Dutch Q fever epidemic in a 'One Health' context:

outbreaks, seroprevalence and occupational risks

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Dutch Q fever epidemic in a 'One Health' context:

outbreaks, seroprevalence and occupational risks

De Q-koorts epidemie in Nederland in een 'One Health' context:

uitbraken, seroprevalentie en beroepsgerelateerde risico's

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 17 april 2018 des middags te 2.30 uur

door

Barbara Schimmer

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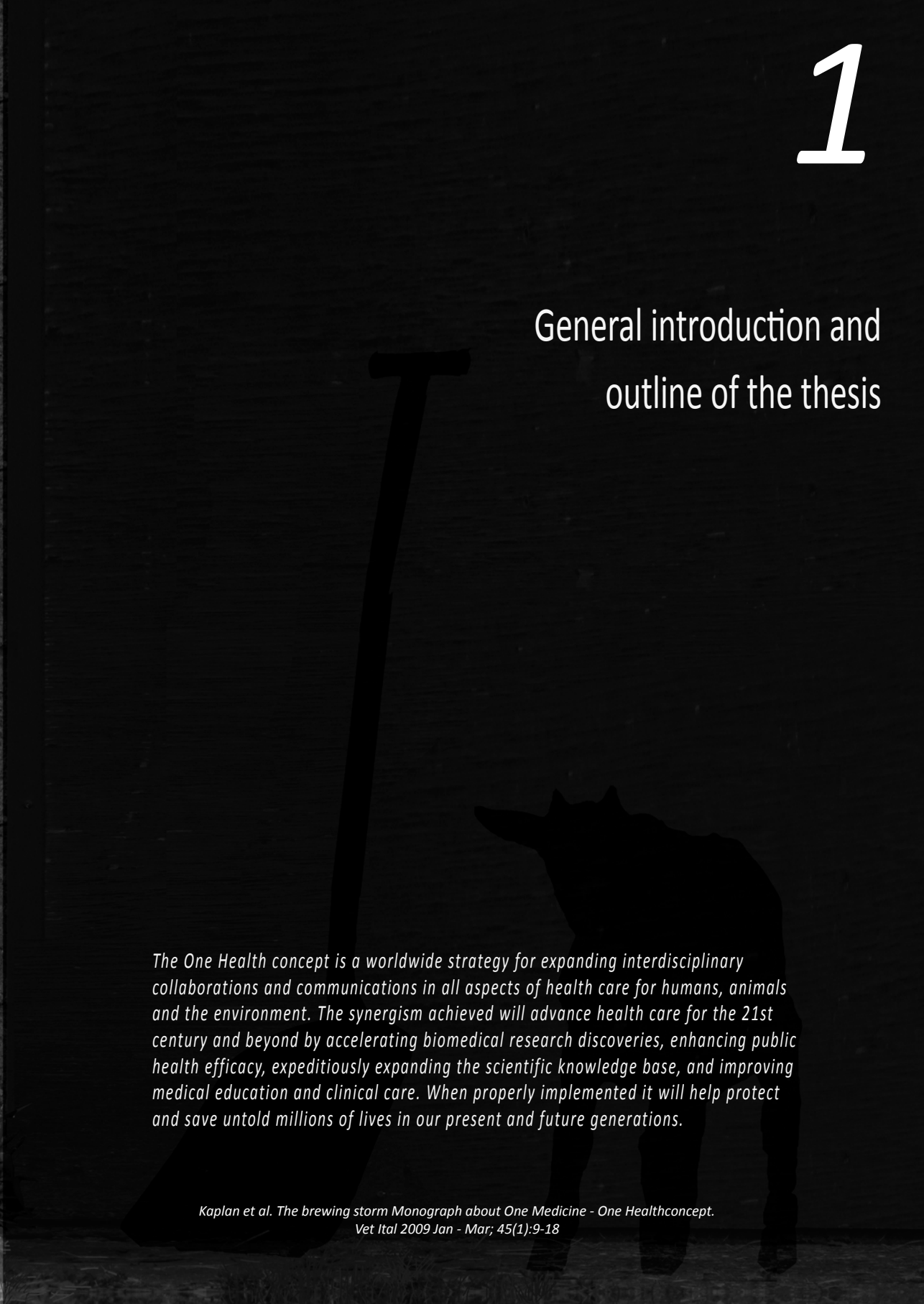


The One Health concept is a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of health care for humans, animals and the environment. The synergism achieved will advance health care for the 21st century and beyond by accelerating biomedical research discoveries, enhancing public health efficacy, expeditiously expanding the scientific knowledge base, and improving medical education and clinical care. When properly implemented it will help protect and save untold millions of lives in our present and future generations.

Kaplan et al. The brewing storm Monograph about One Medicine - One Healthconcept. Vet Ital 2009 Jan - Mar; 45(1):9-18

General Introduction

Coxiella burnetii and Q fever

Q fever is an almost ubiquitous zoonotic disease (1) caused by *Coxiella burnetii (C. burnetii)*, a small, obligate, intracellular bacterium with a Gram-negative cell wall (2). Q fever was first described by Edward Holbrook Derrick during his investigation of an outbreak of febrile illness with an unknown cause in abattoir workers in Brisbane, Australia in 1933 (3). The illness was given the name 'Query' (Q) fever and was applied at a time when the causative agent was unknown. Four years later, Herald Rea Cox discovered the bacterium in ticks in Montana in the United States (4), while Frank Macfarlane Burnet isolated the same pathogen from the blood and urine of the Australian patients (5). Both researchers are honored in the name *Coxiella burnetii.*

Animal reservoirs and transmission routes for human Q fever

C. burnetii can infect a broad range of vertebrate and invertebrate hosts. Domestic ruminants such as sheep, cattle and goats are considered to be the main reservoirs for human infection in most parts of the world. Cats, dogs, birds, rodents and other wildlife could also represent potential sources of human infections (6, 7). *C. burnetii* infections in domestic ruminants are often asymptomatic, but spontaneous abortions in late gestation (8), premature delivery, stillbirth and weak offspring in pregnant goats and sheep, do occur as well (9). *C. burnetii* is excreted in milk, urine, vaginal fluids and feces of infected ruminants, but most abundantly, up to 1 billion bacteria per gram, in amniotic fluids and the placenta released at abortion or parturition. Shedding routes and duration depend on ruminant type and presence of clinical signs (10-12), but can persist for months. Polymerase chain reaction (PCR)-testing of bulk tank milk (BTM) is a useful method to detect shedding of *C. burnetii* at farm level (13).

