EPIDEMIOLOGY OF Q FEVER AMONG DAIRY CATTLE FARMS AND FARMERS, CHIANG MAI, THAILAND 2015

Pawinee Doung-ngern

A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology in the Gillings School of Global Public Health.

Chapel Hill
2016

Approved by:

Steven Meshnick

Michael Emch

Gilbert Kersh

Gary Koch

David Weber

ABSTRACT

Pawinee Doung-ngern: Epidemiology of Q fever among dairy cattle and dairy farmers, Chiang Mai, Thailand 2015
(Under the direction of Steven Meshnick)

Q fever is a zoonosis, caused by the gram negative bacteria *Coxiella burnetii*. Knowledge of the epidemiology of Q fever in Thailand is limited. This study was conducted to determine the burden and the risk factors of *C.burnetii* infection in dairy cattle farms and farmers in Chiang Mai, Thailand. A prospective cohort study was conducted in five dairy cooperatives where evidence of *C.burnetii* was reported. The project included three components 1) a cohort study among farmers, 2) bulk tank milk (BTM) screening, and 3) farm investigation and specimen collection from cows and their environments in milk positive farms. Samples and data collection were obtained at baseline, 6, and 12 month intervals. Human sera were tested using Indirect Immunofluorescense Assay; cow sera and BTM were tested using Enzyme Linked Immunosorbent Assay; and vaginal swab and environmental samples were tested using Polymerase Chain Reaction. Baseline data were analyzed using logistic regression and Generalized Estimating Equation models to estimate the odds ratio (OR) and 95% confidence interval (CI). Among 306 randomly selected farms, 282 farms (92.2%) and 532 from 637 randomly selected farmers (83.5%) participated. The prevalence of *C.burnetii* antibodies in BTM was 40.8% (115/282) and the *C.burnetii* seroprevalence among farmers was 16.9% (90/532). Investigation in BTM positive farms showed *C.burnetii* seroprevalence was 28.4% (224/790) at the individual cow level and 91.9% (91/99) at the farm level. Multivariate analysis showed that having more than 80% of cows ≥ 2 years of age (OR 2.34, 95%CI 1.09 - 5.06) and having an infected farms within 1 km (OR 2.88, 95%CI 1.17 – 7.06) were positively associated with the odds of *C.burnetii* antibodies in BTM. Cleaning the birthing area (OR 0.27, 95%CI 0.08 - 0.86) and quarantining newly purchased animals (OR 0.54, 95%CI 0.30 - 0.97) provided protection. Working in a milk positive farm and exposure to birth products during calving were associated with seropositivity among farmers. This study provides useful information for Q fever prevention and control. Health education regarding Q fever prevention should be provided to farmers and public health and

animal health officers in high risk areas in Thailand.

To livestock farmers in Thailand. This work could not be accomplished without their support. I hope this work will contribute to an improvement in the awareness of occupational risk to prevent the occurrence of Q fever among this high risk population.

ACKNOWLEGDEMENTS

This work was made possible with support from several people and organizations. I would like to first express the deepest appreciation to all my committee members, especially Dr. Steven Meshnick, my dissertation chair, who has been very supportive and encouraging. When I first discussed this project with him, he was very enthusiastic and provided his insightful comments about conducting research in the field. He trained me to be a researcher and allowed me to work and think independently, but he made sure that my ideas were feasible and practical. With his guidance, I have learned the skills necessary to conduct scientific research in the real world. I am also very fortunate to work with Dr. Gary Koch. As a student in his Categorical Data Analysis class, I am glad to apply the knowledge I have learned from the class to my real data set. Dr. Koch provided critical suggestions on sampling and data analyses, as well as sharing his experiences about cattle farms. He has always provided intuitive comments and clear answers for all the questions that I had in a timely fashion even when I was far away in the field. I have learned a lot from him. I am grateful for Dr. Gilbert Kersh for his support and suggestions, not only for this project, but also to other Q fever researchers in Thailand. As a Q fever expert, he has always provided insightful advice; he was one of the key persons to push this research forward. I am thankful for Dr. Michael Emch for his suggestions on spatial analysis. I am always curious to learn about spatial epidemiology and with Dr. Emch's guidance I have gained more knowledge in spatial analysis. I am also grateful for Dr. David Weber, who always provided valuable feedback and suggestions. His enthusiasm about my project and his helpful critiques on the questionnaires and the research results has always been appreciated. It has been a privilege to work with all these splendid mentors.

I cannot thank enough my mentors in Thailand, Dr. Pawin Padungtod, who introduced me to the Q fever project and guided me through the process of developing the proposal, submission for funding, and conducting research in the field. As public health is multidisciplinary, Dr. Pawin has shown me the power of networking and collaboration, as well as the importance of the "One Health" principle and its application

to real world research practice. Although he moved to work in another country, he consistently gave comments or suggestions whenever I asked for help. My sincere thank also goes to Dr. Saowapak Hinjoy, my colleague and mentor, who has always provided mental and academic advice whenever I needed it. I would like to express my deepest gratitude to P'Mae, my colleague, my sister, and my dear friend who has always been supportive and believed in me. She encourages me to pursue my dream and teaches me to be optimistic, whatever the challenges in life I may have faced. She was one of the key persons who helped me run this project even when I was far away from Thailand.

It would not be possible to conduct this project without the collaboration and precious support from the dairy cooperatives and Chiang Mai One Health Team consisting of the staff from the regional, provincial, and district health offices and the livestock office. I am grateful for the dairy cooperative members and the farmers who participated in our study. This project would not be accomplished without their support and participation. In addition, I would like to thank Drs. Somphorn Phornwisetsirikun, Pranee Rodtian, Phruetthiphon Sukpom, P'Yee, and P'Mai from Chiang Mai One Health Team, Drs. Natinee Kittiwan, Phornpan Laoleu, and staff from the Veterinary Research and Development Center in Lampang, Drs. Pattarin Opaschaitat and Tapakorn Chamchoy from the Thai National Institute of Animal Health, Dr. Decha Pangjai and his team from the Thai National Institute of Health, Ms. Dararat Danklai from the immunology laboratory at Maharaj Nakorn Chiang Mai Hospital, the veterinarians from Mae-on animal hospital, and Drs. Wittawat Modethed, Kanin, Puttipol Kongsook, Narin, and all the staff from the herd health units for all their support and suggestions during data collection and field works. Further acknowledgements go to the staff of the IEIP-Q team; Dr. Teerasak Chuxnam, Dr.Charatdao Bunthi, Ms. Somreuthai Na Nan, Apiladee Soonngam, Pajaree Kongsoon, Somkid Kongyu, and Janjao Rodchangphuen who helped with logistics and coordination, and Mr. Benz and his family for taking us to the field. The generous assistance and oversight of the Bureau of Epidemiology, Ministry of Public Health, and Chiang Mai livestock and health offices administration is also acknowledged, with thanks. I would also like to thank to the Centers for Disease Control and Prevention, USA for their financial support of this project.

From the beginning of my PhD, I would like to thank Drs. Sopon Iamsiritaworn, Sujarti Jetanasen, Pasakorn Akarasewi, Chuleeporn Jiraphongsa, Potjaman Siriarayaporn, Tanarak Plipat, and the staff

from the Field Epidemiology Training Program, and the Bureau of Epidemiology, Department of Disease Control for their support and for giving me the opportunity to pursue the doctoral degree in epidemiology. I am also grateful for the Royal Thai Ministry of Public Health which provided me with the scholarship. My profound appreciation also goes to Dr. Pradya Somboon from the Department of Parasitology, Faculty of Medicine, Chiang Mai University; and Ajarn Surachai, Leepitakrat, and Dr. Alongkot Polawat from the Armed Forces Research Institute of Medical Sciences (AFRIMS) for their kind support in tick identification; the students and staff from the Biomedical Consulting Laboratory, Department of Biostatistics, University of North Carolina at Chapel Hill for their suggestions and help with sampling methods; Dr. Sharon Weir for her supports during my first year in Chapel Hill, and our department student service specialists-Nancy Colvin, Carmen Woody, Valerie Hudock, and Jennifer Joyce Moore, who always supported us and were there whenever we had questions. My sincere thanks also go to Dr. Leedom Lefferts for proofreading my dissertation and Dr. Rungsima Kullapat for her kind hospitality.

Most importantly, I would like to thank my family who always supports me with love and encouragement although thousands of miles away from the United States. Thanks to my dad, mom, sisters, and brother who are always on my side. I could not go this far without all their support. In addition, I would like to acknowledge my friends and classmates here at UNC-CH for all the good times we spent together and that made me feel that Carolina was like home. My special thanks to Joann Gruber, Xiaojuan Li, Mugdha Gokhale, Jean Strelitz, Angela Lui, Jabulani Ncayiyana, Nalyn Siripong, Thatsanee, Wasin, Natnaree, Prapaporn, Pornpun, Pattawee, Panitan, Panit, and Amp for listening to me, taking care of me when I was sick, studying with me, and sharing the joy and laughs together.

TABLE OF CONTENTS

LIST OF TABLES xi

LIST OF FIGURES xii

LIST OF ABBREVIATIONS xiii

CHAPTER 1: SPECIFIC AIMS 1

CHAPTER 2: INTRODUCTION AND LITERATURE REVIEW 4

Q fever (Query fever) 4

Clinical manifestations 5

Laboratory diagnosis 7

Epidemiology of Q fever 10

Prevention and control of Q fever 12

Treatment of Q fever 14

Thailand: overview of the country 16

Q fever in Thailand and research gaps 16

CHAPTER 3: RESEARCH DESIGN AND METHODS 19

Study design 19

Study area 21

Study population 23

Eligibility criteria 24

Enrollment procedure 24

Sampling methods 25

Data collection 26

Laboratory procedures and quality control 30

Data management and analyses 32

Sample size and power 35

Timeline 37

Ethical considerations 37

Significant and impact 37

CHAPTER 4: COHORT PROFILE: A LONGITUDINAL STUDY OF Q FEVER AMONG DAIRY COW FARMS AND FARMERS IN CHIANG MAI, THAILAND 2015 – 2016 39

Introduction 39

Cohort description 40

Measurements 46

Preliminary finding at baseline 49

Strengths and limitations 49

CHAPTER 5: EPIDEMIOLOGY OF *C.BURNETII* INFECTION AMONG DAIRY CATTLE FARMS, CHIANG MAI, THAILAND 2015 51

Introduction 51

Methods 52

Results 56

Discussion 62

CHAPTER 6: SEROPREVALENCE AND FACTORS ASSOCIATED WITH *COXIELLA BURNETII* INFECTION AMONG DAIRY FARMERS, CHIANG MAI, THAILAND 2015 71

Introduction 71

Methods 72

Results 74

Discussion 82

Future works 83

CHAPTER 7: SUMMARY 84

Discussion 84

Public health impact 87

Future works 87

APPENDIX 1. QUESTIONNAIRE FOR FARM REPRESENTATIVE 89

APPENDIX 2. QUESTIONNAIRE FOR INDIVIDUAL FARMER 93

REFERENCES 101

LIST OF TABLES

Table 1. Number of farms and dairy cows by dairy cooperative (Data from Chiang Mai Provincial livestock office report, May 2014) 23

Table 2. Time line of the project 37

Table 3. Distribution of farms and number of cows by dairy cooperatives, Chiang Mai, Thailand 41

Table 4. Outcome definitions used in the study of the Epidemiology of Q fever among dairy farms and farmers in Chiang Mai, Thailand 2015 – 2016 47

Table 5. Summary of exposure variables collected for the study of Epidemiology of Q fever among dairy farms and farmers in Chiang Mai, Thailand 2015 - 2016 48

Table 6. Results of univariate analyses of factors associated with dairy farms with milk positive, Chiang Mai, Thailand 2015 59

Table 7. Results of multivariate analysis of factors associated with dairy farms with seropositive vs. farms with non-positive milk, Chiang Mai, Thailand 2015 61

Table 8. Results of multivariate analysis of factors associated with dairy farms with seropositive vs farms with non-positive milk (Explore the effect of percentage of cow age > 2 year in categorical variable), Chiang Mai, Thailand 2015 62

Supplemental Table1. Farm characteristics and seroprevalence of *C.burnetii* in BTM in the farms participated in the study, Chiang Mai, Thailand 2015 66

Supplemental Table 2. Results of multivariate analysis of factors associated with dairy farms with milk seropositive (Comparing farms with positive vs farms with negative milk) (N=249) 70

Table 9. Characteristics of farmers participated in the study by the IFA results, Chiang Mai, Thailand, 2015 75

Table 10. Univariate analysis of factors associated with seropositivity among farmers 80

LIST OF FIGURES

Figure 1.The cohort study protocol 19

Figure 2. Study areas and study cooperatives, Chiang Mai Province, Thailand 22

Figure 3. Power analysis of cohort study by relative risk and number of exposed subjects 36

Figure 4. Power analysis of the ecological study of Q fever in the dairy cow farms 36

Figure 5. A protocol of the study 42

Figure 6. Examples of farm by farm size 43

Figure 7. Distribution of dairy farms by Bulk Tank Milk (BTM) results 58

Figure 8. Examples of farm environment 59

Figure 9. Distribution of BTM results and the proportion of seropositive farmers, Chiang Mai, Thailand 2015 82

LIST OF ABBREVIATIONS

BTM:	Bulk Tank Milk
CI:	Confidence Interval
DLD:	Department of Livestock Development, Thailand
ELISA:	Enzyme-Linked Immunosorbent Assay
GEE:	Generalized Estimating Equation
IFA:	Indirect Immunofluorescense Assay
IgG:	Immunoglobulin G
IgM:	Immunoglobulin M
NIAH:	National Institute of Animal Health, Thailand
NIH:	National Institute of Health, Ministry of Public Health Thailand
OR:	Odds ratio
PCR:	Polymerase Chain Reaction
TUC:	Thai-Ministry of Public Health – US.CDC Collaboration
US.CDC:	Centers for Disease Control and Preventions, United States
VRDC:	Veterinary Research and Development Center

CHAPTER 1

SPECIFIC AIMS

Q fever is a zoonosis, caused by the gram negative bacteria *Coxiella burnetii.* It is highly contagious and classified as a category B bioterrorism agent (1). It is capable of causing a large outbreak, resulting in high morbidity in humans, loss of several animal lives, and can have potential impact on an economy as reported in an outbreak in the Netherlands (2, 3). The main route of transmission is by inhalation of aerosols contaminated with infected animal secretions. Q fever can infect a variety of hosts such as humans, ruminants, dogs, cats, and ticks. Human exposure to host animals creates a high risk of infection (4). Most of the infections are asymptomatic, but Q fever can cause abortions and weak offspring in animals, and a flu-like symptom as well as pneumonia and hepatitis in humans (5, 6). Moreover, it can cause chronic sequelae in humans including endocarditis in 1 – 5% and chronic fatigue syndrome in 10 – 20% of infections (7-10). Q fever diagnosis relies on laboratory analyses, particularly serological tests. In Thailand, information about Q fever is limited. It is not a notifiable disease, laboratory capacities are limited, and no epidemiological study has been conducted to understand the risk factors of the disease (11-14). Studies conducted in 2012 suggested that Q fever might be endemic among ruminants in Thailand and the burden of Q fever in humans might be higher than expected. The most recent studies found evidence of *C. burnetii* DNA in ruminants in 9 provinces in Thailand (15), while the seroprevalence of Q fever was 16% among dairy farmers in Chiang Mai (16). A genotyping study of animal placentas in Thailand showed that dairy cows had positive ST18 (Pattarin et al., unpublished), the strain associated with acute disease in humans (17). Thus, dairy cows could be a significant source of Q fever in humans, and dairy farmers in Thailand might have an increased risk of infection in Thailand. To be better prepared and to prevent outbreaks of Q fever, the epidemiological study of Q fever is essential. The specific aims of this study are to:

1. Describe the burden and factors associated with *C. burnetii* infection in dairy cow farms using bulk tank milk (BTM) as a proxy for Q fever infection at the herd level.

1.1. Describe the prevalence of antibodies to *C. burnetii* infection in dairy farms

1.2. Identify factors associated with Q fever in dairy cow farms (Q fever positive milk)

Hypothesis: Not cleaning the birthing area after birth increases the chance of Q fever positive in the farm

Rationale: Livestock, including cattle, are the most frequent source of Q fever infection in humans (6, 8). A previous study in Chiang Mai, Thailand, showed that dairy cows could be a significant source of Q fever (Pranee, Padungtod poster). The study reported on here was conducted in five dairy cooperatives in Chiang Mai Province where a previous study showed evidence of Q fever during June 2015 – August 2016 (16). Farms were randomly selected from the list of the dairy cooperatives. BTM was tested at baseline, with two additional follow ups at 6 and 12 month intervals. Farm representatives were interviewed about farm characteristics and farm management. If the screening was positive, a farm investigation including sample collection from 8 cows per farm, other animals, and the farm environment was performed. This dissertation is part of the main project, but only baseline information was used for data analysis. Prevalence and factors associated with the antibody to *C. burnetii* in BTM were evaluated using logistic regression.

2. Describe the burden and factors associated with Q fever among dairy farmers

2.1. Describe the seroprevalence of Q fever among dairy farmers

2.2. Identify factors associated with *C.burnetii* infection or Q fever among the dairy farmers

Hypothesis: Being close contact with dairy cows, particularly during calving, increases the risk of acquiring Q fever.

Rationale: Q fever can cause serious outbreaks and pose chronic life threatening sequelae, such as endocarditis in humans (18). Understanding the epidemiology of Q fever among high risk populations is crucial for targeting and implementing intervention. A prospective cohort study among dairy farmers was conducted. Farmers were randomly selected from participating farms, as described in aim 1. Face to face interviews by trained interviewers using a tested questionnaire were performed to collect all necessary information. Interviews and blood collections were conducted at the same time as the study of BTM (aim1). Sera were tested using Indirect Immunofluorescence Assay (IFA). Information about farmers,

farms, and the environment were incorporated and analyzed using the Generalized Estimating Equation (GEE) to evaluate factors associated with seroprevalence of Q fever infection among dairy farmers.

CHAPTER 2

INTRODUCTION AND LITERATURE REVIEW

Q fever (Query fever)

Q fever was first described in 1937 in Australia after an outbreak of acute febrile illness among abattoir workers. It was named Q (query) fever since the origin was unknown at that time. Q fever is a zoonotic disease, caused by gram negative intracellular bacteria called *Coxiella burnetii*. (8, 19). The bacteria are highly contagious and require a biosecurity level 3 laboratory. *C. burnetii* is classified as a category B bioterrorism agent (second highest priority) because it is resistant to severe environments, moderately easy to disseminate, can cause a high morbidity rate, and requires specific management and surveillance (1). The bacterium can form a spore-like structure, called a small-cell variant (SCV), which can resist extreme environments such as heat or aridity and chemical agents such as disinfectants. In addition, the bacteria also have large-cell variant (LCV) components which will multiply in the host immune response cells including monocytes and macrophage (4, 20).Previous studies have shown that *C. burnetii* can survive up to two weeks in contaminated aerosols, more than one month on meat in cold storage, and up to five months in contaminated soil (8, 21, 22). A study by Kersh et al. showed that *C. burnetii* can persist in the environment even one year after an outbreak (23) and viable *C. burnetii* were found in the environment in the areas with and without livestock such, as a bank, a city hall, a community center, etc. (24). *C. burnetii* has two antigenic variations when cultured in eggs or cell cultures. Phase I, a virulent form, is the natural phase found directly in infected humans or animals. Phase II, a non-virulent form, is obtained in the laboratory after repeated passages through cell culture (4, 8).

Infection in animals mainly spreads via air-borne transmission. Infected animals shed the bacteria via their secretions such as birth products, milk, urine, and feces, regardless of clinical symptoms (6, 25). The shedding might persist for several months, especially in vaginal mucus, feces, and milk (26). Cows and goats shed the bacteria through milk more often than do sheep (27). Ticks are suspected as the source of infection among animals (20, 28). In addition, ticks are suspected to be a sustaining source of infection in

both animals and humans because they can shed a large number of bacteria via their feces which then can become contaminated dust when dry (20, 28, 29). Ingestion of placenta or milk from infected ruminants or infected rats is another possible source of infection, as reported in dogs and cats (20).

Humans are infected mainly by inhaling aerosol particles or dust contaminated with infected animal products such as amniotic fluid, urine, or feces (4, 30). Studies have shown that wool, shoes, clothing, straw, and other materials contaminated with animal excreta are suspected vehicles for disease dissemination (22, 31, 32). Other less common routes of transmission include food-borne transmission, blood transfusion, bone marrow transplants, mother to child transmission, and sexual transmission (10, 20, 22, 30, 33-37). Person-to-person transmission is very rare. One example of such transmission is the report of Q fever infection after attending an obstetrical procedure on a patient and fetus with Q fever (36, 38). Investigations of previous outbreaks have shown that infection can occur under several other conditions, for example, wind-borne contamination from a slaughter house (39-41), likely infection from contaminated clothes among laundry workers at a Q fever laboratory (31), and ingestion of milk and cheese (42, 43). Most Q fever infections in humans are reported to be associated with ruminants contact; however, some studies report that close contact with domestic pets including cats and dogs can be associated with Q fever infection (44, 45).

Clinical manifestations

Infection in animals is usually asymptomatic. Nonetheless, Q fever can cause miscarriages, stillbirths, weak offspring, retention of the placenta, metritis, and infertility in mammals (6). These clinical symptoms are usually presented in sheep and goats. Q fever infection in cattle is mostly asymptomatic, but metritis is the more common clinical manifestation of the disease in cattle and can last for several months (46-48). *C. burnetii* infection caused a big outbreak of abortions and stillbirths among dairy goats in the Netherlands in 2007 (49) and was attributed to 0.5 – 3.8% of abortions in cattle in Germany (27). Regardless of clinical manifestation, infected animals can shed bacteria through their mucosal discharges and secretions. Infected cows and goats can shed the bacteria for a longer time in milk, e.g., infected cows can shed bacteria in milk for 13 months. Additionally, infected sheep can shed bacteria more through vaginal secretions (47). One study showed that 65% of infected cattle shed bacteria by one route;

milk is the most frequent route of shedding (33.6%) compared to vaginal mucus (10.9%) or feces (20.9%) (50). Combined shedding routes are also possible. For example, 15% of infected cattle shed bacteria through their feces and vaginal secretion, 10.0% shed via their milk and vaginal secretions, 3.6% shed in both their milk and feces, and 6.4% shed the bacteria in milk, vaginal mucus and feces (50). In addition, cows developed chronic infections more frequently than sheep. As a result, cows tend to shed the bacteria for a longer period than sheep (48).

Similar to infection in animals, the majority of Q fever infections in humans are asymptomatic. The incubation period of Q fever in humans usually ranges from 2–3 weeks after exposure, with the longest reported incubation period of 2 months (8, 51). Incubation periods and clinical manifestations in humans are influenced by host factors such as the immune system status of the patients, bacteria strain specificity, dose of the bacteria, and the route of infection to which each individual has been exposed (30). One study of human subjects showed that subcutaneous and intramuscular injections need a smaller inoculum to cause the disease; for these individuals the incubation period was as fast as 1–2 days among the subjects who received intramuscular injections (52). About 40% of humans infected with Q fever develop acute Q fever which usually presents with a fever, headache, myalgia, chills, and fatigue. Some patients develop severe symptoms such as pneumonia, hepatitis, meningitis, encephalitis, or myocarditis (8). The case fatality rate is approximately 1 – 2% (4). Approximately 2 – 5 % of acute Q fever infections require hospitalization (30, 53). However, in the outbreak in the Netherlands, the largest outbreak of Q fever reported to date, the percentage of hospitalizations could reach 20% (49). Infection in pregnant women can cause serious pregnancy complications (38, 54-56). Approximately 80% of untreated pregnancies with Q fever have obstetric complications including abortions, fetal deaths in utero, preterm deliveries, and intrauterine growth retardation (56). Furthermore, Q fever can cause chronic infections in humans regardless of acute stage clinical manifestations. Chronic infections can develop within a few months or several years after infection (10). Approximately 1–5 % of infections develop endocarditis, a chronic life-threatening condition (9, 57). In addition, approximately 10–20% of patients develop chronic fatigue syndrome which can last for years and reduce quality of life (2, 4). People with underlying valvular heart disease, immunocompromised patients, and pregnant women are at high risk of developing chronic infection (10, 30).

Q fever infection in children has been reported, but they are less likely to develop symptoms when compared to adults (53). Children with Q fever infections can present with fever, weakness, coughing, vomiting, diarrhea, abdominal pain, rashes, and pneumonia. Severe symptoms in children, including hepatitis, hemolytic uremic syndrome, myocarditis, pericarditis, osteomyelitis, meningoencephalitis, and rhabdomyolysis are rare, but possible (10, 53). Chronic Q fever in children has been reported in patients presenting with endocarditis and osteomyelitis. A review by Maltezou et al. in 2002 found 5 reported cases of children with Q fever endocarditis. The median age was 7 years (minimum and maximum age range 3.5–11 years) (53). In addition, a review by Nourse et al. in 2004 showed that 14 cases of Q fever osteomyelitis were reported worldwide and 6 of them were children with ages ranging from 2 – 9 years (58).

Laboratory diagnosis

Since clinical presentations of Q fever are nonspecific and exposure history with animals may or may not be helpful, diagnosis of Q fever in animals and humans is usually based on laboratory analysis. Serology is the most common diagnostic tool since it is less complicated when compared to culture and molecular biology techniques which have lower sensitivity and require a more sophisticated laboratory capacity (at least BSL3) (8, 10). Indirect Immunofluorescence Assay (IFA) is the current reference laboratory method for Q fever serological diagnosis (6, 8, 53). IFA can detect both phase I, a virulent form found in patients with chronic infection, and phase II, a non-virulent form found in patients with acute infection (4, 8). Therefore, IFA is also used for monitoring the progression of the disease to chronic infection and the effectiveness of treatment in chronic Q fever patients (8, 10). However, IFA has certain disadvantages as the interpretation is more subjective and tiring, and is expensive. Enzyme-linked immunosorbent assay (ELISA) is another serological method more widely used especially for seroepidemiological studies because it is easier to perform and has high sensitivity (8). ELISA is a qualitative test, hence, changes in antibody titer cannot be measured. The compliment fixation test (CF test) is another common laboratory method used in the past. It has high specificity, but lower sensitivity and is more time consuming when compared to IFA or ELISA (8). In addition, CF tests fail to detect seroconversion in the early stages of infection. Previous studies have shown that the CF test can detect

seroconversion 14–21 days after onset whereas IFA can detect it in 10–15 days (59). Some studies determine the sensitivity and specificity of these serological tests for Q fever diagnosis. Nonetheless, the interpretation of sensitivity and specificity can be challenging because there is no good gold standard laboratory diagnosis for Q fever. As a result, reference test bias might be possible. Most studies used another serological test as the gold standard. For example, the study by Meekelenkamp et al. showed that the sensitivity of IFA was 100.0%, the specificity was 95.3%, the positive predictive value was 95.5%, and the negative predictive value was 100.0% using a combination of IFA and ELISA results as the gold standard (60). The sensitivity of a commercial ELISA ranges from 85.7–99% and the specificity ranges from 87.6 – 97.6% using IFA as a reference (60, 61).

Polymerase Chain Reaction (PCR) is useful for *C. burnetii* DNA detection in a timely manner. However, PCR is only sensitive when the specimens are collected within the first two weeks of onset and before antibiotic administration (10). PCR is very sensitive for detecting bacteria shedding in animal secretion, milk, and tissue samples such as heart valves, placenta, and fetal tissue (4). However, the sensitivity of PCR tests on human sera are inconsistently reported. A 2003 study showed that the sensitivity of PCR was only 24% among patients who had symptoms clinically compatible with Q fever and had seroconversion (62). A more recent study conducted during a large outbreak of Q fever in the Netherlands in 2010 showed that the sensitivity of PCR was 98% in the acute serum (fever day 5 or earlier) of patients with serologically proven Q fever infection whose acute phase sera were also seronegative. Moreover, the positivity of PCR was lower when the antibodies were developed (63).

The US National Notifiable Diseases Surveillance System (NNDSS) has defined laboratory diagnostic criteria for acute and chronic Q fever as confirmed or supportive conditions (64). Laboratory confirmed acute Q fever is defined as:

- Having serological evidence of a fourfold increase in immunoglobulin G (IgG)-specific antibody titer to *C. burnetii* phase II antigen by indirect immunofluorescence assay (IFA) between acute and convalescent sera taken 3 – 6 weeks apart OR
- Detection of *C. burnetii* DNA in a clinical specimen via amplification of a specific target by polymerase chain reaction (PCR) assay, OR
- Demonstration of *C. burnetii* in a clinical specimen by immunohistochemical methods (IHC), OR

- Isolation of *C. burnetii* from a clinical specimen by culture.

Laboratory supportive acute Q fever is defined as:

- Having a single supportive IFA IgG titer of ≥1:128 to phase II antigen OR
- Having serologic evidence of elevated phase II IgG or immunoglobulin M (IgM) antibody reactive with *C. burnetii* antigen by ELISA, dot-ELISA, or latex agglutination.

For chronic Q fever, laboratory confirmed chronic Q fever is defined as any patient who has clinical symptoms compatible with culture-negative endocarditis, or suspected infection of a vascular aneurysm/prosthesis, or chronic hepatitis, osteomyelitis, osteoarthritis, or pneumonitis with one or more of the following laboratory results:

- IgG titer ≥ 1:800 to *C.burnetii* phase I antigen by IFA
- Detection of *C. burnetii* DNA in a clinical specimen by PCR
- Demonstration of *C. burnetii* in a clinical specimen by IHC
- Isolation of *C. burnetii* from a clinical specimen by culture

In addition, laboratory supportive chronic Q fever is defined as IFA IgG titer to *C. burnetii* phase I antigen ≥ 1:128 and < 1:800.

A Q fever diagnostic criterion using IFA in a single serum can vary by country depending on the baseline prevalence of the disease. A guideline in France suggested that a single IFA IgG titer to *C. burnetii* phase II antigen ≥1:200 is considered recent acute Q fever infection (8). IgM is not recommended for diagnosis of acute Q fever because IgM may persist at a low level for a long period of time and IgM alone might not be a good indicator for active infection (65).

