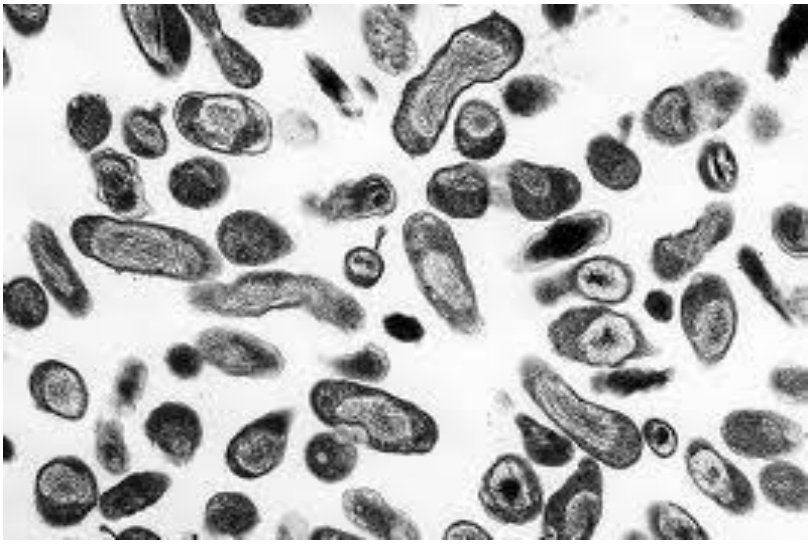
FACULTY OF HEALTH AND MEDICAL SCIENCES
UNIVERSITY OF COPENHAGEN
DEPARTMENT OF LARGE ANIMAL SCIENCES

Omar Faruk Miazi

Master's Thesis (Veterinary epidemiology)

Coxiella burnetii antibodies in Danish dairy cattle: Prevalence, incidence, recovery, and association of dam status with calf status and calf mortality when accounting for diagnostic test quality



Coxiella burnetii; Source: Rocky Mountain Laboratories

Principal advisor: Jens Frederik Gramstrup Agger

Co-advisors: Bo Markussen and Jørgen Steen Agerholm

Omar Faruk Miazi
Student ID-VLR 576

Submitted 28 September 2012


DEDICATED
TO
MY MOTHER

Table of Contents

Acknowledgements

At first I remember my almighty Allah blessing me to carry out this work smoothly. I am utmost delightful to Jens Frederik Gramstrup Agger, Associate Professor, Department of Large Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, for critical guidance during the project period. I would also like to express my honest and sincere gratitude to Bo Markussen, Anna-BodilChristoffersen and Jørgen Steen Agerholm for privileging to have support in the manuscripts and in data analysis.

I would like to thank all members of the Epi-group at Gronnegårdsvej 8. Special thanks to Jeanne Talchow Oakman for her instrumental support and in official things with a smile. I extend my gratitude to Syed Sayeem Uddin Ahmed and SharminChowdhury for many sorts of help offered during the project tenure. Special thanks and my deepest sense of gratitude to Suman Paul for encouraging and conducting this research work with valuable suggestions.

The study was funded by Kvaegafgiftsfonden (grant number) and the Faculty of Life Sciences, University of Copenhagen as part of a PhD project. The author gratefully acknowledgesthe farmers for participating in the study and the technical staff at the National Veterinary Institute, Technical University of Denmark for analysing the samples. I also acknowledge Jørgen Nielsen who supplied our target data form the Danish Cattle Database.

At the end, I would like to thank my wife and coming son, who tolerated missed schedules and many others, demands of this project. I am also grateful to my family members and my friends for giving me continuous support in several ways during research work and preparation of this thesis successfully.

Omar Faruk Miazi
September, 2012

SUMMERY

Coxiella burnetii is an obligate intracellular zoonotic bacterium and domesticated ruminants including dairy cattle are considered the main reservoir for human exposure. *C. burnetii* was regarded as an economically insignificant pathogen for domestic livestock, but the recent outbreak of Q fever in the Netherlands have shown that Q fever infection in animals may have extensive economic implications. Q fever infection in cattle is usually subclinical although sporadic abortions occur. The almost worldwide occurrence of *C. burnetii* has been documented by serological studies. The infection is generally subclinical in animals (e.g. cattle, sheep and goat), although abortions in late pregnancy, stillbirths and the delivery of weak offspring, retained placenta, endometritis, infertility and low birth rates may occur.Until recently, Q fever was notified in very low numbers annually in Denmark. But during recent years in Denmark higher rates of antibodies to *Coxiella burnetii* have been detected in animals and humans than previously reported. So we did two studies with the aim of the first work was to 1) study the relationship between levels of *C. burnetii* antibodies in offspring and their dam, 2) to estimate the prevalence, incidence and recovery of *C. burnetii* antibody positivity under the assumption of perfect and imperfect tests, 3) to estimate the sensitivity and specificity of the CHEKIT Q-Fever Antibody ELISA TEST Kit, and 4) to estimate associations of age groups, herd status and breed with prevalence, incidence and recovery in Danish dairy cattle. The objective of the second study was to evaluate the relationship of antibody status of *C. burnetii* in Danish dairy cows with calf death, delivery condition of cow and birth size of calf.

The thesis consists of four chapters:

Chapter 1 presents a brief introduction on the history and general bacteriology of the *Coxiella burnetii;* a brief overview of the dairy sector in Denmark and the situation of *Coxiella burnetii* in Denmark; and background and objective of this thesis.

Chapter 2 estimates prevalence, incidence, recovery and relations between dam and offspring when accounting for diagnostic test quality for *Coxiella burnetii* antibody levels in Danish dairy cattle. Prevalence and incidence of seropositive animals were medium in young calves, low in older calves and heifers and again high in cows. Recovery was higher in young animals than in cows. Antibodies in offspring blood and dam milk showed positive relationship: strongest early in life and ceased with increasing age of offspring. Sensitivity and specificity of ELISA test was standard enough for a good test. The cut-off value S/P ≥68% was specified for differentiation between infected and non-infected animals in Hidden Markov Model. Prevalence and incidence were high in calf for taking colostrum but in young and heifer the antibody disappears due to develop resistance system in this animals. But in cows the prevalence and incidence increases again for continuous exposed by organisms from environment and contacts with infected animals more. Recovery was high in young and heifer because of body immune system is start working in this time which is a normal biological phenomenon. Antibodies in offspring blood and dam milk showed positive relationship and it strongest in young calf and low in old calf. This indicates that calves have short lasting colostral antibodies and later decreasing in nature and finally disappears.

In chapter 3, for calf deatha total of 3974 milk samples from 2103 dams from the selected herds were then collected in three different time periods: August-October 2008 (Time 1), January-February 2009 (Time 2) and April-June 2009 (Time 3). The general results provide relationship of *Coxiella burnetii* antibody status of dam with calf death in individual level of Danish dairy cattle. There was no significant association betweencalf deaths, delivery condition or birth size with parity and breed but a significant association was found betweendelivery condition andherd condition. No significant association was found between antibody status of the dam and calf death, birth size and delivery condition. A significant random effect of herd in different models was observed. It is concluded that dam antibody status of *C. burnetii* appears to be non-related with calf deaths,

delivery condition and birth size. These results indicate that, in Denmark, *C. burnetii* antibody level is not an important factor for calf deaths and these calf deaths are related with other herd level variables.

Chapter 4 synthesizes the knowledge derived from the previous chapters and discusses their practical relevance to the estimation of general frequencies of *Coxiella burnetii* antibody in Danish dairy cattle and association between *Coxiella burnetii* antibody of dam and their offspring. This thesis suggests that the inference from prevalence, incidence and recovery as well as association between calf death and dam antibody status in Denmark. These results could be useful for the making decision for control and prevention of *Coxiella burnetii* as well as reproductive disorder management in Denmark.

CHAPTER 1
GENERAL INTRODUCTION

SYNOPSIS

This chapter provides a brief introduction on the history and general bacteriology of the *Coxiella burnetii* bacteria; the global scenario of *Coxiella burnetii* prevalence; abrief overview of the prevalence in Denmark mainly in dairy industry. The chapter also presents the background and the objective of this thesis.

GENERAL INTRODUCTION

1. ANOVERVIEWOF*COXIELLA BURNETII* BACTERIUM

1.1. History

Coxiella burnetii is an obligate intracellular bacterium that is causal agent for widespread zoonotic disease Q fever (Arricau-Bouvery and Rodolakis, 2005; Baca and Paretsky, 1983; Behymer and Riemann, 1989; Kazar, 2005). Q fever as a disease affecting slaughterhouse workers was first observed in Australia in 1933 (Derrick, 1937). Frank Macfarlane Burnet who, along with Mavis Freeman, reproduced the characteristic febrile reaction in guinea pigs from Derrick submitted infected guinea pig tissue (Burnet and Freeman, 1937). In Hamilton, Montana, USA, an unknown agent was discovered nearly simultaneously as part of field study on the ecology of Rocky Mountain spotted fever.*Dermacentorandersoni*ticks collected in Nine Mile, Montana, were fed on guinea pigs, one of which developed a febrile illness that did not mimic Rocky Mountain spotted fever in presentation (Davis and Cox, 1938). According to Davis and Cox (1938), the agent had both bacterial and viral characteristics. Link between the Nine Mile and Q fever agents was discovered serendipitously due to a laboratory-acquired infection by the Nine Mile agent. Subsequent cross protection studies confirmed that the Q fever and Nine Mile agents were very likely the same pathogen (Dyer, 1939), and the patient displayed signs and symptoms strikingly similar to Q fever (Dyer, 1938). The organism did not behave exactly like a typical rickettsia, the agent was placed in a new genus in the family *Rickettsiaceae*, the genus "*Coxiella*" and species " *burnetii* " in honor of Cox and Burnet, respectively. Until 1948 that the classification and nomenclature of the Q fever agent was finalized and Cornelius B. Philip of RML proposed that the current status of Coxiella (at the time being a subgenus of *Rickettsia*) be elevated to full genus status and that the name be changed from *Rickettsia burneti*to *Coxiella burnetii* (Philip, 1948).The

discovery and past milestones of early history of *C. burnetii* are described in detail by McDade (1990).

1.2 The Bacterium

C. burnetii is of the Kingdom: Bacteria, Phylum: Proteobacteria, Class: γ-Proteobacteria, Order: Legionellales, and Family: Coxiellaceae taxonomically. Thisis an obligate intracellular, Gram-negative rod that exhibits pleomorphic characteristics having a distinct poly-phasic life cycle generalized to correspond to stages inside and outside of the host cell (Varghees et al., 2002).

C. burnetii have been documented morphologically distinct forms of in the literature from as early as 1959 when B. Babudieri reported his light microscopy observations of "a very short rod, frequently with a bipolar appearance and sometimes as a minute paired coccus" (Babudieri, 1959). The true status of *C. burnetii* was debated until 1981 when transmission electron microscopy (TEM) showed *C. burnetii* as having distinct forms termed large cell variants (LCV), small cell variants (SCV), and spore-like particles (SLP). The approximate sizesfor these forms are ~1μm for the LCV with the SCV ranging from 0.2 – 0.5μm and the 16SLP varying from 0.13 – 0.17μm (McCaul and Williams, 1981; McCaul, T.F., 1991; Heinzen et al., 1999). The organism is thought to be an extracellular survival form with enhanced resistance to environmental stressors such as desiccation and heat due to its small cell variant, with its characteristic condensed chromatin (Waag, 2007).

1.3 Phase Variation

The genetic diversity of *C. burnetii* is further evidenced by the presence of antigenicallyand structurally unique LPS molecules. Some distinct LPS chemotypes havebeen described that are associated with specific genomic groups (Toman et al., 2009). A potential link between LPS chemotype and virulence potential has been proposed (Hackstadt, 1990;Skultety et al., 1998). An

LPS phase variation occurs in the laboratory. Virulent *C. burnetii* isolated from natural sources and infections all produce a full-length LPS that is serologically defined as "phase I" or the smooth variant, which is virulent to its host. Serial in vitropassage of phase I *C. burnetii* in embryonated eggs or tissue culture results in LPS molecules with decreasing molecular weights and distinct constituent sugar compositions. This culminates in the truncated LPS of avirulent "phase II" organisms or the rough type. Phase II LPS contains a lipid, identical to that of phase I LPS, and has some core sugars. However, at present, the genetic lesion(s) leading to the severely truncated LPS of phase II organisms is unknown.

1.4 Coxiellaburnetii: Epidemiology

Vary widely reservoir's*C. burnetii* has been demonstrated to infect livestock and wildlife (Enright, 1971; Gardon, 2001; Marrie, 1989).The seroprevalence among goat, sheep, and cattle, are 41.6%, 16.5%, and 3.4% respectively, in the United States(McQuiston et al., 2002).A high prevalence of *C. burnetii* exposure has been reported in humans and domesticated animals in several European countries in recent years (Gilsdorf et al., 2008; McCaughey et al., 2010; Guatteo et al., 2011). In Denmark, an increasing number of Q fever cases have been reported in humans (Villumsen et al., 2009). And Agger et al. (2010) reported infection with *C. burnetii* may be considered an increasing problem in Denmark. It has been reported as an endemic infection throughout the world except for New Zealand (Maurin and Raoult, 1999).Sheep are the most commonly reported source for human infection (McQuiston, et al., 2002). Chronically infected animals shed bacteria in milk and urine (Berri and Rodolakis, 2000; Berriet al.,2002; Winn et al., 1953). Though it does not commonly present itself as an overt disease in goats, sheep, and cattle, *C. burnetii* has a tropism for placental/birthing tissues and causes many spontaneous abortions (Waldhalm et al., 1978; Palmer et al., 983). Domesticated animals present a means by which the animal and human worlds conjoin for

C. burnetii infections. In summary, *C. burnetii* agent are plentiful in both natural and domestic environments for potential zoonotic transmission.

1.5 Coxiella burnetii: Global distribution

C. burnetii has been firmly established in globally (Babudieri, 1959) with the notable exception of New Zealand (Hilbink et al., 1993;Kazar J., 2005;Maurin, and Raoult, 1999). Norway remained Q fever free until 1997 when four Norwegian tourists returned from travel to Bhutan, the Canary Islands, and Morocco. Upon reentry to Norway, each presented with acute Q fever (Zvizdic et al., 2002). It is likely only a matter of time until the first case of Q fever will be found in New Zealand on base of ease and speed of modern travel. All major areas of the globe will have confirmed *C. burnetii* or Q fever cases, when this occurs.