Humans usually become infected through inhalation of aerosolized particles released by *C. burnetii* infected animals. *C. burnetii* can persist for prolonged periods in the environment in an extracellular spore-like form being resistant to heat, drying, and many disinfectants. Dry and windy weather conditions can facilitate airborne spread of these bacterial aerosols over considerable distances (2, 14), resulting in patients with acute Q fever that do not report recent livestock contact and residing far from the original *C. burnetii*-contaminated areas. A possible, though controversial, mode of transmission is the digestive route through ingestion of raw-milk dairy products from *C. burnetii* infected livestock (15, 16). *C. burnetii* DNA has been detected in dairy products in various European countries. However this route seems to constitute a limited public health threat as no viable bacteria could be isolated in these products (17). *C. burnetii* and *Coxiella*-like bacteria have been detected in many tick species, however arthropod transmission has not been proven in humans (18, 19). Human-to-human transmission is only described in anecdotal reports and has rarely followed after transfusion of blood collected from Q fever patients with bacteremia, after bone marrow transplantation (20) and in nosocomial settings through respiratory spread or contact with *C. burnetii*-infected birth products of parturient women (21-23). Finally, sexual transmission via infected sperm has been suspected (24).

Clinical manifestations, laboratory diagnosis and sequelae of acute Q fever

Human Q fever can present with a wide spectrum of acute and chronic clinical manifestations. After exposure, 60% of infected persons will stay asymptomatic, while the other 40% usually develop symptoms within an incubation period of 2 to 3 weeks. The clinical presentation of acute Q fever is often non-specific, and likely to be underdiagnosed (25).

Acute Q fever usually presents as a self-limiting mild flu-like illness with abrupt onset of fever, chills, headache, fatigue, myalgia, cough, dyspnea, nausea or vomiting, and may be accompanied by pneumonia or hepatitis (14). In literature, approximately 2-5% of all symptomatic patients develop severe clinical manifestations that require hospitalisation, such as atypical pneumonia and acute hepatitis or infrequently, pericarditis, meningitis or myocarditis (14, 26).

Geographical and seasonal variations are observed in the main clinical presentations of acute Q fever. Pneumonia is the predominant presenting symptom in Germany, the Netherlands and Basque Territory while mainly hepatitis cases are diagnosed in France and southern Spain (14, 27, 28). These differences are probably based on different routes of infection (29), host factors, circulating *C. burnetii* strains and the infectious dose.Most primary infections respond well to antibiotic therapy with a two-week course of doxycycline (30), resulting in a reduced risk of hospitalisation if initiated

within the first week of symptoms (31). The case fatality is reported around 1% in hospitalized acute Q fever cases (26, 32), but has not been thoroughly studied in large epidemic settings. Symptomatic cases are more likely to occur in adults, and more in males than in females. Pregnant women are less likely to be symptomatic (33). Children seem to have milder disease (34) but may also have a lower rate of infection after community exposure compared to adults (35). Q fever fatigue syndrome (QFS) is the most frequently reported sequela in approximately 20% of patients with symptomatic acute Q fever (36). It is characterised by long-term incapacitating fatigue, according to some definitions at least during 6 months, with no sign of a persistent *C. burnetii* infection. The severity of the primary infection appears a predictor of long-term reduced health status (36). A randomized clinical trial comparing the efficacy of doxycycline and cognitive behavioral therapy (CBT) versus placebo for the treatment of QFS shows that CBT is effective in reducing fatigue severity. Long-term treatment with doxycycline does not reduce fatigue severity compared to placebo (37).

Laboratory diagnosis of acute Q fever

Various serological methods, such as immunofluorescence assay (IFA) test, complement fixation test (CFT) or enzyme-linked immunosorbent assay (ELISA) can be used to establish the diagnosis of acute Q fever (38). *C. burnetii* displays antigenic phase variation. Antibodies are expressed against phase 2 antigens during the acute infection and against phase 1 antigens in an established infection. For both antigens, IgM antibody production precedes IgG production, and thus three phases are distinguished in acute Q fever: a seronegative phase of 7 to 15 days after symptom onset, followed by IgM and IgG phase 2 seroconversion during the acute *C. burnetii* infection and subsequent IgM and IgG phase 1 seroconversion in the established infection. Molecular detection of *C. burnetii* DNA by PCR is an indispensable method for early diagnosis of acute Q fever as PCR is highly sensitive in serum during the first days after symptom onset, but rapidly declines to undetectable levels as the serologic response develops (39). Persistently high titers of IgG phase 1 antibodies are associated with chronic Q fever.

Chronic Q fever and persistent *C. burnetii* infection

Approximately 1-2% of symptomatic acute Q fever cases will develop chronic Q fever, a potentially lethal disease that manifests itself usually within the first year after primary infection, but can also present years later (40). Persons with underlying conditions, especially those with pre-existing cardiac valve defects, vascular grafts or aneurysms, immunocompromised and pregnant women, are at risk for progression to persistent focalized *C. burnetii* infection manifested as vascular infections or as latent endocarditis that may take 10-15 years to develop (41).
During the Dutch Q fever epidemic, vascular infections were the most common manifestation of a persistent infection, followed by endocarditis (42), in contrast to France where endocarditis is most common (43). There is a current lack of consensus whether the distinction between ‘acute’ and ‘chronic’ Q fever is still valid, as both the virulence of the *C. burnetii* strain and host-specific risk factors increasing host susceptibility, may influence the clinical presentation. Some researchers advocate for more precise qualifications of different clinical forms of Q fever and long-term complications of persistent infection (43).