For animals, the CF test is more widely used for diagnosis of Q fever particularly in a routine diagnosis at the herd level for abortive diseases (6). Interpretation of the serology test in animals needs to be cautious. The animals might seroconvert after exposure to *C. burnetii,* but not shed the bacteria (4, 6, 66). Moreover, infected animals can also shed the bacteria without having seroconversion (66). Thus, a serological test is useful to detect the prevalence of Q fever, but it is not a reliable method to determine whether specific animals are the potential sources of transmission of *C. burnetii* to humans or other animals. To detect shedding sources, the PCR test of animal body fluids such as milk, vaginal mucus, and feces is more reliable (10).

Epidemiology of Q fever

Q fever is a worldwide zoonotic disease. It has been reported in at least 51 countries globally (67). Nonetheless, an understanding of the actual prevalence of Q fever in humans and animals is unknown because the diagnosis of Q fever is based on laboratory tests and laboratory capacities are not available in many parts of the world (68). In addition, a comparison between serological studies can be challenged because of the differences in serological methods and the cut-off points used in each study (66).

In animals

C. burnetii can infect a variety of hosts including domestic and wild animals, such as livestock, cats, dogs, rodents, birds, fish, reptiles, and ticks (20). However, cattle, sheep, goats, and domestic pets are the most likely sources of infection in humans (8, 25). The presence of Q fever in ruminants varies by country. For example, Q fever was more prevalent in goats in Iran (69), France, and the United States, but was more prevalent in cows in Japan and Zimbabwe (27, 70, 71). Domestic pets such as dogs and cats can be infected with Q fever. The seroprevalence study in the United States found that dogs have a higher prevalence of Q fever than cats (53% vs 9%) (71), whereas a Japanese study found similar proportions of Q fever in cats and dogs (15% vs 10%) (70). The role of dogs in Q fever transmission to humans is not clear (45), but having close contact with a cat's parturient was suspected to be associated with Q fever infection in humans in Nova Scotia, Canada (72) , and in Maine, United States (73). A seasonal pattern of Q fever has been observed in European studies where Q fever was more prevalent in the summer or during lambing and calving seasons (74-76). Seroprevalence studies showed that increases in age, number of parity, specific breeds such as Danish Holstein, herd size, and farm practices including having loose housing in the stable, no quarantine of newly purchased animals on the farm, and a lack of hygienic precautions of the veterinarian while on the farm were positively associated with the seropositivity of Q fever among dairy cattle (74, 77).

C. burnetii has been found in more than 40 species of ticks (8, 48, 78). The role of ticks in Q fever transmission is not clear. Ticks were suspected to have a role in the transmission of *C. burnetii* between wild animals and from wild to domestic animals (71, 79). Nonetheless, previous studies in endemic areas of Q fever in Germany and Spain showed that the proportion of ticks testing positive by PCR was very small or zero (80, 81). Despite the suspicious role of ticks in Q fever transmission, infected ticks can

excrete the bacteria through their saliva and feces. The study showed that infected ticks excrete high amounts of bacteria in their feces, reaching 10^{12} organisms per gram (48). When they dry, the feces can contaminate dust particles and, therefore, possibly transmit the disease to other animals and humans.

In humans

A surveillance system for Q fever has not been widely put in place. Most of the available data about Q fever has come from seroprevalence studies. Thus, comparisons of prevalence among different areas can be difficult, since such prevalence may vary depending on the time of the study, the geographical location, population tested, laboratory methods, and cut-off points. Seroprevalence studies in the United States and Japan found that approximately 3% of the healthy adult population and 10–20% of persons in high risk occupations such as farmers and veterinarians had antibodies to *C. burnetii* (70, 82). A report from the Netherlands showed that the seroprevalence of *C. burnetii* was 2.4% among the general population, 12.2%-24.0% among blood donors, and 83.8% among high risk groups. In contrast, the seroprevalence of *C. burnetii* among blood donors was 22.0% in Germany and 38.0% in Bulgaria (27). Q fever outbreaks were reported in several countries (8, 70). However, the largest outbreak ever reported took place in the Netherlands from 2007 – 2010 where goats were the source of the outbreak and over 3,500 human cases were identified. More than 20% of the patients were hospitalized and several goats were killed for outbreak control purpose (3). The estimated loss from this outbreak was 307 million Euro (2).

Q fever can be considered an occupational hazard for people whose work involves close contact with animals; livestock farmers and veterinarians are at a high risk of infection (8, 10). A study of national disease surveillance in the United States showed that persons who had a history of livestock contact, including farmers, veterinarians, slaughterhouse workers, and tannery workers, had higher prevalences of antibodies to *C. burnetii* when compared to those who did not have a history of livestock contact (71). Nonetheless, close contact is not necessary to acquire Q fever infections; evidence of windborne transmission is indicated for several outbreaks of Q fever (40, 83-85). Studies have shown that the closer a residential area is to a farm with a history of abortions, the higher the proportion of Q fever infection (84, 86). The study in the Netherlands describing the serious outbreak of Q fever showed that people living

within 5 kilometers of infected farms were at an increased risk of getting Q fever infection (86). In 2014, an unusual outbreak of Q fever was reported among six people who received intramuscular injections of fetal sheep cells in Germany. This live cell therapy was believed to improve these people's general health, but no published articles to date have supported such a notion (87).

Q fever can affect people of all ages, but the majority of reported cases are in people of working age, particularly those aged >40 (88, 89). A study in the United States found that the number of Q fever cases increases with age; the peak was in those in the 50–59 year-old group (89). This finding could be explained by an increase in exposure to bacteria with age. Q fever is rarely reported in children, but is possible. The youngest reported age was 8 months (53). The majority of reported Q fever cases are male (8). Previous studies have shown that males have a higher risk of developing symptomatic Q fever than females. This might be explained by the protective effects of estradiol in females as shown in animal studies (90) and sex-associated occupational exposure in males (91).

Unlike Q fever in animals, a seasonal pattern of Q fever in humans is inconclusive. A review of the national disease surveillance in the United States showed evidence of a seasonal pattern with almost 40% of cases occurring from April to June (89), similar to the findings in France (92) and England (93). However, no definite seasonal pattern has been observed in Australia (94).

Prevention and control of Q fever

Vaccines, strengthening of environmental cleaning and hygiene, and the use of personal protective equipment are recommended to prevent and control Q fever.

Prevention

Vaccines against Q fever are available for humans and animals. Nonetheless, vaccines are not widely available or used in many countries. The most common type of vaccine is the whole-cell inactivated vaccine because it causes fewer adverse reactions and fewer complications when compared to the live attenuated vaccine. Vaccine in animals, particularly ruminants, is mainly used in Australia and Europe (95). Studies have shown that a vaccine can provide protection for *C. burnetii* negative animals and can partly prevent the shedding of the bacteria in milk, animal fluids, and birth products (50, 95). A

study of cows also showed that vaccination before pregnancy reduced a significant amount of bacteria shedding compared to vaccination during pregnancy and a placebo (50).

Vaccines are not widely used in humans because of serious side effects, the complexity of vaccine administration, and cost. Q-vax is the most recent vaccine used in humans. This vaccine can cause severe adverse effects such as severe local inflammatory or general systematic reaction in previously infected individuals. Therefore, vaccination is not recommended for those who have been previously infected. In addition, vaccine administration requires a prescreening for previous infection by either a skin or serology test (25). As a result, a vaccine is not feasible to use in a number of settings, especially in countries where laboratory capacities for Q fever diagnosis are limited. Vaccines are used in Australia among high risk populations such as abattoir workers and veterinarians (25, 96). Clinical trials and cohort studies on the efficacy of Q fever vaccine conducted in Australia showed that the vaccine efficacy in humans ranged from 83–100% (25, 96). However, the randomization and double-blind methods were not clearly explained in these clinical trial studies (96).

The use of personal protective equipment (PPE) including gloves, boots, and masks whenever an individual is exposed to animal secretions is also recommended to prevent the disease. Post exposure prophylaxis is not recommended for the general population. No scientific study has been conducted to evaluate the efficacy of post-exposure prophylaxis. However, doxycycline 100 mg twice daily or tetracycline 500 mg orally every 6 hours for 5 – 7 days, started 8 – 12 days past exposure is recommended for post exposure prophylaxis for essential personnel or for those considered at high risk. Early administration of the antibiotic within 7 days after exposure is not useful and will lengthen the onset of the disease (10, 35, 97).

Control

Like other zoonotic diseases, Q fever control in animals is vital to manage outbreaks for both animals and humans. Bacterial decontamination is essential once Q fever infection is evident. To reduce bacterial contamination of the environment, good hygiene practices on the farm are advocated. 0.4 – 0.6% calcium cyanamide or lime is recommended for treatment of manure, aborted fetuses, infected fetal fluids, and membranes before incineration or field burial. This process must be done in the absence of wind to avoid spreading bacteria (20, 26, 48). United Kingdom Health Protection Agency guidelines recommend use of

2% formaldehyde, 1% Lysol, 5% hydrogen peroxide, 70% ethanol, or 5% chloroform for surface decontamination. In addition, spills of contaminated materials should be cleaned immediately using hypochlorite (5000 p.p.m. available chlorine), 5% peroxide, or phenol-based solutions (65). Determination of *C. burnetii* and its viability after decontamination is recommended, but might not be feasible. Milk from infected dairy animals should be discarded. Milk from uninfected animals on the same farm can be used if pasteurized at 72°C for 15 minutes or by a similar thermal treatment, unless it is highly suspected that dairy products are dangerous for human consumptions (48). Antibiotic treatment with tetracycline injections the last month of gestation is used to reduce the occurrence of abortion and bacteria shedding into the environment during parturition. However, no study has confirmed its effectiveness (26). Although the roles of ticks in Q fever transmission are not clearly identified, tick control is suggested to reduce the sustainability of the bacteria in the environment (20, 98).

Human-to-human transmission is infrequent and secondary transmission among humans is very rare. Nevertheless, standard hospital infection control practices such as wearing masks, gloves and gowns are recommended when taking care of *C. burnetii* infected patients. Staff members who participate in the obstetric and gynecological procedures of pregnant women with Q fever or who carry the specimens suspected of *C. burnetii* contamination must wear N-95 masks, surgical gloves, and protective gowns. Decontamination of materials spilled with Q fever patient's secretions should use 0.05% hypochlorite, 5% peroxide, or phenol-based-solutions performed immediately. Contaminated equipment or instruments can be decontaminated by autoclaving or boiling for 10 minutes (35).

Treatment of Q fever

Treatment of Q fever is controversial in animals since the majority of infections are asymptomatic. To date, no effective treatment to eradicate the shedding of bacteria from infected animals has been discovered (97). Parental treatment of individual animals with oxytetracycline at 20 mg/kg given 20 days apart in late gestation to prevent adverse pregnancy outcomes might be useful when abortions are active in the herd (97). Nonetheless, information on the effectiveness of antibiotic treatment for animals is limited.

According to the most recent guideline for the treatment of Q fever in humans (2013), doxycycline is the drug of choice for acute Q fever in adults, children aged greater than 8 years, and patients with severe illness regardless of their ages. Doxycycline 200 mg per day for 14 days is recommended for acute infection (10). Fluoroquinolones, erythromycin, trimethoprim/sulfamethoxazole, and rifampin are alternative drugs (8, 10). Treatment is not recommended for asymptomatic individuals or for those whose symptoms have resolved (10). As infections can become chronic, recommendations on the follow-up for Q fever infections have been devised. Patients who are at high risk of developing chronic Q fever, including those who have valvular heart defects, aneurysms, vascular grafts, or pregnancies at the time of acute infection, should be monitored closely at 3, 6, 12, 18, and 24 months. Physical examinations and serological studies of phase I and phase II IgG and IgM antibodies should take place. If no evidence of rising phase I IgG antibody titers to 1:1024 or greater after 2 years and no clinical evidence of chronic infection such as endocarditis, vascular infection, or osteomyelitis occurs, serological monitoring could cease or be continued less frequently. For healthy acute Q fever patients with no identified risk factors for chronic infection, clinical and serological follow-up approximately 6 months after diagnosis should be performed. If the patient does not have serological or clinical evidence suggestive of the progression of chronic Q fever, follow-up can be discontinued.

Treatment of chronic Q fever should not be based on serological results alone. Identification of potential organ infection should be performed if the patient develops any symptoms with evidence of phase I IgG antibody ≥ 1:1024. Doxycycline (100 mg twice daily) in combination with hydroxychloroquine (200 mg three times daily) is the drug of choice for treatment of chronic Q fever infection. Duration of treatment depends on the site of infection; for example, treatment is recommended for 18 months or longer in patients with chronic Q fever endocarditis. Serological follow-up of sera after treatment is required to determine treatment effectiveness. Cured chronic Q fever is defined as the patient having phase I IgG titers less than or equal to 1:200 (10).

THAILAND:

Overview of the country

Thailand is the third largest country in Southeast Asia, after Indonesia and Myanmar. It shares borders with the Lao PDR to the northeast, Myanmar to the north and northwest, Cambodia to the east, and Malaysia to the south. The country is administratively divided into 4 regions including north, northeast, central, and south (99). The annual calendar includes three seasons: summer (second half of February to mid-May), rainy (second half of May to mid-October), and cool (second half of October to mid-February) (100). The population was approximately 66,785,000 in 2011 (101). The majority of people work in agriculture, for example, rice and livestock farming (99). A public health infrastructure has been in place in Thailand for more than 70 years (99). To date, health care facilities are available nationwide, including hospitals at all provincial and district levels and health centers at the sub-district level. Universal health care coverage applies to all Thai people.

A national disease surveillance system was established in 1960 after a cholera epidemic. To date, there are 87 notifiable infectious diseases. These are divided into seven groups comprising food and water borne diseases, respiratory diseases, vector borne diseases, sexually transmitted diseases, central nervous system infection, vaccine preventable diseases, and zoonotic diseases. For zoonotic diseases, diseases under the surveillance system are rabies, leptospirosis, melioidosis, brucellosis, S*treptococcus suis* infections, avian influenza, and anthrax. Q fever is not currently included under the surveillance system.

Q fever in Thailand and research gaps

Q fever was first reported in Thailand in 1967 by a US-Southeast Asia Treaty Organization (SEATO) medical research study on rickettsial diseases (12). However, even today little is known about the disease. Q fever diagnosis in Thailand is limited. The serology test for Q fever diagnosis was not available before 2012. Laboratory capacity for Q fever diagnosis using IFA has been strengthened since 2012 with the support from the Thai Ministry of Public Health – U.S.CDC collaboration (TUC). To date, five institutes can perform IFA for Q fever diagnosis. PCR for Q fever diagnosis is available at the national

level [National Institute of Health (NIH) and National Institute of Animal Health (NIAH)] and at some veterinary research centers and university hospitals.

Because of limitations in laboratory diagnosis, as well as the low level of awareness among doctors and health personnel, few studies of Q fever have been conducted in Thailand. No studies explored risk factors associated with the disease. A study of the etiologies of acute fever in four hospitals from different regions of Thailand in 2001 – 2002 showed that Q fever was responsible for approximately 1% of the patients presented with acute undifferentiated fever (12, 14). Subsequently, in 2012, the first two cases of chronic Q fever endocarditis were reported in the northeastern region (13). The first case was a farmer who worked on a cattle farm, while the second was a patient with underlying aortic valvular heart disease who lived in an area surrounded by cattle farms. No additional studies were conducted to identify the possible source of infection (13). The most recent study published in 2014 showed that *C. burnetii* was responsible for approximately 8% (5/60) of patients with culture negative infective endocarditis admitted to Srinagarind Hospital, Khon Kaen, northeastern Thailand (102).

Interest in Q fever research increased after the first two reported cases of Q fever endocarditis. In 2012, a study of Q fever among ruminants with tuberculin positive results using the ELISA test in three districts of Chiang Mai Province found the seroprevalence of Q fever among cows was 4.8% (28/581) at the individual cow level and 62% (13/21) at the farm level (103). Furthermore, a different seroprevalence study showed the prevalence of Q fever was 15.6% (24/154) among 154 farmers and veterinarian-related personnel (16). Another study in Bangkok showed seroprevalence of Q fever among dairy cattle was 7% (9/130) and seroprevalence of Q fever among chickens was 1% (1/141) Q fever surveillance of ruminant placentas in 2012 using PCR showed the presence of *C. burnetii* in beef, dairy cattle, goats, and buffaloes in 9 provinces from 3 regions of Thailand (15). In addition, a genotyping study from animal placentas showed positive results for strain ST18 in dairy cattle placenta (Pattarin et al., unpublished data). Strain ST18 was reported to be associated with acute disease in humans (17). This evidence suggests that Q fever might be endemic in Thai animals and that the burden of the disease in humans might be higher than expected.

In Thailand, the dairy cow business is growing. The most recent report in 2014 by the Office of Agricultural Economics found that the number of dairy cows has increased every year since 2008 (104).

The majority of Thai farms are small to medium. Thus, all farms are either part of a dairy cooperative supported by the government or part of a private company. Considering that farmers work closely with animals and that the virulent strain is found in dairy cows, dairy farmers are one of the populations at greatest risk of acquiring Q fever.

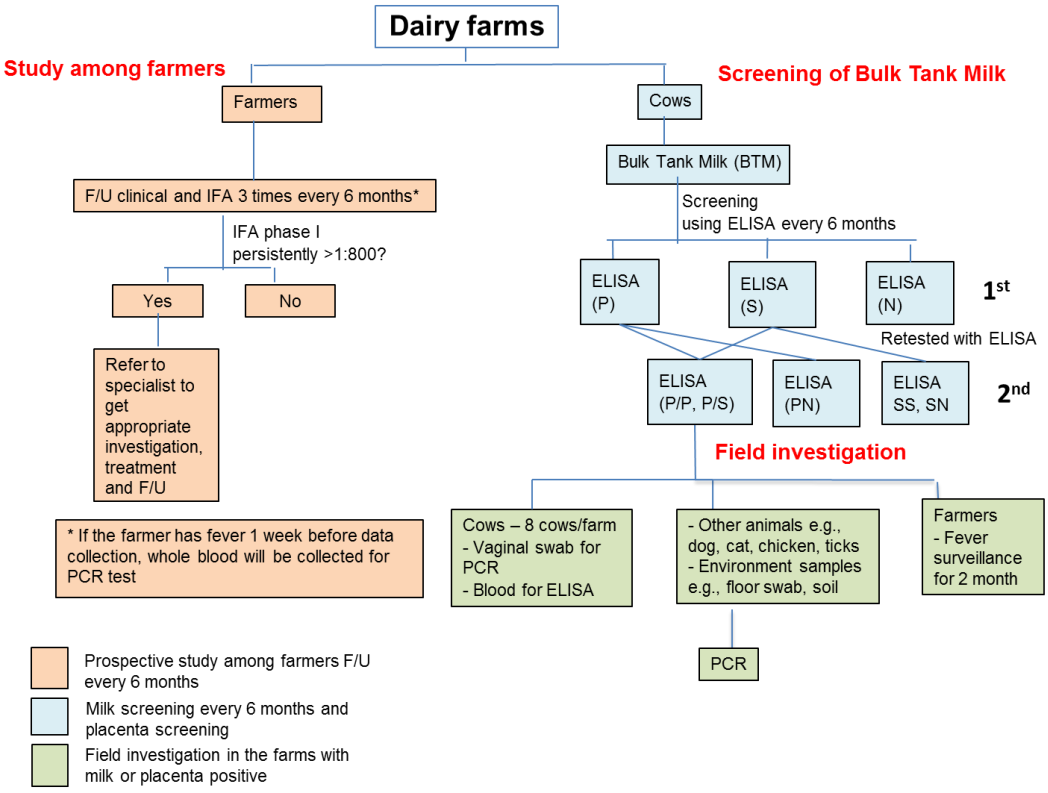
Since no study has been conducted to understand the magnitude and risk factors associated with Q fever, we proposed an epidemiological study to better assess the risk of Q fever, particularly among this high-risk population. The outcomes of this study can provide scientific evidence for the disease burden and factors associated with Q fever in dairy cattle farms and farmers. Following from these results, prevention and control strategies of Q fever can be established.

CHAPTER 3

RESEARCH DESIGN AND METHODS

Study design

The main project was a one-year longitudinal study of dairy cattle farms and farmers conducted in Chiang Mai Province from June 2015 to August 2016. The study was conducted in three dairy cooperatives where Q fever was reported in 2012 and other two dairy cooperatives in adjacent areas. It was divided into three parts: 1) a prospective study among farmers, 2) screening bulk tank milk (BTM), and 3) field investigation of farms with positive BTM (Figure 1). The study included data and specimen collection at three time points every 6 months. This dissertation involves describing the study design and the analyses of baseline data of farms and farmers.



P = positive, N = negative, S = suspect

Figure 1. The cohort study protocol

There are two parts to the animal study: 1) bulk tank milk (BTM) screening for *C. burnetii* antibodies using ELISA at 3 time points including baseline with 2 more collections 6 months apart) (Figure 1: diagram in blue), and 2) field investigation on the farms with positive BTM to identify active shedders; this will provide information for the implementation of control measures and prevention (Figure1: diagram in green). Field investigation was conducted by the investigator in collaboration with public health and animal health officers. The investigation included vaginal swabs and blood collection from cows on the farms with positive antibodies in the milk. During the investigation, we provided health education regarding Q fever prevention to farmers and conducted active case finding. If the farmer had fever within one week, we collected a whole blood sample for PCR testing.

Although Q fever transmission through milk is very rare, milk is a good and feasible sample to use as an indicator to detect Q fever infection in dairy animals as compared to feces and vaginal swabs. Previous studies show that, among infected cows, 38.0% of positive PCR samples were found in milk, 32.3% in feces, and 29.7% in vaginal mucus (50). A study published in 2014 showed that cows shed bacteria mainly in milk and PCR positive milk is correlated with seropositivity in cows (105). Other studies have used BTM screening to detect farms with possible Q fever infection (80, 105, 106), but had never before been used in Thailand. Since our study used milk as the indicator for further investigation, it is possible that we might have missed some infected cows that did not secrete the antibody through their milk, or infected heifers, or infected cows that did not give birth during the study period. Nonetheless, this selection bias was minimized since previous studies had shown that cows shed the bacteria mainly through milk (50, 107) and the risk of Q fever in animals increases when age and parity number increases (74, 77).

For the human study, a prospective cohort study among farmers on dairy cooperative farms at 3 time points was performed (Figure1: diagram in orange). Two farmers from participating farms were randomly selected. Individual information and serum samples were collected at baseline (month 0). Two additional follow-ups on clinical symptoms and blood samples at 6 and 12 months were performed. Milk collection was performed on the same day as specimen collection in humans.

Study area

Previous seroprevalence studies among dairy farms registered in dairy cooperatives in Chiang Mai Province in 2012 showed evidence of seropositive Q fever in dairy farms in Sanpatong (6 out of 6 farms positive), Mae-on (2 out of 4 farms positive), and Mae-wang districts (5 out of 11 farms positive) (103). In addition, a serosurvey of Q fever among dairy farmers in the three dairy cooperatives in 2012 showed the prevalence of Q fever among farmers was 16.7% (2/12) in Mae-on, 13.3% (4/30) in Sanpatong, and 0 (0/11) in Mae-wang districts (Rodtian et.al. unpublished data). Therefore, the study was conducted on the dairy farmers in these three dairy cooperatives, and two other dairy cooperatives in adjacent areas.

Chiang Mai was ranked as the province with the 5th highest number of dairy cattle in Thailand in 2012 (108). Most Chiang Mai farms are small to medium. All farms in this province join or sell their milk product either to dairy cooperatives or private companies. Since a dairy cooperative is a local business open to all farms using the same business agreement, farm members of each dairy cooperative can come from any area. The Chiang Mai provincial livestock office has registered 9 diary cooperatives. The report of the Department of Livestock Development in May 2014 notes that a total of 1,004 dairy farms existed in Chiang Mai, with 796 farms registered to 9 dairy cooperatives (79.3%) (109). The farms from 5 dairy cooperatives involved in this study accounted for 44.9% (451/1,004) of all farms and 45.6% (15,995/35,054) of all cows in Chiang Mai Province (109).

The farm members of the dairy cooperatives in our study come from 10 districts: Mae-on, Sankampaeng, Sansai, Doi-saked, Mae-rim, Mae-taeng, Sarapee, Sanpatong, Mae-wang, and Doi-lor (Figure 2).

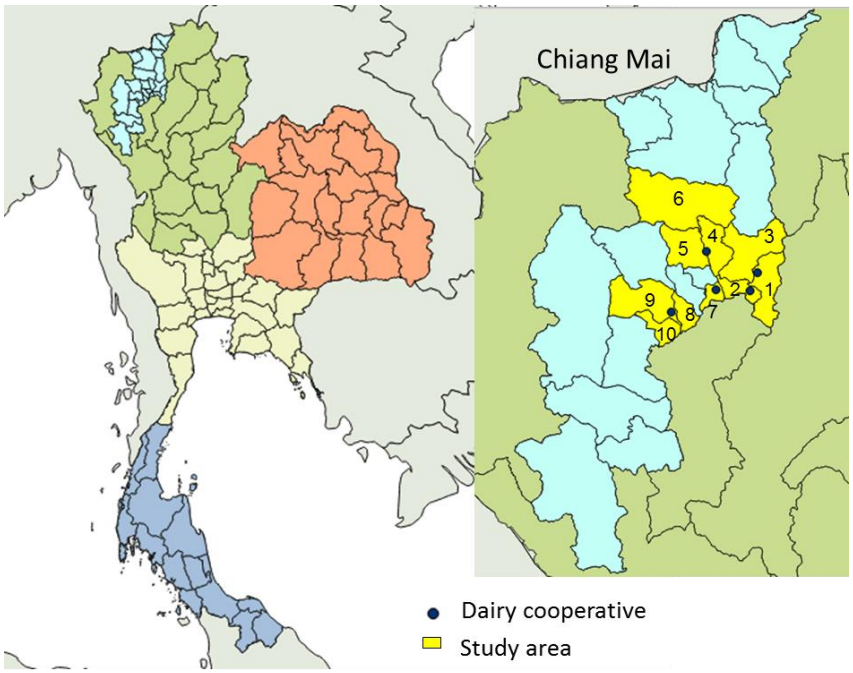


Figure 2. Study areas and study cooperatives, Chiang Mai Province, Thailand (1: Mae-on, 2: Sankampaeng, 3: Doi-saked, 4: Sansai, 5: Mae-rim, 6: Mae-taeng, 7: Sarapee, 8:Sanpatong, 9: Mae-wang, 10: Doi-lor

Mae-on District covers an area of approximately 442 km^2 with a population of 21,363 people in 2012. The majority of people work on livestock farms, particularly dairy farms (110). Sankampaeng is a suburban district, adjacent to Mae-on. It covers 197.8 km^2 and had a population of 79,072 in 2012. The majority of population work on farms growing crops such as rice and fruits, and on dairy cattle farms (111). Doi-saked is a district adjacent to Mae-on and Sankampaeng, covers an area of approximately749 km^2 with a population of 70,282 in 2012. The majority of people work on rice and fruit producing farms. Sansai district covers an area of 285.0 $km^2$with a population of 114,223. The majority of people there work on rice and potato farms. Mae-rim is a district to the west of Sansai. It covers an area of 495 km^2 with a population of 81,047. The majority of people work on non-livestock farms that produce vegetables and rice. Mae-taeng covers an area of 1,326.78 km^2 and had a population of 75,538. The majority of people work on rice and fruit farms (111). Sarapee district covers an area of 97.45 km^2, and had a population of 75,454. The majority of people work on non-livestock farms (111). Sanpatong district covers an area of 178.18 km^2 with a population of 75,875. The majority of people work in non-livestock farms such as rice and tobacco farms (111). Mae-wang district is the area to the west of Sanpatong district. It covers an area of 601.68 km^2, and had a population 31,045. The majority of people work in non-livestock

farms such as rice farms. Doi-lor is the district to the west of Mae-Wang, covering an area of 219 km^2, with a population of 26,975. The majority of people work on fruits and rice farms (111).

Study population

The study subjects included dairy farms, cows, and all adult farmers who work in the participating farms of the five dairy cooperatives.

Animals: The population was divided into two levels, the individual cow and the farm. In Thailand, all dairy cooperatives are registered to two government organization: the Cooperative Promotion Department and the Department of Livestock Development (DLD). The DLD is responsible for milk quality control and animal health. In 2013, there were 98 dairy cooperatives nationwide, with approximately 26,000 farm members or 80% of all the dairy farms in Thailand (112). This study included 5 out of 9 (55.6%) dairy cooperatives in Chiang Mai province. Dairy farms registered at any of the five dairy cooperatives before June 2015 were eligible for the study. Data from a May 2014 DLD survey is presented in Table 1.

Table 1. Number of farms and dairy cows by dairy cooperative (Data from Chiang Mai Provincial livestock office report, May 2014)

Dairy cooperative	Number of farms	Number of dairy cows
Coop1	136	4,357
Coop2	113	4,055
Coop3	81	3,063
Coop4	61	2,566
Coop5	60	1,954
Total	**451**	**15,995**

The Department of Livestock Development has specified three categories of farm size: small (number of cows less are 20 or lower), medium (number of cows between 21 – 100), and large (the number of cows greater than 100) (108). Data from the Chiang Mai Artificial Insemination and Biotechnology Research Center in 2012 showed that the majority of the farms in the five dairy cooperatives are medium size farms (113). Coop1 had no large farms, 71 (60.2%) medium farms, and 47 (39.8%) small farms; Coop2 had 6 (3.1%) large farms, 139 (72.0%) medium farms, and 48 (24.9%) small farms; Coop3had 1 (1.9%) large farm, 31 (58.5%) medium farms, and 21 (39.6%) small farms; Coop4 had 1 (1.5%) large

farm, 44 (65.7%) medium farms, and 22 (32.8%) small farms; and Coop5 had no large farm, 44 (62.0%) medium farms and 27 (38.0%) small farms (113).

Each farm has one unique bulk tank milk (BTM) number. Farmers sell BTM to the dairy cooperative daily. The cooperative tests the BTM from all the farms for milk quality assurance including somatic cell count and antibiotic contamination.

Humans: No information was available at the dairy cooperatives on the number of farmers. However, the local animal health officer, in an interview, stated that there were approximately 2 – 5 farmers per farm because the majority of farms in the study areas are small to medium. Any person 18 or older who worked on the participating farms when the study was conducted were eligible to be in the study.

Since the study population was specific to dairy farms and farmers of the five dairy cooperatives, extrapolation of results to other farms in the Kingdom or elsewhere might be limited. Nonetheless, the results from this study, which highlight the risk of Q fever for this specific group, can be beneficial to other dairy cattle farms in the country because more than 80% of these farms are part of dairy cooperatives. In addition, the study might be useful for other settings with similar farm practices.

