

2 x 2 Tables for Sample Comparisons

	Positive (Fecal Material)	Negative (Fecal Material)
Positive (Genital Swab)	105	224
Negative (Genital Swab)	0	39
	Positive (Milk)	Negative (Milk)
Positive (Genital Swab)	89	240
Negative (Genital Swab)	2	37
	Positive (Fecal Material)	Negative (Fecal Material)
Positive (Milk)	26	79
Negative (Milk)	65	198

General Discussion

The primary aim of my study was to investigate the role of wildlife in the epidemiology of *C. burnetii* at the livestock-wildlife interface in Ontario, Canada. Although *C. burnetii* antibodies have been detected previously in wild animals, their role in the transmission of *C. burnetii* is not well understood. In the first chapter, I explored the role of wildlife in the transmission dynamics of *C. burnetii* by comparing infection prevalence among wildlife and livestock species on 16 Ontario dairy goat farms and 14 adjacent natural areas. In the second chapter I assessed the utility of different non-invasive biological sample types for the detection of *C. burnetii* DNA. I compared the prevalence, sensitivity of each sample type (genital swab, fecal swab, fecal material and milk) for DNA detection, and level of agreement between sample types in the detection of *C. burnetii* DNA from domestic and wildlife species.

Coxiella burnetii at the Livestock-Wildlife Interface

Similar to previous studies identifying *C. burnetii* in a variety of wildlife species (Astobiza et al., 2011; Enright et al., 1971; Kazar, 2005; Ho et al., 1995l; Meredith et al., 2014; Mcquiston & Childs, 2002; Reusken et al., 2011), I found *C. burnetii* in all wildlife species sampled in this study. The prevalence ranged from 33% (n = 3) for red-backed voles to 80% (n = 57) for eastern chipmunks. Although Thompson et al. (2012) did not detect *C. burnetii* in eastern chipmunks sampled in Algonquin provincial park, serological studies have identified evidence of exposure to *C. burnetii* in chipmunks, including least chipmunks (reviewed in Meerburg & Reusken, 2011). Only 12 eastern chipmunks were sampled in Thompson et al. (2012), thus it is likely *C. burnetii* would have been detected in these species given a larger sample size.

As predicted in chapter one, I found no significant difference in the prevalence of *C. burnetii* in wildlife trapped on dairy goat farms compared to adjacent natural areas (Figure 1.3). These findings suggest that the wildlife may be able to maintain *C. burnetii* independent of livestock infection. In chapter one, I also predicted that prevalence of *C. burnetii* in wildlife would be the same on adjacent farm and natural area sites, regardless of the distance between sites. Deer mice and raccoons were the only species with large enough sample sizes to investigate this prediction. There was no significant difference between infection prevalence among either species with respect to the distance between sites (Figure 1.4 and 1.5 respectively). Thus, these results further support wildlife as potential maintenance hosts for *C. burnetii*; however, without further comparison of strain types, I cannot confirm wildlife as reservoir species in this study (Pavio et al., 2010). The epidemiology of zoonotic pathogens at the livestock-wildlife interface are often complex and difficult to disentangle (reviewed in Simpson, 2002). Some pathogens, such as Brucellosis in the greater Yellowstone area, are maintained by both domestic and wild populations (Cheville et al., 1998) and this may be the case for *C. burnetii* in Ontario as well.

Strain typing will allow us to determine if transmission of *C. burnetii* between wildlife and livestock may be occurring (Archie et al., 2009; Pavio et al., 2010). To date, 35 different Multi-Spacer Sequence Typing strain types have been identified (Hornstra et al., 2011). Strain type ST20 has been attributed to dairy goats, inclusive of all sample types considered; however, strain types have not been successfully identified for any wildlife species. Wildlife species may be infected with a different strain of *C. burnetii* than domestic animals and dairy goats. In which case, even though wildlife may serve as

maintenance species for some strain types, they may be spill-over hosts for livestock associated *C. burnetii* (Nugent, 2011; Daszak et al., 2000). As well, bridge-species have recently been proposed in systems with multi-host pathogen transmission, whereby a host species provides a link through which a pathogen can be transmitted from a maintenance host to a target species or population (Caron et al., 2015). Although the results from this study support wildlife as potential maintenance hosts, the role of wildlife in the transmission of *C. burnetii* at the livestock-wildlife interface is still unclear and further experimental investigations are required to determine the true role of wildlife in the transmission of *C. burnetii*.