Occupational Q fever and seroprevalence studies

Q fever is a recognized occupational zoonosis, as illustrated by the first description of Q fever in a population of slaughterhouse workers (3). Sporadic cases or occupational Q fever clusters have occurred in persons working with livestock or animal products, such as farmers, veterinarians, animal attendants, hunters, sheep shearers, wool workers and slaughterhouse workers. Also laboratory staff cultivating *C. burnetii* or doing animal experiments using sheep or sheep placentas (44) have occupational risk to acquire *C. burnetii* infection. Imported Q fever has been reported in military personnel returning from field missions in endemic areas (45). In occupational groups working with infected livestock or in a high-risk environment for *C. burnetii* transmission, infection may occur for example through exposure during parturition, manure and waste removal, slaughter, animal product handling and consumption of unpasteurized milk. However, the probability of transmission depends on the animal health status, different activities carried out by the employee, contact frequency with animals and animal products and preventive measures taken (46). Appropriate health education in veterinary-associated populations might also reduce the risk of infection (47).

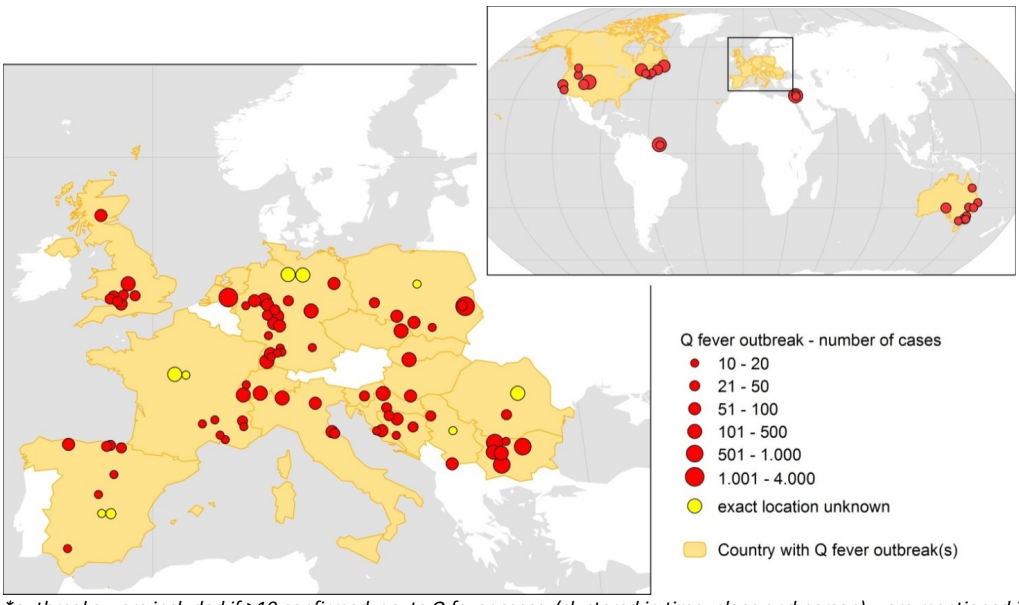
Physicians should consider the risk of infection with *C. burnetii* in patients with Q fever compatible symptoms, especially in those with potential occupational exposures (48). Seroprevalence studies assessing the presence of *C. burnetii* IgG phase 1 and phase 2 antibodies using IFA or ELISA have been carried out in various livestock-associated occupational groups, such as in farmers (49), agricultural workers (50), slaughterhouse workers (51), shepherds (52), veterinarians (53, 54), veterinary students (55, 56), foresters (57), hunters (58) and in workers processing livestock-associated products such as wool or hides (59). In general, seroprevalence estimates in occupational groups associated with livestock are much higher than estimates in the general population or in blood donors, reflecting the inherent occupational risks (44). However, it remains difficult to compare these estimates between occupational groups as different population samples, serological methods, diagnostic algorithms and cut-off values are used, reflecting a lack of standardization in interpretation of serology results (60, 61).

The epidemic potential of *C. burnetii*

C. burnetii is a pathogen with the potential to cause epidemics due to its relative ease of transmission, environmental stability, high virulence, low infective dose and airborne route of transmission (62). Because of these features, the Centers for Disease Control and Prevention (CDC) in the United States has classified *C. burnetii* as a category B biological threat agent (63). Seasonal patterns of human Q fever usually show a spring peak in endemic European countries reflecting the lambing season or peak after the rainy season in tropical areas, as the epidemiology of this zoonotic infection in humans reflects the circulation of *C. burnetii* in livestock and wildlife. Long-term changes in Q fever seasonal patterns may reflect changes in animal husbandry (64). Q fever outbreaks in community and occupational settings have been reported in Europe, North America, the Middle East and Australia *(Figure 1)*.

Infected sheep or goats herds are the most implicated animal outbreak source (65). To a much lesser extent, cattle, parturient cats and dogs, pigeons, fallow deer and wild rabbits have caused small Q fever clusters, mainly in household settings (66-70). Community outbreaks can occur in isolated rural regions, but can also affect numerous persons living in semi-rural or urban areas downwind from nearby ruminant grazing areas, livestock farms or abattoirs where *C. burnetii* has become aerosolized (71, 72).

*Figure 1. Location and size of acute Q fever outbreaks in Europe and worldwide, 1980-2015 (n=111)**



**outbreaks were included if ≥10 confirmed acute Q fever cases (clustered in time, place and person) were mentioned in published outbreak reports or research articles in English-language medical literature*

Point-source outbreaks have been reported after the annual Muslim sheep feast (73) or after large public gatherings where pregnant small ruminants were exhibited and lambed at the occasion, shedding large amounts of *C. burnetii* (74). Geographical clusters of human Q fever in the community are also reported where no definite animal source could be identified (33, 75, 76). Epidemiological investigations can be complicated by so-called universal exposure, by which most or all persons are exposed to the same suspected sources of infection (77). Occupational outbreaks have occurred in various high-risk settings, such as in abattoirs (71, 78, 79), research laboratories using sheep as experimental animals (80-82) or recently in staff working in a 'live cell therapy' clinic using sheep fetal cells for human injection (83). Outbreaks have also occurred in industrial settings where occupational exposure was unexpected such as in a renovated office due to contaminated strawboard (84), a cosmetics supply factory (85) or a waste-sorting plant with exposure to animal carcasses as urban waste (86).