1.6 Coxiella burnetii: Transmission

Primary mode of human acquired Q fever is aerosol transmission of *Coxiella burnetii*. *C. burnetii* aerosols are typically generated as contaminated dust from soils becomes disturbed and airborne (Gardon et al., 2001; Zvizdic et al., 2002). Fomite *C. burnetii* aerosols are generated in zoological associated activities such as animal processing at abattoirs (Carrieri et al., 2002; Riemann et al., 1975) and the birthing of chronically infected livestock which typically exposes veterinarians (Noah and Crowder, 2002; Macellaro et al., 993) and animal handlers (McQuiston et al., 2002) to this organism . Actively growing *C. burnetii* in laboratory research settings presents another potential source for aerosolized agent. Ingestion of *C. burnetii* contaminated materials is a second mode of bacterial transmission, though in relation to aerosols this is a far less common. The shedding of *C.burnetii* in milk(Enright et al., 1957; Biberstein, et al., 1974; McQuiston et al., 2003),has led to cases of Q fever (Fishbein and Raoult, 1992). *C. burnetii* shed via urine and fecal material (Maurin and Raoult, 1999) and runoff under unsanitary conditions can present a potential fecal-oral

infectious route from contaminated water stores. The transmission of *C. burnetii* via fecal-oral and aerosol mechanisms between livestock is likely considering the relative proximity of living conditions in feedlots, stockyards, and barns. It has been speculated that *C. burnetii* infection via ingestion may present a primary mode for hepatic Q fever (Maurin and Raoult, 1999; Fishbein and Raoult, 1992).Historically it was thought that *C. burnetii* cycled in ticks via transovarial and transstadial transmission (Pandurov and Zaprianov, 1975; Walker and Fishbein, 1991)prior to being vectored into humans or animals (Walker and Fishbein, 1991).Venereal transmission of *C. burnetii* has been demonstrated in animal models (Kruszewska and Tylewska-Wierzbanowska, 1997). Cutaneous transmission of *C. burnetii* was suggested in a 1993 report (Duvalet al., 1993). Above informationgive the possible route of *C.burnetii* transmission; however, it remains an extremely unlikely route for obtaining Q fever.

2. OVERALL BACKGROUND OF THE PROJECT

The bacterium *Coxiella burnetii* (*C. burnetii*), was detected as the causative organism of Q fever. Q fever was first described in 1935 as an outbreak of febrile illness among abattoir workers in Brisbane, Australia. *C. burnetii* was first identified in 1937 in Australia (Derrick, 1937).

Thehe causative agent of Q fever, *C. burnetii* is an obligate, intracellular, pleomorphic bacterium (Maurin and Raoult, 1999; Arricau-Bouvery and Rodolakis, 2005). Since first identification *C. burnetii* has been reported as an endemic infection throughout the world except for New Zealand (Maurin and Raoult, 1999).Domestic ruminants including dairy cattle are considered the main reservoir for human exposure (Arricau-Bouvery and Rodolakis, 2005; Cutler et al., 2007; Kim et al., 2005). Infection in dairy cows mostly remains clinically unrecognized (Rodolakis, 2009) and has a long persistence (Lang, 1990) with shed large amounts of bacteria via birth fluids and placenta during parturition (Rodolakis et al., 2007). Dairy cattle with infection may shed bacteria via milk for a longer period (Rodolakis et al., 2007). The risk of *C. burnetii* infection in ruminants varies

with individual animal traits such as age and parity, breed, gender, level of milk production and lactation stage (McCaughey et al., 2010; Garcia-Ispierto et al., 2011).

C. burnetii infection is generally subclinical in animals (e.g. cattle, sheep and goat), although abortions in late pregnancy, stillbirths and the delivery of weak offspring, retained placenta, endometritis, infertility and low birth rates may occur (Rodolakis, 2006; Arricau-Bouvery and Rodolakis, 2005; McQuiston et al., 2002; Sanford et al., 1994). *C. burnetii* is a cause of sporadic abortion in cattle (Jensen et al., 2007; Rady et al., 1985).In dairy cattle, *C. burnetii,* and antibody seropositivity is related with reproductive problems (Khalili et al., 2011).

Higher prevalence of C. burnetii expo-sure has been reported in humans and domesticated animals in several European countries in recent years (Gilsdorf et al., 2008; McCaughey et al., 2010; Guatteo et al., 2011). In Denmark, an increasing number of Q fever cases has been reported in humans (Villumsen et al., 2009), and Agger et al. (2010) reported a high seroprevalence (59%) in BTM samples from 100 randomly selected Danish dairy cattle herds. This means during recent years in Denmark higher rates of antibodies to *C. burnetii* have been detected in animals and humans than previously reported.

Considering above discussion about *C. burnetii* control must be priority in Denmark. For the purpose of effective control and containment of the disease, Denmark needs to know about epidemic behavior, spread and persistence of *C. burnetii* in Denmark. However, lack of epidemiological knowledge on *C. burnetii* in Denmark is hampering the control programme. The endeavor of the Master's project is to address the knowledge gaps. Epidemiological knowledge generated from the basic as well as advanced analysis of data might help in planning of the control, spread and associations' status. In addition, the outcome of this thesis may form the basis for designing surveillance for C. *burnetii* in Denmark.

3. OBJECTIVES

During recent years in Denmark higher rates of antibodies to *C. burnetii* have been detected in animals and humans than previously reported.*Coxiella burnetii* is a well-known cause of placentitis and subsequent abortion in ruminants.

But there are no reports on prevalence, incidence and recovery in individual level of dairy cattle as well as the relationship with calf death and dam antibody status.Thus the aim of this work was to A.1) study the relationship between levels of *C. burnetii* antibodies in offspring and their dam, 2) to estimate the prevalence, incidence and recovery of *C. burnetii* antibody positivity under the assumption of perfect and imperfect tests, 3) to estimate the sensitivity and specificity of the CHEKIT Q-Fever Antibody ELISA TEST Kit, and 4) to estimate associations of age groups, herd status and breed with prevalence, incidence and recovery in Danish dairy cattle. B.To evaluate the relationship of antibody status of *C. burnetii* in Danish dairy cows with calf death, delivery condition of cow and birth size of calf.

4.REFRENCES

Agger JF, Christoffersen AB, Rattenborg E, Nielsen J, Agerholm JS: 2010. Prevalence of Coxiella burnetii antibodies in Danish dairy herds. Acta Veterinaria Scandinavica. 2010, 52, 1-5.

Angelakis E, Raoult D: Q fever. Vet. Microbiol. 2010,140, 297–309.

Arricau-Bouvery N, Rodolakis A: Is Q fever an emerging or re-emerging zoonosis? Vet. Res. 2005, 36, 327–350.

Babudieri B:*Q fever: a zoonosis.* Advances in Veterinary Science. 1959, 5, 81-181.

Baca OG, Paretsky D: Q fever and *Coxiella burnetii*: a model for host-parasite interactions,Microbiol. Rev. 1983, 47, 127–49.

Behymer D, Riemann H:*Coxiella burnetii* infection (Q fever), J. Am. Vet. Med. Assoc.1989,194, 764–767.

Berri M, et al.:*Shedding of Coxiella burnetii in ewes in two pregnancies following an episode of Coxiella abortion in a sheep flock.* Vet.Microbiol. 2002, 85(1), 55-60.

Berri M, Laroucau K, Rodolakis A:*The detection of Coxiella burnetii from ovine genital swabs, milk and fecal samples by the use of a single touchdown polymerase chain reaction.* Vet.Microbiol. 2000, 72(3-4), 285-93.

Biberstein EL, et al.:*A survey of Q fever (Coxiella burnetii) in California dairy cows.* Am. J. Vet .Res. 1974, 35(12) 1577-82.

Burnet FM, Freeman M (1937) Experimental studies on the virus of "Q" fever. Med. J. Aust. 1937, 2, 299–305.

Carrieri MP, et al.:*Investigation of a slaughterhouse-related outbreak of Q fever in the French Alps.* Eur. J.Clin.Microbiol. Infect. Dis. 2002, 21(1), 17-21.

Cutler SJ, Bouzid M, Cutler RR:Q fever. J. Infect. 2007, 54, 313-318.

Davis GE, Cox HR: A filter-passing infectious agent isolated from ticks. I. Isolation from *Dermacentorandersonii*, reactions with animals, and filtration experiments. Public. Health. Rep. 1938, 53, 2259–2276.

Derrick EH: "Q" fever, a new fever entity: clinical features, diagno-sis and laboratory investigation. Med. J. Aust. 1937, 2, 281–299.

Duval X, et al.:*Q fever with cutaneous and encephalitic involvement.* Lancet. 1993, 341(8852), 1094-1095.

Dyer RE: (1938) A filter-passing infectious agent isolated from ticks. IV. Human infection. Public. Health. Rep. 1938, 53, 2277–2282.

Dyer RE: Similarity of Austrailian “Q” fever and a disease cause by an infectious agent isolated from ticks in Montana. Public. Health. Rep. 1939, 54, 1229–1237.

Enright JB, et al.:*Coxiella burneti in a wildlife-livestock environment. Antibody response of ewes and lambs in an endemic Q fever area.* Am. J.Epidemiol. 1971, 94(1), 62-71.

Enright JB, Sadler WW, Thomas RC:*Pasteurization of milk containing the organism of Q fever.* Am. J. Public. Health. 1957, 47(6), 695-700.

Fishbein DB,Raoult D:*A cluster of Coxiella burnetii infections associated with exposure to vaccinated goats and their unpasteurized dairy products.* Am. J.Trop. Med.Hyg. 1992, 47(1), 35-40.

Garcia-Ispierto I, Almeria S, Lopez-Gatius F: Coxiella bur-netii seropositivity is highly stable throughout gestation in lactating high-producing dairy cows. Reprod. Domest. Anim. 2011, http://dx.doi.org/10.1111/j.1439-0531.2011.01788.x.

Gardon J, et al.:*Suburban transmission of Q fever in French Guiana: evidence of a wild reservoir.* J. Infect.Dis. 2001, 184(3), 278-284.

Gilsdorf A, Kroh C, Grimm S, Jensen E, Wagner-Wiening C, Alpers K: Large Q fever outbreak due to sheep farming near residential areas, Germany, 2005. Epidemiol. Infect., 2008, 136, 1084–1087.

Guatteo R, Beaudeau F, Berri M, Rodolakis A, Joly A, Seegers H: 2011. Shedding routes of Coxiella burnetii in dairy cows: implications for detection and control. Vet. Res. 2011, 37, 827–833.

Hackstadt T: (1990) The role of lipopolysaccharides in the virulence of *Coxiella burnetii* . Ann. NY Acad. Sci.1990, 590, 27–32.

Heinzen RA, Hackstadt T, Samuel JE:*Developmental biology of Coxiella burnettii.* Trends Microbiol. 1999, 7(4), 149-54.

Hilbink F, et al.:*Q fever is absent from New Zealand.* Int. J.Epidemiol. 1993, 9(2), 213-216.

Jensen TK, Montgomery DL, Jaeger PT, Lindhardt T, Agerholm JS, Bille- Hansen V, Boye M:Application of fluorescent in situ hybridisation for demonstration of Coxiella burnetii in placentas from ruminant abortions. APMIS. 2007, 115, 347-353.

Kazar J:*Coxiella burnetii* Infection, Ann. NY Acad. Sci. 2005, 1063,105–114.

Khalili M, Sakhaee E, Babaei H: Frequency of anti-*Coxiella burnetii* in cattle with reproductive disorders. Comp Clin Pathol. 2011, 1-3:DOI:10.1007/s00580-011-1199-2.

Kim SG, Kim EH, Lafferty CJ, Dubovi E:Coxiella burnetii in bulk tank milk samples, United States. Emerg. Infect. Dis. 2005, 11, 619-621.

Kruszewska D,Tylewska-Wierzbanowska S:*Isolation of Coxiella burnetii from bull semen.* Res. Vet. Sci. 1997, 62(3), 299-300.

Lang GH: Coxiellosis (Q fever) in animals. T.J. Marrie (Ed.), Q Fever. The Disease, CRC Press, Boca. Raton. 1990, pp. 23–48.

Macellaro A, Akesson A, Norlander L:*A survey of Q-fever in Sweden.* Eur. J.Epidemiol. 1993, 9(2), 213-216.

Marrie TJ, et al.:*Truckin' pneumonia--an outbreak of Q fever in a truck repair plant probably due to aerosols from clothing contaminated by contact with newborn kittens.* Epidemiol. Infect. 1989,102(1), 119-27.

Maurin M, Raoult D:*Q fever.* Clin.Microbiol. Rev. 1999, 12(4), 518-553.

McCaughey C, Murray LJ, McKenna JP, Menzies FD, McCullough SJ, O'Neill HJ, Wyatt DE, Cardwell CR, Coyle PV:Coxiella burnetii (Q fever) seroprevalence in cattle. Epidemiology and Infection. 2010, 138, 21-27.

McCaul TF, Williams JC:*Developmental cycle of Coxiella burnetii: structure and morphogenesis of vegetative and sporogenic differentiations.* J.Bacteriol. 1981, 147(3), 1063-1076.

McCaul, TF:*The developmental cycle of Coxiella burnetii*, in *Q Fever: The Biology of Coxiella burnetii*, J.C. Williams and H.A. Thompson, Editors. CRC Press: Boca. Raton. Fl. 1991, pp. 223-258.

Mcdade, J.E., 1990. Historical aspects of Q fever. In: Marrie TJ (ed) Q fever, vol 1, The disease. CRC Press, Boca Raton.

McQuiston JH, Childs JE:*Q fever in humans and animals in the United States.* Vector Borne Zoonotic. Dis. 2002,2(3), 179-191.

McQuiston JH, Childs JE, Thompson HA: Q fever. Journal of the American Veterinary Medical Association. 2002, 221, 796-799.

McQuiston JH, et al.:*Prevalence of antibodies to Coxiella burnetii among veterinary school dairy herds in the United States, 2003.* Vector Borne ZoonoticDis. 2005, 5(1), 90-91.

Noah, DL, Crowder HR:*Biological terrorism against animals and humans: a brief review and primer for action.* J. Am. Vet. Med. Assoc. 2002, 221(1), 40-43.

Palmer NC, Kierstaed M, Key WD:*Placentitis and abortion in goats and sheep in Ontario caused by Coxiella burnetii.* Can. Vet. J. 198324, 60-63.

PandurovS,Zaprianov M:*[Studies on the retention of R. burneti in Rh. bursa and H.detritum ticks]*. Vet. Med.Nauki. 1975, 12(4), 43-48.

Philip CB:*Comments of the name of the Q fever organism.* Public Health Rep. 1948, 63, 58.

Rady, M., Glavits, R., Nagy, G., 1985. Demonstration in Hungary of Q-Fever Associated with Abortions in Cattle and Sheep. Acta Veterinaria Hungarica 33, 169-176.

Riemann HP, et al.:*Toxoplasma gondii and Coxiella burneti antibodies among Brazilian slaughterhouse employees.* Am. J.Epidemiol. 1975, 102(5), 386-393.