Eligibility criteria

All farm members of the five dairy cooperatives are qualified. Dairy farmers whose age was 18 years or older, worked on a farm in the five dairy cooperatives, had no contraindication for drawing blood such as infected or scarred tissues, and were willing to participate in the study were eligible to be included in the study. A dairy farmer was defined as any person who worked in the dairy farms regardless of gender or job description. For the study in cows (the farm investigation), all cows on the farms for which the milk tested positive for *C. burnetii* antibodies were eligible.

Enrollment procedure

A meeting between the investigators and all stakeholders including dairy cooperative managers, public health and animal health officers in the study areas, and public health and animal health officers from the Chiang Mai Provincial Health Office and Chiang Mai Provincial Livestock Office was arranged to discuss the project and solicit feedback from these stakeholders. After the cooperatives agreed to

participate, separate meetings between the investigators and farmers were conducted to provide health education regarding Q fever and to inform them about the project. Lists of dairy cooperative farm members were made available from the dairy cooperative offices. The number of farmers on each farm was obtained from a short survey taken during the meeting. Two stage probability sampling was conducted to select farms and farmers. Selected farms and farmers were contacted later by telephone. Data collection dates and times were arranged after these farmers agreed to participate in the study.

Sampling methods

We selected dairy cooperatives based on the evidence of Q fever from the previous study (103) and two other dairy cooperatives in their neighborhoods. Two-stage probability sampling was performed for the cohort study among farmers. The primary sampling unit was the farms on the list provided by the dairy cooperatives. To select the farms, we used stratified sampling according to farm size. The farms were stratified into three categories: 1) farms with 1–20 cows, 2) farms with 21–40 cows, and 3) farms with > 40 cows. All farms in the third category and 50% of the farms in the first two categories were selected. This method took into account differences in farm size. From the selected farms, we planned to randomly select two farmers.

The advantages of our sampling strategy were to increase specificity and to improve feasibility. Our study was specific to a high risk population since we created a specific cohort of dairy farmers. Consequently, specific prevention and control could be implemented. Moreover, this design was feasible since Chiang Mai Province has a well-established 'One Health team' which includes networks of both animal and public health professionals. As a result, the dairy cooperatives were more likely to agree to participate in the study. Two-stage sampling within the dairy cooperative allowed us to infer the results for all farms and farmers within these dairy cooperatives. The disadvantage of the purposive sampling was because it was non-probability sampling; thus we could not generalize the results to dairy farms and farmers in other areas or to the general population. Nevertheless, the study among this specific population can generate new knowledge of Q fever among high risk people in Thailand. Therefore, it can provide policy makers with scientific evidence of Q fever prevention and control.

Data collection

Data were collected as follows concerning dairy cattle: 1) bulk tank milk (BTM) screening for antibodies to *C. burnetii* using ELISA at 3 time points including baseline and 2 follow-ups at 6 month intervals, and 2) field investigation on farms with positive BTM. Field investigation included blood and vaginal swabs collected from 8 cows on each of the positive farms. We provided health education about Q fever prevention and control to farmers during the farm visit. We also collected whole blood samples for a PCR test if the farmer had had fever within 7 days before the visit; this whole blood was collected to identify for *C. burnetii* DNA using real time PCR. For a cohort study among farmers, face-to-face interviews were conducted by trained interviewers using tested questionnaires to collect exposure data from all farmers who participated in the study. Data and sample collection of farmers were conducted at the same time as the BTM screening.

- Exposure assessments

Two questionnaires, a questionnaire to measure exposure history of each farmer and one to assess farm practice and farm management were used to assess farm exposure. These were developed and tested with the group of farmers in the study area before they were used to assure that the questions were clear and easy to understand. A face-to-face interview was conducted by trained interviewers using the tested questionnaires. All participating farmers were interviewed about their job descriptions and other exposure history on the farm. Farm owners or farm representatives were also interviewed about farm practices and farm management using the questionnaire. The interviews were conducted 3 times – at baseline and two follow-ups at 6 month intervals. The interviewers were health officers and nurses. Training sessions on the questionnaire and the interview were held before data collection to ensure that all interviewers understood the questionnaire and procedures.

The exposure assessment was divided into three components: animal, human, and ecological.

1) Animal component:
    - Bulk tank milk number and the somatic cell count
    - Demographic information on cows: ages and breed
    - Reproductive history: number of parity, history of abortions, stillbirths, having weak offspring, placenta retention, and metritis

- Length of stay on farm, such as newly moved to or born and raised on the farm
- History of antibiotic use for 2 months before interview

2) Human component: since the data were collected 6 months apart, exposure history was an estimation of the history in the past 6 months before the interview.
   - Demographic information: age, gender, address, occupation, educational level, underlying diseases, and pregnancy status for women.
   - History of exposure to cattle: job descriptions and personal protective equipment used on the farm such as masks and boots. Previous studies showed that Q fever infection is dose dependent, hence, the average duration of exposure to cows (days/week and hours/day) was collected.
     - Definition of main exposure: close contact with cow secretions, particularly attending calving, and handling cow's birth products. Exposures were classified as binary variable (yes or no), as well as the degree of exposure, defined by the number of cows farmer helped with calving, hours of work per day, and days per week.
     - Other exposure covariates: milking and culling
   - History of exposure to other animals and other possible risk factors: history of raising or having any of these animals: dogs, cats, rats, chickens, ducks, and history of abortion among them, history of traveling to other animal farms, histories of tick bites, drinking raw milk, cooking and eating raw placenta, and receiving blood transfusions.
   - Clinical signs and symptoms such as fever, headache, myalgia, fatigue, nausea, vomiting, abdominal pain, jaundice, cough, dyspnea. Moreover, laboratory information such as complete blood count, liver function test, and chest X-ray were extracted from the medical record review if the patient has visited the hospital.
   - History of antibiotic use one month before the interview: name of antibiotic, dosage, and duration of antibiotic use.

3) Ecological component: other studies have shown that environmental factors such as farm practices, other animals raised on the farm, and wind direction were associated with Q fever

infection (39-41). Therefore, information regarding these ecological factors was gathered by interview and data extraction. We collected information at baseline and at the follow-ups every 6 months. Ecological factors and data sources include:

- Farm characteristics: e.g., number of cows on the farm, number of farmers, duration of farm business. These data were obtained from interviews with farmers and a review of the district livestock office logbook.
- Practices in the farm: e.g., having loose housing in the stable, no quarantine of newly purchased animals coming onto the farm, cleaning practices, and tick control practices. This information was gathered by interviews with the farm representative.
- The main exposure of interest for the farm study was cleaning practices in the birthing area right after birth. The degree of exposure was classified into binary variables (yes or no) and categorical variables such as never, rarely, sometime, often, always etc.
- Geographical location of the farm and other animal farms in the area. This information was obtained from the provincial livestock office and during the farm visit.
- Number and types of animals raised on the farm: for example dogs, cats, chickens, and vaccines used on the farms. This information was obtained from interviews with farmers.
- We planned to collect meteorological factors such as wind patterns and humidity during the study period. Unfortunately, no meteorological stations are located in the study areas.

- Outcome assessment

Study outcomes were evaluated at baseline (month 0) and 6 and 12 month intervals. For the human study, interview and blood collection for IFA was performed at 3 time points. If a farmer had a fever without an antibiotic administration within 7 days before the interview, whole blood samples were collected for the PCR test. The outcomes of interest are Q fever infection in humans and animals, determined by clinical presentations and laboratory results. Definitions of Q fever infection in humans were adapted from the US National Notifiable Diseases Surveillance System (64) and classified as follows:

Clinical suspicion of acute Q fever: any person who had a fever and at least one of the following signs or symptoms: severe headache, pneumonia, acute hepatitis, or elevated liver enzymes.

Confirmed acute Q fever infection: a person who had clinical suspicion of acute Q fever and a four-fold increase in IgG specific antibody titer to *C. burnetii* phase II antigen by indirect immunofluorescence assay (IFA) or had a PCR positive for *C. burnetii* DNA.

Probable acute Q fever infection: a person who had clinical suspicion of acute Q fever and a single serum of IgG antibody titer to *C. burnetii* phase II antigen ≥ 1:128 by IFA.

Suspected acute Q fever infection: clinical symptoms compatible with clinical suspicion of acute Q fever and had IgG antibody titer to *C. burnetii* phase II antigen ≥ 1:64 by IFA.

Asymptomatic infection: a person who did not have fever during the study period and had a four-fold increase in the IgG specific antibody titer to *C. burnetii* phase II antigen by IFA.

Q fever seropositive: an individual who had a single serum of IgG antibody titer to *C. burnetii* phase II antigen ≥ 1:64 by IFA.

Laboratory confirmation of chronic Q fever infection: any farmer who had serum of IgG antibody titer to *C. burnetii* phase I antigen ≥ 1:800 by IFA.

Laboratory suspect of chronic Q fever infection: any farmer who had IgG antibody titer ≥1:128 and <1:800 to *C. burnetii* phase I antigen by IFA.

Outcomes of the animal study were assessed from laboratory results through BTM screening using ELISA at the farm level and ELISA and PCR tests at the individual cow level. BTM screening was performed two times. We recollected and retested BTM samples from farms with positive or suspected BTM found at the first screening. Definitions of the outcomes of interest in animals were defined as the following:

Farm with active Q fever shedder: farm had evidence of *C. burnetii* DNA from vaginal swabs using PCR.

Farm with evidence of Q fever infection: farm had evidence of *C. burnetii* antibody in milk using ELISA.

C. burnetii seropositive: cow tested positive for antibody to *C. burnetii* by ELISA.

Active Q fever shedder: cow had positive *C. burnetii* DNA from vaginal swabs using PCR.

BTM negative result: BTM tested negative at the first screening using ELISA.

BTM positive result: farm with a first positive and positive or suspected positive second test and with suspected first test and positive second test.

BTM uncertain result: farm with first suspect and suspect or negative second test and with first positive and negative second test.

Laboratory procedures and quality control

- Specimen collection, storage and shipment

Data collection dates were arranged when farmers agreed to participate in the study. Participants were interviewed and had blood drawn on the same day. Specimen collections were performed at 3 time points: baseline and 2 additional follow-ups at 6 month intervals. Five milliliters of blood were collected each time by well-trained nurses using sterile techniques and stored in clot blood tubes before transferring to Maharaj Nakorn Chiang Mai Hospital on the same day they were collected. Sera were extracted and stored at Maharaj Nakorn Chiang Mai Hospital's laboratory below -20°C. Sera were later transferred to Thai-NIH for IFA testing. All human sera were tested for IgG and IgM antibodies to *C. burnetii* phase I and phase II antigen. In addition, if the participant had had a fever within 7 days before the interview, 5 ml of blood were collected in Ethylene diamine tetraacetic acid (EDTA) tubes for PCR testing.

All the BTM samples were screened at 3 time points, 6 months apart using ELISA. Twenty milliliters of milk from the BTM were collected at the dairy cooperatives in the morning by a research assistant on the same day or within one week of data collection of the farmers. All milk samples were labeled and transferred to the Veterinary Research and Development Center in ice boxes on the same day the specimens are collected. If the milk was positive, blood specimens for ELISA and vaginal swabs for PCR from 8 cows on that positive farm were collected. In addition to specimen collection from cows, sera of domestic animals such as dogs and cats raised on the farm and from cow ticks were collected to evaluate the existence of Q fever infection. Animal blood samples were tested for *C. burnetii* antibody using ELISA. Ticks were collected to identify the species and the presence of *C. burnetii* by Polymerase Chain

Reaction (PCR). Laboratory tests for all animal specimens were performed at the Veterinary Research and Development Center (Northern Region) in Lampang Province.

Specimen collection in animals and humans was performed by trained veterinarians and nurses. Laboratory analyses were performed by trained technicians following industry and CDC guidelines for ELISA and IFA respectively. In addition, PCR studies were validated following US-CDC protocol.

- Q fever diagnostic testing

(1) *Molecular Detection of C. burnetii*

Polymerase Chain Reaction (PCR) is a useful tool and has become more popular for Q fever diagnosis because it provides a result in a timely fashion (63). Previous studies have shown that using PCR to detect *C. burnetii* DNA is more sensitive within the first week after a fever (10). Therefore, blood specimens from farmers who had a fever one week before the interview were collected in EDTA tubes. Real time PCR was used to detect the evidence of *C. burnetii* DNA. The transposase gene of insertion element, IS1111, of *C. burnetii* was the target gene. Previous studies showed that IS1111 is the sensitive target for PCR since they identified 75 isolates of *C. burnetii* from all over the world (114). Real time PCR was performed at Thai-NIH, following the protocol of the Q fever Laboratory, Ricketsial Zoonoses Branch, Division of High Consequence Pathogens and Pathobiology, National Center for Emerging and Zoonotic Infectious Diseases, US-CDC. The forward primer was 5'-ccgatcatttgggcgct-3' and the reverse primer 5'-cggcggtgtttaggc-3'. The hybridization probe method was used in real time PCR and the probe sequence was 5'-ttaacacgccaagaaacgtatcgctgtg-3' (115). Strand-specific detection using a labelled nucleic acid probe enhanced specificity of the PCR since the probe demonstrated the presence of the target sequence.

Vaginal secretion from vaginal swabs, swabs from the birthing area, soil, leftover food, whole blood samples from other animals on the farm, and ticks were tested using real time PCR. DNA extractions were performed using a Qiagen QIAmp kit, following the Qiagen Standard Tissue Extraction Protocol. Real time PCR for all animal and environment samples was performed at the Veterinary Research and Development Center (Northern Region), using the same protocol as for humans.

(2). *C. burnetii Serology*

Indirect Immunofluorescence Assay (IFA) is the current reference laboratory method for Q fever serological diagnosis. IFA can detect both phase I, a virulent form found in subjects with chronic infection, and phase II, a non-virulent form found in patients with acute infection (10). The capability to perform IFA for Q fever diagnosis was developed in Thailand in 2012 with support from the US.CDC. The *C. burnetii* phase I and phase II antigens used for IFA slides were the synthesis of *C. burnetii* Nine Mile strain. IFA were performed following the US.CDC protocol. Our study determined the presence of IgG and IgM specific antibody to *C. burnetii* for both phase I and phase II antigen. Serological positive results were defined according to the case definitions mentioned previously in the outcome assessment section (64).

The Enzyme-Linked Immunosorbent Assay (ELISA) test has become more popular tool for use in serological surveys for Q fever since it has high sensitivity and specificity and is easy to perform (8, 10). ELISA has also been used for milk screening in other studies, which found that ELISA results corresponded with PCR results and can be a good tool for screening at the population level (105, 107). ELISA kit (IDEXX CHECKIT™ Q-fever Test Kit) was used for the serological study in milk and cow sera. The tests were carried out at the Veterinary Research and Development Center in Lampang Province following industry protocol.

Data management and analysis

Data management and quality control

Interviewers were trained about the questionnaire before interviewing to ensure the quality of data collection. Questionnaires were rechecked for completeness after the interview by research assistants. Data were entered using EpiData3.1 (EpiData Association, Odense, Denmark). Data checking and cleaning were conducted. If there was a missing value or uncertain information, we rechecked the questionnaire or communicated with the participants to make sure that the information was correct or changed, as appropriate. Quality control for laboratory methods followed standard criteria of the reference laboratory.

Data analysis

Data and sample collection started in June 2015 and finished August 2016. Laboratory work was delayed from the planned schedule. Therefore, this dissertation focusses only on the analyses of the baseline data.

Aim1. Describe the burden and factors associated with Q fever in dairy cattle farm

1.1 Describe farms with Q fever positive in BTM and prevalence of Q fever in dairy cattle on the farms with positive BTM.

To illustrate the magnitude of Q fever among dairy cattle farms in the five dairy cooperatives, the proportion of farms according to the ELISA results were calculated from the BTM screening data. Characteristics of these farms, such as number of cows, number of other animals, and farm practices by BTM results were calculated. Prevalence and 95%CI were calculated using sampling weight. Sampling weight was calculated from the number of eligible farms divided by the number of selected farms. Since only cows on farms with positive BTM were further investigated, the proportion of individual cows testing positive among all the cows being tested was calculated. Within-herd seroprevalence was also calculated. Clinical manifestations and characteristics of cows with serological positive and vaginal swab positive and negative were portrayed. In addition, geographical distribution of the farms and environmental factors such as distance from the positive farms were explored.

1.2 Identify factors associated with the prevalence of C. burnetii antibody in BTM.

BTM was used as the indicator for *C. burnetii* infection at the farm level. To identify factors associated with prevalence of *C. burnetii* antibody in BTM, logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI). A literature review was performed to determine the factors that might be associated with *C. burnetii* infection. Distances from the farms with positive BTM were calculated using ArcGIS. Univariate analysis of each variable controlling for sampling variables (cooperative and farm size) was performed. Variables with p-value ≤ 0.2 were considered important. Collinearity between variables was assessed and only informative variables were included in the multivariate analysis. The age and number of cows on the farms were reported as significant factors associated with the prevalence of *C. burnetii*; hence, these variables were always included in the model. In addition, sampling variables

including cooperative and farm size were always included in the multivariate analyses. Since the number of cows and farm size were highly correlated, only farm size was included in the model. Manual backward elimination was performed. The Likelihood Ratio Test (LRT) was performed to determine the importance of included variables in the model and a p-value < 0.05 was considered significant. An ecological fallacy was possible since the analysis only involved group level data, such as environmental factors and farm management practices; individual data were unavailable for analysis. Nevertheless, this objective underscored the potential factors associated with the prevalence of *C. burnetii* infection at the farm level, which is crucial in terms of prevention and control.

Aim2. Describe the burden and factors associated with Q fever among dairy farmers

2.1 Describe the prevalence, incidence, and clinical spectrum of Q fever among dairy farmers

The outcomes of interest were the seroprevalence of *C. burnetii* infection and prevalence of Q fever among farmers. Definitions of seropositivity and Q fever infection have been mentioned above. The prevalence of seropositive individuals were those who had IFA positive results regardless of clinical signs and symptoms at baseline. The prevalence proportion was calculated using the number of prevalent cases as a numerator and the number of the study population as a denominator.

Descriptive statistics (frequencies, mean, standard deviation, median, interquartile range, maximum, and minimum) concerning cases and exposure variables by person, place, and time were performed. The geographical distribution of cases by farms, number of dairy cattle, and other possible sources of infection, such as other animal farms were explored.

2.2 Identify factors associated with Q fever among dairy farmers and their family members

To determine factor(s) associated with seropositivity among farmers, the Generalized Estimating Equation (GEE) model was applied. Since Q fever infection among the farmers working on the same farms could be influenced by both individual factors, such as age, gender, and underlying health conditions, and group factors, for example, the number of *C. burnetii* reservoirs on the farm and the proportion of active shedder animals, an independent assumption for traditional regression was violated. GEE is a statistical model to deal with correlated data by modeling the covariance structure. The GEE

provides a population average of the effect as a function of covariates over group-to-group heterogeneity, while accounting for within group non-independence of observations. The GEE requires a sufficiently large number of clusters for a robust estimation of standard errors. The benefit of GEE is that it allows robust inference even if the correlation model is mis-specified (116).

Each farm has a unique tank number which was used as a unique identifier. The main exposure of interest was assisting in calving and coming into contact with a cow's birth products. Univariate analyses controlling for sampling variables were conducted. Variables with GEE type 3 p-value ≤ 0.20 were considered important and were included in the multivariate analysis. Farm variables (aim 1.2) such as cleaning practices, proximity to positive farms, and BTM results were also incorporated into the analysis. Confounder assessment was performed using the change in beta estimation of the main exposure to the outcome in the full model compared to the reduced model or $|\beta full - \beta reduced|$. The estimate of the main exposure in the full model is the gold standard. Change greater than 10% meant that the variable dropped from the full model (the reduced model) was a potential confounder; hence, that variable was kept in the model. The covariate with the highest p-value in the full model would be dropped first. The Odds Ratio (OR) and the 95% confidence interval (CI) were estimated from the model. Data analyses were performed using SAS version 9.3.

Possible challenges and plan:

- The study is subject to measurement errors as the outcome specification is based on serological tests which depend on the immune responses of each individual and the sensitivity and specificity of the diagnostic tests. Therefore, outcome misclassification is possible. To account for measurement errors, sensitivity analyses using a different antibody cutoff titer will be performed.

Sample size and power

Sample size justification was performed using information from a previous study where the prevalence of Q fever among dairy farmers was 16% (16). The CSurvey program was used for sample size calculation (117). For sample size calculation for the whole project, estimated one year incidence of Q fever among farmers (seroconversion) was 1.6%, one-half length of the confidence interval as 1.6%, the level of confidence as 95%, the design effect of 2, and the number of farmers per farm as 2, the

number of clusters needed as 260 farms and 520 farmers. The expected *C. burnetii* seroprevalence among farmers at baseline was 16%. To account for prevalence at baseline, the sample size we needed was 310 farms and 620 farmers. EPISHEET was applied to estimate the power using the sample size calculated from the CSurvey (118). We assumed that the risk of *C. buretii* infection in un-exposed humans with no history of assisting in calving was 0.018, the ratio of unexposed to exposed was 1, alpha was 0.05, the expected RRs range was from 2.0 – 3.0, and the estimated power was 23% – 78% if the number of exposed farmers was 260 (Figure 3).

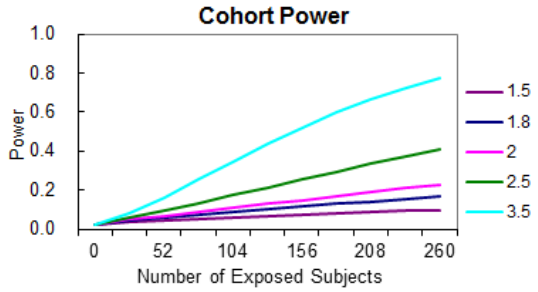


Figure 3. Power analysis of cohort study by relative risk and number of exposed subjects

For the animal study, to identify factors associated with *C. burnetii* infection in dairy cow farms (aim 1.2), the prevalence ratio was estimated from the ecological data. The main exposure was cleaning practice right after calving. To calculate the power based on the number of farms we have from the study in humans, we assumed that 260 farms participated in the study, the ratio of exposed to unexposed farm was 1, the risk of Q fever in un-exposed farm was 0.05, alpha was 0.05, and the expected prevalence ratio ranged from 2 – 3, the estimated power was 32% - 79% (Figure 4).

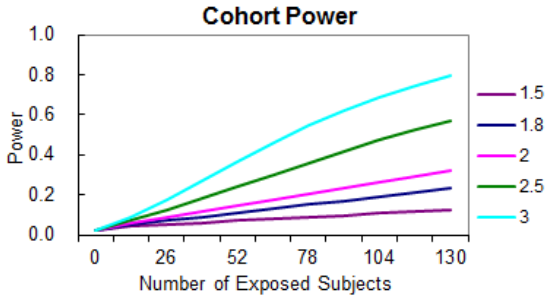


Figure 4. Power analysis of the ecological study of Q fever in the dairy cattle farms

Timeline

The on-site project was initiated in May 2015 and baseline data collection started in June 2015. The first month was field preparation

Table 2. Time line of the project

Project Aspect	May-15	Jun-15	Jul-15	Aug-15	Sep-15	Oct-15	Nov-15	Dec-15	Jan-16	Feb-16	Mar-16	Apr-16	May-16	Jun-16	Jul-16	Aug-16	Sep-16
Study preparation																	
Finalize study protocol and materials																	
Contact and prepare field work																	
Data collection																	
Meeting with stakeholders e.g., public and animal health officers																	
Train interviewers																	
Sampling and recruitment																	
Bulk tank milk surveillance		Baseline							1st F/U								
Data and specimen collection in human		Baseline							1st F/U								
Laboratory work																	
Data management, analysis, and manuscript																	
Data cleaning																	
Data analysis																	
Manuscript preparation																	
Submit papers for publication																	

Ethical considerations

This project was reviewed and approved by the Institutional Review Board (IRB) of the Department of Disease Control, Ministry of Public Health Thailand, the University of North Carolina at Chapel Hill, and the U.S. CDC. Informed consent forms for individual farmers and each farm were used. On enrollment day, each farmer was informed about the project, potential risks and benefits, and given the consent form. Farmers were allowed to ask any questions they might have had regarding the project. Consents were obtained from farm owners and farmers before data and sample collection. This project was exempted from the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill review.

Significant and impact

This study was the first longitudinal study to explore the epidemiology of Q fever among dairy cow farms and farmers in Thailand. Considering its longitudinal design, we have evaluated the incidence and risk factors associated with Q fever infection. Therefore, the outcomes of this study can provide scientific evidence for Q fever prevention and control among the high risk Thai population. In addition, this study

has clinical implications as it will highlight clinical spectrums and possible factors associated with developing signs and symptoms of Q fever patients; hence, it can provide a guideline for physicians in the diagnosis of Q fever. The results of this study will be presented to the Thai infectious disease medicine council so that it can also improve physicians' awareness of Q fever. Moreover, this study demonstrates the importance of the "One Health principle" in which the capacity of Thai government partners including animal and public health sectors to conduct a field investigation and laboratory testing of Q fever will be strengthened.

CHAPTER 4

COHORT PROFILE: A LONGITUDINAL STUDY OF Q FEVER AMONG DAIRY CATTLE FARMS AND FARMERS IN CHIANG MAI, THAILAND 2015 – 2016

INTRODUCTION

Q fever is a zoonotic disease, caused by Gram-negative intracellular bacteria *Coxiella burnetii.* It has a low infectious dose and is classified as a category B bioterrorism agent (1). It is capable of causing large outbreaks, which may result in high morbidity in humans and animals. It can also have serious economic impacts, as was reported in the Netherlands (3, 119). The main route of transmission is by inhalation of aerosols contaminated with the bacteria from infected animal secretions such as birth products. *Coxiella burnetii* can infect a variety of hosts such as humans, ruminants, dogs, cats, and ticks. Exposure to these animals can lead to a high risk of infection (4). Q fever can cause abortion and weak offspring in animals, and flu-like symptoms, pneumonia and hepatitis in humans (5, 6). In addition, it can cause chronic sequelae in humans including endocarditis in 1 – 5% and chronic fatigue syndrome in 10 – 20% of the infections (7, 9, 10). Since the clinical manifestations of acute Q fever are non-specific, Q fever diagnosis relies on laboratory tests, particularly serology.

Q fever was first reported in Thailand in 1966 by the US SEATO Medical Research Study on Rickettsial Diseases (120). However, little was known about it because Q fever diagnostic capacity was limited in Thailand. After two reported cases of chronic Q fever endocarditis in Thailand in 2012, laboratory capacities for Q fever diagnosis using Indirect Immunofluorescense Assay (IFA) and Polymerase Chiang Reaction (PCR) were strengthened with the support of a Thai Ministry of Public Health – U.S.CDC Collaboration (TUC). In 2012, a seroprevalence study of Q fever in ruminants and people who were occupationally exposed to animals in two provinces of Thailand using ELISA showed the seroprevalence to *C. burnetii* among farmers at 43% (68/159) in the northern and 3% (15/502) in the

northeastern provinces (unpublished). In addition, the seroprevalence of *C. burnetii* in animals was higher in the North (5.5% vs 0%); however, the prevalence in sheep and goats was higher in the northeastern province (5.9% vs 1.0%). Another study in Chiang Mai found the seroprevalence of Q fever among farmers and livestock officers was 16% using IFA (unpublished).

In Thailand, the dairy business is growing. The most recent report by the Office of Agricultural Economics found that the number of dairy cows has increased every year since 2008 (104). No previous study done in Thailand examined risk factors associated with Q fever infection. Therefore, the Bureau of Epidemiology, Ministry of Public Health (MOPH), Thailand, in collaboration with Chiang Mai provincial health and livestock offices, the Department of Livestock Development (DLD), the National Institute of Health (NIH), the National Institute of Animal Health, and the Thai-MOPH – US. CDC Collaboration (TUC) launched a longitudinal epidemiological study to quantify the risk and identify risk factors associated with *C. burnetii* infection among dairy farmers and the prevalence and factors associated with *C. burnetii* infection in dairy cattle herds. The aims of the study were to describe the prevalence, incidence, and factors associated with Q fever and *C. burnetii* seropositivity in dairy farmers, to describe clinical manifestations of Q fever cases, and to describe the prevalence and factors associated with *C. burnetii* infection in dairy cattle farms using milk as a proxy for *C. burnetii* infection at the herd level. In addition, the prevalence of *C. burnetii* infection and factors associated with *C. burnetii* shedding and *C. burnetii* seropositivity in cows on farms with positive milk were explored. The outcomes of this study provide scientific evidence for the burden and factors associated with Q fever in dairy cattle farms and farmers in Thailand. Further, these data aid the development of prevention and control policies for Q fever.

COHORT DESCRIPTION

Setting

A longitudinal study among dairy cattle farms and farmers was conducted in Chiang Mai Province from January 2015 – December 2016. The study was conducted in five dairy cooperatives including three dairy cooperatives where Q fever was reported in 2012 (103) and two other dairy cooperatives in adjacent areas. Chiang Mai was ranked as the province with the 5th highest number of dairy cattle in 2012 (108). Nine diary cooperatives were registered to the Chiang Mai provincial livestock office. According to a

report from the Department of Livestock Development in May 2014, a total of 1,004 dairy farms were registered in Chiang Mai with 796 farms registered to nine dairy cooperatives (79.3%). The farms in this study were part of the five study dairy cooperatives and included 44.9% (451/1,004) of all farms and 45.6% (15,995/35,054) of all cows in Chiang Mai Province. The distribution of farms and cows by the study dairy cooperative is described in Table 3.

Table3. Distribution of farms and number of cows by dairy cooperatives

	Number of farms (number of cows)			
Cooperative	Farm with 1 - 20 cows	Farm with 21 - 40 cows	Farm with > 40 cows	Total
Coop1	41(582)	54(1572)	38(2203)	136(4357)
Coop2	24(331)	48(1381)	41(2343)	113(4055)
Coop3	21(274)	33(1029)	27(1760)	81(3063)
Coop4	7(115)	28(926)	26(1525)	61(2566)
Coop5	16(204)	26(779)	18(971)	60(1954)
Total	109(1506)	189(5687)	150(8802)	451(15995)

Meetings between the investigators and all stakeholders including dairy cooperative managers, public health and animal health officers in the study areas, and public health and animal health officers from the Chiang Mai Provincial Health Office and the Chiang Mai Provincial Livestock Office were held in Chiang Mai from January 20th – 23rd, 2015 to engage with stake holders and develop the project work plan. Separate meetings between the investigators and farmers from the five dairy cooperatives were conducted from March 16th – 20th, 2015 to provide health education regarding Q fever and to inform them about the project. The study was divided into three parts: 1) a prospective study of farmers, 2) screening of bulk tank milk produced by each farm, and 3) field investigation of milk positive farms (Figure 5). For the farmer study (Figure 5: diagram in orange), randomly selected farmers who were willing to participate and agreed to have blood drawn were interviewed at 3 time points including baseline and two more follow-up points at 6 months and 12 month intervals after the baseline.