Sporadic Q fever cases in the Netherlands: the situation until 2006

In 1955, Kaplan and Bertagna reported the existence of Q fever in 51 countries on five continents (87). It was concluded that the disease was not present in the Netherlands as 2411 human sera including 664 patients with atypical pneumonia, 524 cattle sera and 294 sera from guinea-pigs and rabbits tested negative in a complement fixation test (CFT) (88). The first case report describing Q fever in the Netherlands dates from 1956, describing 3 human cases, including one butcher, with an atypical pneumonia (89-91). Acute Q fever is a notifiable disease in the Netherlands since 1975. Until 2006, Q fever was rarely notified in The Netherlands, with an average of 17 cases (range 1-32) annually (92). The majority of these cases occurred in persons with an occupational risk, such as farmers and veterinarians. Unlike other European countries, such as France, Spain, Germany and the United Kingdom, no Q fever outbreaks had occurred in the Netherlands. Only a small cluster of imported Q fever occurred in Dutch tourists returning from a farm holiday in France in 2000 (93).

The Dutch Q fever epidemic, 2007-2009

An unprecedented large epidemic of Q fever caused by *C. burnetii* occurred in the Netherlands during three consecutive years from 2007 until 2009, resulting in the notification of more than 4,000 acute Q fever cases and a substantial disease burden in the community (94, 95) *(Figure 2).* On the basis of the notifications and seroconversions in blood donors, there was a ratio of one Q fever notification to 12.6 incident *C. burnetii* infections, corresponding to at least 50,000 individuals infected with *C. burnetii* during the Dutch Q fever epidemic (96).
End of May 2007, a general practitioner in the small rural village of Herpen in Noord-Brabant province was confronted with a rapidly increasing number of patients presenting with respiratory illness and atypical pneumonia in his practice, initially attributed to *Mycoplasma pneumoniae*. After more in-depth diagnostics it turned out to be Q fever as the majority of these patients had a positive serology for acute *C. burnetii* infection (92). This village outbreak was the start of a large and protracted human Q fever epidemic in the southeastern part of the country for three consecutive epidemic years. In 2007, a total of 168 cases were notified, followed by 1,000 notified cases in 2008, mainly in residents of the province of Noord-Brabant. In 2009, a total of 2,354 cases was notified as the epidemic further expanded in the provinces of Noord-Brabant and Gelderland and spread to the neighboring provinces Limburg and Utrecht *(Figure 2-3)*.

Following drastic and ultimate veterinary control measures, a sharp decline in notified human cases was observed with 504 notified cases in 2010. The annual epidemic peaks occurred synchronic with the kidding and lambing seasons of dairy goats and sheep. The densely populated province of Noord-Brabant with 2.4 million inhabitants was mostly affected *(Figure 3)*. The risk to acquire an acute *C. burnetii* infection seemed related to the proximity of nearby dairy goat farms to the residential addresses of cases. In 2005, veterinary *C. burnetii* was diagnosed for the first time as a cause of abortion at two dairy goat farms, using immunohistochemistry on sections of placenta (97). During the period 2005-2009, abortion waves due to *C. burnetii* were confirmed on 26 dairy goat farms and in addition cases of *C. burnetii*-related abortion were confirmed at two dairy sheep farms *(Figure 4)*.
Retrospective syndromic surveillance using hospital discharge diagnosis data suggested sporadic occurrence of human Q fever clusters (pneumonia and hepatitis) in the southern region of the Netherlands that remained unrecognized since 2005 until May 2007 (98).

More human clusters probably have remained undetected as was shown in the Livestock Farming and Neighbouring Residents' Health study that found high *C. burnetii* IgG phase 2 prevalence, up to 10%, in residents of municipalities with no notified cases of acute Q fever during the Dutch Q fever epidemic (99).

Figure 2. Acute Q fever notifications by month of illness onset, the Netherlands, 2007 –2016. (source: OSIRIS, RIVM)

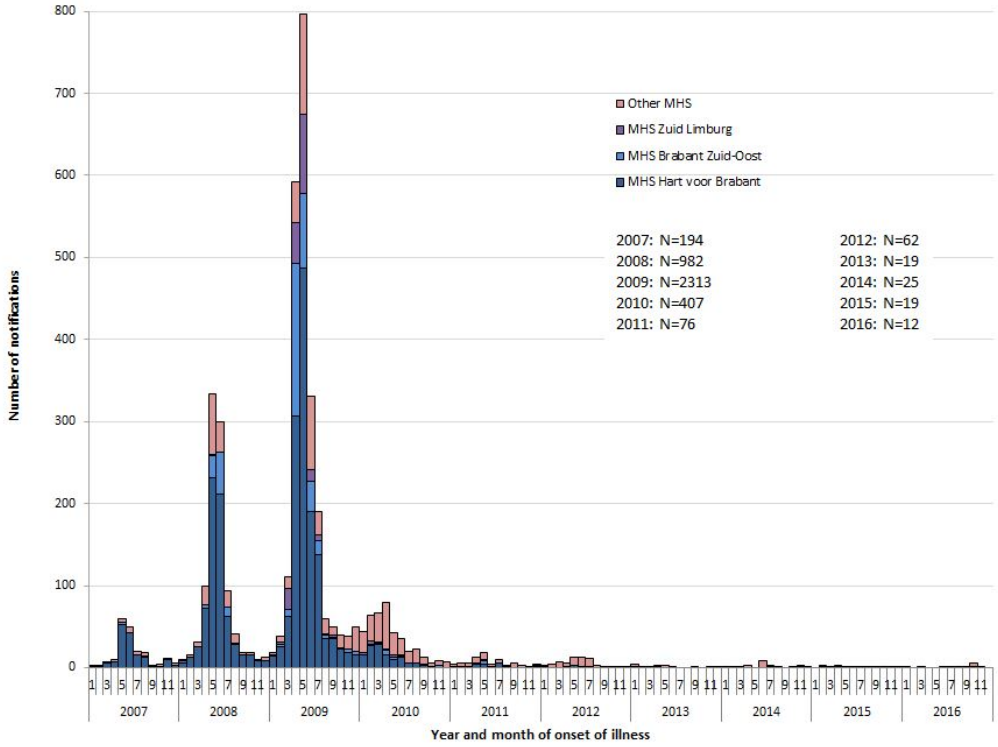


Figure 3. Incidence of notified Q fever cases per 100,000 inhabitants by municipality, the Netherlands, 2007- 2010. (source: OSIRIS, RIVM).