While most studies have investigated the serological prevalence of *C. burnetii* in domestic and wildlife species (Meredith et al., 2014; Minor et al., 2013; Kirchgessner et al., 2012; Komiya et al., 2003; Reusken et al., 2011; Schimmer et al., 2011), I investigated the prevalence of *C. burnetii* in wildlife species based on DNA detection. This method is a more robust method when investigating current infection compared to serological results, which can provide false positives in the case of individuals with past infections (Berri et al., 2001; Fournier & Raoult, 2003). Since adjacent sites were sampled simultaneously throughout the study period, the comparison of wildlife infection status between adjacent farm and natural area sites is reliable, in the sense that infection prevalence among species in adjacent sites was compared during the same time period.

Comparison of Sample Types for Detection of Coxiella burnetii in Wildlife Species

In chapter two, I investigated the impact of sample type on the detection of *C. burnetii* DNA from five wildlife species. Since my sampling methods included the collection of fecal material for wildlife species and the collection of fecal swabs in the

absence of fecal material, I was unable to compare these sample types in parallel. Instead, I compared fecal swabs with genital swabs (Table 2.1) and fecal material with genital swabs in a separate analysis (Table 2.2). Taking into account prevalence, sensitivity for *C. burnetii* DNA detection, and level of agreement, genital swab and fecal swab sample types were suitable sample types for detecting *C. burnetii* DNA for deer mice, eastern chipmunks and raccoons (Table 2.4). On the other hand, genital swab, fecal swab and fecal material sample types were suitable sample types in the detection of *C. burnetii* DNA for house mice and red squirrels (Table 2.4). It is important to acknowledge that the sample size of individual red squirrels ($n < 10$) and house mice ($n < 15$) were low, which likely reduced statistical power. As such, we may not have had a sufficient sample size to detect a difference in sample types for these species if one was present. Similar studies need to be conducted to confirm my results and potentially identify a single sample type that is optimal for each of the wildlife species included in this study.

Comparison of Sample Types for Detection of Coxiella burnetii in Dairy Goats

Sample types for dairy goats including genital swabs, fecal material, and milk, were assessed in parallel. Taking into account prevalence, sensitivity for *C. burnetii* DNA detection, and level of agreement, genital swabs were optimal sample types in the detection of *C. burnetii* DNA for dairy goats. Previous studies have shown that dairy goats shed highest amounts of *C. burnetii* DNA in birthing tissues (Fournier et al., 1998; Roest et al., 2011). Since my sampling methods included sampling dairy goats that had most recently kidded, it is not surprising that genital swabs would present as the optimal sample type. Because our study focused on recently kidded dairy goats, care should be taken in generalizing these findings to goats at other stages of production. Consequently,

further investigations of optimal sampling types for dairy goats in different sampling scenarios need to be conducted to identify the single most effective sample type for the detection of *C. burnetii* DNA.

Directions for Future Research - Missing Epidemiological Information

This study identified *C. burnetii* in all wildlife species sampled (deer mice, eastern chipmunks, house mice, opossums, raccoons, red-backed voles, red squirrels and skunks) as well as dairy goats and other domestic animals sampled on farms (cats, cows, dogs, horses and pigs). Due to laboratory limitations, it is unclear whether these animals are infected with the same bacterial strains of *C. burnetii*. Without these strain data, it is not possible to determine if transmission may be occurring among livestock, other domestic animals and wildlife. As a result, it is important that future studies investigate the bacterial strains of *C. burnetii* responsible for infecting different wildlife and domestic animal species on adjacent study sites to better understand whether wildlife are involved in the transmission of *C. burnetii* among livestock and other domestic animals (Archie et al., 2009; reviewed in Foley et al. 2009). Moreover, it is important to experimentally investigate the transmission of *C. burnetii* at the livestock-wildlife interface in order to determine the direction of transmission (Archie et al., 2009). Such experimentations should involve the experimental infection of both wildlife and livestock species to observe the transmission pathway. At the same time, it is equally important to discern the role of environmental factors in the epidemiology of *C. burnetii*. *Coxiella burnetii* is known to remain viable in the environment for extended periods of time (Azad & Radulovic, 2003), and has also been identified in soil, dust and air samples (Kersh et al., 2010; Yanase et al., 1998; De Bruin et al., 2013). Thus, it is important to identify the

role of environmental factors in the transmission of *C. burnetii* at the livestock-wildlife interface.