Rodolakis A: Q fever, state of art: Epidemiology, diagnosis and prophylaxis. Small Ruminant Research- 2006, 62, 121-124.

Rodolakis A: Q fever in dairy animals. Rickettsiology and Rickettsial Diseases. Fifth International Conference. Ann. N. Y. Acad. Sci. 2009, 1166, 90–93.

Rodolakis A, Berri M, Hechard C, Caudron C, Souriau A, Bodier CC, Blanchard B, Camuset P, Devillechaise P, Natorp JC, Vadet JP, Arricau-Bouvery N: Comparison of Coxiella burnetii shedding in milk of dairy bovine, caprine, and ovine herds. J. Dairy Sci. 2007, 90, 5352–5360.

Sanford SE, Josephson GKA, Macdonald A : Coxiella-Burnetti (Q-Fever) Abortion Storms in Goat Herds After Attendance at An Annual Fair. Canadian Veterinary Journal-Revue Veterinaire Canadienne. 1994, 35, 376-378.

Skultety L, Toman R, Patoprsty V: A comparative study of lipopolysaccharides from two *Coxiella burnetii* strains considered to be associated with acute and chronic Q fever. Carbohyd. Polymers. 1998, 35,189–194.

Toman R, Skultety L, Ihnatko R: *Coxiella burnetii* glycomics and proteomics–tools for linking structure to function. Ann. N Y Acad. Sci. 2009, 1166, 67–78.

Varghees S, et al.:*Cloning and porin activity of the major outer membrane protein P1 from Coxiella burnetii.* Infect. Immun. 2002, 70(12), 6741-50.

VillumsenS, Jorgensen CS, Smith B, Uldum S, Schiellerup P, KrogfeltKA: Determination of new cutoff values for indirect immunofluorescence antibody test for Q fever diagnosis in Denmark. Diagn. Microbiol. Infect. Dis. 2009, 65, 93–98.

Waag DM: (2007) *Coxiella burnetii* : host and bacterial responses to infection. Vaccine. 2007, 25, 7288–7295.

WaldhalmDG, et al.:*Abortion associated with Coxiella burnetii infection in dairy goats.* J Am Vet Med. Assoc. 1978, 173(12), 1580-1.

Walker DH,Fishbein DB: *Epidemiology of rickettsial diseases.* Eur. J.Epidemiol. 1991, 7(3), 237-245.

Winn JF, et al.:*Q fever studies. XVII. Presence of Coxiella burnetii in the feces of naturally infected sheep.* Am. J.Hyg. 1953, 58(2), 183-187.

Zvizdic S, et al.:*Q-fever, human and animal morbidity in some regions of Bosnia and Herzegovina, in 2000.* Med.Arh. 2002, 56(3), 131-133.

CHAPTER 2 (MANUSCRIPT 1)

COXIELLA BURNETII ANTIBODY LEVELS IN DANISH DAIRY CATTLE: PREVALENCE, INCIDENCE, RECOVERY AND RELATIONS BETWEEN DAM AND OFFSPRING WHEN ACCOUNTING FOR DIAGNOSTIC TEST QUALITY

SYNOPSIS

Background

The frequency estimation especially the general epidemiology like the estimation of prevalence, incidence and recovery indicate disease pattern, and helps to hypothesize causal inferences of that pattern. There for estimation of these frequencies is the first step of analysis of any infectious disease in an area. Without this the transferring pattern of Coxiella *burnetii* antibody from dam to offspring is also helpful for disease control management. During recent years Coxiella *burnetii* antibody seropositive individuals have been detected at increasing frequency in dairy cattle. Yet now the individual level prevalence, incidence and recovery of this antibody in Danish dairy cattle were not detected. Thus the aim of this work was to 1) study the relationship between levels of *C. burnetii* antibodies in offspring and their dam, 2) to estimate the prevalence, incidence and recovery of *C. burnetii* antibody positivity under the assumption of perfect and imperfect tests, 3) to estimate the sensitivity and specificity of the CHEKIT Q-Fever Antibody ELISA TEST Kit, and 4) to estimate associations of age groups, herd status and breed with prevalence, incidence and recovery in Danish dairy cattle.

The study

The present study is a continuation of a study by *Agger et al.* (2010), who determined the level of *C. burnetii* antibodies in one BTM sample from each of 100 randomly selected dairy herds (see Figure 1). The samples were tested using the CHEKIT Q-Fever Antibody ELISA TEST Kit (IDEXX) based on *C. burnetii* inactivated phase 1 and phase 2 antigens. Then we randomly selected 10 positive, 10 negative and 4 intermediate herds. Blood samples were collected from within herd randomly selected animals; i.e. 10 young calves (age≤6 months), 10 old calves (6<age≤11months), 10 heifers (11<age<1st calving) and 30 cows (>1st calving) from August-October 2008 (T1).Milk samples from dams of all age groups animals were collected at the same time if possible cases. A total of 2113 blood samples were collected from 1278 animals at times T1, T2 = January-February 2009 and T3 = April-June 2009.Repeated measurements were modelled by a Hidden Markov Model and the unobserved states were modelled by logistic regressions with a random effect of herd. In this model we used cut-off value S/P ≥68%. The output of this analysis was compared with that of another logistic regression model where the unobserved states were not considered. In the later model the cut-off value was S/P ≥40%. Finally the estimation of prevalence, incidence and recovery were determined by classical or conventional estimation method where the cut-off value was S/P ≥40%.The age specific prevalence at T1 and incidence risk in T1 to T3 as well as incidence risks for the periods T1-T2 and T2-T3 were calculated. An animal was considered a positively seroconverted i.e. infected by *C. burnetii* if it tested negative at the beginning of a period and positive at the end of the same period. The incidence risks were calculated by dividing the number of new cases with the total number of negative animals at the beginning of the period. An animal was considered negatively seroconverted if it tested positive at the beginning of the period and negative at the end of the period. The probability of negative seroconversion was calculated as the number of negatively seroconverted animals during the period divided by the total number of positive animals at the beginning of the period. These calculations assumed 100% test sensitivity and specificity, respectively.

Principal findings and Significance

There was a positive relationship between antibodies in offspring blood and dam milk, which was strongest early in life and ceased with increasing age of offspring.The pattern of prevalence, incidence and recovery as well as association was same in both analyses. The prevalence and incidence varied from medium in calves to null in young and in heifers up too high in parity groups. Recovery was higher in young calves, old calves and heifers than in cows. Age groups and herd status had a significant impact on prevalence. Age groups have significant effect on incidence and recovery. Negative and intermediate herds' animals had lower odds of prevalence of animal seropositive herds in both studies. Sensitivity and specificity of ELISA test was standard enough for a good test. The cut-off value S/P ≥68% was specified for differentiation between infected and non-infected animals in Hidden Markov Model. The pattern of prevalence and incidence is logical in biologically .Prevalence is high in calf for consuming colostrum low in young and heifer for starting work of immune system and again high in cow due to more exposed by contaminated environment. Recovery is high in young and heifer for neutralizing antibody by time as well as neutralized by body immunity. The cut-off value S/P ≥68% was in Hidden Markov Model because unobserved states were considered for modelling.

Coxiella burnetii antibody levels in Danish dairy cattle: Prevalence, incidence, recovery and relations between dam and offspring when accounting for diagnostic test quality

ABSTRACT

Although infection of cattle with *Coxiella burnetii* occurs worldwide, detailed knowledge on infection frequencies in individual dairy cattle is not studied before. The aim of this study was to estimate prevalence, incidence and recovery as well as relationship between offspring and dam serostatus in Danish dairy cattle. By this way we also differentiated two tests with separate cut-off values. Initially a study of herd categories on the basis of the level of *C. burnetii* antibodies in bulk tank milk (BTM) was done. Then we randomly selected 10 positive, 10 negative and 4 intermediate herds. Blood samples were collected from within herd randomly selected animals; i.e. 10 young calves (age≤6 months), 10 old calves (6<age≤11months), 10 heifers (11<age<1st calving) and 30 cows (>1st calving) from August-October 2008 (T1).Milk samples from dams of all age groups animals were collected at the same time if possible cases. A total of 2113 blood samples were collected from 1278 animals at times T1, T2 = January-February 2009 and T3 = April-June 2009.Repeated measurements were modelled by a Hidden Markov Model and the unobserved states were modelled by logistic regressions with a random effect of herd. In this model we used cut-off value S/P ≥68%. The output of this analysis was compared with that of another logistic regression model where the unobserved states were not considered. In the later model the cut-off value was S/P ≥40%. Finally the estimation of prevalence, incidence and recovery were determined by classical or conventional estimation method where the cut-off value was S/P ≥40%.

There was a positive relationship between antibodies in offspring blood and dam milk, which was strongest early in life and ceased with increasing age of offspring. The pattern of prevalence, incidenceand recovery as well as association was same in both studies. Prevalence and incidence varied from medium in calves to null in young and in heifers upto high in parity groups. Recovery was higher in young calves and heifers than in cows in both studies. Age groups and herd status had a significant impact on prevalence. Age groups have significant effect on incidence and recovery. Negative and intermediate herds' animals had lower odds of prevalence of animal seropositive herds in both studies. Sensitivity and specificity of ELISA test was standard enough for a good test. The cut-off value S/P ≥68% was specified for differentiation between infected and non-infected animals in Hidden Markov Model.

Prevalence and incidence of seropositive animals were medium in young calves, low in older calves and heifers and again high in cows. Recovery was higher in young animals than in cows. Antibodies in offspring blood and dam milk showed positive relationship: strongest mainly in age group 90 days ≤ age <120 days and ceased with increasing age of offspring. This indicatse that calves have short lasting colostral antibodies and remain seronegative until first calving.

Key words:*Coxiella burnetii,* dairy cattle, antibody, prevalence, incidence and recovery

INTRODUCTION

Coxiella burnetii is an obligate intracellular zoonotic bacterium (Maurin and Raoult, 1999) and domesticated ruminants including dairy cattle are considered the main reservoir for human exposure (Arricau-Bouvery and Rodolakis, 2005; Cutler et al., 2007; Kim et al., 2005). *C. burnetii* was regarded as an economically insignificant pathogen for domestic livestock (Palmer et al., 1983; Raju et al., 1988; Waldham et al., 1982; Zeman et al., 1989), but the recent outbreak of Q fever in the Netherlands (ref) have shown that Q fever infection in animals may have extensive economic implications. Q fever infection in cattle is usually subclinical (Arricau-Bouvery and Rodolakis 2005) although sporadic abortions occur (Jensen et al. APMIS JFA has the ref). The almost worldwide occurrence of *C. burnetii* has been documented by serological studies (There is a recent French review that can be used as ref but studies on incidence and recovery of the infection in dairy cattle has apparently not been published. In Denmark, 59.0% of dairy herds were *C. burnetii* antibody positive in bulk tank milk (BTM), (Agger et al., 2010), and 2-86 % of dairy cows were antibody positive and 2-93 % of dairy cows were PCR test positive in 12 randomly selected BTM positive herds (Angen et al 2011). In a Canadian study 67% of dairy herds were found seropositive (Lang, 1988). The prevalence was 20.0% and 37.7% in cattle at animal and herd levels respectively in a French study(Guatteo et al., 2011). In Northern Ireland 64.5 % of dairy cattle herds and 10.4% of dairy cattle were found seropositive for *C. burnetii*(McCaughey et al., 2010). The seroprevalence in individual cattle has been reported as 12%, 21%, 39% and 46% in Germany, the USA, the Netherlands and Japan, respectively (Biberstein et al., 1974; Houwers and Richardus, 1987; Htwe et al., 1992a; Htwe et al., 1992b; Rehacek, 1993). In The Netherlands the *C. burnetii* antibody blood seroprevalence was reported to be 16.0 % in lactatingdairy cows and 1.0% in cattle below 21 months of age (J.Muskens, 2011).

Previous studies have shown that the prevalence of *C. burnetii* antibodies in individual animal varies with herd size (McCaughey et al., 2010), farming system (Capuano et al., 2001; Ruiz-Fons et al., 2010) breed and age groups (McCaughey et al., 2010).

The aim of this work was to 1) study the relationship between levels of *C. burnetii* antibodies in calf, young and heifer with their dam, 2) to estimate the prevalence, incidence and recovery of *C. burnetii* antibody positivity under the assumption of perfect and imperfect tests, 3) to estimate the sensitivity and specificity of the CHEKIT Q-Fever Antibody ELISA TEST Kit, and 4) to estimate associations of age groups, herd status and breed with prevalence, incidence and recovery in Danish dairy cattle.

MATERIALS AND METHODS

Study population and sampling strategy:

The present study is a continuation of a study by *Agger et al.* (2010), who determined the level of *C. burnetii* antibodies in one BTM sample from each of 100 randomly selected dairy herds (see Figure 1). The samples were tested using the CHEKIT Q-Fever Antibody ELISA TEST Kit (IDEXX) based on *C. burnetii* inactivated phase 1 and phase 2 antigens. The results were expressed as S/P values and estimated as

$$S/P = (\text{optical density (OD)}_{\text{sample}} - \text{OD}_{\text{negative control}})/(\text{OD}_{\text{positive control}} - \text{OD}_{\text{negative control}})) \times 100\%$$

According to the producer recommendations S/P ≥40% is positive, S/P <30% is negative, and results in the interval 30%≤ S/P <40% are intermediate. Accordingly the 100 herds were categorized as test positive, test negative or test intermediate.

We randomly selected 10 positive, 10 negative and 4 intermediate herds within the three groups for a prospective cross sectional study with follow up during a period of 11 months from August 2008 to June 2009. All lactating cows were milk sampled at 3 time points (Figure 1).