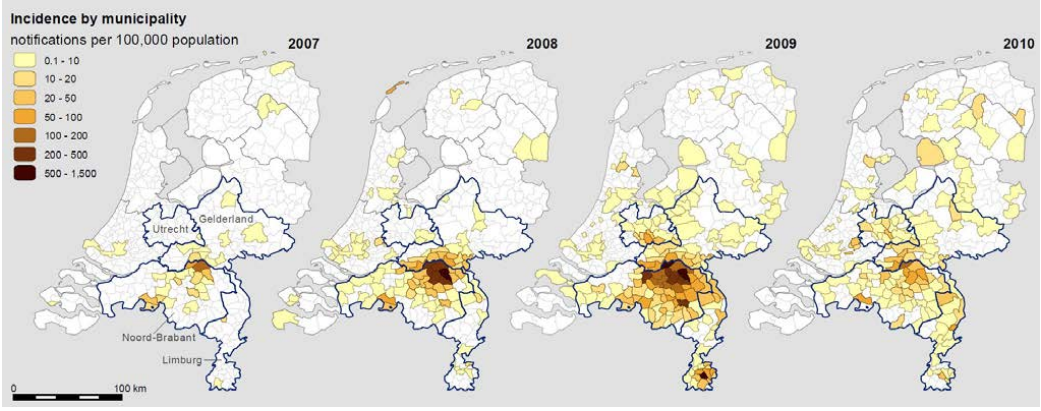
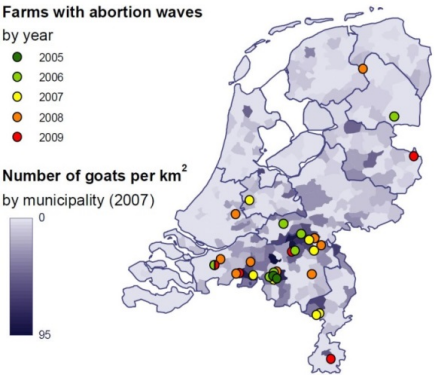


Figure 4. Locations of dairy goat farms (n=27) and dairy sheep farms (n=2) with reported waves of spontaneous abortions caused by Coxiella burnetii, 2005–2010 (source: GD Animal Health).



Veterinary and public health control measures

The Dutch Q fever epidemic emerged as an important nationwide human and veterinary public health challenge and gained worldwide attention due to its size, disease burden and high societal costs (100). On the European level, concerns were raised about contributing factors to the development of this large protracted Q fever epidemic (65, 94). Since the start of the Dutch epidemic, dairy goats at commercial goat farms reporting abortion waves due to *C. burnetii* were considered the primary animal source, as they were mainly located in the aforementioned areas where human cases occurred. The cyclic nature of the epidemic with multiple Q fever clusters in different areas in the south of the country, and the peaks during dry periods in spring strongly supported this hypothesis (101).

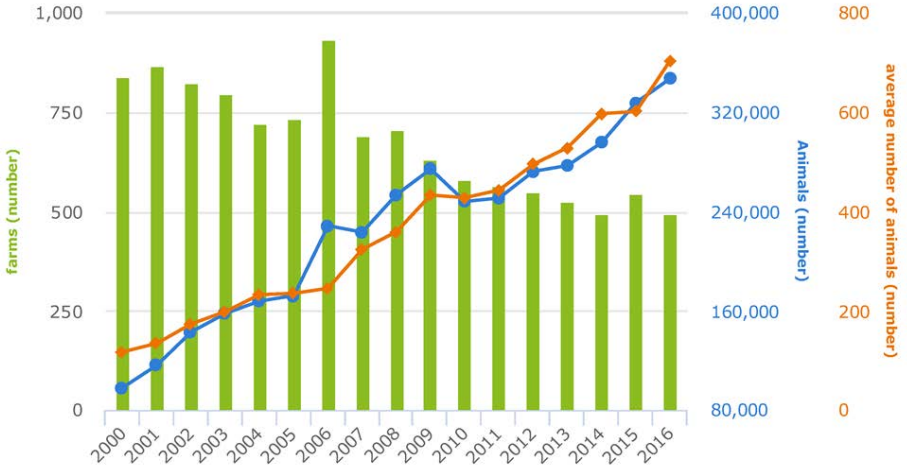
During the past decades, goat farming in the Netherlands had undergone a large shift from mostly hobbyists to commercial dairy goat farms (*Figure 5*). In 1995, 76,000 goats were housed in the Netherlands, of which 56% stayed in commercial dairy goat farms. In comparison, in 2009, 375,000 goats were housed in the Netherlands, of which 80% stayed on about 350 commercial farms with between 200 and 10,000 adult goats. These farms are mainly located in the densely populated province of Noord-Brabant, close to one another and often border villages and cities.

On these commercial farms, herd sizes had increased on average by about 600 animals per farm in 2007 when the Dutch Q fever epidemic started. Therefore, one of the putative causes of the emergence of Q fever in goats in the Netherlands was the intensive husbandry system in which dairy goats are kept. Before June 2008, abortion waves on small ruminant farms were reported on a voluntary basis to the Animal Health Service. It then became mandatory for farmers and private veterinarians to notify an unusual occurrence of abortions in dairy small ruminants. This mandatory notification could potentially facilitate the early detection of related human cases or clusters. A voluntary veterinary vaccination campaign with an inactivated phase I vaccine (Coxevac®,CEVA Santé Animale) started during the fall of 2008 in the south of the Netherlands, targeting the high-risk Q fever areas to lower abortion rates and limit the spread of the infection by decreasing the bacterial load in vaginal mucus, feces and milk in dairy goats.

The new upsurge and expansion of Q fever cases in 2009 warranted the implementation of extended veterinary control measures, such as a nationwide mandatory hygiene protocol and BTM-monitoring as an additional criterion for veterinary notification. In December 2009 it was decided to cull all gestating goats on 88 BTM-positive small ruminant farms determined by PCR while the remaining dairy goats and sheep nationwide were given compulsory vaccination.