Coxiella burnetii vaccines for dairy goats are used in several countries, excluding Canada, and studies have assessed the effectiveness between vaccines targeting the phase I and phase II *C. burnetii* antigens (Arricau-Bouvery et al., 2005; Hogerwerf et al., 2011). However, studies investigating the correlation between vaccinated dairy goats and wildlife infection prevalence are lacking. Before implementing such studies, it is imperative to first determine whether wildlife are capable of transmitting to and from livestock and other domestic animals, through strain comparisons (Archie et al., 2009). Furthermore, the majority of studies investigating the clinical and subclinical symptoms of *C. burnetii* infection focus primarily on humans, livestock and other domestic animals (Angelakis & Raoult, 2010; Marrie, 2003; Maurin & Raoult, 1999). To my knowledge, there are no studies aimed at determining the clinical and subclinical symptoms of *C. burnetii* infection among wildlife species. If further investigations support wildlife as maintenance and reservoir species in the transmission of *C. burnetii*, it will be important to investigate the clinical and subclinical impacts of infection on wildlife and identify bacterial shedding routes among affected wildlife species. This information will provide a more comprehensive understanding of the epidemiology of *C. burnetii* at the livestock-wildlife interface.

Overall, this study provides evidence to support wildlife, specifically, small and medium sized mammals, as potential maintenance hosts of *C. burnetii* in southern Ontario. Optimal sample types for the detection of *C. burnetii* DNA vary depending on the species and the context of the sampling method. Although this study adds valuable

information to our understanding of the epidemiology of *C. burnetii* at the livestock-wildlife interface, there are still many unknowns, including the potential for shared bacterial strains among wildlife and livestock species, as well as the direction of the transmission pathway, and clinical/subclinical symptoms of infected wildlife. It is important for future studies to investigate these unknowns so the epidemiology of *C. burnetii* may be better understood in order to limit transmission and prevent future outbreaks by increasing farm biosecurity through more rapid and routine monitoring of livestock infections.

Literature Cited

Angelakis, E., & Raoult, D. (2010). Q fever. *Veterinary Microbiology*, *140*(3-4), 297–309. http://doi.org/10.1016/j.vetmic.2009.07.016

Archie, E. A., Luikart, G., & Ezenwa, V. O. (2009). Infecting epidemiology with genetics: a new frontier in disease ecology. *Trends in Ecology & Evolution*, *24*(1), 21–30. http://doi.org/10.1016/j.tree.2008.08.008

Arricau-Bouvery, N., Souriau, A., Bodier, C., Dufour, P., Rousset, E., & Rodolakis, A. (2005). Effect of vaccination with phase I and phase II *Coxiella burnetii* vaccines in pregnant goats. *Vaccine*, *23*, 4392–4402. http://doi.org/10.1016/j.vaccine.2005.04.010

Astobiza, I., Barral, M., Ruiz-Fons, F., Barandika, J. F., Gerrikagoitia, X., Hurtado, A., & García-Pérez, A. L. (2011). Molecular investigation of the occurrence of *Coxiella burnetii* in wildlife and ticks in an endemic area. *Veterinary Microbiology*, *147*(1-2), 190–194. http://doi.org/10.1016/j.vetmic.2010.05.046

Azad, A. F., & Radulovic, S. (2003). Pathogenic rickettsiae as bioterrorism agents. *Annals of the New York Academy of Sciences*, *990*, 734–738. http://doi.org/10.1086/518147

Berri, M., Souriau, A., Crosby, M., Crochet, D., Lechopier, P., & Rodolakis, A. (2001). Relationships between the shedding of *Coxiella burnetii*, clinical signs and serological responses of 34 sheep. *The Veterinary Record*, *148*(16), 502–505. http://doi.org/10.1136/vr.148.16.502

Caron, A., Cappelle, J., Cumming, G. S., de Garine-Wichatitsky, M., & Gaidet, N. (2015). Bridge hosts, a missing link for disease ecology in multi-host systems. *Veterinary Research*, *46*(1), 1–11. http://doi.org/10.1186/s13567-015-0217-9

Cheville, N. F., McCullough, D. R., & Paulson, L. R. (1998). *Brucellosis in the Greater Yellowstone Area*. Washington, DC: National Academy Press.