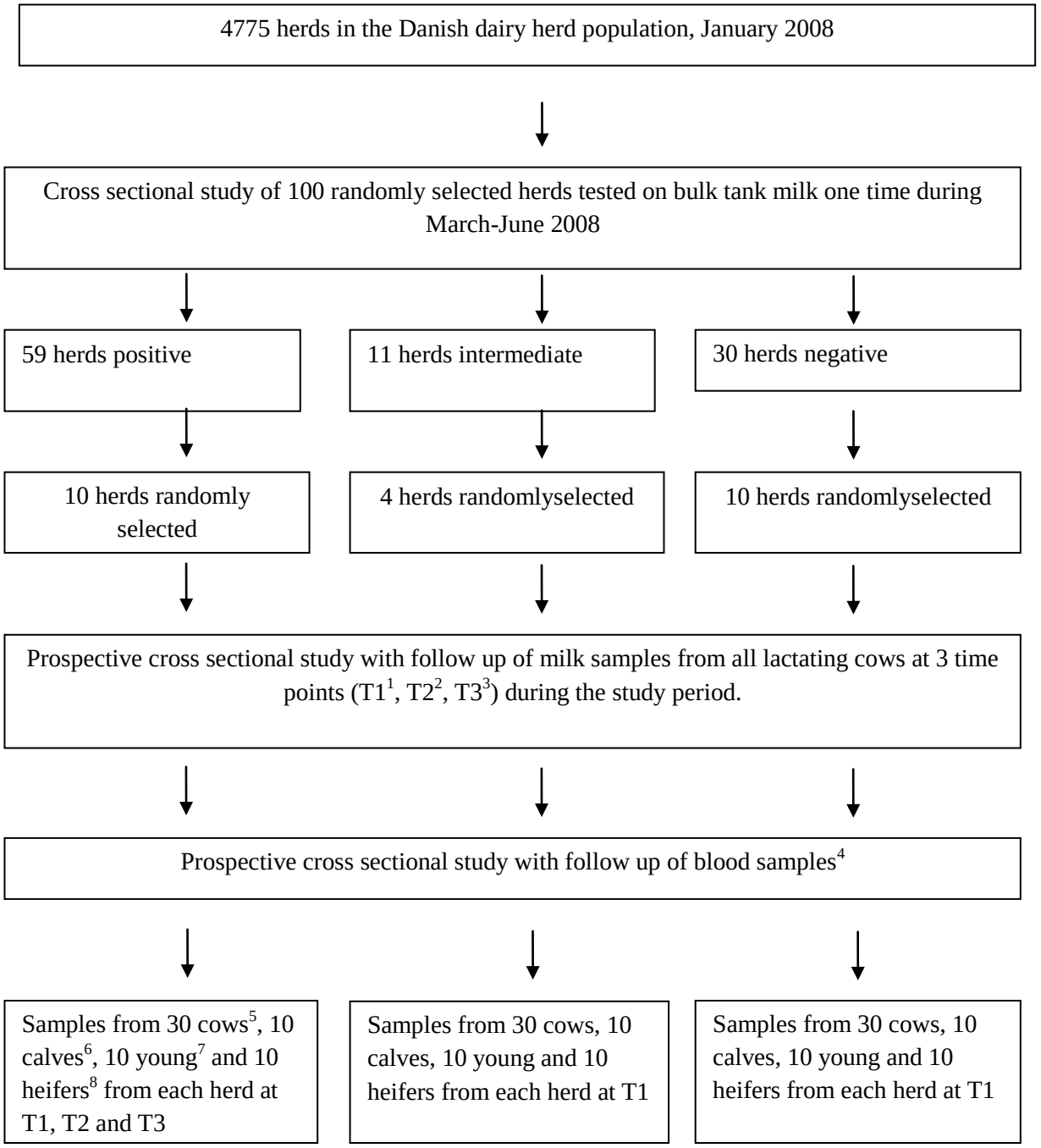


Figure 1 Diagram of study design for sampling

Figure 1 All samples were tested for *C. burnetii* antibodies.[1]T1: Sampling one time during August-October 2008; [2]T2: Sampling one time during January-February 2009; [3]T3: Sampling one time during April-June 2009; [4]The planned number of blood samples was not always obtained due to practical limitations. [5]Cows: Cattle that have calved; [6]Calves: age ≤6 months; [7]Young: 6 months< age ≤ 11 months; [8]Heifer: 11 months < age < 1st calving.

Blood samples were collected from 10 young calves (age<6 months), 10 old calves (6 months≤ age≤11 months), 10 heifers (11 months<age<1st calving), and 30 cows (parity≥ 1st parity) at T1, T2 and T3 in the 10 positive herds. Then in cow we divided four groups that were parity-1, parity-2, parity-3 and parity-4 which were combined given name parity groups. Blood samples from similar age groups in the 10 negative and the 4 intermediate herds were collected only at T1 (see Figure 1).

If possible, the same animal was sampled at all three time points to maximize the number of repeated measurements. However, as dairy herds are dynamic units, previously sampled but subsequently culled animals, were replaced by new randomly sampled animals within the same age strata as the culled animal belonged to, i.e. 1-3 samples were collected per animal. The study included 1278 cattle from 24 dairy herds.

Blood sampling and laboratory analysis:

Five to eight ml of blood was drawn in Vacutainer® serum tubes and tested at the Danish National Veterinary Institute for *C. burnetii* antibodies. The samples were centrifuged at 3000 g during 10 minutes; serum was stored at 5^0 C and shortly after tested for *C. burnetii* antibodies. All samples were tested in duplicate by the CHEKIT Q-Fever Antibody ELISA TEST Kit and the OD of each sample were averaged and expressed as S/P values. Cut off values for categorising these results are presented in the following section.

$$S/P\,value = \frac{OD\,Sample - OD\,negative\,control}{OD\,positive\,control - OD\,negative\,control}\,X100$$

Statistical Analysis:

The aims of the statistical analysis was to estimate the *C. burnetti* antibody prevalence, incidence and recovery according to the *SP-value*; to test hypothesis for their relations to the explanatory variables (*breed, age, initial herd status*), and to study the dam and off spring antibody relationship.

The data were analysed in two ways.

In the first approach the sensitivity and specificity of the diagnostic test was not known. Thus, data were analysed as apparent test results by categorising samples as positive when S/P>=40 and as negative when S/P<40. The age specific prevalence at T1 and incidence risk in T1 to T3 as well as incidence risks for the periods T1-T2 and T2-T3 were calculated. An animal was considered a positively seroconverted i.e. infected by *C. burnetii* if it tested negative at the beginning of a period and positive at the end of the same period. The incidence risks were calculated by dividing the number of new cases with the total number of negative animals at the beginning of the period. An animal was considered negatively seroconverted if it tested positive at the beginning of the period and negative at the end of the period. The probability of negative seroconversion was calculated as the number of negatively seroconverted animals during the period divided by the total number of positive animals at the beginning of the period. These calculations assumed 100% test sensitivity and specificity, respectively. The pattern of prevalence, incidence and recovery was expressed as medium, low and high on the base of values. Descriptive, univariable and multivariable analyses were performed including evaluation of a random effect of herd. In this approach data was analyzed in SAS 9.2., using PROC FREQ and PROC GLIMMIX.

In the second approach incidence and recovery were estimated taking the risk of false positive or false negative results into account. In order to do this the repeated measurements within animals were modelled by a Hidden Markov Model (HMM), (Rabiner 1989). The implementation of this method is described in the following paragraphs. The unobserved states in this HMM are the unobserved serostatus at T1, T2, and T3. The prevalence at T1 and the incidence and recovery (from T1 to T2 and from T2 to T3) inferred from the unobserved states were modelled by separate logistic regressions with a random effect of herd identity, where the models for incidence and recovery are allowed to depend on whether the transition is from T1 to T2 or from T2 to T3. Given the infection status of an animal at one of the sampling times we assume that the logarithm of one plus the observed *SP-value* is normally distributed with mean and standard deviation that only depends on the infection status and not on the associated explanatory variables.

Denoting these means and standard deviations by $\mu_{healthy}$, $\mu_{infected}$ and σ_{healty}, $\sigma_{infected}$, respectively, and the probability density function for the standard normal distribution by φ, the conditional probability for

infection of an animal with *SP-value x* and prior prevalence *p* (as predicted from the logistic regressions) is given by Bayes' formula

$$P(infected|x,p) = \frac{p * \varphi\left(\frac{\log(1+x) - \mu_{infected}}{\sigma_{infected}}\right)}{(1-p) * \varphi\left(\frac{\log(1+x) - \mu_{healthy}}{\sigma_{healthy}}\right) + p * \varphi\left(\frac{\log(1+x) - \mu_{infected}}{\sigma_{infected}}\right)}$$

The assumption of a conditional normal distribution of *log (1+SP)* may be validated comparing the observed *SP-values* with the model predictions. The *HMM* is estimated using the *Expectation-Maximization algorithm* (Dempster et al. 1977) initialized with a threshold for infection at SP≥40, and where the expectation step is evaluated at the maximum a posteriori predictions of the random effects logistic regressions. The hypothesis tests for the relations of prevalence, incidence and recovery on the explanatory variables are done as likelihood ratio Chi square tests in the weighted logistic regressions, where the predictions from the *HMM* are taken as known weights on the inferred infection statuses. Effects that are non-significant on the 5% level are removed in a backward selection procedure. Results for the significant effects are given on two different forms. Firstly, estimated odds ratios against a reference level are given with their 95% profile likelihood confidence intervals. Secondly, the prevalence, incidence, recovery, sensitivity (at T1) and specificity (at T1) as predicted by the statistical model are stated for the different age groups corresponding to the division given by the significant effects. Since the latter is not reported as standard we give more details on this calculation. Consider for instance the estimates for sensitivity stratified according to animal age, and in particular the calves, say. If the true infection status at T1 for the observed calves were known, then we would estimate the sensitivity as the proportion of infected animals that were diagnosed as infected by the ELISA test. However, since the true infection status is unknown it is replaced by the conditional probability $P(infected|x,p)$ derived from Bayes' formula. Since this also is the probability for a positive diagnosis the estimated sensitivity is given by

$$Sensitivity = \frac{\sum_i (P(infected|x_i,p_i))^2}{\sum_i P(infected|x_i,p_i)},$$

where the sums are taken over all calves in the dataset. The numerator in this quotient may be interpreted as the sample size for the estimation in question. These sample sizes will be given together with the estimates and serves as a replacement for confidence intervals we have not been able to derive (larger sample size corresponds to higher confidence). Moreover, estimates for the variance components for the random effects in the logistic regressions and estimates of the normal distributions of the logarithmic SP-values for seronegative and seropositive animals, respectively, are given.

Linear regression analyses for the relationship between antibody levels in offspring age groups (response) and their dams (exposure) were conducted using log(1+offspring SP) and log(1+dam SP) to normalise the distributions.

RESULTS

Offspring and dam relationship:

Table 1 shows the relationship between calf, young and heifer *C. burnetii* antibody status and their respective dam's antibody status.

Table 1 Linear regression analysis if the relationship between S/P values of *Coxiella burnetii* antibody levels in samples of offspring blood and of milk from their dams. The data are analysed separately by age stratum.

Offspring age group*	Age interval	Sample size	Beta	Intercept	P-values
Calves	Age≤6 months	133	0.14	0.92	0.00
Calves	Age <90 days	15	0.06	0.98	0.62
	90 days ≤ age <120 days	25	0.55	0.78	0.001
	120 days ≤ age <150days	56	0.14	0.87	0.03
	150 days ≤ age < 180 days	37	0.04	0.99	0.70
Young animals	6 months<age≤11 months	62	-0.01	1.05	0.90
Heifers	11 months<age<1st calving	42	0.16	0.63	0.15

*Calf: age≤6 months; young: 6 months<age≤11 months; heifer: 11 months<age<1st calving

Each offspring was represented only one time in the analyses; i.e. mainly from T1 and the newly added offspring at T2 and at T3. A significant relation between the antibody level of dam and offspring was only founds for young calves. A further age subgrouping of these (Table 1) shows that only antibody status. In calves 90-150 days old were significantly associated with the dam

level. The lack of significance for calves <90 days old is most likely due to the small number (15) (Table 1).

Prevalence estimation:

The results of prevalence from our 1st approachare presented in Table 2.

Table 2 Apparent prevalence of *Coxiella burnetii* antibody test positive blood samples from dairy cattle at time point T1. The results are stratified by age group and by the herd S/P level of antibodies in an initial BTM sample.

Age group*	Dairy herd category of S/P level in the initial BTM sample								
	Ten positive herds			Four intermediate herds			Ten negative herds		
	Positive[2]	Total	Prevalence	Positive	Total	Prevalence	Positive	Total	Prevalence
Calf	5	30	16.7	1	18	5.6	0	24	0
Young	4	85	4.7	0	20	0	0	38	0
Heifer	0	104	0	0	0	0	0	5	0
Parity 1	24	83	28.9	3	46	6.5	1	89	1.1
Parity 2	18	74	24.3	5	39	12.8	1	86	1.1
Parity 3	16	60	26.7	5	16	31.3	1	45	2.2
Parity≥4	12	58	20.7	4	17	23.5	7	50	14.0

*Calf: age≤6 months; young: 6 months<age≤11 months; heifer: 11 months<age<1st calving; Parity≥4: parity 4 and above; [2]A sample was considered test positive when SP≥40.

Compared to the herd status based on BTM antibody level, the overall picture showed a high prevalence of seropositive cattle in BMT positive herds, slightly lower in BTM intermediate herds and low in BTM negative herds. Seroprevalence in different age groups generally showed a medium prevalence in calves, a low – zero prevalence in young animals and in heifers, and a high level in parity groups Table 2.

Incidence risk estimation:

The results of incidence risk from our 1st approachare presented in Table 3.

Table 3 Incidence risk of *Coxiella burnetii* antibody test positive cases based on blood samples from 236 dairy cattle tested three times (T1-T3) during an 11 months period. The results are stratified in seven age groups.

Age group	No. of (-)veanimal at T1*	No. of animals seroconverted** during T1-T2*	Incidence risk T1-T2 (C.I. 95%)	No. of animals seroconverted during T2-T3*	Incidence risk T2-T3 (C.I. 95%)	No. of animals seroconvert eted during T1-T3	Overall Incidence risk (C.I. 95%)
Calf	15	0	0 (0-0)	1	6.7 (.002-.32)	1	6.7 (.002-.32)
Young	46	1	2.2 (.001-.12)	0	0	1	2.2 (.001-.12)
Heifer	61	1	1.6 (.0004-.09)	1	1.7 (.0004-.09)	2	3.3 (.004-.11)
Parity1	41	3	7.3 (.015-.12)	2	5.3 (.006-.17)	5	12.2 (.041-.26)
Parity2	29	5	17.2 (.058-.36)	1	4.2 (.001-.18)	6	20.7 (.08-.40)
Parity3	19	1	5.3 (.0013-.26)	2	11.1 (.013-.33)	3	15.8 (.034-.40)
Parity≥4	25	0	0 (0-0)	0	0 (0-0)	0	0 (0-0)
Total	236						18.0

*T1: August-October 2008; T2: January-February 2009 and T3: April-June 2009; Calf: age≤6 months; young: 6 months<age≤11 months; heifer: 11 months<age<1st calving; Parity≥4: parity 4 and above; **A sample was considered test positive when SP≥40.

A total of 236 animals were tested three times (T1-T3) during 11 months and they were the basis for calculating the incidence risk of seroconversion from seronegative to seropositive (Table 3). The overall incidence risk among calves was 6.7%, and among young calves and heifers 2.2% and 3.3%, respectively. The incidence risk in parity 1, parity 2 and parity 3 were 12.2%, 20.7% and 15.8%, respectively. There were no new cases among parity 4 cows. The incidence risks varied between the periods T1-T2 and T2-T3, e.g. an increase from 0% to 6.7 % among calves and a decrease from 17.2% to 4.2% among parity 2 cows (Table 3).