Limited human control options were available to prevent clinical human disease during this Q fever epidemic. There is only one human Q fever vaccine (Q-VAX) that is currently licensed in Australia. Since 2002, this vaccine has been used in Australia in a targeted Q fever vaccination program offered to persons with an occupational risk to acquire a *C. burnetii* infection, such as livestock farmers and veterinarians (102), with different uptake (103). The Dutch government decided to offer this unlicensed Q-VAX vaccine to patient groups at high risk of Q fever complications in 2010-2011 (104) as a one-off vaccination campaign. Vaccination of high-risk occupational groups such as ruminant farmers, culling workers and veterinarians was not recommended during the Dutch Q fever epidemic.

*Figure 5. Number of dairy goats, dairy goat farms and average herd size, The Netherlands, 2000-2016. (source: Statistics Netherlands)**



* There were 343 professional dairy goat farms (≥32 dairy goats) in 2015 and 358 professional dairy goat farms in 2016 (median: 1.032 goats; mean: 1.226 goats) in the Netherlands. (source: *M. Gonggrijp, H. Brouwer, R. van den Brom, P. Vellema. Data analysis small ruminants 2016. Final report.Aug 2017, GD Animal Health*)

The aftermath: 2011-2016

The number of notified Q fever cases further decreased to 81 cases in 2011 and 66 cases in 2012, suggesting that the intervention measures, including continued annual vaccination of all dairy goat and sheep herds, were effective. From 2013 until 2016, the number of notified cases stayed low at pre-epidemic levels, with 19 in 2013, 28 in 2014, 22 in 2015 and 9 in 2016, respectively (*Figure 2)*. During this period, no patient clusters with acute Q fever have been notified.

A molecular link to goats and sheep based on MLVA-genotyping was not established until the aftermath of the Dutch epidemic (105, 106). A single clone of *C. burnetii* designated CbNL01 was responsible for the Dutch outbreak (105). The same clone was observed in Belgium without an increase in incidence of human Q fever (107). In 2016, the complete genome sequence of the *C. burnetii* outbreak strain in goats (NL3262) and that of an epidemiologically linked strain of a Dutch patient with a chronic Q fever infection, both having the outbreak-related CbNL01 MLVA genotype, was published (108).

Nationwide compulsory vaccination for all commercial dairy small ruminant farms and for small ruminant farms with a public function has been in place since 2010, and BTM-monitoring has continued. In the aftermath of the Dutch Q fever epidemic, the focus shifted from diagnosing acute Q fever patients to early identification and treatment of patients with chronic Q fever. Even though chronic Q fever is not notifiable, a separate national database is maintained for research purposes, including over 300 patients with confirmed or probable chronic Q fever patients (42).

Recommendations for serological and clinical follow-up in acute Q fever patients regardless of compatible clinical presentation have been made (109, 110). Screening of the Dutch general population for chronic *C. burnetii* infection is under debate based on ongoing cost-effectiveness analyses and expected benefit.

The need and benefit of the One Health approach

The most important lesson learned from the Dutch Q fever epidemic is that a close cooperation between the human and veterinary fields is essential for responding to outbreaks of zoonotic diseases (111). The Dutch Q fever epidemic intensified professional collaboration of multiple institutes and joined infectious disease control managers, policymakers and researchers from the human and veterinary domains. The involvement of two different ministries in this Q fever epidemic demonstrated key organizational differences in response structures, with a highly centralized veterinary domain around the Ministry of Agriculture and a strongly decentralized operational public health response. The Dutch Q fever epidemic has ignited fierce discussions about the perceived lack of cross-sectoral collaboration in the approach of zoonotic outbreaks, like occurred in other zoonotic outbreaks or major food safety events abroad such as the national HUS outbreak in Germany in 2014 (112). This prerequisite of a 'One Health' approach was one of the conclusions made by the official Q fever outbreak evaluation committee in 2010 that evaluated the process and actions of the Dutch government with respect to the Q fever crisis (113). A national zoonosis structure was implemented with an interdisciplinary early warning forum that meets monthly since 2011 to early identify signals and carry out risk assessments of emerging zoonoses. This regular and more informal contact has shortened communication lines and facilitated exchange of specific disease knowledge and diagnostic tools, benefiting overall outbreak management (114, 115).

Outline and aim of the thesis

This thesis describes the main results of an integrated human-veterinary research project in all three relevant ruminant species and their farm households carried out between 2009 and 2011 with the aim to study the prevalence and risk factors for *C. burnetii* infection. This so-called 'Q-VIVE' project included dairy goat farms (141 participating farms), dairy sheep farms (14), non-dairy sheep farms (119), and dairy cattle farms (311). In contrast to many previous studies, where only human cases or animals were studied in isolation, this study uniquely integrated data collection for humans, animals and the environment. The presence of *C. burnetii* antibodies was studied in a subset of small ruminants (dairy goats, dairy sheep, non-dairy sheep) or bulk milk (dairy cattle) and in a maximum of three persons of the farm household, including employees. For some dairy sheep and dairy goat farms, also the barn and the barn environment were sampled for presence of the bacterium. In addition, the seroprevalence and risk factors for presence of *C. burnetii* antibodies in other livestock-associated occupational groups such as in culling workers, veterinarians and veterinary students were studied in separate cross-sectional studies. Furthermore, we estimated the seroprevalence of *C. burnetii* antibodies in the general Dutch population just before the start of the Dutch Q fever epidemic in 2006-2007. Several in-depth outbreak investigations of small ruminant-associated Q fever clusters and community outbreaks carried out in 2008 and 2009, as part of the protracted Dutch Q fever epidemic, are reported in this thesis.