The other two parts of the study in animals included screening of bulk tank milk (BTM) for the *C. burnetii* antibody using ELISA at three time points using the same time frame as the farmer study (Figure 5: diagram in blue) and field investigation on the farms with positive milk to identify the source of

C. burnettii. These were taken so that control measures and prevention could be implemented (Figure 5: diagram in green).

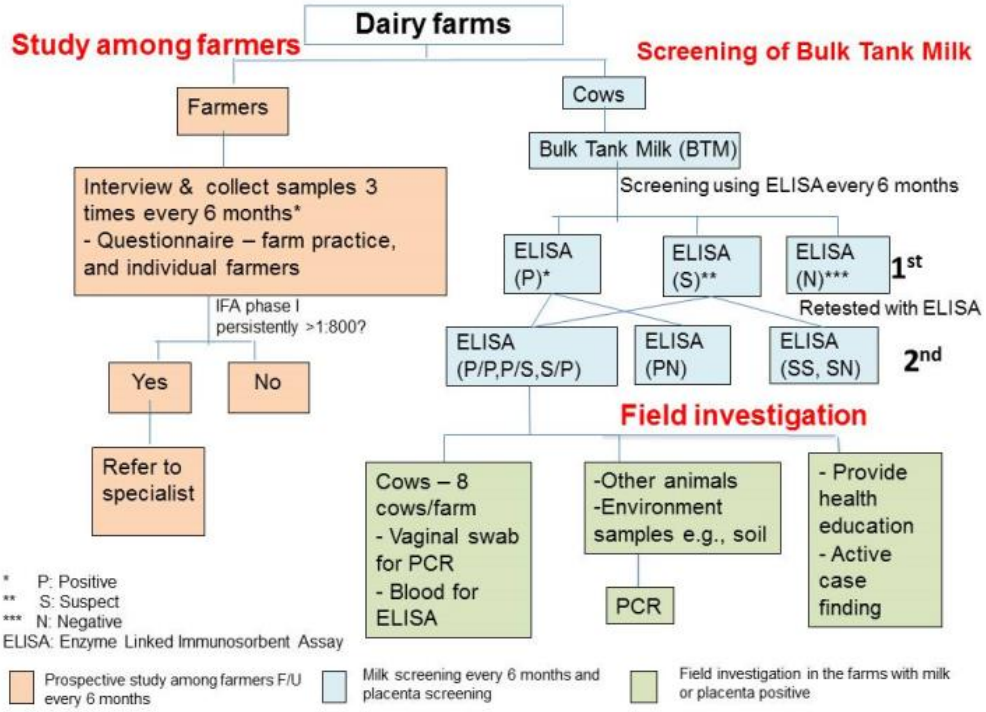


Figure 5. A protocol of the study

Eligibility criteria

Farms, farmers, and cows in the five dairy cooperatives were eligible for the study. All Thai farmers who worked in the participating farms, whose age was 18 or older when the study was conducted, and had no contraindication for blood drawn were eligible. A dairy farmer was defined as any person who worked in the dairy farms regardless of their gender and job description. In addition, all cows on ELISA positive milk farms were eligible.

Sample size and sampling

Sample size justification was performed based on the results of a previous study where the prevalence of Q fever among dairy farmers was 16%.[16] The CSurvey program was used for sample size calculation (117). The estimated one year incidence of seroconversion of *C. burnetii* infection among farmers was 1.6%. The half-length of the 95% confidence interval was specified as 1.6%, with a design

effect of 2, for an average of 2 farmers per farm. Hence, the number of clusters needed for the study was 260 farms and 520 farmers. We assumed that the prevalence at baseline was 16% for a positive serology to *C. burnetii* at baseline. Thus, to account for the prevalence at baseline, we needed to screen a total sample size of 620 farmers from 310 farms at the beginning of the study.

Two-stage sampling was used to select a farmer cohort. The list of farms was obtained from the databases of the dairy cooperative with the primary sampling unit as the farm. We selected farms using stratified random sampling. All the farms in the five cooperatives were divided into three categories based on the number of cows within the farms; 1) farms with 1–20 cows, 2) farms with 21–40 cows, and 3) farms with > 40 cows. All farms with > 40 cows were selected, about 50% of the farms with 21 – 40 cows were selected, and about 50% of the farms with 1 – 20 cows were selected so that ultimately 306 farms were randomly selected. Figure 6 showed the examples of farm by farm size.

Figure 6. Examples of farm by farm size

The second sampling unit was the farmer. Lists of farmers were obtained from a short survey during the meeting with farmers, in which we asked for lists of the number of farmers at their respective farms. The sampling of farmers was adjusted based on the number of farmers per farm we obtained after the survey. The number of farmers from the selected farms varied from 1 – 8. To account for the variation of number of farmers to reach the targeted sample size, all farmers were selected at farms with 1 – 3 farmers; 3 farmers were randomly selected from farms with more than 3 farmers by simple random sampling and random digit number. Ultimately, 637 farmers were randomly selected.

During the farm visit, 8 cows were selected as randomly as feasible to represent infection at the farm level. Sample size calculation was performed using the CSurvey program; the estimated seroprevalence

of *C. burnetii* infection in dairy cows was 15%, the estimated one-half length of the 95% confidence interval was 4%, the design effect was 3, and the number of clusters expected to have positive milk was 115 farms. The numbers of cows needed per farm was 8.

Data and specimen collection

Selected farms and farmers were contacted by research assistants to arrange the date and time for data collection. Two questionnaires were developed and tested with farmers in the study area to insure that the questions were clear and easy to understand. One questionnaire, asking about the exposure history of each farmer, and a second questionnaire, assessing farm practice and farm management, were used for the interviews. A face-to-face interview was conducted by trained interviewers using both questionnaires to collect exposure data from all farmers on participating farms. In addition, farm owners or farm representatives were interviewed about farm management and farm practice. The interviews were conducted 3 times – at baseline and at 2 follow-up interviews 6 months apart using the same questionnaires. The interviewers included health officers and nurses who were trained before data collection to ensure that all interviewers understood the questionnaires.

For Bulk Tank Milk (BTM) screening, farmers store milk in containers and sell it to the dairy cooperative in the morning and in the evening. Research assistants collected 20 ml of milk from containers on the morning of the same day or within one week of data collection of farmers. All milk samples were labeled and transferred to the Veterinary Research and Development Center at temperatures under 4°C on the same day that the specimens were collected. We recollected and retested BTM from farms with positive milk or suspect ELISA on the first screening.

Field investigations were conducted by investigators in collaboration with public health and animal health officers in the area. Eight cows on each positive milk farm were randomly selected for blood draws and vaginal swab collection. Information for sampled cows such as breed, gravidity, and history of reproductive disorder were obtained by a review of the cow's record and an interview with the farmer during the farm visit. A record card for each cow included its name, breed, and reproductive history. Moreover, a swab from the birthing areas, soil at the end of the sewage area, leftover food on trays, ticks, and blood from other animals in the farm were collected. Active case finding of farms with positive milk

was conducted to evaluate if the farmers had fever. Farmers who reported feeling ill or having fever were interviewed and clinical presentations were assessed by the researcher. If the fever occurred within 7 days before the interview, a whole blood sample was collected for PCR testing. Only newly positive milk farms were visited.

Ethical considerations

The study protocol was reviewed and approved by the Institutional Review Board (IRB) of the Department of Disease Control, Ministry of Public Health – Thailand, and the University of North Carolina at Chapel Hill. Informed consents were obtained from farm owners and farmers before data collection. The baseline data were collected from June to August 2015, the first follow up took place from January to March 2016, and the second follow up was done from June to August 2016.

Laboratory study

- Serological study

Indirect Immunofluorescence Assay (IFA) is the current reference laboratory method for Q fever serological diagnosis. IFA can detect antibodies against Nine Mile Phase I *C. burnetii*, which are typically found at high levels in subjects with chronic Q fever, and antibodies against Nine Mile Phase II *C. burnetii*, which are typically found in patients with acute infections (10). The capability to perform IFA for Q fever diagnosis was developed in Thailand in 2012 with the support of the US CDC. *C. burnetii* Nine Mile phase I and II antigens were provided by the US CDC. Human sera were kept at - 20°C before transfer to the Thai National Institute of Health (Thai-NIH) for IFA testing. IFA will be performed to determine IgM and IgG specific antibody to *C. burnetii* for both phase I and phase II antigens following the he US CDC. protocol (121).

ELISA kits (IDEXX CHECKIT™ Q-fever Test Kit) were used for the serological study in milk and cow sera. The tests were carried out at the Veterinary Research and Development Center (VRDC) in Lampang Province following the industry protocol. Optical Density (OD) was evaluated. Sample per Positive percentages (S/P%) was calculated by 100 x ($OD_{sample} – OD_{negative\ control}$)/($OD_{positive\ control} - OD_{negative\ control}$). The S/P% <30% was defined as negative, S/P% between 30% to less than 40% was defined as

suspect, and S/P% ≥ 40% was defined as positive. We collected second BTM samples from the farms with positive or suspect milk results to retest for *C. burnetii* antibodies using ELISA. Farms with the first positive and positive or suspected second test were defined as positive BTM. In addition, farms with a suspected the first test, and positive second test were also defined as positive BTM. BTM samples with negative ELISA results were defined as negative. The rest of the results were defined as uncertain.

- Molecular Detection of *C. burnetii*

Real time PCR was used to detect the evidence of *C. burnetii* DNA in cow placenta, vaginal secretion, other animal whole blood, soil, ticks, and swab from the birthing area in BTM positive farms. In addition, 50 milk samples from each round of sample collection were randomly selected for PCR analysis to determine the correlation between serological evidence for *C. burnetii* and *C. burnetii* shedding in milk. DNA extractions were performed using QIAmp® genomic DNA and DNeasy® kits (QIagen), following the QIagen Standard Tissue Extraction instructions. Real time PCR was performed at the Veterinary Research and Development Center, following the protocol of the Q Fever Laboratory, Ricketsial Zoonoses Branch, Division of High Consequence Pathogens and Pathobiology, National Center for Emerging and Zoonotic Infectious Diseases, U.S.CDC. The forward primer 5'-ccgatcatttgggcgct-3', the reverse primer 5'-cggcggtgtttaggc-3' and a hybridization probe 5'-ttaacacgccaagaaacgtatcgctgtg-3' were used to amplify the transposase gene of the insertion element IS1111 of *C. burnetii* (115). DNase/RNase free water was used as a negative control and samples of previously confirmed *C. burnetii* DNA were used as positive control. PCR was performed using the Roche, LightCycler® 96 System. PCR positive was defined when the sample had typical amplification curves with a cycle threshold (Ct) value less than 40.

MEASUREMENTS

Outcome assessment

Outcomes of interest in farmers, milk, and cows are defined in Table 4. IFA tests were conducted on sera from all participating farmers throughout the study period. The outcome definitions for famers were based on clinical manifestations and IFA titer. Laboratory criteria were adapted from the case definitions

of Q fever infection in humans used by the US National Notifiable Diseases Surveillance System (NNDSS) (64). Outcomes in milk were defined according to the ELISA results. Milk-negative was defined as ELISA negative on the first test only. Milk positive was defined as a farm with the milk positive by ELISA two times, with a first test positive and suspect second test, or with a first test suspect and positive second test result. The remainders of the milk results were defined as uncertain. Cows in the farm with positive milk were randomly selected for blood drawn and vaginal swab collection. Seropositive cows were defined as the cows with ELISA positive according to the manufacture's guideline. In addition, cows with PCR positive from vaginal swab samples were defined as active shedders.

Table 4. Outcome definitions used in the study of the epidemiology of Q fever among dairy farms and farmers in Chiang Mai, Thailand 2015 – 2016

Outcomes	Definitions
Farmer	
Clinical suspicion of acute Q fever	Fever and at least one of the following clinical findings and signs: severe headache, pneumonia, or elevated liver enzymes
Confirmed acute Q fever infection	Clinical finding(s) & ≥ 4 increase in IgG specific antibody titer to *C. burnetii* phase II antigen, or PCR positive for *C. burnetii* DNA
Probable acute Q fever infection	Clinical finding(s) & single serum of IgG titer to *C. burnetii* phase II antigen ≥ 1:128
Suspected acute Q fever infection	Clinical finding(s) & phase II antigen ≥ 1:64
Asymptomatic infection	No history of fever, but having ≥ 4 increases in the IgG specificantibody titer to either *C. burnetii* phase I or phase II antigen
Seropositive to *C.burnetii* infection	Single serum of IgG antibody titer to either phase I or phase II antigen ≥ 1:64
Laboratory confirmation of chronic Q fever infection	Serum of IgG antibody titer to *C. burnetii* phase I antigen ≥ 1:800 plus a recognizable nidus of infection
Laboratory supportive of chronic Q fever infection	IgG antibody titer to *C. burnetii* phase I antigen ≥1:128 but <1:800 plus a recognizable nidus of infection
Milk (BTM)	
Negative result	Negative ELISA
Positive result	First positive ELISA and second positive or suspect, or first suspect ELISA and second positive
Uncertain result	ELISA positive/negative, suspect/suspect, and suspect/negative
Non-positive result	combination of negative and suspect BTM
Cows	
Seropositive	Cows that tested positive for antibody to *C. burnetii* by ELISA
Active Q fever shedder	Animals that have positive *C. burnetii* DNA using PCR

Exposure assessment

Exposures of interest were divided into the individual famer level and the farm level (Table 5). Individual factors included demographic information, farmers' job descriptions on the farm such as assist in birthing, milking etc., working experiences, working hours, use of Personal Protective Equipment (PPE) on the farms, and other potential risk factors. Examples of other potential risk factors included assisting other animal birthing and contact with other animals. For the farm, we obtained information on farm location, farm characteristics such as number of cows, breed, breeding, and farm management, for example cleaning practices, feeding systems, and tick control systems. In addition, the physical history of individual cows including age, breed, number of calves, and history of reproductive disorders were obtained during the farm investigation.

Table 5. Summary of exposure variables collected for the study of Epidemiology of Q fever among dairy farms and farmers in Chiang Mai, Thailand 2015 – 2016

Farmer Characteristics	Farm Characteristics
Demographic Characteristics	Farm address
Age	Location of the farms
Sex	Number of cows
Address	Breed/Breeding
	Feeding system
History of illness	Air flow
Underlying diseases	Floor/ bedding
History of abortion in women	Cow housing
Signs and symptoms one week before data collection	Cleaning practices in general and after calving
	Quarantine of newly purchased animals
Working history	Raising other animals in the farm
Assist in cow birthing	Tick control practices
Contact with birth product	
Milking	
Cleaning stables	
PPE such as gloves, use of masks, etc.	
Other risk factors	
Help with other animal births	
Drink raw milk	
Eat raw meat	
Travel to other animal farms	
Receive blood transfusion	
Tick bite	

PRELIMINARY FINDINGS AT BASELINE

Among 306 randomly selected farms, 282 farms (92.2%) and 532 farmers from 637 randomly selected farmers (83.5%) participated in the study. In participating farms, the median number of farmers per farm was 2 (ranged from 1 – 8). The median number of cows per farm was 41 (ranged from 4 – 125). Approximately half of participating farms had more than 40 cows per farm (51.8%) and 34% and 14.2% of the farms had number of cows 21 – 40 and 1 – 20, respectively. Participation varied by farm size with 40 out of 58 farms (69.0%) with 1 – 20 cows participating, 96 out of 98 farms (98.0%) with 21 – 40 cows participating, and 148 out of 150 farms (97.3%) with > 40 cows participating in the study. The response rate by coops showed 73/78 (93.6%) for coop1, 39/42 (92.8%) for coop2, 78/88 (88.6%) for coop3, 40/42 (95.2%) for coop4, and 52/52 (100.0%) for coop5. The baseline seroprevalence of *C. burnetii* in milk was 40.8% (115/282); the baseline seroprevalence of *C. burnetii* among farmers was 16.9% (90/532). Among 115 farms with positive milk, 99 farms were visited and samples were collected from 790 cows. The seroprevalence to *C. burnetii* was 28.4% (224/790) at the individual cow level and 91.9% (91/99) at the farm level.

STRENGTHS AND LIMITATIONS

Our study was the first longitudinal study to determine the epidemiology of Q fever among dairy farmers in Thailand. Since information on Q fever in Thailand is limited, this study provided substantial information of *C. burnetii* infection among dairy farms and farmers. We examined the baseline information of Q fever in this high risk population as well as the clinical spectrum of Q fever and serological pattern in response to *C. burnetii* infection. Considering the longitudinal design, we were able to evaluate the incidence and risk factors associated with *C. burnetii* infection. Therefore, the outcomes of this study provide scientific evidence for Q fever prevention and control among the high risk populations in Thailand. In addition, this study highlights clinical spectrums and possible factors associated with developing signs and symptoms of Q fever patients in Thailand; hence, it provides a guideline for physicians to include Q fever as a differential diagnosis. The results of this study were presented to the Thai infectious disease medicine council to improve physicians' awareness of Q fever. Moreover, this study demonstrated the

One-Health concept in which Thai animal and public health sectors can collaborate to conduct a field investigation and laboratory testing of Q fever.

Since the study was conducted in a specific area in Thailand, generalizability of results to other areas might be limited. Nevertheless, the study in this specific population generated new knowledge of Q fever among a high risk population in Thailand. Moreover, the information can be useful for other farms with similar practices. As with other longitudinal studies (122), loss of respondents, in this case farmers, to follow-up meetings was possible. To prevent these losses to follow-up, we explained the study protocol to farmers and answered any questions that they had on the enrollment day. In addition, we collaborated closely with dairy cooperatives to ensure that farmers were informed about data collection date and time.

Moreover, measurement errors could be possible since our study assessed the antibody response to *C. burnetii*. The results of antibody level could depend on several factors such as the immune response of each individual, the laboratory methods, and the experiences of the laboratory staff. To minimize the measurement errors, our study used the same laboratory methods and same readers throughout the study. Moreover, to take the measurement errors into account, sensitivity analyses using different antibody cutoff values were performed to determine possible risk factors for the disease.

CHAPTER 5

EPIDEMIOLOGY OF *C.BURNETII* INFECTION AMONG DAIRY CATTLE FARMS, CHIANG MAI, THAILAND 2015

Introduction

Q fever is a zoonotic disease, caused by obligate intracellular bacteria, *Coxiella burnetii*. It can infect a wide variety of hosts including humans, ruminants, dogs, cats, ticks, and wild animals (123). Q fever transmits mainly via aerosols. Ticks are suspected to be another source of infection in animals (124). Animals infected with *C. burnetii* are mostly asymptomatic. However, infection can cause reproductive problems in ruminants. Previous studies showed that abortions were more common in small ruminants such as goat and sheep. Metritis and infertility might be associated with Q fever infection in cattle which can last for several months (6, 46-48). Infected animals shed bacteria via birth products, milk, urine, and feces regardless of their clinical symptoms (6, 25). This shedding might persist for several months, especially in vaginal mucus, feces, and milk (26). Cattle and goats shed bacteria through milk more frequently than do sheep (27). *C. burnetii* infections are mostly asymptomatic in human; however, it can cause a “flu”-like illness, and some Q fever infections develop chronic sequelae including endocarditis in 1 – 5%, and chronic fatigue syndrome in 10 – 20% (7-10). Since clinical manifestations of Q fever are nonspecific, the diagnosis of Q fever in both animals and humans relies on laboratory tests, particularly serology (10).

Knowledge of Q fever in Thailand is limited. After the first reports of Q fever in Thailand in 1966 (120), some studies were conducted to understand the burden and risk factor for Q fever. Q fever research received more attention after the first two reported cases of human Q fever endocarditis in Thailand in 2012 (13). In 2012, a study examined the presence of *C. burnetii* DNA in ruminants’ placenta from nine provinces of Thailand. This study showed the presence of *C. burnetii* DNA in beef, dairy cattle, goats, and buffalo (15). A separate study was conducted in 2012 – 2013 in two provinces, Chiang Mai and Nakornratchasima, to determine the seroprevalence to *C. burnetii* among occupationally exposed

people and ruminants, using Enzyme Linked Immunosorbent Assay (ELISA) (manuscript submitted). The prevalence of *C. burnetii* seropositivity among farm workers with occupational exposure was 43% in Chiang Mai and 3% in Nakornratchasima. Seropositivity to *C. burnetii* was highest in dairy cattle (4.6%, 45/988), followed by goats (3.5%, 18/516), and sheep (2.1%, 1/48). Because knowledge of Q fever epidemiology in Thailand is limited, the Bureau of Epidemiology, Ministry of Public Health Thailand, in collaboration with Chiang Mai public health and animal health, the National Institute of Animal Health (NIAH), the National Institute of Health (NIH), and the Thailand Ministry of Public Health – U.S. CDC Collaboration (TUC) conducted a longitudinal study of Q fever among dairy cattle farms and farmers in Chiang Mai during 2015 – 2016. Here, we report baseline information to determine the percentage of dairy cow farms where cows show evidence of *C. burnetii* infection using antibodies in Bulk Tank Milk (BTM) as a surrogate outcome for *C. burnetii* infection at the herd level. Risk factors associated with *C. burnetii* infection on dairy cow farms were also determined.

Methods

Study design and study population

This study was part of a one year longitudinal study of Q fever among dairy farms and farmers conducted in Chiang Mai Province; data were collected from June 2015 – August 2016. A full description of the cohort and study design was described elsewhere (manuscript submitted). In brief, a study was conducted in three dairy cooperatives of Chiang Mai where the evidence of Q fever was reported in 2012 (17) and two other dairy cooperatives in the adjacent areas out of the total of nine dairy cooperatives in Chiang Mai. Farms were randomly selected from these five dairy cooperatives based on the number of cows on each farm. Farms were stratified according to the number of cows into three groups: 1) farms with 1–20 cows, 2) farms with 21–40 cows, and 3) farms with > 40 cows. All farms in category 3 were selected and 50% of farms from categories 1 and 2 were randomly selected.

Sample size calculation for the project was based on the estimated incidence of seroconversion to *C. burnetii* among farmers, using the CSurvey program (117). We assumed that the estimated incidence of seroconversion to *C. burnetii* was 1.6% in one year, the half-length of the confidence interval was 1.6%,

the design effect was 2, and the number of selected farmers per farm was 2. The sample size for the farms after taking 16% seroprevalence among farmers at baseline into account was 310.

Data collection

Q fever was largely unknown to farmers. Meetings between the investigating team, local public health and animal health officers, and farmers were set up to inform farmers about the project and to provide health education regarding Q fever. Randomly selected farms were contacted by research assistants. Farm owners and workers provided informed consent before participating in the study. The study was approved by IRBs at the Department of Disease Control, Ministry of Public Health – Thailand, the University of North Carolina at Chapel Hill, and the U.S. CDC. Face-to-face interviews with farm owners or farmers who knew about farm practices were performed by trained public health officers and nurses. Questionnaires were tested with farmers in the study area to ensure that the questions were clear and easy to understand. General information about the farm such as number of cows, breed, breeding methods, feeding systems, farm practices (i.e., cleaning, and personal protective equipment used in the farm), and farm management (i.e., waste management) were collected.

Each farm registered with a Coop had its own unique Bulk Tank Milk number. Farmers sell milk to dairy cooperatives in the morning and in the evening. The research assistants collected 20 ml samples of BTM at the dairy cooperatives in the morning on the same day or within one week after the interview. All milk samples were labeled, stored in ice boxes, and transferred to the Veterinary Research and Development Center in Lampang Province (VRDC-Lampang) on the day they were collected.

Farms with a positive BTM ELISA were further investigated to identify the possible source of the infection. During the farm visit, we collected sera and vaginal swabs from 8 cows per farm, swabs from the birthing area, soil at the end of the water drainage, leftover food on the tray, whole blood of other animals in the farm, and ticks if available. In addition, health education was provided to farmers regarding Q fever prevention.

Laboratory analysis

Laboratory tests were carried out at the Veterinary Research and Development Center in Lampang Province (VRDC-Lampang). VRDC-Lampang is a regional branch of the National Institute of Animal Health where staff members are trained for *C. burnetii* testing. We used Enzyme Linked Immunosorbent Assay (ELISA) - IDEXX CHECKIT™ Q-fever Test Kit to detect antibodies in milk and cow sera. ELISA was performed following the IDEXX Q fever manufacturer's protocol. ELISA results were determined according to sample by positive control percentages (S/P%) calculated from the optical density (OD) values ($OD_{sample} - OD_{negative\ control}$)/ ($OD_{positive\ control} - OD_{negative\ control}$) x 100. The S/P% <30% was defined as negative, S/P% between 30% or more to less than 40% was defined as suspect, and S/P% ≥ 40% was defined as positive according to the manufacturer's protocol. All BTM samples were first screened by ELISA. Farms with BTM suspected or positive results were retested using ELISA in new BTM samples. BTM positive was defined as farms with ELISA positive in both tests or positive in one and suspect in the second test. BTM negative was defined as ELISA negative from the first screening. All others were defined as uncertain.

Vaginal swab samples, whole blood from other animals, soil, swabs from birthing areas, leftover food on trays, and ticks were tested for evidence of *C. burnetii* DNA using real time Polymerase Chain Reaction (PCR). DNA extraction was performed using QIAGEN ® following the manufacturer's instructions. Real-time Taqman PCR was performed at the VRDC-Lampang following the protocol from the US CDC Q fever laboratory. The target gene was the IS1111 insertion sequence. The forward primer is ccgatcatttgggcgct and the reverse primer is cggcggtgtttaggc. The hybridization probe sequence is FAM-ttaacacgccaagaaacgtatcgctgtg-BHQ1 (115).

Data analysis

Data were entered using EpiData3.1 (EpiData Association, Odense, Denmark). Data cleaning and analyses were performed in SAS version 9.3 (SAS Institute, Cary, NC, USA). Only baseline information is reported in this paper. Descriptive statistics describing prevalence of the antibodies in BTM by farm characteristic, such as number of cows, breed, breeding, farm practices, and farm management were calculated. In addition, mean, median, and measures of dispersion including standard deviation,

minimum, maximum, and interquartile ranges were calculated for continuous variables. The prevalence of antibody to *C. burnetii* in BTM and its 95% Confidence Intervals (95%CI) were calculated using weighted estimates. Sampling weights were calculated using the number of eligible farms divided by number of selected farms.

For farm investigation data, the antibody prevalence and prevalence of *C. burnetii* DNA were calculated at the individual cow and at the herd level. In addition, the median within-herd antibody prevalence and the median within-herd prevalence of *C. burnetii* DNA were calculated from the proportion of positive cows per farm. The proportions of positive test results were also calculated for environmental samples.

The logistic regression method was used to estimate the Odds Ratio (OR) and the 95% Confidence Interval (CI) of factors associated with *C. burnetii* antibody positivity in BTM. We developed two logistic regression models comparing BTM positive vs. BTM non-positive (combination of negative and uncertain BTM results), and BTM positive vs. BTM negative. All exposure variables of interest were evaluated in the univariate analyses adjusted for sampling variables including cooperative and farm size. Variables with p-value ≤0.2 in a univariate model were considered important and were included in the multivariate analysis. Exposure variables that met the criteria were assessed for their collinearity. If there was strong correlation between two exposure variables, only the variable that was more informative was included in the multivariate model. In multivariate logistic regression analyses, the manual backward stepwise elimination technique was applied. Sampling variables (dairy cooperative and farm size) were always kept in the model. Number of cows on the farm and age stratification of cows have frequently been reported as important factors in *C. burnetii* seropositivity in ruminants and were evaluated in the model (125, 126). Number of cows was explained by farm size. However, information about the cows' mean or median age on the farm was not available. Therefore, we used the number of cows by age category to describe the age distribution of cows on the farm. Age of cows was divided into 3 groups: < 1 year, 1 to < 2 years, and ≥ 2 years. The percentages for the number of cows by age group was calculated and included in the model to take into account the importance of the cows' ages. Percentages for cow's age were explored as both a continuous and categorical variable. The Likelihood Ratio Test (LRT) was performed to

evaluate the importance of included variables in the model and a p-value < 0.05 was considered significant.

Farm location information was obtained from the Chiang Mai Provincial Livestock Office, as well as from the farm visit. The proportions of farms with positive BTM among all farms tested were calculated and portrayed. Spatial autocorrelation among BTM positive and BTM negative farms were examined using Moran's I statistics. The distances from the nearest positive farms were calculated and assessed in the logistic model. Spatial analysis was performed using ArcMap10.3.1 (ArcGIS 10.3.1, Esri, Redlands, CA).

Results

Among 306 randomly selected farms, 282 farms participated in the study (92.2%). For the 24 non-participating farms, 9 farms stopped their business and 15 farms were unwilling to participate. There were 148 BTM's with positive or suspect results at the first ELISA screening and 154 BTM with negative results. After the second ELISA screening, 115 BTM met the positive criteria and 33 farms had uncertain results.

The overall prevalence of antibodies to *C. burnetii* in BTM was 38.98% (95%CI 32.91 – 45.05%). The prevalence was highest in coop1 at 55.16% (95%CI 42.99 – 67.34%), followed by coop2 at 37.36% (95%CI 25.53 – 49.18%), coop3 at 36.51% (95%CI 21.94 – 51.08%), coop4 at 25.69% (95%CI 7.91 — 43.47%), and coop5 at 22.48% (95%CI 7.64 – 37.32%) (Supplemental Table 1). The prevalence of antibodies to *C. burnetii* in BTM was higher on farms with higher numbers of cows in all cow age ranges. In addition, farms that used both artificial insemination and natural breeding had a higher prevalence of *C. burnetii* antibody compared to those using artificial insemination alone (79.04% vs 38.33 %).

Prevalence varied on farms with different cow bedding types: farms using concrete with rubber mats had the highest prevalence (58.95%, 95%CI 39.80% - 78.11%), followed by farms with concrete only (40.68%, 95%CI 33.14% - 48.23%), and concrete and soil (19.576%, 95%CI 8.73% - 30.40%). Farms with separate birthing stables and separate stables for quarantine had a lower prevalence compared to those that did not.