Recovery estimation:

In the 1st approach among animals that were tested positive all three sampling points (T1-T3), the serocoversionfrom positive to negative of 2 young animals out of 2 at (T1-T2) which was 100%. In young and heifer at T2 was 1 positive and seroconverted from positive to negative that's at T3 was also 100%. Animals that are tested only in two repeated groups at (T1-T2) seroconverted in young and heifer was 5 out of 5 and 2 out of 2 positive animals. At (T2-T3) there were 5 animals seroconverted out of five positive in

Logistic regression model with random effect:

The results of the final model of logistic regression with random effect of herd are shown in Table 4.

Table 4 Logistic regression result of *Coxiella burnetii* antibody for prevalence based on a cut off SP≥40.

Response	Effect	Comparison	Odds ratio (95% CI)	P_value
			OR (LCL; HCL)	
Prevalence	Age groups*	Calf	0.37 (0.21; 0.65)	<0.0001
		Young	0.05 (0.02; 0.16)	
		Heifer	0.02 (0.00; 0.06)	
		Parity-2	1.04 (0.69;1.57)	
		Parity-3	1.16 (0.72; 1.85)	
		Parity≥4	1.20 (0.75; 1.91)	
		Parity-1	1.00	
	Herd status	Negative	0.10 (0.03; 0.35)	0.0015
		Intermediate	0.53 (0.03; 2.21)	
		Positive	1.00	

*: Calf: age≤6 months; young: 6 months<age≤11 months; heifer: 11 months<age<1st calving; Parity≥4: parity 4 and above

There were significant effects of age groups and herd status (p<0.0001 and 0.0015) on the risk of animals being test positive. Odds ratio was 1.20 when comparing parity 4 with parity 1. The odds ratios were very low 0.02 and 0.05 when comparing parity 1 with heifers and young animals and moderate low in calves.

The results of prevalence, incidence risk and recovery from our 2ndapproachare presented in Table 5. When we looked the results from 2nd approach we find the same pattern of prevalence and incidence risk like medium prevalence and incidence risk in calves, a low – zero prevalence in young animals and in heifers, and a high level in parity groups. In the herd condition based prevalence we also found the same pattern like high prevalence of seropositive cattle in BMT positive herds, slightly lower in BTM intermediate herds and low in BTM negative herds. In the 2nd approach in initially BTM positive herdsthe recovery was higher in young and heifer 100% than in adult cows and this pattern is like the results of 1st approach. In parity groups 1-4 the recovery rates were 24.0%, 23.0%, 6.0% and 14.0% respectively Table 5.

Table 5 Model based empirical prevalence, incidence risk and recovery according to age group and initial herd status (positive, intermediate or negative) based on a previously selected BTM sample.

.

	Prevalence in 24 herds at T1			Incidence in 10 positive herds between T1-T2 or T2-T3	Recovery (clearance) in 10 positive herds between T1-T2 or T2-T3
Herd status Age groups	Positive herds	Intermediate herds	Negative herds	Positive herds	Positive herds
Calf	0.13 (30.0**)	0.03 (18.0)	0.01 (24.0)	0.00 (3.0)	NA (0.0)
Young	0.00 (85.0)	0.00 (20.0)	0.00 (38.0)	0.00 (80.9)	1.00 (5.1)
Heifer	0.00 (104.0)	NA (0.0)	0.00 (5.0)	0.00 (291.0)	1.00 (1.0)
Parity 1	0.25 (83.0)	0.09 (46.0)	0.02 (89.0)	0.04 (87.4)	0.24 (19.6)
Parity 2	0.24 (74.0)	0.07 (39.0)	0.01 (86.0)	0.03 (116.3)	0.23 (41.7)
Parity 3	0.26 (60.0)	0.09 (16.0)	0.01 (45.0)	0.03 (54.8)	0.06 (19.2)
Parity 4+	0.24 (58.0)	0.15 (17.0)	0.04 (50.0)	0.00 (88.9)	0.14 (21.1)

*: Calf: age≤6 months; young: 6 months<age≤11 months; heifer: 11 months<age<1st calving; Parity 4+: parity 4 and above. **: Estimated sample size in parenthesis

The final three models of logistic regression with prevalence, incidence and recovery as the respective responses and the explanatory variables age group and breed and random effect of herd are shown in Table 6.

Table 6 Logistic regression result of *Coxiella burnetii* antibody for prevalence, incidence and recovery in 10 dairy herds that were initially test positive on one bulk tank milk sample.

Response	Effect	Comparison	Odds ratio (95% CI)**	P_value
Prevalence	Age groups*	Calf	0.49 (0.15;1.58)	<0.0001
		Young	0.00 (NA)	
		Heifer	0.00 (NA)	
		Parity-2	0.86 (0.43;1.73)	
		Parity-3	1.04 (0.48;2.25)	
		Parity-4+	1.74 (0.81;3.76)	
		Parity-1	1.00 (ref.)	
	Herd status	Negative	0.03 (0.00; 0.22)	0.0015
		Intermediate	0.36 (0.05; 2.58)	
		Positive	1.00 (ref.)	
Incidence	Age groups*	Calf	0.09 (NA)	0.0061
		Young	0.00 (NA)	
		Heifer	0.00 (NA)	
		Parity-2	0.88 (0.20; 3.93)	
		Parity-3	0.77 (0.12; 5.04)	
		Parity-4+	0.00 (NA)	
		Parity-1	1.00 (ref.)	
Recovery	Age groups*	Calf	NA (NA)	0.0074
		Young	>9999 (NA)	
		Heifer	>9999 (NA)	
		Parity-2	0.79 (0.19; 3.27)	
		Parity-3	0.20 (0.02; 2.18)	
		Parity-4+	0.48 (0.08; 2.98)	
		Parity-1	1.00 (ref.)	

*Calf: age≤6 months; young: 6 months<age≤11 months; heifer: 11 months<age<1st calving; Parity 4+: parity 4 and above. ** Estimated odds ratios with confidence intervals based on the standard errors. If standard errors are so large that the resulting confidence errors are exceedingly wide a 'NA' has been inserted.

There were significant effects of age in all three models. The same pattern as seen in the previous analyses is confirmed with relatively high odds of test positivity among parity groups 1-4 compared to young animals and heifers, and a medium risk in calves. There was also a significant effect of initial herd status (as random variable) in the prevalence model.

Table 7 Model based age specific empirical sensitivities and specificities of Chekit Q-fever Antiody ELISA test kit (IDEXX) in 987 cattle from 24 Danish dairy herds at time T1.

Age groups*	Calf	Young	Heifer	Parity-1	Parity-2	Parity-3	Parity-4+
Sensitivity	0.88 (4.2**)	0.11 (0.1)	NA (0.0)	0.97 (26.3)	0.96 (21.6)	0.97 (18.4)	0.93 (18.9)
Specificity	0.99 (67.8)	1.00 (142.9)	1.00 (109.0)	1.00 (191.7)	1.00 (177.4)	0.99 (102.6)	0.99 (106.1)

*: Calf: age≤6 months; young: 6 months<age≤11 months; heifer: 11 months<age<1st calving; Parity 4+: parity 4 and above. **: Estimated sample size in parenthesis.

The sensitivity of the test for estimation of prevalence was 88 % in calves and 97-93 % in parity groups 1-4 (Table 7). The sensitivity in heifers was 0, because there were no positive animals in this group. The specificity of the test was 99-100 % for estimating the prevalence.

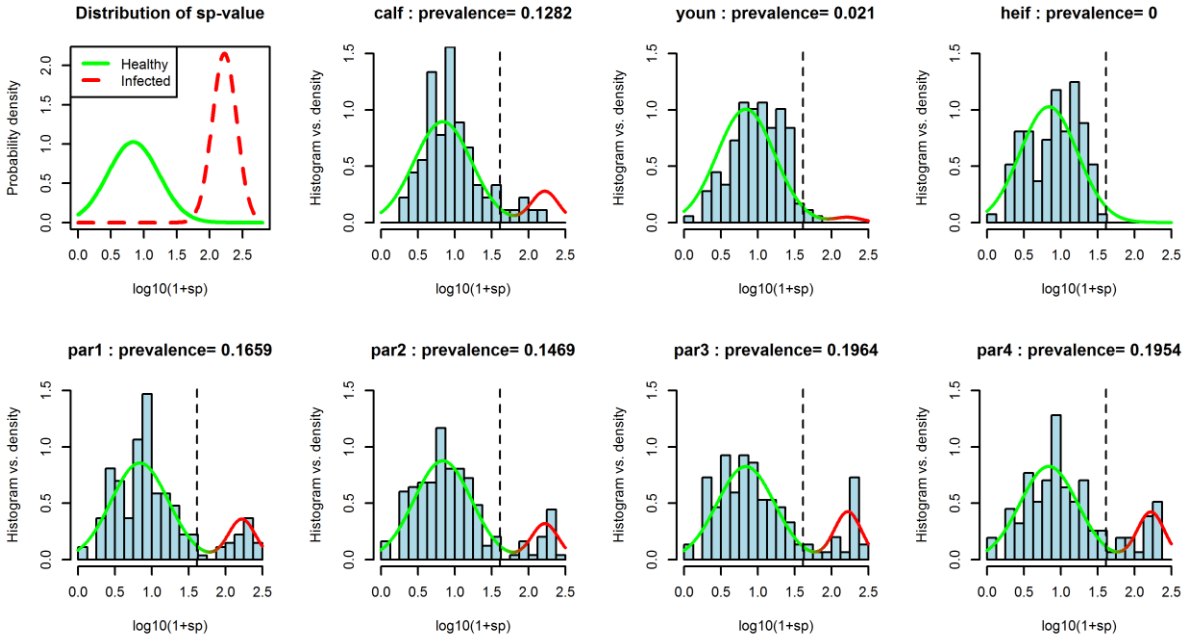


Figure 2 Infected and non-infected levels of *Coxiella burnetii* antibody S/P value.

To validate the assumed logarithmic normal distributions for the *SP-values* we compare the histograms for the observed logarithmic *SP-values* in the seven age groups at time T1 against the fitted two component mixture of normal distributions. The fit is reasonable good speaking in favour of the normality assumption.

Figure 2 shows the fitted probability densities for the SP values in the different age groups, where the colour signifies the posterior probability for being seronegative (green) and seropositive (red). The vertical dashed

line display the cut-off value at SP≥40.The EM algorithm fits a larger change point between seronegative and seropositive animals (for prevalence=0.20 the cut-off value is estimated at *SP>68.08*). The prevalence varied from high in calves (13.0%) to low in young's (2.0%) and in heifers (0%) and again high among parity 1-4 groups with 17.0%, 15.0%, 20.0% and 20.0% respectively at T1.

DISCUSSION

The results of this study as presented in the patterns of the estimated prevalences, incidences and "recovery" (Tables 2-7) leads indicates that the neonatal calf receives maternal colostral antibodies that subsequently disappear within the first months of life. The calf may be protected against infection with *C. burnetii* during this period, but it is surprising that cattle remain seronegative until calving, especially because *C. burnetii* is most likely present in the environment of infected cattle herds, its high resistance to breakdown and the supposed low infection dose.

One explanation could be that neonatal dairy calves are often removed from their dams within a few hours after birth and thus are removed from a contaminated cow barn environment and raised in a non-contaminated environment. The female calves usually live in other farm buildings until they deliver their own first calf and then "re-enter" the cow barn as lactating cows. At that time they may be highly exposed to a contaminated environment (provided the herd is infected) and contract the infection. An alternative but less likely hypothesis is that the non-mature cattle are latent infected and that the pregnancy and calving process re-activates the infection.

The rise of *C. burnetii*antibodies in calves indicates the transfer of maternal antibodies through colostrum.

This is in agreement with the findings of Garry et al., (1996) and Mahmood et al., (2007).There is a general knowledge about intra-uterine transmission of antibodies is not possible for cow because of the structure of uterus. But it is common for most organisms' antibodies are generally transferred by colostrum.

A comparison among the different age groups of prevalence shows that the prevalence high in parity groups and increasing in nature in parity 1-3 (Table 3).That makes an argument risk of antibody positivity increase with age. Howeverin the year 2010 by McCaughey et al. also found the same result, the prevalence

increased with age. As well as by Ruiz-Fons et al., (2010) showed that age-associated seroprevalence differed between ruminant species (Beef cattle, sheep and goat) but generally increased with age. The dairy cattle is also under ruminant species so our study result is supported by the above mentioned study results. The parity groups are higher seropositive, because these groups of cows are keeping in the same house for long time. That tended to be a risk factor for being a high seropositivity. On the other hand the farmers enter new cows in the herds and make some chance to introduce seropositive animals in herds which is a risk factor for higher seropositivity. Earlier research provides same evidence introducing new animals in the herd was always found as a classical risk factor for introducing disease (Marano et al., 2007).

Finally in 1st and 2nd approaches we found the similarity in results in prevalence, recovery and in associations. The cut-off value was high in Hidden Markov Model because unobserved states of data were considered for analysing. In our 2nd approach sensitivity of ELISA test was 93% to 97% in parity groups and specificity of the test was 99% to 100% in all the age groups. On the other hand (Horigan et al., 2011) estimated the ELISA test to 81% sensitivity and 94% specificity for detecting antibodies to *C. burnetii* infection when using a cut-off value of SP>=40 as specified by the manufacturer. However, ELISA is increasingly being used for the testing of a wide range of animal species (Jaspers et al., 1994) and is shown to be more sensitive and specific than the CFT (Jozwik et al., 2007; Peter et al., 1987).

CONCLUSION

The study indicates that dairy heifers generally are sero-negative from the time where colostrum derived antibodies disappear until parturition. The reason for this is unknown but may reflect e.g. differences in exposure level to the organism, changed susceptibility during the heifer period or reactivation of a latent infection around first parturition. Prevalence of antibodies in calves was medium level, and zero level in young and heifers zero level however a high and increasing level in parity groups. Similar pattern in incidence risk and recovery across the age groups were found. ELISA test was standard enough for a good test on based of sensitivity and specificity value. In Hidden Markov Model, specified cut-off value was S/P ≥68% for differentiation between infected and non-infected animals.

Acknowledgments

The study was funded by Kvaegafgiftsfonden and the Faculty of Life Sciences, University of Copenhagen as part of a PhD project. The authors gratefully acknowledge the breeders for participating in the study and the technical staff at the National Veterinary Institute, Technical University of Denmark for analysing the samples.

References

Agger, J.F., Christoffersen, A.B., Rattenborg, E., Nielsen, J., Agerholm, J.S., 2010. Prevalence of *Coxiella burnetii* antibodies in Danish dairy herds. Acta Vet. Scand. 52, 1-4.

Angen, Ø., Ståhl, M., Agerholm, J.S., Christoffersen, A.-B., Agger, J.F. 2011. Dynamics of relationship between the presence of Coxiella burnetii DNA, antibodies, and intrinsic variables in cow milk and bulk tank milk from Danish dairy cattle. J. Dairy Sce. 94: 5750-5759. doi:10.3168/jds.2011-4197.