In **Chapter 2,** the seroprevalence and associated risk factors for *C. burnetii* infection in the general population during the pre-epidemic period (2006-2007) is described and a longitudinal study in a cohort of acute Q fever patients in which we obtained more insight in the time course of IgM and IgG antibody responses to *C. burnetii*. In **Chapter 3**, several outbreak investigations that were performed during the Q fever epidemic in 2007-2009 are described, including seroprevalence and risk factors for acquisition of acute Q fever. In **Chapter 4** the results are presented of the Q-VIVE integrated human-veterinary sero-epidemiological studies on *C. burnetii* infection and associated risk factors on commercial farms in four ruminant sectors, both for farm households and their herds. In **Chapter 5,** the seroprevalence and associated risk factors of *C. burnetii* infection in Dutch livestock veterinarians and veterinary students are described in two seroepidemiological studies to assess the potential impact of Q fever. A general discussion of the findings of the present thesis is given in **Chapter 6**. Several recommendations are given in targeted approaches for future Q fever control, focusing on both primary (reduction of circulation in the ruminant reservoir) and secondary (reduction of transmission from infected animals to humans) preventive measures. In **Chapter** 7 the main findings of this thesis are summarized.

References (Chapter 1)

1. Hilbink F, Penrose M, Kovacova E, Kazar J. Q fever is absent from New Zealand. International journal of epidemiology. 1993;22(5):945-9.
2. Cutler SJ, Bouzid M, Cutler RR. Q fever. The Journal of infection. 2007;54(4):313-8.
3. Derrick EH. "Q" fever, a new fever entity: clinical features, diagnosis and laboratory investigation. Reviews of infectious diseases. 1983;5(4):790-800.
4. Davis GE, Cox HR. A filter-passing infectious agent isolated from ticks. I. Isolation from Dermacentor andersoni, reactions in animals, and filtration experiments. Public Health Rep. 1938;53:2259-67.
5. Burnet FM, Freeman M. Experimental studies on the virus of "Q" fever. Reviews of infectious diseases. 1983;5(4):800-8.
6. Meredith AL, Cleaveland SC, Denwood MJ, Brown JK, Shaw DJ. *Coxiella burnetii* (Q-Fever) Seroprevalence in Prey and Predators in the United Kingdom: Evaluation of Infection in Wild Rodents, Foxes and Domestic Cats Using a Modified ELISA. Transboundary and emerging diseases. 2014.
7. Davoust B, Marie JL, Pommier de Santi V, Berenger JM, Edouard S, Raoult D. Three-toed sloth as putative reservoir of *Coxiella burnetii*, Cayenne, French Guiana. Emerging infectious diseases. 2014;20(10):1760-1.
8. Hogerwerf L, Courcoul A, Klinkenberg D, Beaudeau F, Vergu E, Nielen M. Dairy goat demography and Q fever infection dynamics. Veterinary research. 2013;44:28.
9. Agerholm JS. *Coxiella burnetii* associated reproductive disorders in domestic animals--a critical review. Acta veterinaria Scandinavica. 2013;55:13.
10. Arricau-Bouvery N, Rodolakis A. Is Q fever an emerging or re-emerging zoonosis? Veterinary research. 2005;36(3):327-49.
11. Rodolakis A, Berri M, Hechard C, Caudron C, Souriau A, Bodier CC, et al. Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine, and ovine herds. Journal of dairy science. 2007;90(12):5352-60.
12. Rodolakis A. Q Fever in dairy animals. Annals of the New York Academy of Sciences. 2009;1166:90-3.
13. Bauer AE, Olivas S, Cooper M, Hornstra H, Keim P, Pearson T, et al. Estimated herd prevalence and sequence types of *Coxiella burnetii* in bulk tank milk samples from commercial dairies in Indiana. BMC veterinary research. 2015;11:186.
14. Raoult D, Marrie T, Mege J. Natural history and pathophysiology of Q fever. The Lancet Infectious diseases. 2005;5(4):219-226.
15. Krumbiegel ER, Wisniewski HJ. Q fever in the Milwaukee area. II. Consumption of infected raw milk by human volunteers. Archives of environmental health. 1970;21(1):63-5.
16. Signs KA, Stobierski MG, Gandhi TN. Q fever cluster among raw milk drinkers in Michigan, 2011. Clinical infectious diseases. 2012;55(10):1387-9.
17. Eldin C, Angelakis E, Renvoise A, Raoult D. *Coxiella burnetii* DNA, but not viable bacteria, in dairy products in France. Am J Trop Med Hyg. 2013;88(4):765-9.
18. Cooper A, Stephens J, Ketheesan N, Govan B. Detection of *Coxiella burnetii* DNA in wildlife and ticks in northern Queensland, Australia. Vector borne and zoonotic diseases. 2013;13(1):12-6.
19. Duron O, Sidi-Boumedine K, Rousset E, Moutailler S, Jourdain E. The Importance of Ticks in Q Fever Transmission: What Has (and Has Not) Been Demonstrated? Trends Parasitol. 2015;31(11):536-52.
20. Kanfer E, Farrag N, Price C, MacDonald D, Coleman J, Barrett AJ. Q fever following bone marrow transplantation. Bone Marrow Transplant. 1988;3(2):165-6.
21. Amit S, Shinar S, Halutz O, Atiya-Nasagi Y, Giladi M. Suspected person-to-person transmission of Q fever among hospitalized pregnant women. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;58(11):e146-7.
22. Osorio S, Sarria C, Gonzalez-Ruano P, Casal EC, Garcia A. Nosocomial transmission of Q fever. J Hosp Infect. 2003;54(2):162-163.
23. Raoult D, Stein A. Q fever during pregnancy--a risk for women, fetuses, and obstetricians. The New England journal of medicine. 1994;330(5):371.
24. Milazzo A, Hall R, Storm PA, Harris RJ, Winslow W, Marmion BP. Sexually transmitted Q fever. Clinical infectious diseases : 2001;33(3):399-402.
25. Porter SR, Czaplicki G, Mainil J, Guatteo R, Saegerman C. Q Fever: current state of knowledge and perspectives of research of a neglected zoonosis. Int J Microbiol. 2011;2011:248418.
26. Maurin M, Raoult D. Q fever. Clinical microbiology reviews. 1999;12(4):518-53.
27. Tissot Dupont H, Raoult D, Brouqui P, Janbon F, Peyramond D, Weiller PJ, et al. Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. Am J Med. 1992;93(4):427-34.
28. Espejo E, Gil-Diaz A, Oteo JA, Castillo-Rueda R, Garcia-Alvarez L, Santana-Baez S, et al. Clinical presentation of acute Q fever in Spain: seasonal and geographical differences. Int J Infect Dis. 2014;26:162-4.
29. Marrie TJ, Stein A, Janigan D, Raoult D. Route of infection determines the clinical manifestations of acute Q fever. J Infect Dis. 1996;173(2):484-7.
30. Kersh GJ. Antimicrobial therapies for Q fever. Expert review of anti-infective therapy. 2013;11(11):1207-14.
31. Dijkstra F, Riphagen-Dalhuisen J, Wijers N, Hak E, Van der Sande MA, Morroy G, et al. Antibiotic therapy for acute Q fever in The Netherlands in 2007 and 2008 and its relation to hospitalization. Epidemiology and infection. 2011;139(9):1332-41.
32. Kampschreur LM, Wegdam-Blans MC, Thijsen SF, Groot CA, Schneeberger PM, Hollander AA, et al. Acute Q fever related in-hospital mortality in the Netherlands. The Netherlands journal of medicine. 2010;68(12):408-13.