The prevalence of BTM positivity was 47.35% (95%CI 39.46 – 55.25 %) in farms located within 1 kilometer of other BTM positive farms and 23.52% (95%CI 9.62 – 37.41%) and 17.48% (95%CI 6.53% - 28.43%) for farms within 1 –2 km and more than 2 km, respectively. Median length of farm business among positive farms was 18.5 years (range 1 – 40, IQR 6 – 21 years) and 13 years (range 1 – 43, IQR 10 – 26 years) among non-positive farms. The median percentage of cows aged ≥ 2 years on milk positive farms was 65.3% (range 0 – 100%, IQR 53.6% - 83.9%) and the median percentage of cows aged >2 years on milk negative farms was 62.5% (range 0 – 100%, IQR 53.3% - 71.7%).

The univariate analyses after adjusting for sampling variables (dairy cooperative and farm size) showed that years of farm business, cow breed, breeding methods, cow bedding type, frequency of floor cleaning, having separate birthing stables and separate stables for quarantine, newly purchased animals in the past 6 months, having more than 80% of cows ≥ 2 years of age, the quarantine of newly purchased animals, practices of cleaning after birth, and distances from the nearest milk positive farms were important factors (Table 6). These variables were determined for collinearity. The results showed that ever quarantining newly purchased animals and having separate stables for quarantine; ever cleaning the cow birthing area right after calving and having separate birthing stables; and farm size and number of cows age 2 years or older were highly correlated. Thus, only the quarantine of newly purchased animals and cleaning of the birthing area right after birth were included in the multivariate analysis. The percentage of cows age ≥ 2 years was used in the model instead of the number of cows aged > 2 years to account for age.

The multivariate logistic regression model showed that dairy cooperative, farm size, cow bedding type, distance from positive farms, cleaning after birth, and quarantining newly purchased animals were associated with the odds of having antibodies in BTM with each variable adjusted for all others (Table 2). Cleaning the cow birthing area right after birth (OR 0.27, 95%CI 0.08 - 0.87) and quarantining newly purchased animals (OR 0.52, 95%CI 0.29 - 0.93) were negatively associated with the presence of antibodies to *C. burnetii* in BTM. In addition, farms with >40 cows and a farm located within 1 kilometer of a milk positive farm were positively associated with the antibodies to *C. burnetii* in BTM.

Different types of stall surfaces seemed to be an important factor in our analysis. Farms with a concrete and rubber mat base (OR 3.90, 95%CI 1.34 – 11.39) and concrete base (OR 2.07, 95%CI 0.97

– 4.43) were positively associated with the odds of antibodies in BTM compared to concrete and soil base. Although the percentage of cows aged ≥ 2 years within the farm as a continuous variable was not statistically significant, further exploratory analysis found that having more than 80% of cows aged ≥ 2 years of age was positively associated with the odds of positive milk (OR 2.3, 95%CI 1.09 - 5.06) (Table 3). In addition, having newly purchased cows in the past 6 months was negatively associated with the odds of antibodies to *C. burnetii* in BTM (OR 0.48, 95%CI 0.24 – 0.97) (Table 8).

A separate analysis comparing farms with positive and negative BTM was performed. There were a totally of 249 farms composed of 115 positive farms and 134 negative farms included in the analysis. The multivariate analysis (Supplemental Table 2) showed similar results compared to the analysis of positive vs. non-positive farms. Cooperative, farm size, ever quarantining of a newly purchased animal in the past 6 months, and proximity to positive farms were significantly associated with seropositivity in BTM. Although having more than 80% of cows aged ≥ 2 years of age, ever cleaning the birthing area right after birth, and stall base were not statistically significantly associated with the prevalence of antibodies to *C. burnetii* in BTM, the odds ratio showed a pattern similar to the analysis of positive vs non-positive farms.

Positive farms were clustered in particular areas (Figure 7). Spatial analysis showed the evidence for spatial autocorrelation for BTM positive and negative farms using Morans I statistic (z-score was 2.35 and p-value 0.02).

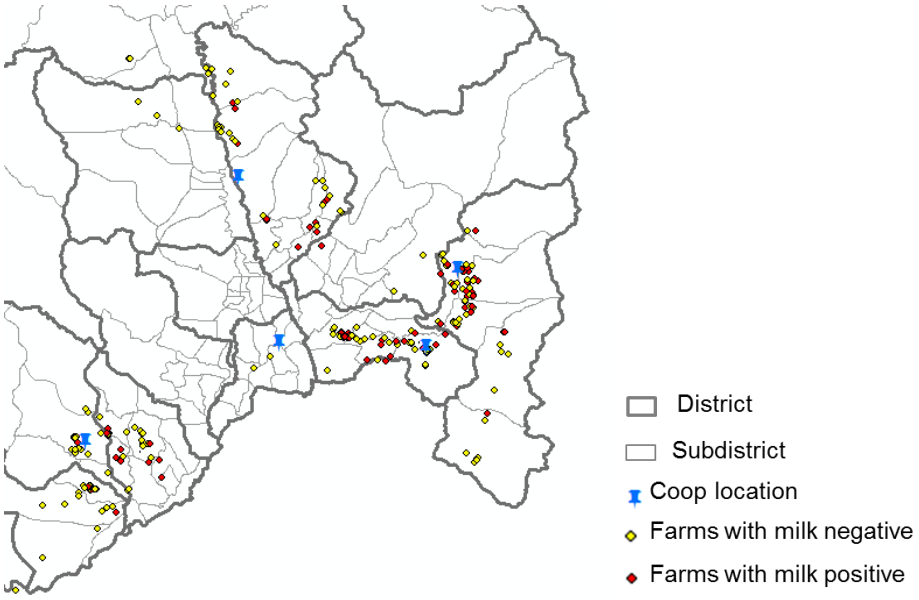


Figure 7. Distribution of dairy farms by Bulk Tank Milk (BTM) results

Farm investigation

Ninety-nine out of 115 milk positive farms were visited (86.08%). Of the 16 missing farms, 12 farms could not be visited because of a disease outbreak in the cows, 3 farms stopped their business, and 1 farm was unwilling to participate. Vaginal swabs and sera from 790 cows, 99 swab samples from birthing areas, 99 samples of soil at the end of the water drainage, leftover food samples in trays from 57 farms, ticks from 53 farms, and 39 whole blood samples of other animals in the farms were collected during these farm visits. Samples from other animals included 30 dogs, 4 cats, 4 chickens, and 1 horse. Figure 8 showed examples of farm environment.

The overall unweighted seroprevalence to *C. burnetii* infection in cow was 28.35% (224/790) and the unweighted prevalence of *C.burnetii* DNA in vaginal swab samples was 18.48% (146/790). At the herd level, 91.9% (91/99) of the farms with positive BTM had at least one seropositive cow and 39.4% (39/99) of the farms had at least one cow with evidence of *C. burnetii* DNA using PCR. The median within-herd seroprevalence was 25.0% (range 0 – 62.50%, IQR 12.50% – 37.50%) and the median within-herd prevalence of *C. burnetii* DNA in vaginal swabs was 0 (range 0 – 100%, IQR 18.40% - 31.60%). In addition, 12.12% (12/99) of the environmental swabs from the cow birthing area, 7.07% (7/99) of the soil samples, and 1.92% (1/52) of ticks from 52 farms were PCR positive. None of the other animals sampled had evidence of *C. burnetii* DNA in their blood.

Figure 8. Examples of farm environment

Table 6. Results of univariate analyses of factors associated with dairy farms with milk positive, Chiang Mai, Thailand 2015

	Positive vs non-positive (N=282)			
Covariates	**OR***	**95% CI**		**p-value**
Cooperatives				0.0003
Farm size				0.0376
Years of farm business	1.021	0.993	1.049	0.1476
Breed (Holstein vs. Holstein and others)	1.512	0.846	2.703	0.1631

Breeding: Artificial Insemination(AI) and natural mating vs. AI only	5.195	0.527	51.207	0.1582
Number of cows age<1	1.03	0.969	1.094	0.3489
Number of cows age 1- < 2years	1.017	0.98	1.056	0.3725
Number of cows age ≥ 2 years	1.032	1.006	1.059	0.0147
Percentage of cow age ≥ 2 years	1.868	0.385	9.06	0.4381
Proportion of cow age ≥ 2 years on the farm				0.0384
- < 80%			Ref.	
- ≥ 80%	2.142	1.041	4.406	0.0384
Barn type (free vs. tie-stall barn)	1.173	0.67	2.054	0.5761
Airflow in the stable (natural airflow vs. natural and fan)	1.289	0.351	4.735	0.7027
Stall bases: (Cow bedding type)				0.0266
- Concrete and ground vs. concrete only	0.494	0.237	1.03	0.0598
- Concrete and rubber mats vs. concrete only	1.945	0.873	4.332	0.1035
Frequency of floor cleaning				0.223
- 1 time/day vs. > 2times/day	2.557	0.384	17.011	0.2266
- 2 times/day vs. >2 times/day	0.687	0.35	1.349	0.0946
Frequency of antiseptic used				0.46
- Never vs. always	1.739	0.675	4.477	0.2137
- Sometime vs. always	1.059	0.56	2.002	0.4454
Having separate birthing stable (Yes vs. No)	0.607	0.301	1.222	0.1622
Having birth at the tiding area (Yes vs. No)	1.346	0.795	2.279	0.2686
Cleaning the birthing area right after birth				0.0494
- Sometimes vs. no	0.278	0.059	1.321	0.1076
- Always vs. no	0.245	0.08	0.754	0.0142
Newly purchased animal in the past 6 months (Yes vs. No)	0.526	0.277	0.996	0.0487
Ever quarantine newly purchased animals	0.566	0.331	0.971	0.0386
Having separate stables for quarantine (Yes or No)	0.46	0.235	0.9	0.0234
Raising other animals on the farm (Yes or No)	1.307	0.78	2.19	0.3098
Raising dog on the farms				0.4289
- No dog but others vs. no animal	1.045	0.493	2.216	0.6953
- Dog or dog with others vs. no animal	1.457	0.816	2.6	0.2405
Raising cat on the farms				0.3829
- No cat but others vs. no animal	1.48	0.84	2.605	0.8468
- Cat or cat with others other vs. no animal	0.938	0.418	2.108	0.25
Rat on a farm (Yes or No)	1.352	0.76	2.403	0.3044
Tick control (Yes or No)	1.033	0.529	2.017	0.9238
History of abortion in other animals (Yes or No)	6.362	0.317	127.64	0.2265
Distance from the positive farms				0.0096
- > 1 to 2 kilometers vs. > 2 kilometers	1.55	0.534	4.499	0.4202
- ≤ 1 kilometer vs. > 2 kilometers	3.164	1.365	7.337	0.0073

*Adjusted for all exposure factors, OR: Odds ratio, 95%CI: 95% confidence interval

Table 7. Results of multivariate analysis of factors associated with dairy farms with seropositive vs. farms with non-positive milk, Chiang Mai, Thailand 2015

Covariates	OR	95%Confidence Limits		chi2 p-value	type3 p-value
Dairy cooperative					0.0315
- Coop5				Ref	
- Coop1	2.419	0.87	6.729	0.0905	
- Coop2	1.558	0.569	4.262	0.388	
- Coop3	1.309	0.441	3.883	0.6277	
- Coop4	0.545	0.162	1.832	0.3263	
Farm size					0.0117
- Farm with 1 - 20 cows				Ref	
- Farms with 21 - 40 cows	1.47	0.604	3.577	0.3957	
- Farms with > 40 cows	3.004	1.281	7.044	0.0114	
Stall base					0.0401
- Concrete and ground				Ref	
- Concrete	2.068	0.965	4.433	0.0619	
- Concrete and rubber mat	3.901	1.337	11.39	0.0127	
Ever clean cow birthing areas					0.028
- No				Ref	
- Yes	0.267	0.082	0.867	0.028	
Ever quarantine newly purchased animals					0.0265
- No				Ref	
- Yes	0.522	0.293	0.927	0.0265	
Distance from positive farm					0.015
- > 2 kms				Ref	
- ≤ 1 km	2.941	1.22	7.088	0.0163	
- > 1 to ≤ 2 kms	1.302	0.42	4.042	0.6475	
Proportion of cows aged > 2 years on farm	2.125	0.404	11.19	0.3739	0.3739

Ref: reference

Table 8. Results of multivariate analysis of factors associated with dairy farms with seropositive vs farms with non-positive milk (Explore effect of percentage of cow aged > 2 year as a categorical variable), Chiang Mai, Thailand 2015

Covariates	OR	95%Confidence Limits		chi2 p-value	type3 p-value
Dairy cooperative					0.0301
- Coop5				Ref	
- Coop1	2.399	0.849	6.781	0.0989	
- Coop2	1.328	0.475	3.709	0.5886	
- Coop3	1.239	0.414	3.706	0.7014	
- Coop4	0.517	0.151	1.772	0.2939	
Farm size					0.0085
- Farm with 1 - 20 cows				Ref	
- Farms with 21 - 40 cows	1.528	0.614	3.803	0.3626	
- Farms with > 40 cows	3.24	1.342	7.825	0.009	
Stall base					0.043
- Concrete and ground				Ref	
- Concrete	2.093	0.959	4.566	0.0635	
- Concrete and rubber mat	3.928	1.324	11.649	0.0137	
Ever clean cow birthing areas					0.0263
- No				Ref	
- Yes	0.268	0.084	0.856	0.0263	
Ever quarantine newly purchased animals					
- No				Ref	
- Yes	0.541	0.301	0.974	0.0406	0.0406
Distance from positive farm					0.0162
- > 2 kms				Ref	
- ≤ 1 km	2.878	1.174	7.056	0.0209	
- > 1 to ≤ 2 kms	1.228	0.39	3.862	0.7253	
Proportion of cows aged > 2 years on farm					0.0302
- < 80%				Ref	
- ≥ 80%	2.344	1.085	5.061	0.0302	
Newly purchased cows in the past 6 months					0.04
- No				Ref	
- Yes	0.477	0.235	0.967	0.04	

Ref: reference

Discussion

This study measured the prevalence of *C. burnetii* antibody in BTM from five dairy cooperatives in Chiang Mai Province, Thailand. Antibodies to *C. burnetii* were present in 39.8% (95% CI 32.74% -

44.83%) of farms. The presence of *C .burnetii* antibodies in milk varied by dairy cooperative, and geographic area where the farms were located. The study shows that the number of cows, the proximity to other milk positive farms, different types of stall bases, cleaning right after cow births, and quarantine practices were associated with the prevalence of *C. burnetii* antibodies in milk. As expected, 92% of farms with antibodies in BTM contained at least one seropositive cow. Seroprevalence of *C. burnetii* infection was 28% at the individual cow level and the median within-herd seroprevalence was 25% (range 0 – 62.5%, IQR 12.5% – 37.5%). These data imply that an antibody in BTM is a good marker of *C. burnetii*-infected herds.

The screening of bulk tank milk by ELISA has been done elsewhere (77, 127), but had not previously been done in Thailand. The prevalence of antibody for *C. burnetii* in dairy cattle BTM in our study was lower compared to studies in the Netherlands (81.6%) (127), Spain (66.9%) (128), Ireland (64.5%) (77), and Iran (45.4%) (129). However, comparing seroprevalence between studies was challenging because each study used different tests and different cut-off values. A review by Guatteo et.al. showed that Q fever has been reported from all five continents and the prevalence of *C. burnetii* infection, regardless of laboratory methods and cut-off values, varied from 4.4% - 100% at the herd level (66). Similar to other studies (77, 125, 127), we found that farms with more cows were more likely to have *C. burnetii* antibodies in milk. Farms with larger numbers of cows might have more susceptible hosts so that infection can circulate within the farms without detection. A previous study showed that age is an important factor associated with seropositivity: the presence of older cows was associated with increased seropositivity (77). Our study showed that farms with the percentages of cows aged ≥ 2 years greater than 80% of the total cow population was significantly more likely to have antibodies to *C. burnetii* in BTM. Older cows with persistent exposure and partial immunity to bacteria might serve as a reservoir.

Previous studies showed that *C. burnetii* is extensively shed during calving and can persist in the environment for years afterwards (107). In infected farms, 12% of swabs from cow birthing areas and 7% of soil at the end of the farm drainage were tested positive for *C. burnetii* DNA. Furthermore, our study showed that cleaning the birthing areas after calving was negatively associated with the presence of the antibody in milk. These findings emphasize the importance of environmental decontamination especially after calving to prevent the spread of infection within herds as well as to other animals and humans.

Quarantining newly purchased animals was negatively associated with the presence of antibodies to *C. burnetii* in milk. Quarantining newly purchased animals is recommended for all farms for biosecurity purposes. Nevertheless, for Q fever, quarantine alone might not be sufficient since *C. burnetii* infected animals are mostly asymptomatic (6). More importantly, *C. burnetii* transmission is mainly airborne and windborne; thus, it does not require close contact to spread. The apparent protective effect of quarantine in our study might reflect that it is practiced more often on farms with more concerns for biosecurity. Newly purchasing cows over the past 6 months was inversely associated with the odds of having an antibody in BTM in our study. This finding can be explained because infection can persist on farms and newly purchased animals could be less likely to be exposed to the pathogen.

Stall bases were found to be significant factors in our study. Having concrete or concrete with rubber mats as the stall base were positively associated with positive BTM. Rubber mats were designed to provide cushions for the cows. However, rubber mats could be a sustainable source of bacteria if cleaning and disinfection is not properly performed. These mats require maintenance and replacement. We observed that some of the farms used old rubber mats without replacements.

Living close to farms that had a history of abortion due to *C. burnetii* infection in ruminants was associated with an increased *C. burnetii* seroprevalence in humans (130). A study in the Netherlands showed that living within 2 kilometers of positive farms was associated with seropositivity in humans. Our study showed that proximity to BTM positive farms, especially within 1 kilometer, was positively associated with the prevalence of *C. burnetii* antibodies at the herd level. In Chiang Mai dairy cattle farms were clustered in particular areas, especially in areas where farms from coop1, coop2, and coop3 were located. This could explain the differences of prevalence in BTM by dairy cooperatives.

Our results presented in this paper have some limitations since they are from a cross-sectional analysis of baseline data; hence, the temporal sequences of exposure and outcome are uncertain. This study was conducted in only one province, Chiang Mai, and generalizability could be limited. Nonetheless, the findings from this study enhance the knowledge of *C. burnetii* infection and its risk factors on dairy cattle farms in Thailand. Farm visits and sample collection from individual cows were only performed on the farms with positive milk. Thus, information at the cow level was limited to positive farms

and cannot be generalized to the farms with negative BTM. Other studies focusing on individual cows would improve the knowledge of the roles of *C. burnetii* infection in dairy cattle in Thailand.

This study was among the first to evaluate the prevalence of antibodies to *C. burnetii* in BTM in Thailand. The overall prevalence was approximately 39%. Among BTM positive farms, seroprevalence of *C. burnetii* infection at the herd level was 91.2%, suggesting that milk would be a favorable choice for screening for *C. burnetii* infection at the herd level. Contrarily, a greater proportion of older cows and proximity to other milk positive farms were positively associated with the presence of *C. burnetii* infection in milk. Farm practices such as cleaning cow birthing areas after birth and quarantining newly purchased animals were associated with protection against antibodies in BTM.

Supplemental Table1. Farm characteristics and seroprevalence of *C. burnetii* in BTM on farms participating in the study, Chiang Mai, Thailand 2015

Characteristics	N*	Prevalence	95%LCI	95%UCI
Overall seroprevalene of *C. burnetii* in milk	282	38.98%	32.91%	45.05%
Cooperatives				
- Coop1	78	55.16%	42.99%	67.34%
- Coop2	73	37.36%	25.53%	49.18%
- Coop3	52	36.51%	21.94%	51.08%
- Coop4	40	25.69%	7.91%	43.47%
- Coop5	39	22.48%	7.64%	37.32%
Farm size				
- 1 - 20 cows	40	31.62%	16.28%	46.96%
- 21 - 40 cows	95	36.67%	26.53%	46.82%
- > 40 cows	147	47.18%	38.96%	55.40%
Breeds				
Holstein				
- Holstein only	203	39.74%	32.39%	47.09%
- Holstein and other breeds	79	36.05%	24.05%	48.05%
Reddane				
- No Reddane on the farm	251	39.23%	32.63%	45.83%
- Had Reddane on the farm	31	35.11%	15.03%	55.19%
Jersey				
- No Jersey on the farm	269	38.57%	32.19%	44.95%
- Had Jersey on the farm	13	44.01%	11.30%	76.73%
Sahiwal				
- No Sahiwal on the farm	267	39.60%	33.17%	46.03%
- Had Sahiwal on the farm	15	23.29%	-3.57%	50.15%
Brown Swiss				
- No Brown Swiss on the farm	264	38.51%	32.09%	44.93%
- Had Brown Swiss on the farm	18	44.47%	17.41%	71.53%
Beef cattle				
- No Beef cattle on the farm	263	38.99%	32.54%	45.44%
- Had Beef cattle on the farm	16	27.59%	0.03%	55.14%
Feeding system				
- Eat inside the barn	264	38.80%	32.36%	45.25%
- Eat inside the barn and free grazing on the farm	18	38.52%	11.82%	65.21%
Barn				
- Free-stall barn	123	30.96%	22.02%	39.89%
- Tie-stall barn	158	43.89%	35.42%	52.35%
Breeding methods				
- Artificial insemination	277	38.33%	32.06%	44.60%

- Mixed (natural & artificial insemination)	5	79.04%	21.51%	136.57%
Number of cows by age				
No of cows age less than 1				
- 0 cows	6	75.73%	29.84%	121.63%
- 1 - 20 cows	272	37.65%	31.37%	43.92%
- > 20 cows (range 21 - 30)	2	46.97%	-586.01%	679.94%
No of cows age 1 to less than 2 years				
- 0 cows	18	36.94%	10.89%	63.00%
- 1 - 20 cows	236	37.96%	31.19%	44.74%
- > 20 cows (range 21 - 35)	25	53.15%	30.37%	75.93%
Number of cows age 2 or older				
- 1 - 20 cows	111	31.52%	22.35%	40.68%
- 21 - 40 cows	127	42.94%	33.49%	52.40%
- >40 cows (range 41 - 81)	40	63.54%	47.94%	79.15%
Air flow system				
- Natural airflow	271	38.67%	32.32%	45.02%
- Natural airflow and fan	11	43.00%	7.98%	78.03%
Stall bases				
- Concrete only	198	40.68%	33.14%	48.23%
- Concrete and earth floor	52	19.57%	8.73%	30.40%
- Concrete and rubber mats	32	58.95%	39.80%	78.11%
Separate birthing stable				
- No	232	41.03%	34.09%	47.98%
- Yes	50	26.62%	13.45%	39.79%
Raising other animals on farm				
- No	155	35.03%	26.91%	43.16%
- Yes	127	43.59%	33.93%	53.24%
Dogs				
- No, but raising other animals	155	35.03%	26.91%	43.16%
- Yes, dog only	41	37.04%	19.98%	54.09%
- Yes, dog with other animals	86	47.09%	35.22%	58.96%
Cats				
- No, but raising other animals	155	35.03%	26.91%	43.16%
- Yes, cat only	93	45.23%	33.81%	56.66%
- Yes, cat with other animals	34	38.61%	19.90%	57.32%
Abortion of other animals on farm				
- No	123	42.99%	33.19%	52.80%
- Yes	4	70.00%	-19.14%	159.14%
Rats				
- No	78	38.05%	26.01%	50.09%
- Yes	204	39.10%	31.76%	46.43%

Wild animals				
- No	267	38.55%	32.17%	44.93%
- Yes	15	43.88%	11.48%	76.28%
Farm management/cleaning				
Cleaning the floor				
- 1 time/day	6	69.43%	15.39%	123.48%
- 2 times/day	224	37.26%	30.24%	44.28%
- >2 times/day	52	41.52%	26.98%	56.06%
Liquid used for cleaning floor				
- Water	261	39.38%	32.88%	45.88%
- Water with chlorine	20	32.40%	8.50%	56.30%
Frequency of antiseptic used for cleaning				
- Never	32	45.48%	24.91%	66.04%
- Ever	250	37.88%	31.33%	44.43%
Waste (feces) management				
Left in the environment				
- No	276	39.20%	32.89%	45.51%
- Yes	6	13.08%	-22.26%	48.41%
Fermenting				
- No	233	37.70%	30.93%	44.47%
- Yes	49	44.68%	28.59%	60.78%
Dry and sell				
- No	5	15.52%	-30.48%	61.51%
- Yes	277	39.10%	32.81%	45.40%
Birthing management				
- Only helped sometimes	167	39.98%	31.81%	48.15%
- Always helped with birthing	115	37.16%	27.39%	46.93%
Birth management				
Having separate birthing stable				
- No	232	41.03%	34.09%	47.98%
- Yes	50	26.62%	13.45%	39.79%
Cleaning the stable right after birthing				
- No	17	73.88%	49.83%	97.92%
- Sometime	14	37.94%	5.98%	69.90%
- Always	251	36.58%	30.08%	43.09%
Newly purchased animals in the past 6 months				
- No	218	42.65%	35.38%	49.93%
- Yes	64	26.62%	15.22%	38.02%
Quarantine newly purchased animals				
- Never	180	42.96%	35.01%	50.90%
- Ever	102	31.05%	21.16%	40.94%

Having separate stable for quarantine				
- No	225	41.94%	34.84%	49.04%
- Yes	56	25.98%	13.61%	38.36%
Tick control				
Practice tick control				
- No	52	35.52%	20.61%	50.42%
- Yes	230	39.56%	32.65%	46.47%
Antibiotic use in the past 6 months				
- No	72	42.28%	29.57%	54.99%
- Yes	208	37.08%	29.91%	44.25%
Distance from milk positive farm				
- ≤ 1 kilometer	190	47.35%	39.46%	55.25%
- > 1 to 2 kilometers	40	23.52%	9.62%	37.41%
- > 2 kilometers	49	17.48%	6.53%	28.43%

*N: number of farms

Supplemental Table 2. Results of multivariate analysis of factors associated with dairy farms with milk seropositive (Comparing farms with positive vs farms with negative milk) (N=249), Chiang Mai, Thailand 2015

Covariates	OR	95%Confidence Limits		chi2 p-value	type3 p-value
Dairy cooperative					0.0131
- Coop5				Ref.	
- Coop1	2.554	0.893	7.306	0.0804	
- Coop2	2.24	0.771	6.512	0.1384	
- Coop3	1.394	0.455	4.27	0.5603	
- Coop4	0.515	0.149	1.788	0.2964	
Farm size					0.0248
- Farm with 1 - 20 cows				Ref.	
- Farms with 21 - 40 cows	2.162	0.816	5.731	0.121	
- Farms with > 40 cows	3.46	1.375	8.706	0.0084	
Stall base (cow bedding type)					0.0834
- Concrete and ground				Ref.	
- Concrete	1.491	0.643	3.458	0.3516	
- Concrete and rubber mat	3.868	1.153	12.971	0.0284	
Ever clean cow birthing areas					
- No				Ref.	
- Yes	0.346	0.108	1.11	0.0744	0.0744
Ever quarantine newly purchased animals					
- No					
- Yes	0.541	0.292	1.002	0.0507	0.0507
Distance from positive farm					0.0064
- > 2 kms				Ref.	
- ≤ 1 km	3.457	1.375	8.692	0.0084	
- > 1 to ≤ 2 kms	1.338	0.412	4.343	0.6278	
Proportion of cows aged > 2 years on the farm					
- < 80%				Ref.	
- ≥ 80%	2.053	0.889	4.743	0.0921	0.0921
Newly purchased cows in the past 6 months					
- No				Ref.	
- Yes	0.549	0.258	1.167	0.1188	0.1188

*Ref: Reference

CHAPTER 6

SEROPREVALENCE AND FACTORS ASSOCIATED WITH *COXIELLA BURNETII* INFECTION AMONG DAIRY FARMERS, CHIANG MAI, THAILAND 2015

Introduction

Q fever, a zoonotic disease, is caused by gram negative intracellular bacteria, *Coxiella Burnetii.* It was first identified in 1937 after an outbreak of an unknown febrile illness among abattoir workers in Australia (8). Domestic ruminants are the common reservoirs for Q fever infection in humans. Inhalation of contaminated dust or particles is the main route of transmission. Other routes of transmission such as food-borne, person-to-person transmission, or sexual transmission are rarely been reported (4). The majority of people infected with *C. burnetii* are asymptomatic. However, about 40% of infected humans develop acute Q fever which may present as acute febrile illness, pneumonia, or hepatitis. Approximately 2–5% of the infected develop endocarditis with up to 20% of the infected developing chronic fatigue syndrome (4, 10).

Similar to human infection, Q fever infection in animals is mostly asymptomatic. Some animals, particularly sheep and goats, develop reproductive problems such as abortions, infertility, and weak offspring. Infection in cows can cause metritis (6). Infected animals can shed bacteria regardless of clinical symptoms (107). *C. burnetii* is classified a category B bioterrorism agent since the pathogen is highly contagious and can result in moderate morbidity rates, but low death rates (1). The largest outbreak of Q fever ever reported was in the Netherlands in 2007 where more than 4,000 cases and major economic loses were reported (119).

Knowledge of the epidemiology of Q fever in Thailand is limited. Laboratory capacity for Q fever diagnosis was not available in Thailand before 2012. With support from the US.CDC, diagnostic capacities were strengthened after the first two reported cases of Q fever endocarditis in 2012 (13). To date, staffs from 5 institutes have been trained for Q fever diagnosis using Indirect Immunofluorescense Assay (IFA). Polymerase Chian Reaction (PCR) for Q fever diagnosis is available at the national level

(National Institute of Health (Thai-NIH) and National Institute of Animal Health (NIAH)) and at some veterinary research centers and university hospitals. Previous studies reported the seroprevalence of Q fever was approximately 1% among patients presenting with acute undifferentiated fever in the two tertiary care hospitals in Thailand (12, 14). A 2012 survey among occupationally exposed people and ruminants using Enzyme Linked Immunosorbent Assay (ELISA) in two provinces of Thailand showed that seroprevalence to *C. burnetii* among farmers was 43% (68/159) in Chiang Mai and 3% (15/502) in Nakornratchasima provinces (unpublished). Another study in Chiang Mai found the seroprevalence of Q fever among farmers and livestock officers was 16% using IFA (unpublished). Since information concerning the epidemiology of Q fever in Thailand is limited and Q fever can cause an outbreak, particularly among occupationally exposed people with subsequent chronic life threatening complications, the Bureau of Epidemiology in collaboration with Chiang Mai public health and animal health offices, the Thai-NIH, the NIAH, and the Thai-US. CDC Collaboration initiated a one-year prospective cohort study to understand the burden, risk factors, and clinical manifestation of Q fever among dairy cattle farms and farmers. This study was conducted from June 2015 to August 2016. We report here the preliminary analysis of baseline information of the seroprevalence and the factors associated with *C. burnetii* infection among farmers.