Arricau-Bouvery, N., Rodolakis, A., 2005. Is Q Fever an emerging or re-emerging zoonosis? Vet. Res. 36, 327-349.

Biberstein, E.L., Behymer, D.E., Bushnell, R., Crenshaw, G., Riemann, H.P., Franti, C.E., 1974. Survey of Q-Fever (*Coxiella-Burnetii*) in California Dairy-Cows. Am. J. Vet. Res. 35, 1577-1582.

Capuano, F., Landolfi, M.C., Monetti, D.M., 2001. Influence of three types of farm management on the seroprevalence of Q fever as assessed by an indirect immunofluorescence assay. Vet. Rec. 149, 669-671.

Christoffersen A-B, 2007. Q fever in Danish cattle herds [in Danish]. Dansk Vet.Tid. 90, 13-15.

Cutler, S.J., Bouzid, M., Cutler, R.R., 2007. Q fever. J. Infect. 54, 313-318.

Garry, F. B., Adams, R., Cattell, M.B., 1996. Comparison of passive immunoglobulin transfer to dairy calves fed colostrum or commercially available colostral-supplement products. J. Amer. Vet. Med. Assoc. 208, 107–110.

Guatteo, R., Seegers, H., Taurel, A.F., Joly, A., Beaudeau, F., 2011. Prevalence of *Coxiella burnetii* infection in domestic ruminants: A critical review. Vet. Microbiol. 149, 1-16.

Guatteo, R., Beaudeau, F., Berri, M., Rodolakis, A., Joly, A., Seegers, H., 2006. Shedding routes of Coxiella burnetii in dairy cows: implications for detection and control. Vet. Res. 37, 827-833.

Houwers, D.J., Richardus, J.H., 1987. Infections with *Coxiella burnetii* in Man and Animals in the Netherlands. Zentralblatt fur Bakteriologie Mikrobiologie und Hygiene Series A-Medical Microbiology Infectious Diseases Virology Parasitology 267, 30-36.

Htwe, K.K., Amano, K., Sugiyama, Y., Yagami, K., Minamoto, N., Hashimoto, A., Yamaguchi, T., Fukushi, H., Hirai, K., 1992a. Seroepidemiology of *Coxiella burnetii* in Domestic and Companion Animals in Japan. Vet. Rec. 131, 490.

Jaspers, U., Thiele, D., Krauss, H., 1994. Monoclonal antibody based competitive ELISA for the detection of specific antibodies against *Coxiella burnetii* in sera from different animal species. ZentralblBakteriol 281, 61–66.

John C. Spitzer, J.C., 1998.Vaccination and Medication Procedures for Cattle. http://www.clemson.edu/extension/livestock/livestock/beef/beef_cattle_db/fact_sheets/bc_7005.html(accessed 03.04.12).

Jozwik, A, Jakubowski, T, Kaba, J, et al., 2007.Evaluation of the agreement of ELISA and complement fixation test in the diagnostics of Q fever in cattle. Med. Weter. 63, 655–657.

Htwe, K.K., Amano, K., Sugiyama, Y., Yagami, K., Minamoto, N., Hashimoto, A., Yamaguchi, T., Fukushi, H., Hirai, K., 1992b. Seroepidemiology of *Coxiella burnetii* in Domestic and Companion Animals in Japan. Vet. Rec. 131, 490.

Muskens, J., Engelen, E. V., Maanen, C.V., Bartels, C., Lam, T.J.G.M., 2011. Prevalence of C*oxiella burnetii* infection in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA. Vet. Rec. 168, 79-82.

Kim, S.G., Kim, E.H., Lafferty, C.J., Dubovi, E., 2005. Coxiella burnetii in bulk tank milk samples, United States. Emerg. Infect. Dis. 11, 619-621.

Lang, G.H., 1988. Q-Fever. Vet. Rec. 123, 582-583.

Mahmood, A.K.,Sheikh, M.A., Akhtar.S., Nabi, G., Rashid, H.B., 2007. Duration of Maternally Derived Antibodies Against *Pasteurella multocida* in Cow Calves. Pakistan. Vet. J. 27, 92-94.

Marano, N., Arguin, P.M., Pappaioanou, M., 2007. Impact of globalization and animal trade on infectious disease ecology. Emerg. Infect. Dis. 13, 1807-1809.

McCaughey, C., Murray, L.J., McKenna, J.P., Menzies, F.D., McCullough, S.J., O'Neill, H.J., Wyatt, D.E., Cardwell, C.R., Coyle, P.V., 2010. *Coxiella burnetii* (Q fever) seroprevalence in cattle. Epidemiol. Infect. 138, 21-27.

Maurin, M., Raoult, D., 1999. Q fever. Clin. Microbiol. Rev. 12(4), 518-553.

Palmer, N.C., Kierstead, M., Key, D.W., Williams, J.C., Peacock, M.G., Vellend, H., 1983. Placentitis and Abortion in Goats and Sheep in Ontario Caused by *Coxiella burnetii*. Can. Vet. J. 24, 60-61.

Peter, O., Dupuis, G., Peacock, M.G., Burgdorfer, W., 1987. Comparison of enzyme-linked immunosorbent assay and complement fixation and indirect fluorescent-antibody tests for detection of *Coxiella burnetii* antibody. J. Clin. Microbiol. 25, 1063–1067.

Raju, N.R., Collings, D.F., Saville, P.H., 1988. Abortion in Black Belly Barbados Sheep in Fiji Caused by *Coxiella burnetii*. Australian Vet. J. 65, 225-226.

Rehacek, J., 1993. Rickettsiae and Their Ecology in the Alpine Region. Acta. Virol. 37, 290-301.

Rousset, E., Durand, B., Berri, M., Dufour, P., Prigent, M., Russo, P., Delcroix, T., Touratier, A., Rodolakis, A., Aubert, M., 2007. Comparative diagnostic potential of three serological tests for abortive Q fever in goat herds. Vet. Microbiol. 124, 286-297.

Ruiz-Fons, F., Astobiza, I., Barandika, J.F., Hurtado, A., Atxaerandio, R., Juste, R.A., Garcia-Perez, A.L., 2010. Seroepidemiological study of Q fever in domestic ruminants in semi-extensive grazing systems. Bmc Vet. Res. 6.

Waldham, D.G., Delong, W.J., Lincoln, S.D., Anderson, B.C., Woodard, L.F., Bulgin, M.S., Hall, R.H., 1982. Food Animal Research at the University-Of-Idaho. Mod. Vet. Pract. 63, 102-105.

Woldehiwet, Z., 2004. Q fever (coxiellosis): epidemiology and pathogenesis. Res. in Vet. Scie. 77, 269.

Zeman, R.K., Dritschilo, A., Silverman, P.M., Clark, L.R., Garra, B.S., Thomas, D.S., Ahlgren, J.D., Smith, F.P., Korec, S.M., Nauta, R.J., Dillon, J.S., 1989. Dynamic Ct Vs 0.5 T Mr Imaging in the Detection of Surgically Proven Hepatic Metastases. J. Comput. Assis. Tomogr. 13, 637-644.

CHAPTER 3 (MANUSCRIPT 2)

RELATIONSHIP OF *COXIELLA BURNETII* ANTIBODY STATUS OF DAM WITH CALF DEATH OF DANISH DAIRY CATTLE

SYNOPSIS

Background

Investigation of association has become state of art and considered as fundamental for epidemiological investigations of diseases. Understanding of association provides information diseases control and prevention strategies, and helps generating hypothesis on risk factors where the mechanism of association between risk factor ant outcomes can be calculated. *Coxiella burnetii* infection in domestic animal is common in whole world. This organism is highly fatal for sheep and goat for producing reproductive disorders. Q fever during pregnancy cause reproductive disorder like spontaneous abortion and low birth weight in humans. In dairy cattle, *C. burnetii*, antibody seropositivity is related with reproductive problems. *C. burnetii* is a cause of sporadic abortion in cattle. *C. burnetii* infection in domestic animal of Denmark is endemic in nature and in dairy cattle it is increasing into herd as well as animal level. However, association between risk factors of*Coxiella burnetii* antibodyand outcome reproductive disorders like calf death, yet now not been studied in individual dairy cattle of Denmark. Moreover the association between *C. burnetii* infection and its impact on reproductive disorders is an important issue for dairy cattle industry.

For these reasons, the present study was done with the objective of to evaluate the relationship of antibody status of *C. burnetii* in Danish dairy cows with calf death, delivery condition of cow and birth size of calf.

The study

Twenty two herds were selected on the basis of the *C. burnetii* antibody level in bulk tank milk (BTM) sample. Ten herds were BTM positive, nine herds were BTM negative and three herds were in BTM intermediate. A total of 3974 milk samples from 2103 dams from the selected herds were then collected in three different time periods: August-October 2008 (Time 1), January-February 2009 (Time 2) and April-June 2009 (Time 3).Data on the calving were collected from Danish Cattle Database. Individual cow information extracted from Danish Cattle Database included record of some target variables related with our hypothesis. These variables in the dataset were birth condition, birth course of cow, birth size of calf, breed and parity. The S/P values of individual milk sample and herd condition were taken from a previous study (Paul et al., 2012). Then the two dataset were merged for finding our target variables related with our hypothesis. Three response variables were selected to evaluate the dam antibody status in the present study. The distribution of all categorical response variables were studied and the variables 'calf death', 'delivery condition of cows' and 'birth size of calf' were re-categorized into two level according to their frequency distribution. Status changes based on the S/P values in between time was considered as main exposure variable and this was named as 'dam antibody statuses'. Univariable and multivariablelogistic regression analyses were performed for quantifying the effect of exposure variables on the categorical response variables using PROC GLIMMIX in SAS version 9.2 (SAS institute Inc., USA). Three different logistic models were constructed for three different outcome variables.

Principal findings and Significance

The proportion for calf death was detected 0.21 (435/2102), for delivery condition with assistance 0.26 (407/1554) and for birth size of small 0.49(769/1571) in total sampled dairy cattle 2103. There was no significant association between dam antibody status or parity or breed on calf death or

delivery condition or birth size in the univariable and multivariablerandom effect logistic regression models. A significant association was found between herd condition and delivery condition in in bothmodels. A significant random effect of herd in different models was observed. It is concluded that dam antibody status of *C. burnetii* appears to be non-related with perinatal calf mortality, delivery condition and birth size. There is significant random effect of herd on calf death, indicates that some herds performed more calf death than the others.The above mentioned results indicates that, in Denmark, *C. burnetii* antibody level is not an important factor for calf deaths and these calf deaths are related with other herd level variables.

Relationship of *Coxiella burnetii* antibody status of dam with calf death of Danish dairy cattle

ABSTRACT

Coxiella burnetii (*C. burnetii*) infection occurs worldwide in dairy cattle. However, no publication was found about association between dam antibody status and calf death, delivery condition and birth size in Danish dairy cattle. Therefore, we aimed to evaluate the relationships of calf death, delivery condition and birth size with dam *C. burnetii* antibody status in individual level of Danish dairy cattle. Twenty two herds were selected on the basis of the *C. burnetii* antibody level in bulk tank milk (BTM) sample. Ten herds were BTM positive, nine herds were BTM negative and three herds were in BTM intermediate. A total of 3974 milk samples from 2103 dams from the selected herds were then collected in three different time periods: August-October 2008 (Time 1), January-February 2009 (Time 2) and April-June 2009 (Time 3). Individual cow information was extracted from the Danish Cattle Database and a cow was considered test positive at S/P values ≥40, and otherwise negative. We conducted univariable and multivariable logistic regression analyses with random effect of herd to evaluate the effect of dam antibody status on different response variables. The proportion for calf death was detected 0.21 (435/2102), for delivery condition with assistance 0.26 (407/1554) and for birth size of small 0.49(769/1571) in total sampled dairy cattle 2103. There was no significant effect of parity or breed on calf death, delivery condition or birth size in the univariable random effect logistic regression models. A significant association was found between herd condition and delivery condition in a similar model. Final multivariable random effect logistic regression models showed no significant effect of antibody status of the dam on calf death, birth size and delivery condition but significant relationship was found between herd condition and delivery condition. A significant random effect of herd in different models was observed. It is concluded that dam antibody status of *C. burnetii* appears to be non-related with perinatal calf mortality, delivery condition and birth size.

Key words: *Coxiella burnetii*, dam antibody status, calf death, delivery condition, calf birth size

1. INTRODUCTION

Q fever is an important zoonotic disease worldwide, caused by *Coxiella burnetii* (*C. burnetii*), which is an obligate intracellular bacterium (Arricau-Bouvery and Rodolakis, 2005). The role of *C. burnetii*in cattle reproduction is still controversial. The infection is generally subclinical in animals (e.g. cattle, sheep and goat), although abortions in late pregnancy, stillbirths and the delivery of weak offspring, retained placenta, endometritis, infertility and low birth rates may occur (Rodolakis, 2006; Arricau-Bouvery and Rodolakis, 2005; McQuiston et al., 2002; Sanford et al., 1994).

In dairy cattle, *C. burnetii*, antibody seropositivity is associated with reproductive problems (Khalili et al., 2011). *C. burnetii* is a cause of sporadic abortion in cattle (Jensen et al., 2007; Rady et al., 1985). Several studies reported abortion, placentitis, infertility, and other reproductive disorders in cattle. Stillbirth, lower birth-weight and delivery of weak offspring are the most common clinical manifestations in neonatal calves (Bildfell et al., 2000; Hassig and Lubsen, 1998; Babudieri, 1959; To et al., 1998). In cattle abortion occurs at the end of gestation without showing other specific clinical signs (Tainturier, 1987). The seroprevalence of *C. burnetii* in aborting cows was estimated higher than that in non-aborting cows (Ni et al., 2011).The seroprevalence of *C. burnetii* in aborted cattle was observed statistically significant in a case-control study when compared the case and control groups (Cabassi et al., 2006). But there was no evidence of association between the seropositivity of *C. burnetii* and reproductive disorders in cows (Gazyagci et al., 2011).

A previous herd level study byNielsen et al. (2011)showed no association between the levels of *C. burnetii* antibodies in BTM and perinatal calf mortality was observed in Danish dairy cattle herds.

Therefore the objective was to study the relationship of *C. burnetii* antibody status in individual cows with calf death, delivery condition of cow and birth size of calf.