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2000). Emerging infectious diseases of wildlife - threats to biodiversity and human health. *Science*, *287*(5452), 443–449. http://doi.org/10.1126/science.287.5452.443

De Bruin, A., Janse, I., Koning, M., De Heer, L., Van der Plaats, R. Q. J., Van Leuken, J. P. G., & Van Rotterdam, B. J. (2013). Detection of *Coxiella burnetii* DNA in the environment during and after a large Q fever epidemic in the Netherlands. *Journal of Applied Microbiology*, *114*(5), 1395–1404. http://doi.org/10.1111/jam.12163

Enright, J. B., Franti, C. E., Behymer, D. E., Longhurst, W. M., Dutson, V. J., & Wright, M. E. (1971). *Coxiella burnetii* in a wildlife-livestock environment distribution of Q fever in wild mammals. *American Journal of Epidemiology*, *94*(1), 79–90.

Foley, S. L., Lynne, A. M., & Nayak, R. (2009). Molecular typing methodologies for microbial source tracking and epidemiological investigations of Gram-negative

bacterial foodborne pathogens. *Infection, Genetics and Evolution*, *9*(4), 430–440. http://doi.org/10.1016/j.meegid.2009.03.004

Fournier, P.-E., Marrie, T. J., & Raoult, D. (1998). Diagnosis of Q Fever. *Journal of Clinical Microbiology*, *36*(7), 1823–1834.

Fournier, P.-E., & Raoult, D. (2003). Comparison of PCR and serology assays for early diagnosis of acute Q fever. *Journal of Clinical Microbiology*, *41*(11), 5094–5098. http://doi.org/10.1128/JCM.41.11.5094

Ho, T., Htwe, K. K., Yamasaki, N., Zhang, G. Q., Ogawa, M., Yamaguchi, T., Fukushi, H., & Hirai, K. (1995). Isolation of *Coxiella burnetii* from dairy cattle and ticks, and some characteristics of the isolates in Japan. *Microbiology and Immunology*, *39*(9), 663–671. http://doi.org/10.1111/j.1348-0421.1995.tb03254.x

Hogerwerf, L., van den Brom, R., Roest, H. I., Bouma, A., Vellema, P., Pieterse, M., Dercksen, D., & Nielen, M. (2011). Reduction of *Coxiella burnetii* prevalence by vaccination of goats and sheep, The Netherlands. *Emerging Infectious Diseases*, *17*(3), 379–386. http://doi.org/10.3201/eid1703.101157

Hornstra, H. M., Priestley, R. a, Georgia, S. M., Kachur, S., Birdsell, D. N., Hilsabeck, R., Gates, L. T., Samuel, J. E., Heinzen, R. A., Kersh, G. J., Keim, P., Massung, R. F., & Pearson, T. (2011). Rapid typing of *Coxiella burnetii*. *PloS One*, *6*(11), e26201. http://doi.org/10.1371/journal.pone.0026201

Kazar, J. (2005). *Coxiella burnetii* infection. *Annals of the New York Academy of Sciences*, *1063*, 105–14. http://doi.org/10.1196/annals.1355.018

Kersh, G. J., Wolfe, T. M., Fitzpatrick, K. A., Candee, A. J., Oliver, L. D., Patterson, N. E., Self, J. J., Priestley, R. A, Loftis, A., & Massung, R. F. (2010). Presence of *Coxiella burnetii* DNA in the environment of the United States, 2006 to 2008. *Applied and Environmental Microbiology*, *76*(13), 4469–4475. http://doi.org/10.1128/AEM.00042-10

Kirchgessner, M. S., Dubovi, E. J., & Whipps, C. M. (2012). Seroepidemiology of *Coxiella burnetii* in wild white-tailed deer (*Odocoileus virginianus*) in New York, United States. *Vector-Borne and Zoonotic Diseases*, *12*(11), 942–947. http://doi.org/10.1089/vbz.2011.0952

Komiya, T., Sadamasu, K., Kang, M.-I., Tsuboshima, S., Fukushi, H., & Hirai, K. (2003). Seroprevalence of *Coxiella burnetii* infections among cats in different living environments. *The Journal of Veterinary Medical Science*, *65*(9), 1047–1048. http://doi.org/10.1292/jvms.65.1047

Marrie, T. J. (2003). *Coxiella burnetii* pneumonia. *European Respiratory Journal*, *21*(4), 713–719. http://doi.org/10.1183/09031936.03.00099703

Maurin, M., & Raoult, D. (1999). Q fever. *Clinical Microbiology Reviews*, *12*(4), 518–553.