Methods

Study design and study population

This study is the analysis of baseline data of a one-year prospective cohort study conducted among dairy farms and farmers of the five dairy cooperatives in Chiang Mai Province. A description of the cohort was given elsewhere (Manuscript submitted). In summary, a cohort study was conducted from June 2015 – August 2016, in areas where seroprevalence to Q fever was reported in animals and humans. The sample size for the project was based on the results from a previous study where the prevalence of Q fever among dairy farmers was 16% (unpublished data). The CSurvey program was used for sample size calculation (117). The estimated one-year incidence of seroconversion of *C. burnetii* infection among farmers was 1.6%. The half-length of the 95% confidence interval was specified as 1.6%, with a design effect of 2 for an average of 2 farmers per farm. Thus, the number of clusters needed for the study was

260 farms and 520 farmers. We assumed that the prevalence of *C. burnetii* infection among farmers at baseline was 16%. To account for the baseline prevalence, we needed to screen a total sample size of 620 farmers from 310 farms at the beginning of the study (Manuscript submitted).

Farms were randomly selected based on information provided by dairy cooperatives. However, no information about farmers was available in the dairy cooperative database. Short surveys to obtain the lists of farmer were conducted. The number of farmers from the selected farms varied from 1 to 8. To account for the variation of number of farmers to reach the targeted sample size, all farmers were selected at farms with 1 to 3 farmers and 3 farmers were randomly selected from farms with more than 3 farmers by simple random sampling and random digit number. Eventually, 637 farmers were randomly selected.

Data collection

Selected farms and farmers were contacted by research assistants to make appointments for data collection date and time. Farmers were informed about the project and provided consent forms before data collection. Data were collected using face-to-face interviews by trained interviewers. Two types of questionnaires, one for individual farmers and another for farms, were used in the project. All enrolled farmers were interviewed using the farmer questionnaire and farm owners or farm representatives were interviewed about farm practices. Individual farmer's demographic information, underlying illnesses, job descriptions, history of personal protective equipment used in the farm, and other exposure history to Q fever risk factors were collected in the farmer questionnaire. Information about farm characteristics, farm practices, and farm management were collected in the farm questionnaire.

Laboratory analysis

Blood samples were obtained on the day of the interview. Sera were extracted and frozen at – 20°C before transference to the Thai-NIH for IFA testing. IgG and IgM antibodies against *C. burnetii* phase I and phase II antigens were analyzed. IFA slides were coated with synthetic *C. burnetii* Nine Mile phase I and phase II antigens, provided by the Ricketsial Zoonoses Branch, CDC, Atlanta, USA. The IFA was performed following the US.CDC protocol (121). Sera were screened at the initial dilution of 1:16. An IFA

seropositive farmer was defined as one who had IgG antibody titer to *C. burnetii* either phase I or phase II antigen ≥ 1:64. All other titers were defined as seronegative.

Data analysis

Descriptive statistics including frequencies, mean, standard deviation, median, interquartile range, maximum and minimum of seropositive farmers, and covariates were calculated. Since farmers working at the same farms share certain common environments, a Generalized Estimating Equation (GEE) model was applied to deal with correlated data from within the farm. Each farm had its unique tank number which was used as a unique identifier in the analysis. The odds ratios (OR) and the 95% confidence intervals (CI) were estimated. Sampling variables including dairy cooperative and farm size were always included in the analysis. We performed univariate analyses to determine whether the covariates after adjusting for sampling variables were sufficiently important to be included in the multivariate model. Age was reported to be an important factor associated with seropositivity to *C. burnetii* in humans and was always included in the multivariate analysis. Variables with a p-value from type 3 GEE analysis ≤ 0.20 were considered important and included in multivariate analysis. If the variables in univariate analyses were correlated, variables that were more informative were included in the multivariate analysis. Multivariate analyses were performed using manual forward elimination. Farm factors that were reported to be associated with the prevalence of antibody to *C. burnetii* infection in BTM, such as the practice of continually cleaning the birthing area immediately after birth, proximity to positive farms, and the presence of *C. burnetii* antibody in BTM were also examined in the multivariate analysis.

Distribution of farmers on positive farms was presented on the map using ArcMap10.3.1 (ArcGIS 10.3.1, Esri, Redlands, CA).

Preliminary Results

Among 637 randomly selected farmers, 532 farmers participated (83.5%) in the study. Among these 532 farmers, 355 (67%) farmers did not have antibodies to *C. burnetii* at baseline, 90 (16.9%) farmers had IgG antibody against either *C. burnetii* phase I or phase II antigen ≥ 1:64, and 50 (9.4%) farmers had IgG antibody against either phase I or phase II antigen ≥ 1:128. In addition, two farmers who had IgG

antibody to phase II antigen ≥ 1:128 reported they had had a fever within the past 4 weeks. One farmer had IgG antibody to *C. burnetii* phase I antigen 1:1024 suggestive of possible chronic Q fever infection, but she was asymptomatic.

Seropositivity among farmers differed by dairy cooperative. The median age of farmers who were seropositive was higher than the seronegative farmers. In addition, the percent of seropositives was higher among those who assisted with calving and came into contact with cow birth product or placenta during calving. The proportion of seropositivity among farmers was higher among those who had never used gloves or masks when assisting with calving or coming into contact with cow birth products or placenta. Gender, dairy farm working experiences, and working hours per week were similar for both seropositive and seronegative farmers (Table 9). The prevalence of seropositive farmers was higher on farms with positive BTM. Moreover, the prevalence of seropositive farmers was higher on farms within a 2 kilometer range of the positive farms (Table 9). Univariate analysis showed that age, having any underlying diseases, having ever assisted in cow birthing, having ever been in contact with cow birth products or placenta, having ever bathed a cow, having ever managed manure, and using gloves on the farms were significant (Table10). However, age, underlying diseases, assisting in calving and coming into contact with a cow's birth products or placenta were related. Therefore, only age and coming into contact with a cow's birth products or placenta were selected for multivariate analysis.

Table 9. Characteristics of farmers participating in the study by the IFA results, Chiang Mai, Thailand 2015

Characteristics	Number of farmer	Seronegative (<1:64)	Seropositive (≥1:64)
Cooperatives			
- Coop1	152	132(86.84%)	20(13.16%)
- Coop2	133	106(79.70%)	27(20.30%)
- Coop3	113	81(71.68%)	32(28.32%)
- Coop4	70	62(88.57%)	8(11.43%)
- Coop5	64	61(95.31%)	3(4.69%)
Farm size			
- 1 - 20 cows	70	59(84.29%)	11(15.71%)
- 21 - 40 cos	190	158(83.16%)	32(16.84%)
- > 40 cows	272	225(82.72%)	47(17.28%)
Age (median, min, max)	532	49.67 (18.27,74.48)	51.91 (22.23,70.48)

Gender			
-Female	207	175(84.54%)	32(15.46%)
-Male	325	267(82.15%)	58(17.85%)
Underlying diseases			
- No	415	348(83.86%)	67(16.14%)
- Yes	117	94(80.34%)	23(19.66%)
Diabetes Mellitus (DM)			
- No	517	431(83.37%)	86(16.63%)
- Yes	15	11(73.33%)	4(26.67%)
Hypertension			
- No	486	402(82.72%)	84(17.28%)
- Yes	46	40(86.96%)	6(13.04%)
Years of working experience	532	11 (0.25,40)	12 (1,39)
Working hours/week	532	42 (0.66, 126)	42 (7,84)
Live on farm			
- No	289	244(84.43%)	45(15.57%)
- Yes	243	198(81.48%)	45(18.52%)
Practices in the farm			
Assist in birthing			
- No	97	86(88.66%)	11(11.34%)
- Yes	435	356(81.84%)	79(18.16%)
Contact with birth products or placenta during birthing			
- No	110	98(89.09%)	12(10.91%)
- Yes	422	344(81.52%)	78(18.48%)
Clean birth area			
- No	103	89(86.41%)	14(13.59%)
- Yes	429	353(82.28%)	76(17.72%)
Milking			
- No	109	88(80.73%)	21(19.27%)
- Yes	423	354(83.69%)	69(16.31%)
Bathing cows			
- No	119	105(88.24%)	14(11.76%)
- Yes	413	337(81.6%)	76(18.4%)
Managing manure			
- No	115	101(87.83%)	14(12.17%)
- Yes	417	341(81.77%)	76(18.23%)
Cleaning stables			
- No	33	23(69.70%)	10(30.30%)
- Yes	499	419(83.97%)	80(16.03%)
Personal Protective Equipment (PPE) use in farm activities			
Use of mask while assisting in cow birthing			

- Never	325	260(80.00%)	65(20.00%)
- Ever	110	96(87.27%)	14(12.73%)
Use of gloves during assisting with cow birthing			
- Never	304	241(79.28%)	63(20.72%)
- Ever	131	115(87.79%)	16(12.21%)
Use of mask during exposure to cow birth products/placenta			
- Never	311	249(80.06%)	62(19.94%)
- Ever	111	95(85.59%)	16(14.41%)
Use of gloves during exposure to cow birth products/placenta			
- Never	304	242(79.61%)	62(20.39%)
- Ever	118	102(86.44%)	16(13.56%)
Use of mask during cleaning the birthing area			
- Never	298	241(80.87%)	57(19.13%)
- Ever	131	112(85.5%)	19(14.5%)
Use of gloves during cleaning the birthing area			
- Never	310	248(80%)	62(20%)
- Ever	119	105(88.24%)	14(11.76%)
Use of mask during milking			
- Never	301	247(82.06%)	54(17.94%)
- Ever	122	107(87.7%)	15(12.3%)
Use of gloves during milking			
- Never	363	303(83.47%)	60(16.53%)
- Ever	60	51(85%)	9(15%)
Use of mask when managing manure			
- Never	270	212(78.52%)	58(21.48%)
- Ever	147	129(87.76%)	18(12.24%)
Use of gloves when managing manure			
- Never	317	251(79.18%)	66(20.82%)
- Ever	100	90(90%)	10(10%)
Use of mask when cleaning stable			
- Never	300	241(80.33%)	59(19.67%)
- Ever	147	128(87.07%)	19(12.93%)
Using gloves when cleaning stable			
- Never	351	283(80.63%)	68(19.37%)
- Ever	96	86(89.58%)	10(10.42%)
Use of mask when feeding cows			
- Never	251	206(82.07%)	45(17.93%)
- Ever	163	136(83.44%)	27(16.56%)
Use of mask when bathing cows			
- Never	301	240(79.73%)	61(20.27%)

- Ever	112	97(86.61%)	15(13.39%)
Use of gloves when bathing cows			
- Never	342	274(80.12%)	68(19.88%)
- Ever	71	63(88.73%)	8(11.27%)
Personal Protective Equipment use in general			
Mask			
- Never	242	201(83.06%)	41(16.94%)
- Sometime	181	147(81.22%)	34(18.78%)
- Almost/always	109	94(86.24%)	15(13.76%)
Glove			
- Never	300	243(81.00%)	57(19.00%)
- Sometime	162	136(83.95%)	26(16.05%)
- Almost/always	70	63(90.00%)	7(10.00%)
Wear boots			
- Never	14	14(100%)	0(0%)
- Sometime	23	19(82.61%)	4(17.39%)
- Almost/always	495	409(82.63%)	86(17.37%)
Raise other animals in the residential area			
- No	91	77(84.62%)	14(15.38%)
- Yes	441	365(82.77%)	76(17.23%)
Dogs			
- No	75	65(86.67%)	10(13.33%)
- Yes	169	137(81.07%)	32(18.93%)
Cats			
- No	173	139(80.35%)	34(19.65%)
- Yes	71	63(88.73%)	8(11.27%)
Water Buffaloes			
- No	241	199(82.57%)	42(17.43%)
- Yes	3	3(100%)	0(0%)
Pigs			
- No	241	199(82.57%)	42(17.43%)
- Yes	3	3(100%)	0(0%)
Chickens			
- No	100	82(82%)	18(18%)
- Yes	144	120(83.33%)	24(16.67%)
Ducks			
- No	227	188(82.82%)	39(17.18%)
- Yes	17	14(82.35%)	3(17.65%)
Other animals			
- No	526	436(82.89%)	90(17.11%)
- Yes	6	6(100%)	0(0%)

Previous work			
Ever worked on animal farms			
- No	340	285(83.82%)	55(16.18%)
- Yes	192	157(81.77%)	35(18.23%)
Other possible risks			
Had rats in the house			
- No	139	108(77.7%)	31(22.3%)
- Yes	393	334(84.99%)	59(15.01%)
Traveled to other dairy cow farms			
- No	230	183(79.57%)	47(20.43%)
- Yes	302	259(85.76%)	43(14.24%)
Traveled to farms selling cow meat			
- No	524	435(83.02%)	89(16.98%)
- Yes	8	7(87.5%)	1(12.5%)
Traveled to zoo			
- No	509	424(83.3%)	85(16.7%)
- Yes	23	18(78.26%)	5(21.74%)
Traveled to a goat farm			
- No	530	440(83.02%)	90(16.98%)
- Yes	2	2(100%)	0(0%)
Helped in birthing other animals			
- No	524	435(83.02%)	89(16.98%)
- Yes	8	7(87.5%)	1(12.5%)
Contact with feces from other animals			
- No	465	385(82.8%)	80(17.2%)
- Yes	67	57(85.07%)	10(14.93%)
Tick bite			
- No/Not sure	443	370(83.52%)	75(16.93%)
- Yes	87	72(82.76%)	15(17.24%)
Drank raw milk			
- No	517	428(82.79%)	89(17.21%)
- Yes	15	14(93.33%)	1(6.67%)
Ate raw meat			
- No	323	269(83.28%)	54(16.72%)
- Yes	209	173(82.78%)	36(17.22%)
Ate raw pork			
- No	304	253(83.22%)	51(16.78%)
- Yes	228	189(82.89%)	39(17.11%)
Ate raw Chicken			
- No	513	425(82.85%)	88(17.15%)
- Yes	19	17(89.47%)	2(10.53%)

Cooked raw placenta			
- No	230	191(83.04%)	39(16.96%)
- Yes	302	251(83.11%)	51(16.89%)
Received blood transfusion			
- No	517	431(83.37%)	86(16.63%)
- Yes	15	11(73.33%)	4(26.67%)
Working areas			
Distance from positive farms			
< 1 km	365	298(81.64%)	67(18.36%)
1 - <2 kms	74	57(77.03%)	17(22.97%)
>= 2 kms	89	83(93.26%)	6(6.74%)
BTM results			
- BTM non-positive	304	271(89.14%)	33(10.86%)
- BTM positive	224	167(74.55%)	57(25.45%)

*No farmer raised sheep or goats in the residential area.

Table10. Univariate analysis of factors associated with seropositivity among farmers, Chiang Mai, Thailand 2015

	GEE (cut-off 1:64) outcome = 90/randomly selected farmers N=532				
Variables	**OR**	**95%LCI**	**95%UCI**	**p-value**	**type3 p-value**
Coops					0.0031
Farm size					0.9599
Age (year)	1.0242	1.0029	1.0459	0.0254	0.0212
Gender (male vs. female)	1.262	0.8221	1.9373	0.2873	0.2796
Underlying disease	1.507	0.9389	2.419	0.0894	0.1143
Diabetic Mellitus (DM)					0.2298
- No underlying disease				Ref.	
- DM	2.1433	0.8470	5.4234	0.1075	
- No DM, but had other disease	1.4111	0.8430	2.3619	0.1901	
Working experience (month)	1.0078	0.9837	1.0325	0.5299	0.5258
Working hours/week	1.0024	0.9914	1.0136	0.6651	0.6725
Live on farm	1.2428	0.7419	2.0817	0.409	0.4005
Farm practices					
Assist in birthing	1.4962	0.7950	2.8159	0.2117	0.1784
Contact with cow birth products/placenta during birthing	1.9437	1.0153	3.7213	0.0449	0.0234
Clean birthing areas after birth	1.2987	0.6671	2.5285	0.442	0.4247
Milking	0.7722	0.4822	1.2365	0.2819	0.3024
Bathe cows	1.5100	0.8399	2.7148	0.1685	0.1545
Manage manure	1.6084	0.8268	3.1287	0.1615	0.1334

Clean stables	1.2177	0.5975	2.4817	0.5876	0.5753
Ever worked on animal farms	1.3880	0.7612	2.5312	0.2848	0.3108
Personal Protective Equipment use					
Use mask on the farms					0.7272
- Sometime vs. never	1.0451	0.6106	1.7888	0.8721	
- Always vs. never	0.8111	0.4080	1.6125	0.5504	
Use gloves on the farms					0.0955
- Sometime vs. never	0.7129	0.4103	1.2384	0.2297	
- Always vs. never	0.4338	0.1736	1.0839	0.0738	
Other factors					
Rats in the house	0.7043	0.4258	1.1650	0.1722	0.2080
Ever traveled to other dairy cow farms	0.7089	0.4528	1.1100	0.1326	0.1410
Ever traveled to farms selling meat	0.7653	0.0548	10.691	0.8424	0.8290
Ever traveled to the zoo	1.1900	0.3599	3.9349	0.7756	0.7669
Ever had contact with other animal feces	0.7399	0.3815	1.4353	0.3729	0.3542
Ever had a tick bite	0.8241	0.4372	1.5535	0.5498	0.5202
Ever drank raw milk	0.3211	0.0545	1.8925	0.2094	0.0855
Ever ate raw meat	0.9564	0.5969	1.5324	0.8530	0.8529
Ever ate raw pork	1.1915	0.7085	2.0039	0.5088	0.5042
Ever cooked raw placenta	0.9372	0.5934	1.4802	0.7810	0.7811
Ever received blood transfusion	1.6715	0.7042	3.9674	0.2441	0.3412
Ever washed your hands after animal contact	0.9417	0.4823	1.8385	0.8602	0.8597
Ever eaten in the farm areas	0.8485	0.3955	1.8202	0.6731	0.6611
Farm factors					
Clean area immediately after birth					
- Always vs. not always	0.4401	0.211	0.9178	0.0286	0.0709
Stall base (cow bedding type)					0.3950
- Cement and ground vs. cement only	0.8399	0.3771	1.8709	0.6695	
- Cement and rubber mat vs. cement only	1.6214	0.8001	3.2859	0.1799	
Had separate stables for quarantine (Yes or No)	1.4735	0.8028	2.7045	0.2110	0.2414
Ever quarantined newly purchased animal (ever vs. never)	0.7828	0.4471	1.3703	0.3913	0.3939
Distance from positive farms					0.1085
- < 1 km vs. ≥ 2 kms	2.4995	0.8658	7.2159	0.0903	
- 1 - < 2 kms vs. ≥ 2 kms	3.2692	1.0237	10.4400	0.0456	
Positive BTM (yes vs. no)	2.8880	1.5830	5.2691	0.0005	0.0009

*Ref.: Reference

Figure 9 shows the distribution of proportion of seropositive farmers by subdistrict and the BTM results by farm location. Some areas, particularly the areas of coops1, 2, and 3, had a higher proportion of seropositive farmers which corresponded with the BTM results.

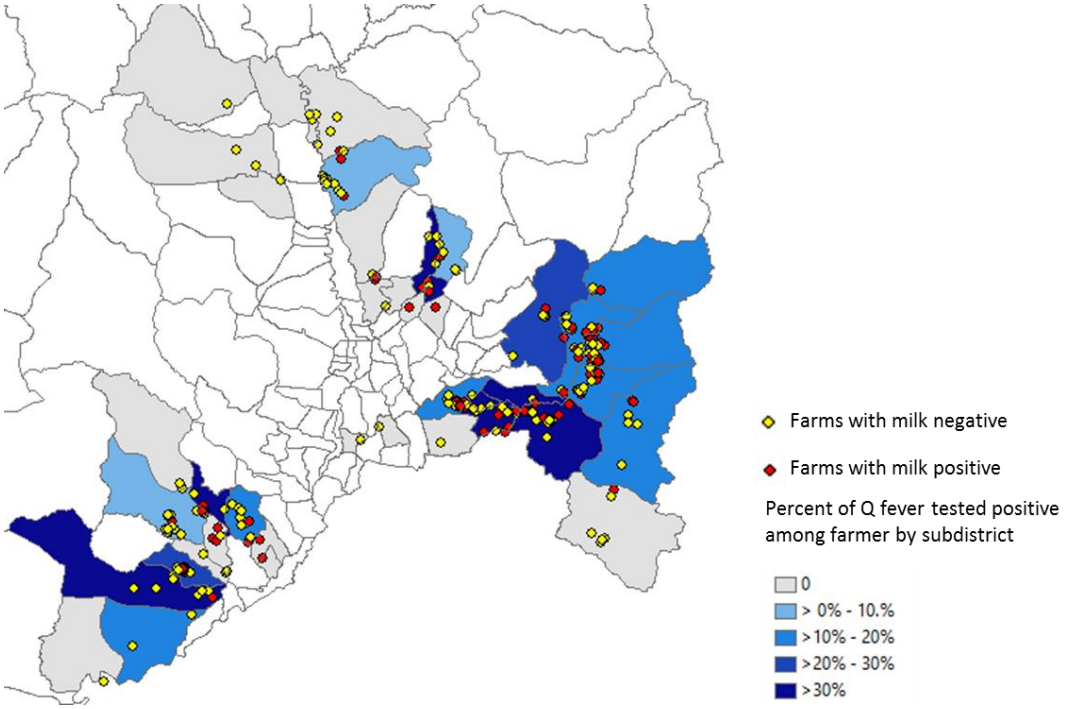


Figure 9. Distribution of BTM results and the proportion of seropositive farmers, Chiang Mai, Thailand 2015

Discussion

The overall seroprevalence of *C. burnetii* infection among farmers in our study was 16.9%. The prevalence was similar to the study in Chiang Mai in 2012 (16%) (unpublished). It is possible that the disease could be endemic in the study areas without detection. Prevalence differed by dairy cooperative. Factors that might be associated with seropositivity among farmers include age, history of coming into contact with cows' birthing products or placenta during calving, assisting in calving, using gloves, and managing manure. Baseline seroprevalence among farmers was similar to the survey conducted in three districts of Chiang Mai in 2012 (unpublished). However, seroprevalence was lower compared to studies among farmers in dairy cattle farms in the Netherlands (72.1%) (125) and Mongolia (35.6%) (126). However, comparing seroprevalence among studies can be challenging, since different cutoff values and sampling methods were used. Seropositivity among Thai farmers was associated with BTM results, suggesting that BTM screening would be beneficial for targeting health education for Q fever prevention among farmers.

Future work

The next step is to incorporate farm factors and perform multivariate analyses using GEE to determine factors associated with the seroprevalence of *C. burnetii* infection among farmers. In addition, sensitivity analysis using different IgG antibody cutoff values will be conducted.

CHAPTER 7

SUMMARY

Discussion

The results of our study add useful information regarding *C. burnetii* infection (Q fever) among a high risk population in Thailand. This is the first longitudinal study to determine the epidemiology of Q fever among dairy cattle farms and farmers in Thailand. Therefore, several outcomes regarding *C. burnetii* infection can be determined, for example, the incidence of Q fever and risk factors, antibodies' response to *C. burnetii* infection, and clinical manifestations of cases. In addition, this study is the first to determine the prevalence of *C. burnetii* antibody in BTM at the herd level in Thailand. The study benefited from more than 80% participation, in which 92% of the randomly selected farms (282/306) and 83% of the randomly selected farmers (532/637) participated.

Analysis of baseline information showed that the unweighted prevalence of *C. burnetii* antibody in BTM was 40.8% (115/282). This is lower when compared to studies from the Netherlands – 78.6% (131) and Spain – 66.9% (128), but similar in range when compared to studies from Iran (45.4%) (129) and Ireland (37.9%) (132). The high prevalence of antibodies in BTM represents past exposure to *C. burnetii,* but it might not signify active infection or active shedding on the farm. Laboratory diagnosis is crucial for *C. burnetii* infection since most infections in cows are asymptomatic and shedding is possible regardless of clinical presentations. A previous study showed no relationship between *C. burnetii* shedding and seroconversion in animals (107). Nonetheless, the prevalence of antibodies to *C.burnetii* could indicate the existence of infection in the area.

Analyses of BTM data showed that positive BTM differed by dairy cooperative and farm size. Farms with positive milk were clustered in specific areas. The odds of positive BTM on farms with > 40 cows was 3 times the odds of positive BTM on the farms with 1–20 cows (OR 3.00, 95%CI 1.28–7.04). However, our study showed that, cleaning the birthing area after calving (OR 0.27, 95%CI 0.08 - 0.87) and quarantining of newly purchased animals (OR 0.52, 95%CI 0.29 - 0.93) were

protective factors.Having more than 80% of cows ≥ 2 years of age (OR 2.3, 95%CI 1.09 - 5.06) and having an infected farm within 1 kilometer (OR 2.94, 95%CI 1.22 – 7.09) were positively associated with the prevalence of *C. burnetii* antibodies in BTM. Our findings are similar to previous studies where increasing herd size, increasing cows' ages, and proximity to positive farms were positively associated with the prevalence of *C. burnetii* (77, 130, 133).

In addition, results from our study showed that the prevalence of positive BTM differed by stall base. Farms with concrete and rubber mat bases (3.90, 95%CI 1.34-11.39) or concrete bases only (2.07, 95%CI 0.97-4.43) were positively associated with the odds of positive BTM compared to farms with concrete and earth bases. Rubber mats are put in place for the comfort of the cows. Most farms did not have separate birthing stables; hence, calving was done in the tiding area. Secretions from calving could remain on the mat or stall bases if cleaning was not appropriately done. *C. burnetii* has a small cell variant stage which can be resistant to harsh environments, such as disinfectants, desiccation, and UV light (123). As a result, completely clearing *C. burnetii* from the environment can be challenging. However, cleaning and disinfection in the birthing area after calving is recommended to reduce the sustainability of the bacteria in the environment (134).

Of the 115 BTM positive farms, 99 were visited (86.1%). Results from farm investigations showed that 91.9% (91/99) of the farms with positive milk had at least one cow seropositive to *C. burnetii.* This information suggests that BTM screening with ELISA could be a good marker for *C. burnetii* infection at the herd level. Furthermore, the environmental investigations during farm visits showed that *C. burnetii* DNA was identified from swabs of the birthing area (12.1%, 12/99), soil recovered at the edge of the water drainage system (7.1%, 7/99), and ticks (1.9%, 1/52). Ticks are suspected as a sustainable reservoir for *C. burnetii* in the environment as they can shed bacteria through their feces (20). Findings from environmental investigations in this study support evidence from BTM analysis and underscore the importance of *C. burnetii* contamination in the farm environment, which then influences the prevalence of *C. burnetii* infection in both animals and humans. Therefore, appropriate cleaning, disinfection, particularly during calving, and tick controls on farms are essential to *C. burnetii* control and prevention.

For the farmers' study, 532 of 637 randomly selected farmers participated (response rate 83.5%). Preliminary analysis shows that *C. burnetii* seroprevalence among the farmers was approximately 17%,

similar to the previous survey conducted in Chiang Mai in 2012 (16%) (unpublished data). Seroprevalence was lower compared to studies among veterinary students or farmers in Japan (35.6%) (70), Taiwan (26.3%) (135), Mongolia (35.6%) (126), and the Netherlands (72.1%) (125). However, different cutoff values were used which make comparison between studies difficult. Our preliminary analysis shows that seropositive farmers were likely to become exposed to the pathogen by direct contact with a cow's birth products (OR 1.9, 95%CI 1.0 - 3.7). Furthermore, other farm factors such as proximity to positive farms and BTM results might influence seropositivity among farmers. A positive BTM was positively associated with the odds of seropositivity among farmers in the univariate analysis (OR 2.9, 95%CI 1.6 - 5.3). Further multivariate analyses will be done to confirm this hypothesis.

Measurement errors could be possible since we measured antibody response to *C. burnetii* infection in milk and farmers. Antibody levels depend on several factors, such as the immune response of each individual, laboratory methods, and experiences of the laboratory staff. To control for these errors, we used the same laboratory methods and the same readers throughout the study. In addition, sensitivity analysis using a different cutoff value was performed to determine possible risk factors.

Before this study was launched, Q fever was unknown to farmers and rarely known to public health and animal health officers. Our baseline analysis shows that *C. burnetii* infection is prevalent in the study areas. The findings from our study provide evidence for Q fever prevention and control. Similar to another study (136), the results from our study suggests that BTM screening is a good indicator for *C. burnetii* infection at the herd level. Screening of BTM periodically can be an important tool to determine the prevalence of *C. burnetii* infection at the herd level. Therefore, health education for Q fever prevention can be targeted. Health care professionals and public health and animal health officers should be informed about Q fever in their areas. In addition, health education regarding Q fever prevention and biosecurity concerns on the farms need to be strengthened among livestock farmers in general.

Vaccines are available for both humans and animals and are used in some countries, such as Australia (137). The most recent vaccine for humans can cause severe adverse reactions if it is given to an individual who has previously been infected. Therefore, prescreening, either by skin test or serological test, is required (25). In cattle, the review showed that vaccination before pregnancy resulted in the reduction of a significant amount of bacteria shedding when compared to vaccination during pregnancy

and a placebo (95). This study highlights the extent of Q fever in dairy cattle farms and farmers; however, the information from this study alone cannot provide evidence to support the importance of Q fever vaccination in general. Since vaccines are costly and vaccine administration is not feasible when laboratory facilities are limited, further information such as the prevalence of chronic Q fever endocarditis, the prevalence of Q fever in other ruminants, and vaccine cost effectiveness are needed in order to ascertain if vaccination is beneficial.

Public health impact

Since information on the epidemiology of Q fever in Thailand is limited, our study provides crucial information about the prevalence of *C. burnetii* infection among dairy cow farms and farmers. Considering its longitudinal design, we are able to evaluate the incidence and risk factors associated with *C. burnetii* infection and clinical presentations of Q fever patients. Therefore, the outcomes of this study provide scientific evidence for Q fever prevention and control in dairy cow farms and farmers in Thailand. Our analyses of baseline information among this high risk population underline the existence of *C. burnetii* in the study areas and factors associated with the prevalence of *C. burnetii* antibodies in BTM and farmers. Farmers and veterinary officers who work closely with ruminants are at risk of contracting *C. burnetii* infections. The results of this study will be presented to policy makers in the animal and public health sectors, hence appropriate prevention and control messages can be distributed to high risk populations in high risk areas. In addition, these results can be presented to the Thai Infectious Disease Medicine Council to improve physicians' awareness of Q fever. Furthermore, this study highlighted the One-Health concept in which animal and public health sectors in Thailand collaborate to conduct a field investigation and laboratory testing of Q fever.