2. MATERIALS AND METHODS

2.1. Study population and sampling strategy

The study included 2103 lactatingcows from 22 herds (Figure1) and was a continuation of the study conducted by Agger et al. (2010). In the initial study the level of *C. burnetii* antibodies were determined in one bulk tank milk (BTM) sample from 100 randomly selected Danish dairy herds (Figure 1). The samples were tested using the CHEKIT Q-Fever Antibody ELISA Test Kit (IDEXX, Liebefeld-Bern, Switzerland) based on *C. burnetii* inactivated phase 1 and phase 2 antigens. The results were expressed as S/P values and estimated as S/P = (optical density (OD) $_{\text{sample}} - \text{OD}_{\text{negative control}}$)/ ($\text{OD}_{\text{positive control}} - \text{OD}_{\text{negative control}}$)) x 100. According to the manufacturer's recommendation herds with S/P ≥40 in BTM samples were considered positive and herds with BTM S/P <30 were considered negative, whereas herds with BTM S/P between 30 to 39 were considered to be intermediate.

After this initial study we selected 10 positive, 9 negative and 3 intermediate herds from three groups following stratified systematic random sampling procedure. This study was a prospective cross sectional study with follow up.Individual milk samples were collected in all lactating cows in August-October 2008 (Time 1 or T1), in January-February 2009 (Time 2 or T2) and in April-June 2009 (Time 3 or T3); i.e.Cows were sampled at intervals of three to seven months.

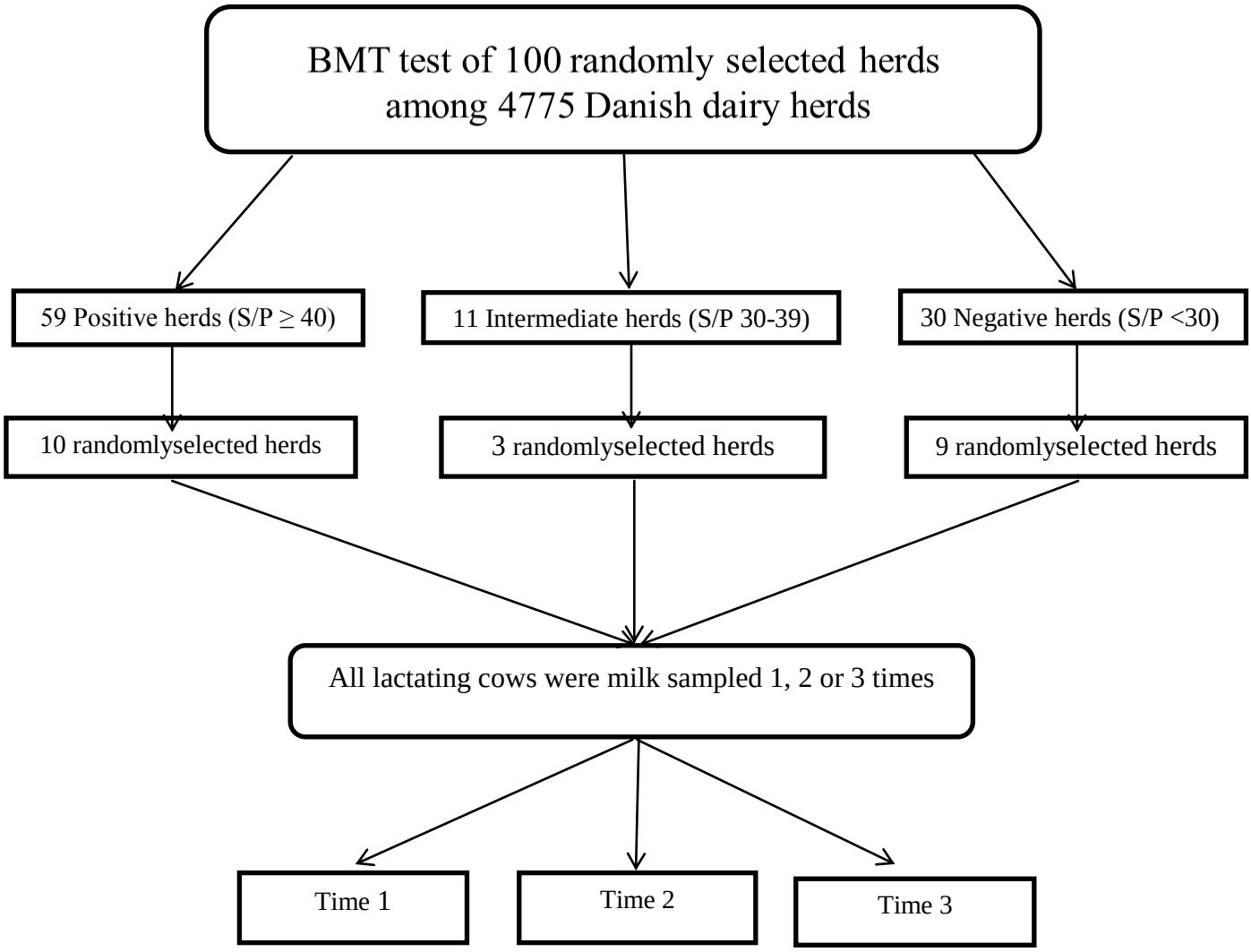


Figure 1 Diagram of design and data collection of prospective cross sectional study from August 2008 to June 2009 involving 2103 cows from 22 Danish dairy herds.

The milk samples were tested for the level of *C. burnetii* antibodiesby using the same test as used for BTM samples. A cow milk sample with S/P ≥40 was considered positive, and otherwise negative. All lactating cows in each herd were thus sampled one, two or three times. A total of 3974 milk samples were collected and analyzed from August 2008 to June 2009.

2.2 Data collection and management

Calving data were collected retrospectively from the Danish Cattle Database. According to Danish legislation, calving data must be recorded continuously by the farmer as part of the routine herd

management, and stored in the Danish Cattle Database. Individual cow information extracted from Danish Cattle Database included record of some target variables related with our hypothesis. These variables in the dataset were birth condition, birth course of cow, birth size of calf, breed and parity. The S/P values of individual milk sample and herd condition were taken from a previous study (Paul et al., 2012). Then the two dataset were merged for finding our target variables related with our hypothesis.

Three response variables were selected to evaluate the dam antibody status in the present study. The distribution of all categorical response variables were studied and the variables 'calf death', 'delivery condition of cows' and 'birth size of calf' were re-categorized into two levels according to their frequency distribution. The calves which got the eartag number within one week were considered as live (1667) and all other groups were considered as dead (435) for variable 'calf death'. Calving without help was considered as without assistance (1147) and calving of all other groups considered as with assistance (407) for variable 'delivery condition of cows'. Birth size of calf was considered as small size (769) and large size (802) based on less than normal size and more than normal size respectively for variable 'birth size of calf'. All of these above mentioned categories were registered in Danish Cattle Database. There were some missing values for variables of our final dataset, 1 for 'calf death', 549 for 'delivery condition of cows' and 532 for 'birth size of calf'.

Status changes based on the S/P values in between time was considered as main exposure variable and this was named as 'dam antibody statuses'. Each cow was considered to change their antibody status in 4 ways according to S/P values in the time between T1 and T2, in-between T1 and T3, and between T2 and T3. The conversion of antibody status was considered 'persistently negative' with no conversion from negative to positive infection status. When a cow status was converted from negative to positive infection, it was considered as 'incidence group', Conversion from a positive to

negative status was given the status 'recovery group' and when there was no conversion from positive to negative, 'Persistently positive'. In addition todam antibody status other explanatory variables were selected for inclusion in to the model to account for their confounding effect. These were parity with two categories (Parity ≤2 and Parity>2), herd condition with three categories (positive, intermediate and negative) and breed with two categories (Holstein and other).

Calving records were restricted to a period of 13th August 2008 to 30th June 2009 because first sampling was started from 13th August 2008 and we considered the last sampling date for our research that was 30th June 2009. By this time we were found 268 calving between T1 and T2, 232 calving between T2 and T3 and 90 calving between T1 and T3. Animals that were only sampled in T1 and in T3 was considered for between T1 and T3 calving. Finally we found 590 between times calving combined.

2.3 Statistical analysis

Descriptive analyses of the qualitative and quantitative exposure variables (parity, breed and herd condition) were performed to explore the distribution of different exposure variables according to dependent variables. Exposure variables were selected according to the study objective and hence included in the analysis. Correlation among the exposure variables was checked for the multivariable analysis to avoid multicollinearity. Univariable and multivariablelogistic regression analyses were performed for quantifying the effect of exposure variables on the categorical response variables using PROC GLIMMIX in SAS version 9.2 (SAS institute Inc., USA). Hierarchical structure of the data was accounted for in the logistic regression analyses where 'herd' was used as random variable. In multivariable analysis, backward elimination procedures were used. During backward elimination procedures dam antibody status was restricted but use of others variables for backward elimination. Though the analysis of univariable model did not give the significant relationship between explanatory and response variable we did multivariable model

analysis because small number of variables were used in the total analysis. Finally in backward elimination procedures we kept dam antibody status restricted and use of others variables for backward elimination. Three different logistic models were constructed for three different outcome variables. Then finally these models were used for these analyses.

i)Logit (Prob. for calf death=2) = Fixed (parity$_i$, breed$_i$ and dam antibody status$_{i,}$) + A (herd$_j$).

ii) Logit (Prob. for delivery condition=2) = Fixed (parity$_i$, breed$_i$ and dam antibody status$_{i,}$) + A (herd$_j$).

iii)Logit (Prob. for birth size=1) = Fixed (parity$_i$, breed$_i$ and dam antibody status$_{i,}$) + A (herd$_j$).

Results are reported as odds and compared using odds ratio.

3. RESULTS

In the descriptive analysis, we found that the proportion for calf death was 0.21 (435/2102), for delivery condition with assistance 0.26 (407/1554) and for birth size of small 0.49 (769/1571) in total sampled dairy cattle 2103 (Table 1).

Table 1 Proportion of perinatal dead calf, assistance delivery of cow and small size of calf in prospective cross sectional study from August 2008 to June 2009 involving 2103 cows from 22 Danish dairy herds.

Variables	Success	Total	Proportion
Calf death	435	2102	0.21
Delivery condition of cow	407	1554	0.26
Birth size of calf	769	1571	0.49

3.1. Estimation of associations

Results of the univariable logistic regression analysis with random effect of herd are shown in Tables 2, 3 and 4 for presenting the parameter estimates of the fixed effects of parity, herd condition and breed on calf death, delivery condition and birth size.

Table 2 Univariable logistic regression analysis of relationship between calf deaths with parity, herd condition and breed adjusted for random effect herd in prospective cross sectional study from August 2008 to June 2009 involving 590 cows from 22 Danish dairy herds.

Exposure	Comparison	Total	Success	**Odds ratio (95% *CI)	P-value
Parity	Parity ≤2	250	36	1	0.09
	Parity>2	340	63	1.61(0.92-2.82)	
Herd condition	Negative	126	30	1	0.47
	Intermediate	51	2	0.16(0.01-3.48)	
	Positive	413	67	0.79(0.13-4.95)	
Breed	Other	221	76	1	0.68
	Holstein	369	23	0.77(0.18-3.28)	

**Odds ratio result was taken from logistic regression model;* CI, Confidence interval

Table 3 Univariable logistic regression analysis of relationship between delivery conditions with parity, herd condition and breed adjusted for random effect herd in prospective cross sectional study from August 2008 to June 2009 involving 590 cows from 22 Danish dairy herds.

Exposure	Comparison	Total	Success	**Odds ratio (95% *CI)	P-value
Parity	Parity ≤2	212	39	1	0.58
	Parity>2	284	47	0.87(0.48-1.53)	
Herd condition	Negative	122	1	1	0.03
	Intermediate	47	11	40.07(1.20-806.20)	
	Positive	327	74	43.39(3.01-624.70)	
Breed	Other	179	9	1	0.45
	Holstein	317	77	1.78(0.29-10.77)	

**Odds ratio result was taken from logistic regression model;* CI, Confidence interval

Table 4 Univariable logistic regression analysis of relationship between birth size with parity, herd condition and breed adjusted for random effect herd in prospective cross sectional study from August 2008 to June 2009 involving 590 cows from 22 Danish dairy herds.

Exposure	**Comparison**	**Total**	**Success**	****Odds ratio(95% *CI)**	**P-value**
Parity	Parity ≤2	214	95	1	0.67
	Parity>2	286	127	1.09(0.72-1.66)	
Herd condition	Negative	122	53	1	0.70
	Intermediate	49	18	0.79(0.15-4.18)	
	Positive	329	151	1.42(0.42-4.82)	
Breed	Other	182	81	1	0.83
	Holstein	318	141	0.90(0.29-2.86)	

**Odds ratio result was taken from logistic regression model;* CI, Confidence interval

There were no significant effect of parity and breed on calf death; delivery condition and birth size. But in one model of that's we found significant relationship between delivery condition and herd condition. The odds of being Parity>2 was 1.61 times higher compared to Parity ≤2 in case of calf death.There was 1.78 times higher odds of Holstein breed compared to other breed in delivery condition. And in birth size the odds of being positive herds were 1.42 times higher compared to negative herds.

The final models of logistic regression with calf death, birth size and delivery condition as the respective responses and the explanatory variable dam antibody status and herd condition with random effect of herd are shown in Table 5.

Table 5 Final multivariable logistic regression analysis of relationship between calf death, delivery conditions and birth size with dam antibody statuses of *Coxiella burnetii* and herd condition adjusted for random effect herd in prospective cross sectional study from August 2008 to June 2009 involving 590 cows from 22 Danish dairy herds.

Exposure variables		Perinatal calf death				Delivery condition				Birth size			
		Total	Success	**Odds ratio (95%*CI)	P-value	Total	Success	Odds ratio (95%CI)	P-value	Total	Success	Odds ratio (95%CI)	P-value
Dam antibody status													
	Persistently negative	392	65	1		342	63	1		346	159	1	
	Incidence	53	14	1.10(0.40-3.08)	0.41	47	6	0.99(0.31-3.11)	0.81	46	18	0.72(0.32-1.63)	0.60
	Recovery	52	12	0.87(0.36-2.07)		38	7	1.60(0.54-4.69)		37	12	0.86(0.38-1.93)	
	Persistently positive	93	8	0.48(0.19-1.22)		69	10	0.90(0.37-2.21)		71	33	1.30(0.68-2.46)	
Herd condition													
	Negative					122	1	1					
	Intermediate					47	11	40.02(1.91-837.18)	0.03				
	Positive					327	74	43.12(2.92-637.94)					

**Odds ratio result was taken from logistic regression model;* CI, Confidence interval

There were no significant effects of dam antibody status oncalf death, birth size and delivery condition. However, we found a significant relationship between delivery condition and herd conditions (P <0.02) in this model. Later we have done some further analysis by splitting the dataset based on herd condition positive, negative and intermediate with an assumption that the positive herd's animals have more chance of calf death. There was no significant effect of dam antibody status on calf death, delivery condition, and birth size in positive herds. However, significant relationship between dam antibody status and delivery condition in negative herds was observed (P <0.0001). There was another significant relationship between dam antibody status and calf death in intermediate herds (P <0.0001). The odds were higher for incidence and lower for other cases when compared with persistently negative in calf death. In delivery condition the odds was 1.60 times higher for recovery than persistently negative. On the other hand in birth size the persistently positive group had 1.30 times higher odds than persistently negative group. There was a significant random effect of herds (P<0.0001) for calf death, delivery condition and birth size in relation with persistently *C. burnetii* antibody status.