Mcquiston, J. H., & Childs, J. E. (2002). Q fever in humans and animals in the United States. *Vector Borne and Zoonotic Diseases*, *2*(3), 179–191. http://doi.org/10.1089/15303660260613747

Meerburg, B. G., & Reusken, C. B. E. M. (2011). The role of wild rodents in spread and transmission of Coxiella burnetii needs further elucidation. *Wildlife Research*, *38*, 617–625. http://doi.org/10.1071/WR10129

Meredith, A. L., Cleaveland, S. C., Denwood, M. J., Brown, J. K., & Shaw, D. J. (2014). *Coxiella burnetii* (Q-fever) seroprevalence in prey and predators in the united kingdom: evaluation of infection in wild rodents, foxes and domestic cats using a modified ELISA. *Transboundary and Emerging Diseases*, *62*(6), 639–649. http://doi.org/10.1111/tbed.12211

Minor, C., Kersh, G. J., Gelatt, T., Kondas, A. V., Pabilonia, K. L., Weller, C. B., Dickerson, B. R., & Duncan, C. G. (2013). *Coxiella burnetii* in Northern fur seals and Steller sea lions of Alaska. *Journal of Wildlife Diseases*, *49*(2), 441–446. http://doi.org/10.7589/2012-09-226

Nugent, G. (2011). Maintenance, spillover and spillback transmission of bovine tuberculosis in multi-host wildlife complexes: a New Zealand case study. *Veterinary Microbiology*, *151*(1), 34–42. http://doi.org/10.1016/j.vetmic.2011.02.023

Pavio, N., Meng, X.-J., & Renou, C. (2010). Zoonotic hepatitis E: animal reservoirs and emerging risks. *Veterinary Research*, *41*(6), 46–66. http://doi.org/10.1051/vetres/2010018

Reusken, C., van der Plaats, R., Opsteegh, M., de Bruin, A., & Swart, A. (2011). *Coxiella burnetii* (Q fever) in *Rattus norvegicus* and *Rattus rattus* at livestock farms and urban locations in the Netherlands; could *Rattus* spp. represent reservoirs for (re)introduction? *Preventive Veterinary Medicine*, *101*(1-2), 124–130. http://doi.org/10.1016/j.prevetmed.2011.05.003

Roest, H. I. J., Tilburg, J. J. H. C., van der Hoek, W., Vellema, P., van Zijderveld, F. G., Klaassen, C. H. W., & Raoult, D. (2011). The Q fever epidemic in The Netherlands: history, onset, response and reflection. *Epidemiology Infection*, *139*(1), 1–12. http://doi.org/10.1017/S0950268810002268

Schimmer, B., Luttikholt, S., Hautvast, J. L. A., Graat, E. A. M., Vellema, P., & van Duynhoven, Y. T. H. P. (2011). Seroprevalence and risk factors of Q fever in goats on commercial dairy goat farms in the Netherlands, 2009-2010. *BMC Veterinary Research*, *7*, 81–95. http://doi.org/10.1186/1746-6148-7-81

Simpson, V. R. (2002). Wild animals as reservoirs of infectious diseases in the UK. *The Veterinary Journal*, *163*(2), 128–146. http://doi.org/10.1053/tvjl.2001.0662

Thompson, M., Mykytczuk, N., Gooderham, K., & Schulte-Hostedde, A. (2012). Prevalence of the bacterium *Coxiella burnetii* in wild rodents from a Canadian natural environment park. *Zoonoses and Public Health*, *59*(8), 553–560.

http://doi.org/10.1111/j.1863-2378.2012.01493.x

Yanase, T., Muramatsu, Y., Inouye, I., Okabayashi, T., Ueno, H., & Morita, C. (1998). Detection of *Coxiella burnetii* from dust in a barn housing dairy cattle. *Microbiology and Immunology*, *42*(1), 51–53. http://doi.org/10.1111/j.1348-0421.1998.tb01969.x