Future work

Further analyses of individual cow data from the farm investigation will be performed to understand the clinical presentation and the factors associated with the prevalence of seropositive cows and *C. burnetii* shedding. In addition, further analyses will be performed to understand the potential risk factors associated with the prevalence of seropositivity among farmers. Sensitivity analyses using different IFA

cutoff values will be applied in multivariate analysis using GEE to determine factors associated with seroprevalence of *C. burnetii*.

Data collection and laboratory tests were still in process when the dissertation was written. Further analyses for the whole project will be performed when data collection and laboratory tests are completed. Analyses will be performed to determine the incidence of Q fever and risk factors associated with the disease. If there is evidence of acute or chronic Q fever infection, clinical presentations of Q fever cases will be described and factors associated with clinical presentation will be explored. In addition, analyses of BTM screening for all three time points will be done to determine factors associated with persistently positive or newly positive BTM. Information for all cows and environmental samples during the farm investigations will be determined to understand factors associated with seropositivity and shedding in cows and the roles of environmental contamination.

Our study used ELISA screening of BTM as a proxy for *C. burnetii* infection at the herd level. Nonetheless, the presence of antibodies suggests previous exposure to *C. burnetii* and may not represent the evidence of shedding of bacteria. A previous study showed that seropositive animals might not shed the bacteria (50). Further laboratory study and analyses to understand the correlation between the antibodies to *C. burnetii* and the presence of *C. burnetii* DNA in BTM will be performed.

Previous studies showed that *C. burnetii* infection related to dairy cows had a milder clinical presentation compared to sheep or goats (125, 138). Other research involving molecular epidemiology to identify *C. burnetii* genotypes should be done to understand the roles of C. *burnetii* genotypes and their clinical manifestation in both cows and humans. In addition, the study of Q fever among other ruminants and other groups of occupationally exposed people will enhance our knowledge of the extent of Q fever in Thailand.

APPENDIX 1: QUESTIONNAIRE FOR FARM REPRESENTATIVE

QFarm Date of interview……/ ……../ ……

Questionnaire for the farm

General information

1 Farm's name	2 Registration number .....
3 Name of interviewee.....	4 Status [0] owner [1] employee
5 Date of starting the farm business ...../...../.....	6 Contact number.....
7 GIS coordinate X..... Y.....	8 Number of farmers in the farm.....

Farm characteristics

Characteristics of the cows

9 How many bulls are in the farm?.....			10 How many dairy cows are in the farm?		
11 Breeding					
Holstein	[0] no	[1] yes, how many.....	Red dane	[0] no	[1] yes, how many.....
Jersey	[0] no	[1] yes, how many.....	Sahiwal	[0] no	[1] yes, how many.....
Others	[0] no	[1] yes, please specify species..... and how many.....			
12 Feeding system?		[0] free grazing only	[1] inside the barn only		[2] both systems
13 Reproduction method?		[0] natural	[1] artificial insemination		[2] both methods
14 Share feeding areas with other animals in the farm?				[0] no	[1] yes
15 Share feeding areas with other animals outside the farm?				[0] no	[1] yes

16 Please specify the number of cows by age group and the housing type for each particular each group

	Number	***Housing type***		
a. age < 1 year		[0] free	[1] inside the barn only	[2] both
b. age1 – 2 year		[0] free	[1] inside the barn only	[2] both
c. age 2 – 4 year		[0] free	[1] inside the barn only	[2] both
d. age > 4 year		[0] free	[1] inside the barn only	[2] both
d. Bull		[0] free	[1] inside the barn only	[2] both

Housing

17 How many stables are in the farm?.....		18 How many cows are in each stable?	
19 Design of housing	[0] bedded resting barn 	[1] frees tall barn 	[2] tie stall barn 
20 Type of enclosure	[0] open (post-and-rail fence)	[1] partially open (partially walled)	[2] closed (mud or cement walled)
21 Type of ventilation	[0] natural wind flow	[1] machine	
22 Type of floor /bedding	[0] none	[1] straw	[2] cement
	[3] saw dust	[4] rubber	[5] others, specify.....
23 Type of roofing	[0] thatched roof	[1] tiles or metal sheet	[2] others, specify.....

1 PD

24 Separate stable for birthing	[0] no	[1] yes, how far is it from other stable …… meter
25 Separate stable to quarantine the newly purchased cows	[0] no	[1] yes, how far is it from other stable …… meter

Other domestic animals in the farm

26 Do you raise other animals in the farm? [0] No, skip to Q 27 [1] yes, please specify in the table

Animals	If yes, raise any of these or not?	Number	Have the animal ever had history of this condition?			Are they raised in the same area with the cows?
			Abortion	Retained placenta	Unknown death	
Dog	[0] no [1] yes		[0] no [1] yes	[0] no [1] yes	[0] no [1] yes	[0] no [1] yes
Cat	[0] no [1] yes		[0] no [1] yes	[0] no [1] yes	[0] no [1] yes	[0] no [1] yes
Buffalo	[0] no [1] yes		[0] no [1] yes	[0] no [1] yes	[0] no [1] yes	[0] no [1] yes
Sheep	[0] no [1] yes		[0] no [1] yes	[0] no [1] yes	[0] no [1] yes	[0] no [1] yes
Goat	[0] no [1] yes		[0] no [1] yes	[0] no [1] yes	[0] no [1] yes	[0] no [1] yes
Pig	[0] no [1] yes		[0] no [1] yes	[0] no [1] yes	[0] no [1] yes	[0] no [1] yes
Chicken	[0] no [1] yes				[0] no [1] yes	[0] no [1] yes
Duck	[0] no [1] yes				[0] no [1] yes	[0] no [1] yes
Other, specify ……	[0] no [1] yes		[0] no [1] yes	[0] no [1] yes	[0] no [1] yes	[0] no [1] yes
Other, specify ……	[0] no [1] yes		[0] no [1] yes	[0] no [1] yes	[0] no [1] yes	[0] no [1] yes

Rodents and wild animals

27 Have you ever seen any rodents in the farm?	[0] no	[1] yes	
28 Have you seen rodent feces in the farm?	[0] no	[1] yes, sometime	[2] yes, often
29 Is there a rodent control program in place in the farm? If yes, please specify how	[0] no	[1] yes, specify…… ……	
30 Have you ever seen any wild animals in the farm area? If yes, please specify the animal	[0] no	[1] yes, specify…… ……	

<u>Farm management</u>

Cleaning

31 How often did you clean the stable?

[0] Never	[1] < 1 time/month	[2] 1 time/month	[3] >1 to <4/month	[4] every week	[5] everyday

32 What do you usually use to clean the stable/stalls?

[0] Water only	[1] Water with detergent	[2] Chrolox	[3] others, please specify……

33 Did you use disinfectant to clean the farm? If yes, how often did you use it?

[0] Never	[1] rarely	[2] sometime	[3] mostly	[4] always

34 Please specify the name of the disinfectants you used……

QFarm Date of interview……../ ……../ ……

Waste management

35 How did you manage the manures?

Collect in a septic tank	[0] no	[1] yes	used as a fertilizer for crops	[0] no	[1] yes
Leave them in the environment	[0] no	[1] yes	sell them	[0] no	[1] yes
Other, please specify……					

36 What are the practices during and after calving?

a. allow natural birthing, only help when there is a complication				[0] no	[1] yes
b. always help with birthing, and stay with the cows until the birthing finish				[0] no	[1] yes
c. clean the stalls immediately after birthing	[0] no	[1] rarely	[2] sometime	[3] mostly	[4] always
d. leave the stall as it was after birthing	[0] no	[1] rarely	[2] sometime	[3] mostly	[4] always

37 What did you usually do with the placentas and the secretion?

Leave it for the cow	[0] no	[1] rarely	[2] sometime	[3] mostly	[4] always
Keep it for cooking	[0] no	[1] rarely	[2] sometime	[3] mostly	[4] always
Collect in a septic tank	[0] no	[1] rarely	[2] sometime	[3] mostly	[4] always
Bury them in the farm	[0] no	[1] rarely	[2] sometime	[3] mostly	[4] always
Sell them to others	[0] no	[1] rarely	[2] sometime	[3] mostly	[4] always
Other, please specify……					

Quarantine and hygiene control

38 In the past 6 months, have you purchased any new cows	[0] no		[1] yes, if yes how many male…… and female ……		
39 Did the farm quarantine any of the newly purchased animals?	[0] no	[1] rarely	[2] sometime	[3] mostly	[4] always
40 If yes, for how long will the quarantine be? ……					

41 Footwear and clothing on farm when arriving and working at the farm

a. Using specific footwear (e.g. boots)	[0] no	[1] yes	d. Have footwear cleaning station or disinfection bath	[0] no	[1] yes
b. Using specific clothing	[0] no	[1] yes	e.Using masks	[0] no	[1] yes
c. Using gloves	[0] no	[1] yes	f. Using aprons	[0] no	[1] yes

42 Do you have any insect control program in the farm, if yes what methods did you use?

[0] none, skip to question 43	[1] yes, by spraying insecticide
[2] yes, by bathing insecticide	[3] yes, others specify……

43 How often did you use insect control?

[0] every week	[1] every 2 weeks	[2] every month	[3] every 2 – 3 month	[4] every 6 month
[5] others, please specify ……				

44 What are the insecticides you use for insect and tick controls?.....

Animal Health care

45 Did all the cows get routine checkup?	[0] no	[1] yes
46 How often is the routine checkup?	[0] < every 6 months	[1] every 6 months
	[2] every year	[3] every other year
47 Please explain, what are included in the routine checkup?..... …….		
48 What do you do if the cows get sick?	[0] ask the local veterinarian for help	[1] buy antibiotic

Antibiotic Use

49 In the past 6 months, have you ever used antibiotics with the cows? If yes, how often have you used it?

Antibiotics	Use?	If yes, frequency of antibiotics usage		
a. Lincomycin	[0] no	[1] rarely	[2] sometime	[1] mostly
b. Penicillin	[0] no	[1] rarely	[2] sometime	[1] mostly
c. Tetracycline	[0] no	[1] rarely	[2] sometime	[1] mostly
d. Doxycycline	[0] no	[1] rarely	[2] sometime	[1] mostly
e. Sufaniramide	[0] no	[1] rarely	[2] sometime	[1] mostly
f. Other,specify ……	[0] no	[1] rarely	[2] sometime	[1] mostly

Vaccine use

50 Is there any vaccination program to the cows?

[0] no [1] yes, please specify all the vaccines that are applied……

Quality of the milk (on the same day that milk is collected for Q fever screening)

51 Somatic cell count (extract data from the dairy cooperative)……

Date of milk collection (dd/mm/yyyy) ……/……../……

Interviewer's name…… Contact number ……

APPENDIX 2: QUESTIONNAIRE FOR INDIVIDUAL FARMER

QFarmers Date of interview ___/ ____/ _____ ID number _____

Questionnaire for the farmer

Demographic information and underlying diseases

1 Name..... Surname.....	2 Contact number.....
3 Date of birth (dd/mm/yyyy)	4 Farm's name.....
5 Gender [0] female [1] male	6 If female, are you pregnant? [0] no [1] yes, gestational age .....weeks
7 Address.....	8 GIS coordinate: X ..... Y.....
9 Did you live in this house since you were born? [0] no [1] yes	10 If no, please specify when you moved in …….. and where did you live before ……

11 Do you have any underlying diseases? [0] no (skip to Question 13) [1] yes

12 If yes, have you been diagnosed with any of these following diseases?

Insulin dependent Diabetes Mellitus	[0] no	[1] yes	Non-insulin dependent diabetes	[0] no	[1] yes
Chronic renal failure	[0] no	[1] yes	Valvular heart disease	[0] no	[1] yes
Lymphoma	[0] no	[1] yes	Aneurysm	[0] no	[1] yes
Cancer on chemotherapy	[0] no	[1] yes	Other cardiovascular disease	[0] no	[1] yes
Cirrhosis	[0] no	[1] yes	Chronic lung diseases	[0] no	[1] yes
If women, have you ever had history of spontaneous abortion?				[0] No	[1] yes

13 Did you take any oral or intravenous steroid every day in the past 6 months? [0] no [1] yes

Job description in the farm

General description

14 How long have you been working in this farm? ……

15 How many days do you usually work per week? ..... days

16 How many hours do you usually work per day? …….... hours

17 Do you live on the farm? [0] no [1] yes

These questions will ask if the farmers ever had any of the activities in the past 6 months

18 At work (in the farm), have you ever used any of these personal protective equipment and how often did you use them in the **past 6 months**?

Gloves	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
Boots	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
Goggles/ face shield	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
Aprons	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
Masks	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always

19 If you use mask, what kind of mask did you use in the farm?

[0] Surgical mask(from health care workers)	[1] Mask made from cotton	[2] N95

20 Have you done any of these jobs in the farm (can answer more than 1), if yes, how often did you do that in the **past 6 months** (e.g., how many hours per day and how many days per week)?

20.1 Assisting with birthing					
[0] no, skip to 20.2	[1] yes, how many cows did you assist with birthing……				
Did you use mask?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
Did you wear gloves?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
20.2 Cleaning the animal areas after birthing					
[0] no, skip to 20.3	[1] yes, how many times have you done in the past 6 months……				
Did you use mask?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
Did you wear gloves?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
20.3 Handling placentas or birth products					
[0] no, skip to 20.4	[1] yes, how many times have you done in the past 6 months……				
Did you use mask?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
Did you wear gloves?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
20.4 Milking					
[0] no, skip to 20.5	[1] yes, how many hours/day…… , how many days/week……				
Did you use mask?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
Did you wear gloves?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
20.5 Cleaning the cows					
[0] no, skip to 20.6	[1] yes, how many times have you done in the past 6 months……				
Did you use mask?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
Did you wear gloves?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
20.6 Cleaning the stables					
[0] no, skip to 20.7	[1] yes, how many times have you done in the past 6 months……				
Did you use mask?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
Did you wear gloves?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
20.7 Cleaning the farm (excluding the stables)					
[0] no, skip to 20.8	[1] yes, how many times have you done in the past 6 months……				
Did you use mask?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
Did you wear gloves?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
20.8 Culling the cows					
[0] no, skip to 20.9	[1] yes, how many times have you done in the past 6 months……				
Did you use mask?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
Did you wear gloves?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always

20.9 Driving the truck containing the cows or milk	[0] no	[1] yes	Hours/day	Days/week.....

Q11. Uniform and practicing after work in **past 6 months?**

Uniform					
21 Did you need to change the working suit in order to work in the farm?				[0] no	[1] yes
22 Did you need to *change the boot* to work in the farm?				[0] no	[1] yes
Practices after work					
23 Did you wash your hands after contact with animals?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
24 Did you change your clothes and take a shower as soon as possible after work?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always
25 Did you eat in the animal areas?	[0] never	[1] almost never	[2] sometime	[3] mostly	[4] always

Other jobs

26 Besides working in this cattle farm, do you have other jobs? [0] no (skip to question 28) [1] yes
27 If yes, do you work in any of these jobs?

Rice farmer	[0] no	[1] yes	Work in non-livestock farms	[0] no	[1] yes
Work in other animal farms	[0] no	[1] yes	If yes, please specify the name of the farm and the type of animals.....		

28 Have you ever worked in other jobs before working in this dairy cattle farm?

[0] no (skip to 29)	[1] yes, please answer if you ever worked in these following jobs and for how long	
- Rice farmer	[0] no	[1] yes, for how long?.....
- Non-livestock farmer (other than rice farms)	[0] no	[1] yes, please specify the farm and for how long?..... .....
- Livestock farmer	[0] no	[1] yes, please specify the farm and for how long?..... .....

Raising and contact with other animals in the house

29 **In past 6 months,** have you raised any of these animals in your family, and how many of them? If yes, have they ever had history of these illnesses?

Animals	**Raise any of these any animals?**	**How many?**	**Have they ever had history of these illnesses in the past year?**		
		Number	**Abortion?**	**Retain placenta?**	**Death of unknown cause?**
Dog	[0] no [1] yes		[0] no [1] yes	[0] no [1] yes	[0] no [1] yes
Cat	[0] no [1] yes		[0] no [1] yes	[0] no [1] yes	[0] no [1] yes
Buffalo	[0] no [1] yes		[0] no [1] yes	[0] no [1] yes	[0] no [1] yes
Sheep	[0] no [1] yes		[0] no [1] yes	[0] no [1] yes	[0] no [1] yes
Goat	[0] no [1] yes		[0] no [1] yes	[0] no [1] yes	[0] no [1] yes

Pig	[0] no	[1] yes		[0] no	[1] yes	[0] no	[1] yes	[0] no	[1] yes
Chicken	[0] no	[1] yes		[0] no	[1] yes	[0] no	[1] yes	[0] no	[1] yes
Duck	[0] no	[1] yes		[0] no	[1] yes	[0] no	[1] yes	[0] no	[1] yes
Others, specify				[0] No	[1] Yes	[0] no	[1] yes	[0] no	[1] yes
Others, specify				[0] No	[1] Yes	[0] no	[1] yes	[0] no	[1] yes

30 **In the past 6 months**, have you ever contacted with these animals (regardless of the history of raising the animals), and how often did you contact them?

Animals	**Have you ever contact the animals?**			**Have you ever contact this animal's secretion such as placenta, feces?**		
Dog	[0] never	[1] sometime	[2] often	[0] never	[1] sometime	[2] often
Cat	[0] never	[1] sometime	[2] often	[0] never	[1] sometime	[2] often
Buffalo	[0] never	[1] sometime	[2] often	[0] never	[1] sometime	[2] often
Sheep	[0] never	[1] sometime	[2] often	[0] never	[1] sometime	[2] often
Goat	[0] never	[1] sometime	[2] often	[0] never	[1] sometime	[2] often
Pig	[0] never	[1] sometime	[2] often	[0] never	[1] sometime	[2] often
Chicken	[0] never	[1] sometime	[2] often	[0] never	[1] sometime	[2] often
Duck	[0] never	[1] sometime	[2] often	[0] never	[1] sometime	[2] often
Rabbit	[0] never	[1] sometime	[2] often	[0] never	[1] sometime	[2] often
Horse	[0] never	[1] sometime	[2] often	[0] never	[1] sometime	[2] often
31 Have you ever seen the rats in your house?				[0] never	[1] sometime	[2] often
32 Have you ever touched the rats or their secretion such as feces?				[0] never	[1] sometime	[2] often

Travelling history in past 6 months

33 Have you ever traveled to other dairy farms?	[0] no	[1] yes	Please specify the farm name .....
34 Have you ever traveled to the meat cattle farms?	[0] no	[1] yes	Please specify the farm name .....
35 Have you ever traveled to the goat farms?	[0] no	[1] yes	Please specify the farm name .....
36 Have you ever traveled to the sheep farms?	[0] no	[1] yes	Please specify the farm name .....
37 Have you ever traveled to the zoo?	[0] no	[1] yes	Please specify the name .....

QFarmers Date of interview ___/ ____/ _____ ID number _____

Other risk factors

Have you ever had history of these activities **in the past 6 months**?

38 Help in the birthing process of the cats?		[0] no	[1] yes	If yes, please answer the questions in the highlighted row below	
Did you use mask?	[0] never	[1] almost never		[2] sometime	[3] mostly	[4] always
Did you wear gloves?	[0] never	[1] almost never		[2] sometime	[3] mostly	[4] always
39 Help in the birthing process of the dogs?		[0] no	[1] yes	If yes, please answer the questions in the highlighted row below	
Did you use mask?	[0] never	[1] almost never		[2] sometime	[3] mostly	[4] always
Did you wear gloves?	[0] never	[1] almost never		[2] sometime	[3] mostly	[4] always
40 Help in the birthing process of other animals?		[0] no	[1] yes	If yes, specify…… and answer the questions in the highlighted row below	
Did you use mask?	[0] never	[1] almost never		[2] sometime	[3] mostly	[4] always
Did you wear gloves?	[0] never	[1] almost never		[2] sometime	[3] mostly	[4] always
41 Contact the dairy cows' feces?		[0] no	[1] yes	If yes, please answer the questions in the highlighted row below	
How often?	[0] < 1 time/wk	[1] 1 – 2 times/wk		[2] 3 – 4 times/wk	[3] > 4 times/wk
Did you use mask?	[0] never	[1] almost never		[2] sometime	[3] mostly	[4] always
Did you wear gloves?	[0] never	[1] almost never		[2] sometime	[3] mostly	[4] always
42 Contact other animals' feces?		[0] no	[1] yes	If yes, please answer the questions in the highlighted row below	
How often?	[0] < 1 time/wk	[1] 1 – 2 times/wk		[2] 3 – 4 times/wk	[3] > 4 times/wk
Did you use mask?	[0] never	[1] almost never		[2] sometime	[3] mostly	[4] always
Did you wear gloves?	[0] never	[1] almost never		[2] sometime	[3] mostly	[4] always
43 Drink raw milk?		[0] no	[1] yes	If yes, where did you get them from? ……	
How often?	[0] never	[1] rarely		[2] sometime	[3] mostly	[4] always
44 Eat raw cow meat, cow's blood, or intestine?		[0] no	[1] yes	If yes, where did you get them from? ……	
How often?	[0] < 1 time/wk	[1] 1 – 2 times/wk		[2] 3 – 4 times/wk	[3] > 4 times/wk
45 Eat raw pork, pig blood, or intestine?		[0] no	[1] yes	If yes, where did you get them from? ……	
How often?	[0] < 1 time/wk	[1] 1 – 2 times/wk		[2] 3 – 4 times/wk	[3] > 4 times/wk
46 Eat raw chicken, chicken blood, or intestine?		[0] no	[1] yes	If yes, where did you get them from? ……	
How often?	[0] < 1 time/wk	[1] 1 – 2 times/wk		[2] 3 – 4 times/wk	[3] > 4 times/wk
47 Bitten by the cow ticks?	[0] no	[1] yes		[2] do not know	
If yes, how often?	[0] < 1 time/wk	[1] 1 – 2 times/wk		[2] 3 – 4 times/wk	[3] > 4 times/wk
48 Bitten by the dog ticks?	[0] no	[1] yes		[2] do not know	
How often?	[0] < 1 time/wk	[1] 1 – 2 times/wk		[2] 3 – 4 times/wk	[3] > 4 times/wk

49 Receive blood transfusions?	[0] no	[1] yes	When and where did you have it? ……

Clinical signs and symptoms

50 In the last 4 weeks, have you ever had any of these symptoms?

Fever	[0] no	[1] yes	Myalgia	[0] no	[1] yes
Headache	[0] no	[1] yes	Non-productive cough	[0] no	[1] yes
Productive cough	[0] no	[1] yes	Dyspnea	[0] no	[1] yes
Nausea	[0] no	[1] yes	Vomiting	[0] no	[1] yes
Abdominal pain	[0] no	[1] yes	Diarrhea	[0] no	[1] yes
Chest pain	[0] no	[1] yes	Palpitation	[0] no	[1] yes
Fatigue	[0] no	[1] yes	Rash	[0] no	[1] yes
Others, please specify					

The clinical signs from medical record review if the patients visited the hospital or health center							
Splenomegaly	[0] no	[1] yes	[2] unknown	Pneumonia	[0] no	[1] yes	[2] unknown
Jaundice	[0] no	[1] yes	[2] unknown	Liver enlargement	[0] no	[1] yes	[2] unknown

51 If you have fever, when was your first day of fever ...../...../..... (*If fever started **1 weeks** **before the interview**, please refer to the investigator for blood collection for PCR*)

Treatment history

52 If you have any of the symptoms above, did you seek care? If yes, where did you seek care?

[0] No	[1] Yes, at the health center	[2] Yes, at the district hospital	[3] Yes, at other hospital. Please specify……

53 If you seek care at the hospital, were you hospitalized? [0] No [1] Yes

54 Have you ever had any antibiotic in the past 2 weeks? If yes, what is the name of the antibiotics?

[0] No	[1] Yes, doxycycline	[2] Yes, amoxicillin	[3] Yes, cotrimoxazole
[4] Yes, but did not know/remember the name		[5] Yes, but other than 1 – 3, please specify ……	

49 Receive blood transfusions?	[0] no	[1] yes	When and where did you have it? ……

Clinical signs and symptoms

50 In the last 4 weeks, have you ever had any of these symptoms?

Fever	[0] no	[1] yes	Myalgia	[0] no	[1] yes
Headache	[0] no	[1] yes	Non-productive cough	[0] no	[1] yes
Productive cough	[0] no	[1] yes	Dyspnea	[0] no	[1] yes
Nausea	[0] no	[1] yes	Vomiting	[0] no	[1] yes
Abdominal pain	[0] no	[1] yes	Diarrhea	[0] no	[1] yes
Chest pain	[0] no	[1] yes	Palpitation	[0] no	[1] yes
Fatigue	[0] no	[1] yes	Rash	[0] no	[1] yes
Others, please specify					

The clinical signs from medical record review if the patients visited the hospital or health center							
Splenomegaly	[0] no	[1] yes	[2] unknown	Pneumonia	[0] no	[1] yes	[2] unknown
Jaundice	[0] no	[1] yes	[2] unknown	Liver enlargement	[0] no	[1] yes	[2] unknown

51 If you have fever, when was your first day of fever ...../...../..... (*If fever started **1 weeks** **before the interview**, please refer to the investigator for blood collection for PCR*)

Treatment history

52 If you have any of the symptoms above, did you seek care? If yes, where did you seek care?

[0] No	[1] Yes, at the health center	[2] Yes, at the district hospital	[3] Yes, at other hospital. Please specify……

53 If you seek care at the hospital, were you hospitalized? [0] No [1] Yes

54 Have you ever had any antibiotic in the past 2 weeks? If yes, what is the name of the antibiotics?

[0] No	[1] Yes, doxycycline	[2] Yes, amoxicillin	[3] Yes, cotrimoxazole
[4] Yes, but did not know/remember the name		[5] Yes, but other than 1 – 3, please specify ……	

Laboratory results (from medical record review)

55 CBC: Hct..... WBC ..... Neutrophil ..... Lymphocyte .....
Monocyte.....Platelet.....

56 Liver function test (LFT): SGOT ..... SGPT .....
Total bilirubin (TB)..... Direct bilirubin (DB).....

57 Chest X-ray.....

58 Final Diagnosis (check all that applied)

[0] Acute febrile illness [1] Pneumonia [2] Hepatitis
[3] Viral infection [4] Others, please specify.....

.....

Interviewer's name..... Contact number .....

REFERENCES

1. CDC (Centers for Disease Control and Prevention). Emergency Preparedness and Response 2013 [cited 2013 08/10]. Available from: http://www.bt.cdc.gov/agent/agentlist-category.asp.

2. van Asseldonk MA, Prins J, Bergevoet RH. Economic assessment of Q fever in the Netherlands. Preventive veterinary medicine. 2013 Jul 15. PubMed PMID: 23866818.

3. van der Hoek W, Hunink J, Vellema P, Droogers P. Q fever in The Netherlands: the role of local environmental conditions. International journal of environmental health research. 2011 Dec;21(6):441-51. PubMed PMID: 21563011.

4. Parker NR, Barralet JH, Bell AM. Q fever. Lancet. 2006 Feb 25;367(9511):679-88. PubMed PMID: 16503466.

5. Morroy G, Prins J, Bergevoet R, Schneeberger P, Bor HHJ, Hoek Wvd, et al. Of goats and humans; the societal costs of the Dutch Q fever saga. International Congress on Infectious Diseases; Bangkok, Thailand2012. p. Abstract No. 46.042.

6. OIE. Q fever. 2010 [cited 9/1/2013]. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2013 [Internet]. 7. Multiple species, [cited 9/1/2013]. Available from: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.12_Q-FEVER.pdf.

7. Raoult D. Chronic Q fever: expert opinion versus literature analysis and consensus. The Journal of infection. 2012 Aug;65(2):102-8. PubMed PMID: 22537659.

8. Maurin M, Raoult D. Q fever. Clinical microbiology reviews. 1999 Oct;12(4):518-53. PubMed PMID: 10515901. Pubmed Central PMCID: 88923.

9. van der Hoek W, Schneeberger PM, Oomen T, Wegdam-Blans MC, Dijkstra F, Notermans DW, et al. Shifting priorities in the aftermath of a Q fever epidemic in 2007 to 2009 in The Netherlands: from acute to chronic infection. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2012;17(3):20059. PubMed PMID: 22297101.

10. Anderson A, Bijlmer H, Fournier PE, Graves S, Hartzell J, Kersh GJ, et al. Diagnosis and management of Q fever--United States, 2013: recommendations from CDC and the Q Fever Working Group. MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports / Centers for Disease Control. 2013 Mar 29;62(RR-03):1-30. PubMed PMID: 23535757.

11. Ellis RD, Fukuda MM, McDaniel P, Welch K, Nisalak A, Murray CK, et al. Causes of fever in adults on the Thai-Myanmar border. The American journal of tropical medicine and hygiene. 2006 Jan;74(1):108-13. PubMed PMID: 16407353.

12. Suputtamongkol Y, Rolain JM, Losuwanaruk K, Niwatayakul K, Suttinont C, Chierakul W, et al. Q fever in Thailand. Emerging infectious diseases. 2003 Sep;9(9):1186-7. PubMed PMID: 14531384. Pubmed Central PMCID: 3016777.

13. Pachirat O, Fournier P-E, Pussadhamma B, Taksinachanekij S, Lulitanond V, Baggett HC, et al. The first reported cases of Q fever endocarditis in Thailand. Infectious Disease Reports. 2012;4(1):17 - 8. Epub 2012-01-04

14. Suttinont C, Losuwanaluk K, Niwatayakul K, Hoontrakul S, Intaranongpai W, Silpasakorn S, et al. Causes of acute, undifferentiated, febrile illness in rural Thailand: results of a prospective observational study. Annals of tropical medicine and parasitology. 2006 Jun;100(4):363-70. PubMed PMID: 16762116.

15. Yingst SL, Opaschaitat P, Kanitpun R, Thammasart S, Ekgatat M, Jirathanawat V, et al. Q Fever surveillance in ruminants, Thailand, 2012. Emerging infectious diseases. 2013 Dec;19(12):2056-8. PubMed PMID: 24274364. Pubmed Central PMCID: 3840854.