4. DISCUSSION

To the best of our knowledge this is the first study to investigate relationship of calf death, delivery condition and birth size with *C. burnetii* dam antibody status in individual level of Danish dairy cattle. Though C. *burnetii* antibody status is an endemic phenomenon in Denmark but the relationship with birth size and calf death as well as delivery condition related with dam *C. burnetii* antibody status was not studied before. Thus, the present study has been conducted.

Between time *C. burnetii* antibody changing status of dam milk samples were given as dam status of persistentlynegative, incidence group, recovery group and persistently positive based on S/P values status. We did not selected *C. burnetii* antibody positivity as an explanatory variable because of restriction of one state but our interest was looking all states between times. On the other hand,

we have selected the dam antibody status as an explanatory variable for finding more details about every states including *C. burnetii* antibody positive state. Besides, we have looked for between time calving conditions in our research, this also supports for dam antibody status as a main explanatory variable.

There is significant random effect of herd on calf death, indicates that some herds performed more calf death than the others. We have not found significant relationship between calf death and the antibody status of dam. One of the multi-level logistic regressions study was done before in Denmark on stillbirth and perinatal calf mortality related with BTM *C. burnetii* antibody by Nielsen et al., 2011. In this study they have found level of antibodies to *C. burnetii* in BTM were highly correlated within herds but changes in BTM antibody levels have not found to be associated with neither risk of stillbirth nor the risk of perinatal calf mortality. Results of the present study are similar to that of the early study - though it is an individual animal level study and was looked for the association between the perinatal deaths of calf with dam antibody status. This means that C. *burnetii* antibody level is not important factor for calf death. Significant random effect of herds indicates that the calf death is related with other herd level variables.

An earlier study (Khalili et al., 2011) showed that, cattle with reproductive problems showed a higher prevalence (51.35%) in comparison to apparently healthy cattle (10.3%) of anti-*C. burnetii* antibodies, and the association was significant. In Turkey, the seroprevalence of coxiellosis has been found significantly higher in cattle with an abortion history (22.6%) than in those without abortion history (5.6%) by Seyitoglu et al.(2006). The seroprevalence of *C. burnetii* in healthy cattle ranges from 2% to 46%, while in cattle with reproductive disorders; the range was 60% to 84%, in Japan (Hirai and To, 1998). Bildfell et al. (2000) reported that bovine placentitis was highly associated with the presence of *C. burnetii*. Metritis is a unique manifestation of the disease in cattle (To et al., 1998). It seems that high seropositivity obtained in these cattle with reproductive

problems has a correlation with *C. burnetii* antibody. But in this study, all the outcome variables related with reproductive problems were not significant according to dam C. *burnetii* antibody status. These differences may be explained by geographic and climatic varieties, size of sampling population, definition of the cow population, assay type, or criteria used to cut-off positive values. Without this, the hypothesis of present study is not exactly the same like others studies. In this study we are looking for relationships of calf death, delivery condition and birth size with dam *C. burnetii* antibody status in individual level of Danish dairy cattle.

There was a finding by To et al.(1998) that, stillbirth and lower birth-weight are the most common clinical manifestations in neonatal calves. Arricau-Bouvery and Rodolakis (2005) described the infection is generally subclinical in animals, although abortions at late pregnancy, stillbirths and the delivery of weak offspring, and cases of retained placenta, endometritis, infertility and low birth rates may occur. However, in the present study birth size and perinatal calf mortality as well as delivery condition was not found to have significant relationship with dam *C. burnetii* antibody status. This seemingly because of those variables were not only related with *C. burnetii* antibody status but also related with others organism as well as management system of dairy cattle.

The calf death is an obligatory parameter for farmers to record and report to Danish Cattle Database. For this reason we did not find missing value in calf death which is named as birth condition in Danish Cattle Database. On the other hand there are some missing values for birth size and delivery condition in dataset, because it is not mandatory for famers to enlist this information. These missing values may be due to the fact that farmers did not enlist these variable values properly in the Danish Cattle Database. In addition, there is some missing when farmers are not interested to enlist birth size and delivery condition information when calf is dead. However, due to the systemic recording, farmers enlist most of the important parameters in their recording properly, though it is not mandatory. In further analysis where we split the dataset based on herd conditions

(positive, negative and intermediate), we have found some significant results. But those results are not authentic enough for small sample sizes, which indicate that the statistical power is insufficient. There was some selection bias due to not taking the herd samples in exact proportions as proportions of positive, negative and intermediate herds. Further studies based on the isolation of *C. burnetii* are needed to elucidate the etiologic role of this microorganism in the calf death, delivery condition and birth size relation with dam *C. burnetii* antibody status in Denmark.

5. CONCLUSION

There is no significant relationship of calf death, delivery condition and birth size with dam *C. burnetii* antibody status in individual level of Danish dairy cattle. In conclusion, different herds have the significant relationship but animal level variables are not significantly related with perinatal calf mortality, birth size as well as delivery condition. The above mentioned results indicates that, in Denmark, *C. burnetii* antibody level is not an important factor for calf deaths and these calf deaths are related with other herd level variables.

Acknowledgement

The study was funded by Kvaegafgiftsfonden (grant number) and the Faculty of Life Sciences, University of Copenhagen as part of a PhD project. The authors gratefully acknowledge the breeders for participating in the study and the technical staff at the National Veterinary Institute, Technical University of Denmark for analysing the samples. I also acknowledge Jørgen Nielsen who supplied our target data form the Danish Cattle Database.

References

Agger, J.F., Christoffersen, A.B., Rattenborg, E., Nielsen, J., Agerholm, J.S., 2010. Prevalence of Coxiella burnetii antibodies in Danish dairy herds. Acta Veterinaria Scandinavica 52.

Arricau-Bouvery, N., Rodolakis, A., 2005. Is Q Fever an emerging or re-emerging zoonosis? Veterinary Research 36, 327-349.

Babudieri, 1959. Q fever: a zoonosis. Adv. Vet. Sci. 5, 81-154.

Bildfell, R.J., Thomson, G.W., Haines, D.M., Mcewen, B.J., Smart, N., 2000. Coxiella burnetii infection is associated with placentitis in cases of bovine abortion. Journal of Veterinary Diagnostic Investigation 12, 419-425.

Cabassi, C.S., Taddei, S., Donofrio, G., Ghidini, F., Piancastelli, C., Flammini, C.F., Cavirani, S., 2006. Association between Coxiella burnetii seropositivity and abortion in dairy cattle of Northern Italy. New Microbiologica 29, 211-214.

Gazyagci, S., Aktas, M.S., Kilic, S., Babur, C., Celebi, B., Duru, S.Y., 2011. Seroprevalence of Q fever in dairy cattle in the Konya province, Turkey. Revue de Medecine Veterinaire 162, 387-390.

Gilsdorf, A., Kroh, C., Grimm, S., Jensen, E., Wagner-Wiening, C., Alpers, K., 2008. Large Q fever outbreak due to sheep farming near residential areas, Germany, 2005. Epidemiol. Infect. 136, 1084–1087.

Guatteo, R., Seegers, H., Taurel, A.F., Joly, A., Beaudeau, F., 2011. Prevalence of Coxiella burnetii infection in domestic ruminants: a critical review. Vet. Microbiol. 149, 1–16.

Hassig, M., Lubsen, J., 1998. Relationship between abortions and seroprevalences to selected infectious agents in dairy cows. Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health 45, 435-441.

Hirai, K., To, H., 1998. Advances in the understanding of Coxiella burnetii infection in Japan. Journal of Veterinary Medical Science 60, 781-790.

Jensen, T.K., Montgomery, D.L., Jaeger, P.T., Lindhardt, T., Agerholm , J.S., Bille- Hansen ,V., Boye, M., 2007. Application of fluorescent in situ hybridisation for demonstration of Coxiella burnetii in placentas from ruminant abortions. APMIS, 115:347-353.

Khalili, M., Sakhaee, E., Babaei, H., 2011. Frequency of anti-*Coxiella burnetii* in cattle with reproductive disorders. Comp Clin Pathol., 1-3:DOI:10.1007/s00580-011-1199-2

Maurin, M., Raoult, D., 1999. Q fever. Clin. Microbiol. Rev. 12, 518–553.

McCaughey, C., Murray, L.J., McKenna, J.P., Menzies, F.D., McCullough, S.J., O'Neill, H.J., Wyatt, D.E., Cardwell, C.R., Coyle, P.V., 2010. Coxiella burnetii (Q fever) seroprevalence in cattle. Epidemiology and Infection 138, 21-27.

McQuiston, J.H., Childs, J.E., Thompson, H.A., 2002. Q fever. Journal of the American Veterinary Medical Association 221, 796-799.

Muskens, J., Wouda, W., von Bannisseht-Wijsmuller, T., van Maanen, C., 2012. Prevalence of Coxiella burnetii infections in aborted fetuses and stillborn calves. Veterinary Record 170, 260-+.

Ni, H.B., Liu, S.G., Jiang, H.F., Wang, C.R., Qian, A.D., 2011. Seroprevalence of Q fever in dairy cows in northeastern China. African Journal of Microbiology Research 5, 3964-3967.

Nielsen, K.T., Nielsen, S.S., Agger, J.F., Christoffersen, A.B., Agerholm, J.S., 2011. Association between antibodies to Coxiella burnetii in bulk tank milk and perinatal mortality of Danish dairy calves. Acta Veterinaria Scandinavica 53.

Rady, M., Glavits, R., Nagy, G., 1985. Demonstration in Hungary of Q-Fever Associated with Abortions in Cattle and Sheep. Acta Veterinaria Hungarica 33, 169-176.

Rodolakis, A., 2006. Q fever, state of art: Epidemiology, diagnosis and prophylaxis. Small Ruminant Research 62, 121-124.

Sanford, S.E., Josephson, G.K.A., Macdonald, A., 1994. Coxiella-Burnetti (Q-Fever) Abortion Storms in Goat Herds After Attendance at An Annual Fair. Canadian Veterinary Journal-Revue Veterinaire Canadienne 35, 376-378.

Seyitoglu, S., Ozkurt, Z., Dinler, U., Okumus, B., 2006. The seroprevalence of coxiellosis in farmers and cattle in Erzurum district in Turkey. Turkish Journal of Veterinary & Animal Sciences 30, 71-75.

Tainturier, D., 1987. Recurrent Metritis Due to Q-Fever in Cows. Recueil de Medecine Veterinaire 163, 195-198.

To, H., Htwe, K.K., Kako, N., Kim, H.J., Yamaguchi, T., Fukushi, H., Hirai, K., 1998. Prevalence of Coxiella burnetii infection in dairy cattle with reproductive disorders. Journal of Veterinary Medical Science 60, 859-861.

Villumsen, S., Jorgensen, C.S., Smith, B., Uldum, S., Schiellerup, P., Krogfelt, K.A., 2009. Determination of new cut-off values for indirect immunofluorescence antibody test for Q fever diagnosis in Denmark. Diagn. Microbiol. Infect. Dis. 65, 93–98.

CHAPTER 4

GENERAL DISCUSSION

SYNOPSIS

This chapter synthesizes the knowledge derived from the previous chapters and discusses their practical relevance to the estimation of general frequencies of *Coxiella burnetii* antibody in Danish dairy cattle and association between *Coxiella burnetii* antibody of dam and their offspring. The thesis suggests that's in Denmark inference for estimation of general epidemiology prevalence, incidence and recovery of *Coxiella burnetii* antibody in Danish dairy cattle. In this study result also suggest about diagnostic test result with separate cut-off values. In association study make the inference association between *Coxiella burnetii* antibody of dam and their offspring.

In Denmark the above results could be useful for knowing the present status, spread and recovery of *Coxiella burnetii* antibody in Danish dairy cattle as well as ELISA test quality for diagnosis and the best cut-off value. Second study helps to identify the association between *Coxiella burnetii* antibody statuses of dam and calf death.

Finally these all result help to take prevention and control of *Coxiella burnetii* in Danish dairy cattle industry.

DISCUSSION

Coxiella burnetii is a significant pathogen for animal as well as human but, perhaps due to its ubiquity in all geographic locations, there has been no effort to develop integrated detection and control measures in the livestock production industry. The main goal of this study was to identify causes and diagnostic tests for infection with *Coxiella burnetii* in Danish dairy cattle, and associations between antibody status of mother cows and their offspring. The results showed the prevalence, incidence and recovery of *Coxiella burnetii* antibody status in individual level of Danish dairy cattle. Risk factors for *Coxiella burnetii* antibody also observed beside this diagnostic test evaluation and detection of good cut-off value were also detected. Association between reproductive disorder like calf death and dam antibody status of *Coxiella burnetii* was also studied.

This is the first time estimated prevalence, incidence and recovery of *Coxiella burnetii* antibody status in individual level of Danish dairy cattle. We find the available prevalent, incident and recovered dairy cattle. The result also showed prevalence and incidence varied from medium in calves to null in young and in heifers up to high in parity groups. Herd conditions and age groups have the significant effect on prevalence. The results of this also research also suggest that dairy cattle may be the most significant reservoir of *Coxiella burnetii* in Denmark and that dairy cattle may pose a significant threat for zoonotic transfer of this pathogen, particularly the people associated with the Dairy cattle industry. This study also helps to understand the epidemiology of *Coxiella burnetii* antibody status in Danish dairy cattle.

The Diagnostic test ELISA was used to detect *Coxiella burnetii* antibody. High sensitivity and specificity of this test was detected which considered the ELISA test as a good diagnostic test for detecting *Coxiella burnetii* antibody status in dairy cattle. The two different cut-off values of S/P were used for estimating prevalence, incidence and recovery but we did not find significant

difference between two cut-off values results. In this case we can suggest that S/P 40 can be used as a cut-off value which is suggested by the IDEXX Company.

Results from association between reproductive disorder like calf death and dam *Coxiella burnetii* antibody status showed that there were no significant association between calf death and dam antibody status. This result indicates that, in Denmark,*Coxiella burnetii* antibody level is not important factor for calf deaths and these calf deaths were related with other herd level variables.

Overall, these projects have identified frequency estimation, diagnostic test evaluation and association between calf death and dam antibody status of *Coxiella burnetii* antibody. These findings have implications for individual who come into contact with domestic ruminant like dairy cattle and will hopefully lead to new research into the epidemiology of Coxiella *burnetii* antibody in Denmark.Finally, the interplay between *Coxiella burnetii* and animals or human needs to be better understood.