16. Strengthening Survey and Joint Investigation on Tuberculosis and Q fever among Ruminants, Farmers and Zoo Keepers by Cooperation of Livestock, Wildlife and Public Health Sectors in One Health Team, Chiang Mai, 2012. [slides presentation]. In press.

17. van Schaik EJ, Samuel JE. Phylogenetic diversity, virulence and comparative genomics. Advances in experimental medicine and biology. 2012;984:13-38. PubMed PMID: 22711625.

18. Dijkstra F, van der Hoek W, Wijers N, Schimmer B, Rietveld A, Wijkmans CJ, et al. 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. 2012 Feb;64(1):3-12. PubMed PMID: 22066649.

19. Greenslade E, Beasley R, Jennings L, Woodward A, Weinstein P. Has Coxiella burnetii (Q fever) been introduced into New Zealand? Emerging infectious diseases. 2003 Jan;9(1):138-40. PubMed PMID: 12533301. Pubmed Central PMCID: 2873763.

20. Angelakis E, Raoult D. Q Fever. Veterinary microbiology. 2010 Jan 27;140(3-4):297-309. PubMed PMID: 19875249.

21. Kelly P, Angelakis E, Raoult D. Ricketsia and ricketsia-like organisms. In: Cohen J, Powderly WG, Opal SM, editors. Infectious Diseases 3ed. Edinburgh: Mosby/Elsevier; 2012. p. 1813-4.

22. Woldehiwet Z. Q fever (coxiellosis): epidemiology and pathogenesis. Research in veterinary science. 2004 Oct;77(2):93-100. PubMed PMID: 15196898.

23. Kersh GJ, Fitzpatrick KA, Self JS, Priestley RA, Kelly AJ, Lash RR, et al. Presence and persistence of Coxiella burnetii in the environments of goat farms associated with a Q fever outbreak. Applied and environmental microbiology. 2013 Mar;79(5):1697-703. PubMed PMID: 23315737. Pubmed Central PMCID: 3591968.

24. Kersh GJ, Wolfe TM, Fitzpatrick KA, Candee AJ, Oliver LD, Patterson NE, et al. Presence of Coxiella burnetii DNA in the environment of the United States, 2006 to 2008. Applied and environmental microbiology. 2010 Jul;76(13):4469-75. PubMed PMID: 20472727. Pubmed Central PMCID: 2897457.

25. Marmion B. A guide to Q fever and Q fever vaccination: CSL Biotherapies; 2009 [cited 2013 10/4]. Available from: http://meatiesohs.org/files/Q_Fever_booklet.pdf.

26. Rodolakis A. Q Fever in dairy animals. Annals of the New York Academy of Sciences. 2009 May;1166:90-3. PubMed PMID: 19538267.

27. Georgiev M, Afonso A, Neubauer H, Needham H, Thiery R, Rodolakis A, et al. Q fever in humans and farm animals in four European countries, 1982 to 2010. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2013;18(8). PubMed PMID: 23449232.

28. Kovacova E, Kazar J. Q fever--still a query and underestimated infectious disease. Acta virologica. 2002;46(4):193-210. PubMed PMID: 12693856.

29. Thompson HA, Dennis DT, Dasch GA. Q fever. In: Jesse L. Goodman, David T. Dennis, Sonenshine DE, editors. Tick-Borne Diseases of Humans. 1 ed. Washington, D.C: ASM Press,; 2005. p. 328.

30. Raoult D, Marrie T, Mege J. Natural history and pathophysiology of Q fever. The Lancet infectious diseases. 2005 Apr;5(4):219-26. PubMed PMID: 15792739.

31. Oliphant JW, Gordon DA, et al. Q fever in laundry workers, presumably transmitted from contaminated clothing. American journal of hygiene. 1949 Jan;49(1):76-82. PubMed PMID: 18108523.

32. De LP, Lennette EH, Deome KB. Q fever in California; recovery of Coxiella burnetii from naturally-infected air-borne dust. Journal of immunology. 1950 Aug;65(2):211-20. PubMed PMID: 15437000.

33. Milazzo A, Hall R, Storm PA, Harris RJ, Winslow W, Marmion BP. Sexually transmitted Q fever. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2001 Aug 1;33(3):399-402. PubMed PMID: 11438911.

34. Kruszewska D, Lembowicz K, Tylewska-Wierzbanowska S. Possible sexual transmission of Q fever among humans. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1996 Jun;22(6):1087-8. PubMed PMID: 8783716.

35. Madariaga MG, Rezai K, Trenholme GM, Weinstein RA. Q fever: a biological weapon in your backyard. The Lancet infectious diseases. 2003 Nov;3(11):709-21. PubMed PMID: 14592601.

36. Racult D, Stein A. Q fever during pregnancy--a risk for women, fetuses, and obstetricians. The New England journal of medicine. 1994 Feb 3;330(5):371. PubMed PMID: 8277971.

37. Miceli MH, Veryser AK, Anderson AD, Hofinger D, Lee SA, Tancik C. A case of person-to-person transmission of Q fever from an active duty serviceman to his spouse. Vector borne and zoonotic diseases. 2010 Jun;10(5):539-41. PubMed PMID: 20020811.

38. Stein A, Raoult D. Q fever during pregnancy: a public health problem in southern France. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1998 Sep;27(3):592-6. PubMed PMID: 9770161.

39. Hawker JI, Ayres JG, Blair I, Evans MR, Smith DL, Smith EG, et al. A large outbreak of Q fever in the West Midlands: windborne spread into a metropolitan area? Communicable disease and public health / PHLS. 1998 Sep;1(3):180-7. PubMed PMID: 9782633.

40. Tissot-Dupont H, Amadei MA, Nezri M, Raoult D. Wind in November, Q fever in December. Emerging infectious diseases. 2004 Jul;10(7):1264-9. PubMed PMID: 15324547. Pubmed Central PMCID: 3323349.

41. Brouqui P, Badiaga S, Raoult D. Q fever outbreak in homeless shelter. Emerging infectious diseases. 2004 Jul;10(7):1297-9. PubMed PMID: 15324553. Pubmed Central PMCID: 3323311.

42. Fishbein DB, Raoult D. A cluster of Coxiella burnetii infections associated with exposure to vaccinated goats and their unpasteurized dairy products. The American journal of tropical medicine and hygiene. 1992 Jul;47(1):35-40. PubMed PMID: 1636881.

43. Hatchette TF, Hudson RC, Schlech WF, Campbell NA, Hatchette JE, Ratnam S, et al. Goat-associated Q fever: a new disease in Newfoundland. Emerging infectious diseases. 2001 May-Jun;7(3):413-9. PubMed PMID: 11384518. Pubmed Central PMCID: 2631794.

44. Marrie TJ, MacDonald A, Durant H, Yates L, McCormick L. An outbreak of Q fever probably due to contact with a parturient cat. Chest. 1988 Jan;93(1):98-103. PubMed PMID: 3335174.

45. Buhariwalla F, Cann B, Marrie TJ. A dog-related outbreak of Q fever. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1996 Oct;23(4):753-5. PubMed PMID: 8909839.

46. To H, Htwe KK, Kako N, Kim HJ, Yamaguchi T, Fukushi H, et al. Prevalence of Coxiella burnetii infection in dairy cattle with reproductive disorders. The Journal of veterinary medical science / the Japanese Society of Veterinary Science. 1998 Jul;60(7):859-61. PubMed PMID: 9713817.

47. Arricau-Bouvery N, Rodolakis A. Is Q fever an emerging or re-emerging zoonosis? Veterinary research. 2005 May-Jun;36(3):327-49. PubMed PMID: 15845229.

48. Porter SR, Czaplicki G, Mainil J, Guatteo R, Saegerman C. Q Fever: current state of knowledge and perspectives of research of a neglected zoonosis. International journal of microbiology. 2011;2011:248418. PubMed PMID: 22194752. Pubmed Central PMCID: 3238387.

49. van der Hoek W, Dijkstra F, Schimmer B, Schneeberger PM, Vellema P, Wijkmans C, et al. Q fever in the Netherlands: an update on the epidemiology and control measures. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2010 Mar 25;15(12). PubMed PMID: 20350500.

50. Guatteo R, Beaudeau F, Berri M, Rodolakis A, Joly A, Seegers H. Shedding routes of Coxiella burnetii in dairy cows: implications for detection and control. Veterinary research. 2006 Nov-Dec;37(6):827-33. PubMed PMID: 16973121.

51. The Communicable Disease Prevention and Control Unit: Victorian Department of Health. Blue book - Guidelines for the control of infectious diseases Melbourne Victoria2005 [updated 02/10/2007 cited 2014 07/25]. Available from: http://ideas.health.vic.gov.au/bluebook/qfever.asp.

52. Williams JC, Thompson HA. Q fever : the biology of Coxiella burnetii. Boca Raton: CRC Press; 1991. 353 p. p.

53. Maltezou HC, Raoult D. Q fever in children. The Lancet infectious diseases. 2002 Nov;2(11):686-91. PubMed PMID: 12409049.

54. Angelakis E, Million M, D'Amato F, Rouli L, Richet H, Stein A, et al. Q fever and pregnancy: disease, prevention, and strain specificity. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2013 Mar;32(3):361-8. PubMed PMID: 23052984.

55. Carcopino X, Raoult D, Bretelle F, Boubli L, Stein A. Q Fever during pregnancy: a cause of poor fetal and maternal outcome. Annals of the New York Academy of Sciences. 2009 May;1166:79-89. PubMed PMID: 19538266.

56. Jover-Diaz F, Robert-Gates J, Andreu-Gimenez L, Merino-Sanchez J. Q fever during pregnancy: an emerging cause of prematurity and abortion. Infectious diseases in obstetrics and gynecology. 2001;9(1):47-9. PubMed PMID: 11368259. Pubmed Central PMCID: 1784628.

57. Fournier PE, Marrie TJ, Raoult D. Diagnosis of Q fever. Journal of clinical microbiology. 1998 Jul;36(7):1823-34. PubMed PMID: 9650920. Pubmed Central PMCID: 104936.

58. Nourse C, Allworth A, Jones A, Horvath R, McCormack J, Bartlett J, et al. Three cases of Q fever osteomyelitis in children and a review of the literature. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004 Oct 1;39(7):e61-6. PubMed PMID: 15472834.

59. Peter O, Dupuis G, Burgdorfer W, Peacock M. Evaluation of the complement fixation and indirect immunofluorescence tests in the early diagnosis of primary Q fever. European journal of clinical microbiology. 1985 Aug;4(4):394-6. PubMed PMID: 3899636.

60. Meekelenkamp JC, Schneeberger PM, Wever PC, Leenders AC. Comparison of ELISA and indirect immunofluorescent antibody assay detecting Coxiella burnetii IgM phase II for the diagnosis of acute Q fever. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2012 Jun;31(6):1267-70. PubMed PMID: 21997772.

61. Field PR, Mitchell JL, Santiago A, Dickeson DJ, Chan SW, Ho DW, et al. 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. 2000 Apr;38(4):1645-7. PubMed PMID: 10747159. Pubmed Central PMCID: 86512.

62. Fournier PE, Raoult D. Comparison of PCR and serology assays for early diagnosis of acute Q fever. Journal of clinical microbiology. 2003 Nov;41(11):5094-8. PubMed PMID: 14605144. Pubmed Central PMCID: 262519.

63. Schneeberger PM, Hermans MH, van Hannen EJ, Schellekens JJ, Leenders AC, Wever PC. Real-time PCR with serum samples is indispensable for early diagnosis of acute Q fever. Clinical and vaccine immunology : CVI. 2010 Feb;17(2):286-90. PubMed PMID: 20032219. Pubmed Central PMCID: 2815520.

64. CDC (Centers for Disease Control and Prevention). Q fever (*Coxiella burnetii*): 2009 Case Definition [updated 12/7/2012; cited 2013 9/7]. Available from: http://wwwn.cdc.gov/NNDSS/script/casedef.aspx?CondYrID=814&DatePub=1/1/2009%2012:00:00%20AM.

65. Health Protection Agency Centre for Infections. Guidelines for Action in the Event of a Deliberate Release: Q fever UK2010 [updated 14 June 2010; cited 2014 5/17]. 1.7:[Available from: http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1194947387885.

66. Guatteo R, Seegers H, Taurel AF, Joly A, Beaudeau F. Prevalence of Coxiella burnetii infection in domestic ruminants: a critical review. Veterinary microbiology. 2011 Apr 21;149(1-2):1-16. PubMed PMID: 21115308.

67. Williams JC, Thompson HA. Q fever : the biology of Coxiella burnetii. Boca Raton: CRC Press; 1991. 353 p. p.

68. Marrie T, Raoult D. *Coxiella Burnetii* (Q fever) In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 7th ed. Philadelphia, PA: Churchill Livingstone/Elsevier; 2010.

69. Khalili M, Sakhaee E. An update on a serologic survey of Q Fever in domestic animals in Iran. The American journal of tropical medicine and hygiene. 2009 Jun;80(6):1031-2. PubMed PMID: 19478271.

70. Porter SR, Czaplicki G, Mainil J, Horii Y, Misawa N, Saegerman C. Q fever in Japan: an update review. Veterinary microbiology. 2011 May 5;149(3-4):298-306. PubMed PMID: 21146331.

71. McQuiston JH, Childs JE. Q fever in humans and animals in the United States. Vector borne and zoonotic diseases. 2002 Fall;2(3):179-91. PubMed PMID: 12737547.

72. Langley JM, Marrie TJ, Covert A, Waag DM, Williams JC. Poker players' pneumonia. An urban outbreak of Q fever following exposure to a parturient cat. The New England journal of medicine. 1988 Aug 11;319(6):354-6. PubMed PMID: 3393197.

73. Pinsky RL, Fishbein DB, Greene CR, Gensheimer KF. An outbreak of cat-associated Q fever in the United States. The Journal of infectious diseases. 1991 Jul;164(1):202-4. PubMed PMID: 2056206.

74. Paul S, Agger JF, Markussen B, Christoffersen AB, Agerholm JS. Factors associated with Coxiella burnetii antibody positivity in Danish dairy cows. Preventive veterinary medicine. 2012 Nov 1;107(1-2):57-64. PubMed PMID: 22748360.

75. Cantas H, Muwonge A, Sareyyupoglu B, Yardimci H, Skjerve E. Q fever abortions in ruminants and associated on-farm risk factors in northern Cyprus. BMC veterinary research. 2011;7:13. PubMed PMID: 21414196. Pubmed Central PMCID: 3070639.

76. Taurel AF, Guatteo R, Joly A, Seegers H, Beaudeau F. Seroprevalence of Q fever in naturally infected dairy cattle herds. Preventive veterinary medicine. 2011 Aug 1;101(1-2):51-7. PubMed PMID: 21645936.

77. McCaughey C, Murray LJ, McKenna JP, Menzies FD, McCullough SJ, O'Neill HJ, et al. Coxiella burnetii (Q fever) seroprevalence in cattle. Epidemiology and infection. 2010 Jan;138(1):21-7. PubMed PMID: 19480726.

78. Toledo A, Jado I, Olmeda AS, Casado-Nistal MA, Gil H, Escudero R, et al. Detection of Coxiella burnetii in ticks collected from Central Spain. Vector borne and zoonotic diseases. 2009 Oct;9(5):465-8. PubMed PMID: 18945192.

79. Hirai K, To H. Advances in the understanding of Coxiella burnetii infection in Japan. The Journal of veterinary medical science / the Japanese Society of Veterinary Science. 1998 Jul;60(7):781-90. PubMed PMID: 9713803.

80. Astobiza I, Barral M, Ruiz-Fons F, Barandika JF, Gerrikagoitia X, Hurtado A, et al. Molecular investigation of the occurrence of Coxiella burnetii in wildlife and ticks in an endemic area. Veterinary microbiology. 2011 Jan 10;147(1-2):190-4. PubMed PMID: 20580169.

81. Pluta S, Hartelt K, Oehme R, Mackenstedt U, Kimmig P. Prevalence of Coxiella burnetii and Rickettsia spp. in ticks and rodents in southern Germany. Ticks and tick-borne diseases. 2010 Sep;1(3):145-7. PubMed PMID: 21771522.

82. CDC (Centers for Disease Control and Prevention). Q fever: Statistics and Epidemiology 2012 [updated 12/19/2012; cited 2013 10/2]. Available from: http://www.cdc.gov/qfever/stats/index.html.

83. Tissot-Dupont H, Torres S, Nezri M, Raoult D. Hyperendemic focus of Q fever related to sheep and wind. American journal of epidemiology. 1999 Jul 1;150(1):67-74. PubMed PMID: 10400556.

84. Boden K, Brasche S, Straube E, Bischof W. Specific risk factors for contracting Q fever: Lessons from the outbreak Jena. International journal of hygiene and environmental health. 2013 Apr 26. PubMed PMID: 23707055.

85. Wallensten A, Moore P, Webster H, Johnson C, van der Burgt G, Pritchard G, et al. Q fever outbreak in Cheltenham, United Kingdom, in 2007 and the use of dispersion modelling to investigate the possibility of airborne spread. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2010 Mar 25;15(12). PubMed PMID: 20350497.

86. Hackert VH, van der Hoek W, Dukers-Muijrers N, de Bruin A, Al Dahouk S, Neubauer H, et al. Q fever: single-point source outbreak with high attack rates and massive numbers of undetected infections across an entire region. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012 Dec;55(12):1591-9. PubMed PMID: 22918992.

87. Robyn MP, Newman AP, Amato M, Walawander M, Kothe C, Nerone JD, et al. Q Fever Outbreak Among Travelers to Germany Who Received Live Cell Therapy--United States and Canada, 2014. MMWR Morbidity and mortality weekly report. 2015 Oct 2;64(38):1071-3. PubMed PMID: 26421460.

88. Hartzell JD, Wood-Morris RN, Martinez LJ, Trotta RF. Q fever: epidemiology, diagnosis, and treatment. Mayo Clinic proceedings Mayo Clinic. 2008 May;83(5):574-9. PubMed PMID: 18452690.

89. McQuiston JH, Holman RC, McCall CL, Childs JE, Swerdlow DL, Thompson HA. National surveillance and the epidemiology of human Q fever in the United States, 1978-2004. The American journal of tropical medicine and hygiene. 2006 Jul;75(1):36-40. PubMed PMID: 16837706.

90. Leone M, Honstettre A, Lepidi H, Capo C, Bayard F, Raoult D, et al. Effect of sex on Coxiella burnetii infection: protective role of 17beta-estradiol. The Journal of infectious diseases. 2004 Jan 15;189(2):339-45. PubMed PMID: 14722900.

91. Tissot-Dupont H, Vaillant V, Rey S, Raoult D. Role of sex, age, previous valve lesion, and pregnancy in the clinical expression and outcome of Q fever after a large outbreak. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2007 Jan 15;44(2):232-7. PubMed PMID: 17173223.

92. Raoult D, Tissot-Dupont H, Foucault C, Gouvernet J, Fournier PE, Bernit E, et al. Q fever 1985-1998. Clinical and epidemiologic features of 1,383 infections. Medicine. 2000 Mar;79(2):109-23. PubMed PMID: 10771709.

93. Pebody RG, Wall PG, Ryan MJ, Fairley C. Epidemiological features of Coxiella burnetii infection in England and Wales: 1984 to 1994. Communicable disease report CDR review. 1996 Aug 16;6(9):R128-32. PubMed PMID: 8810119.

94. Garner MG, Longbottom HM, Cannon RM, Plant AJ. A review of Q fever in Australia 1991-1994. Australian and New Zealand journal of public health. 1997 Dec;21(7):722-30. PubMed PMID: 9489189.

95. Zhang G, Samuel JE. Vaccines against Coxiella infection. Expert review of vaccines. 2004 Oct;3(5):577-84. PubMed PMID: 15485337.

96. Chiu CK, Durrheim DN. A review of the efficacy of human Q fever vaccine registered in Australia. New South Wales public health bulletin. 2007 Jul-Aug;18(7-8):133-6. PubMed PMID: 17854543.

97. Menzies P, Plummer P. Prevention and Control of Coxiella burnetii Infection among Humans and Animals: Guidance for a Coordinated Public Health and Animal Health Response, 2013. Infection.14:73.

98. Oyston PC, Davies C. Q fever: the neglected biothreat agent. Journal of medical microbiology. 2011 Jan;60(Pt 1):9-21. PubMed PMID: 21030501.

99. Aekajampaka P, Wattanamano N. Basic Information of Thailand. 2012 [cited 9/3/2013]. In: Thailand Health Profile 2008 - 2010 [Internet]. Bangkok, [cited 9/3/2013]; [13 - 8]. Available from: http://www.moph.go.th/ops/thp/thp/index.php?id=36&group_=01&page=view_doc.

100. Season in Thailand: Thai Meteorological Department; 2007 [updated 2007; cited 2013 9/3]. Available from: http://www.tmd.go.th/info/info.php?FileID=53.

101. WHO (World Health Organization). Thailand Country Profile 2013 [updated 2013; cited 2013 9/2]. Available from: www.who.int/countries/tha/en/.

102. Watt G, Pachirat O, Baggett HC, Maloney SA, Lulitanond V, Raoult D, et al. Infective endocarditis in northeastern Thailand. Emerging infectious diseases. 2014 Mar;20(3):473-6. PubMed PMID: 24572588. Pubmed Central PMCID: 3944839.

103. Rodtian P, Nuamjit M, Srijan M, Opaschaitat P, Ekgatat M. Q Fever among Dairy Cattle in Chiang Mai Province, Thailand, 2012: A Preliminary Study. Outbreak Surveillance & Investigation Reports (OSIR) [Internet]. 2013 3/13/14 [cited 2014 1/4]; 6(4):[8-12 pp.]. Available from: http://www.osirjournal.net/archive.php?year=2013.

104. Situation and Trend of Agricultural Products in Thailand, 2013. Office of Agricultural Economics, Extension DoA.

105. Pinero A, Barandika JF, Hurtado A, Garcia-Perez AL. Evaluation of Coxiella burnetii status in dairy cattle herds with bulk-tank milk positive by ELISA and PCR. Transboundary and emerging diseases. 2014 Apr;61(2):163-8. PubMed PMID: 23009342.

106. Kim SG, Kim EH, Lafferty CJ, Dubovi E. Coxiella burnetii in bulk tank milk samples, United States. Emerging infectious diseases. 2005 Apr;11(4):619-21. PubMed PMID: 15829205. Pubmed Central PMCID: 3320343.

107. Rodolakis A, Berri M, Hechard C, Caudron C, Souriau A, Bodier CC, et al. Comparison of Coxiella burnetii shedding in milk of dairy bovine, caprine, and ovine herds. Journal of dairy science. 2007 Dec;90(12):5352-60. PubMed PMID: 18024725.

108. Bunphet S. The number of dairy cattle in Thailand by province in 2012 (in Thai): Information Technology Center, Department of Livestock Development,; [updated 10/15/2012; cited 2013 11/8]. Available from: http://www.dld.go.th/ict/th/.

109. The Fifth Regional Livestock Office. Monthly Report of Dairy Cattle population in the Fifth regional Livestock Office, Thailand (In Thai). In: The Fifth Regional Livestock Office, editor. Chiang Mai2014.

110. Department of Provincial Administration. General information of Mae-onn district, Chiang Mai province: Department of Provincial Administration, Ministry of Interia, Thailand,; [updated 08/23/2012; cited 2014 1/19]. Available from: http://www.amphoe.com/menu.php?mid=1&am=837&pv=13.

111. Department of Provincial Administration. General Information of districts in Thailand (In Thai) Bangkok: Department of Provincial Administration, Ministry of Interia, Thailand; 2010 [updated 04/02/2012; cited 2014 07/25]. Available from: http://www.amphoe.com/menu.php.

112. Dairy Cooperative Quality Assurance in Thailand (article is in Thai). Daily News. 2013 2/9/2013;Sect. Agricultural Issue.

113. Chiangmai Artificial Insemination and Biotechnology Research Center. Number of farms by dairy cooperatives. Chiang Mai, Thailand: Chiangmai Artificial Insemination and Biotechnology Research Center; 2012.

114. Bengston IA. Studies on active and passive immunity in "Q" fever infected and immunized guinea pigs. Washington,: U.S. Govt. print. off.; 1941. 1 p.l., 18 p. p.

115. Kersh GJ, Lambourn DM, Self JS, Akmajian AM, Stanton JB, Baszler TV, et al. Coxiella burnetii infection of a Steller sea lion (Eumetopias jubatus) found in Washington State. Journal of clinical microbiology. 2010 Sep;48(9):3428-31. PubMed PMID: 20592144. Pubmed Central PMCID: 2937693.

116. Hubbard AE, Ahern J, Fleischer NL, Van der Laan M, Lippman SA, Jewell N, et al. To GEE or not to GEE: comparing population average and mixed models for estimating the associations between neighborhood risk factors and health. Epidemiology. 2010 Jul;21(4):467-74. PubMed PMID: 20220526.

117. Farid M, Frerichs RR. Csurvey. 2 ed. Department of Epidemiology, University of California, Los Angeles,2008.

118. Rothman K. Episheet: spreadsheets for the analysis of epidemiologic data. 2002.

119. van Asseldonk MA, Prins J, Bergevoet RH. Economic assessment of Q fever in the Netherlands. Preventive veterinary medicine. 2013 Oct 1;112(1-2):27-34. PubMed PMID: 23866818.

120. Sangkasuwan V, Pongpradit P, Bodhidatta P. SEATO Medical research study on rickettsial diseases in Thailand. US Army-SEATO Medical Research Unit, Bangkok, Thailand, 1967.

121. Kersh GJ, Fitzpatrick KA, Self JS, Biggerstaff BJ, Massung RF. Long-Term immune responses to Coxiella burnetii after vaccination. Clinical and vaccine immunology : CVI. 2013 Feb;20(2):129-33. PubMed PMID: 23192629. Pubmed Central PMCID: 3571276.

122. Rothman KJ, Greenland S, Lash TL. Modern epidemiology. 3rd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008. x, 758 p. p.

123. Murray PR, Baron EJ. Manual of clinical microbiology. 9th ed. Washington, D.C.: ASM Press; 2007.

124. Duron O, Sidi-Boumedine K, Rousset E, Moutailler S, Jourdain E. The Importance of Ticks in Q Fever Transmission: What Has (and Has Not) Been Demonstrated? Trends in parasitology. 2015 Nov;31(11):536-52. PubMed PMID: 26458781.

125. Schimmer B, Schotten N, van Engelen E, Hautvast JL, Schneeberger PM, van Duijnhoven YT. Coxiella burnetii seroprevalence and risk for humans on dairy cattle farms, the Netherlands, 2010-2011. Emerging infectious diseases. 2014 Mar;20(3):417-25. PubMed PMID: 24572637. Pubmed Central PMCID: 3944848.

126. Sun WW, Cong W, Li MH, Wang CF, Shan XF, Qian AD. Coxiella burnetii Seroprevalence and Risk Factors in Cattle Farmers and Farm Residents in Three Northeastern Provinces and Inner Mongolia Autonomous Region, China. BioMed research international. 2016;2016:7059196. PubMed PMID: 26966688. Pubmed Central PMCID: 4757716.

127. van Engelen E, Schotten N, Schimmer B, Hautvast JL, van Schaik G, van Duijnhoven YT. Prevalence and risk factors for Coxiella burnetii (Q fever) in Dutch dairy cattle herds based on bulk tank milk testing. Preventive veterinary medicine. 2014 Nov 1;117(1):103-9. PubMed PMID: 25239684.

128. Astobiza I, Ruiz-Fons F, Pinero A, Barandika JF, Hurtado A, Garcia-Perez AL. Estimation of Coxiella burnetii prevalence in dairy cattle in intensive systems by serological and molecular analyses of bulk-tank milk samples. Journal of dairy science. 2012 Apr;95(4):1632-8. PubMed PMID: 22459811.

129. Khalili M, Sakhaee E, Aflatoonian MR, Shahabi-Nejad N. Herd-prevalence of Coxiella burnetii (Q fever) antibodies in dairy cattle farms based on bulk tank milk analysis. Asian Pacific journal of tropical medicine. 2011 Jan;4(1):58-60. PubMed PMID: 21771417.

130. van der Hoek W, Meekelenkamp JC, Dijkstra F, Notermans DW, Bom B, Vellema P, et al. Proximity to goat farms and Coxiella burnetii seroprevalence among pregnant women. Emerging infectious diseases. 2011 Dec;17(12):2360-3. PubMed PMID: 22172140. Pubmed Central PMCID: 3311170.

131. Muskens J, van Engelen E, van Maanen C, Bartels C, Lam TJ. Prevalence of Coxiella burnetii infection in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA. The Veterinary record. 2011 Jan 22;168(3):79. PubMed PMID: 21257587.

132. Ryan ED, Kirby M, Collins DM, Sayers R, Mee JF, Clegg T. Prevalence of Coxiella burnetii (Q fever) antibodies in bovine serum and bulk-milk samples. Epidemiology and infection. 2011 Sep;139(9):1413-7. PubMed PMID: 21073765.

133. Alvarez J, Perez A, Mardones FO, Perez-Sancho M, Garcia-Seco T, Pages E, et al. Epidemiological factors associated with the exposure of cattle to Coxiella burnetii in the Madrid region of Spain. Veterinary journal. 2012 Oct;194(1):102-7. PubMed PMID: 22534189.

134. Anderson A, Boyer T, Garvey A, Marshall K, Menzies P, Murphy J, et al. Prevention and Control of Coxiella burnetii Infection among Humans and Animals: Guidance for a Coordinated Public Health and Animal Health Response, 2013 2013 08/01/2016 08/01/2016]. Available from: http://www.nasphv.org/Documents/Q_Fever_2013.pdf.

135. Chang CC, Lin PS, Hou MY, Lin CC, Hung MN, Wu TM, et al. Identification of risk factors of Coxiella burnetii (Q fever) infection in veterinary-associated populations in southern Taiwan. Zoonoses and public health. 2010 Dec;57(7-8):e95-101. PubMed PMID: 19968850.

136. Guatteo R, Beaudeau F, Joly A, Seegers H. Performances of an ELISA applied to serum and milk for the detection of antibodies to Coxiella burnetii in dairy cattle. Revue Med Vet. 2007;5:250 - 2.

137. Gidding HF, Wallace C, Lawrence GL, McIntyre PB. Australia's national Q fever vaccination program. Vaccine. 2009 Mar 23;27(14):2037-41. PubMed PMID: 19428827.

138. Bosnjak E, Hvass AM, Villumsen S, Nielsen H. Emerging evidence for Q fever in humans in Denmark: role of contact with dairy cattle. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2010 Aug;16(8):1285-8. PubMed PMID: 19832723.