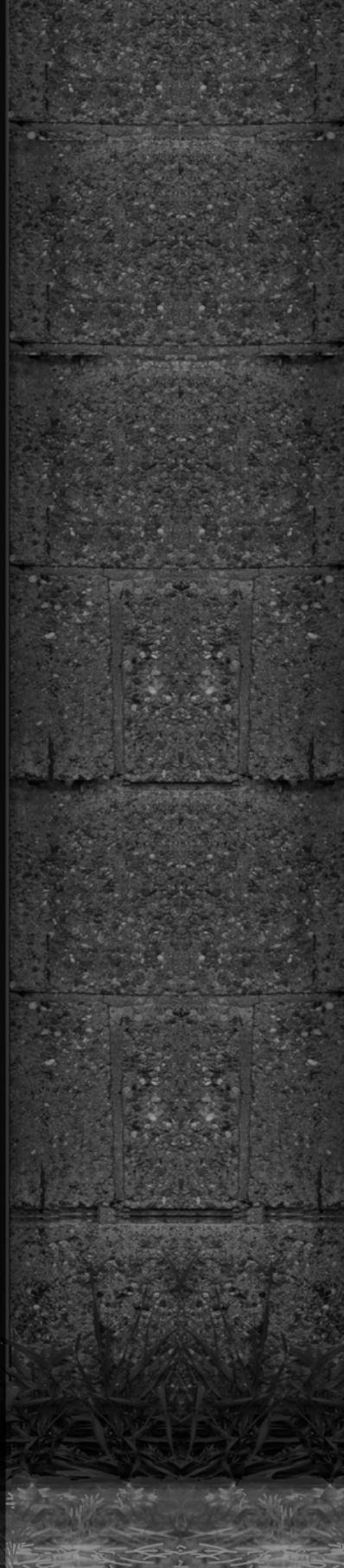
33. Tissot-Dupont H, Vaillant V, Rey S, Raoult D. Role of sex, age, previous valve lesion, and pregnancy in the clinical expression and outcome of Q fever after a large outbreak. Clinical infectious diseases. 2007;44(2):232-7.
34. Maltezou HC, Raoult D. Q fever in children. The Lancet Infectious diseases. 2002;2(11):686-91.
35. Hackert VH, Dukers-Muijrers NH, van Loo IH, Wegdam-Blans M, Somers C, Hoebe CJ. *Coxiella burnetii* infection is lower in children than in adults after community exposure: overlooked cause of infrequent Q Fever reporting in the young. Pediatr Infect Dis J. 2015;34(12):1283-8.
36. Morroy G, Keijmel SP, Delsing CE, Bleijenberg G, Langendam M, Timen A, et al. Fatigue following acute Q-Fever: a systematic literature review. PloS one. 2016;11(5):e0155884.
37. Keijmel SP, Delsing CE, Bleijenberg G, van der Meer JWM, Donders RT, Leclercq M, et al. Effectiveness of Long-term Doxycycline Treatment and Cognitive-Behavioral Therapy on Fatigue Severity in Patients with Q Fever Fatigue Syndrome (Qure Study): A Randomized Controlled Trial. Clinical infectious diseases. 2017;64(8):998-1005.
38. Herremans T, Hogema BM, Nabuurs M, Peeters M, Wegdam-Blans M, Schneeberger P, et al. Comparison of the performance of IFA, CFA, and ELISA assays for the serodiagnosis of acute Q fever by quality assessment. Diagnostic microbiology and infectious disease. 2013;75(1):16-21.
39. Schneeberger PM, Hermans MH, van Hannen EJ, Schellekens JJ, Leenders AC, Wever PC. Real-time PCR with serum samples is indispensable for early diagnosis of acute Q fever. Clin Vaccine Immunol. 2010;17(2):286-90.
40. European Centre for Disease Prevention and Control: Technical Report: ECDC Risk Assessment on Q Fever 2010. Available: http://ecdc.europa.eu/en/publications/Publications/1005_TER_Risk_Assessment_Qfever.pdf.
41. Million M, Thuny F, Richet H, Raoult D. Long-term outcome of Q fever endocarditis: a 26-year personal survey. The Lancet Infectious diseases. 2010;10(8):527-35.
42. Kampschreur LM, Delsing CE, Groenwold RH, Wegdam-Blans MC, Bleeker-Rovers CP, de Jager-Leclercq MG, et al. Chronic Q fever in the Netherlands 5 years after the start of the Q fever epidemic: results from the Dutch chronic Q fever database. Journal of clinical microbiology. 2014;52(5):1637-43.
43. Eldin C, Melenotte C, Mediannikov O, Ghigo E, Million M, Edouard S, et al. From Q Fever to *Coxiella burnetii* Infection: a Paradigm Change. Clinical microbiology reviews. 2017;30(1):115-90.
44. Dorko E, Rimarova K, Pilipcinec E. Influence of the environment and occupational exposure on the occurrence of Q fever. Central European journal of public health. 2012;20(3):208-14.
45. White B, Brooks T, Seaton RA. Q fever in military and paramilitary personnel in conflict zones: case report and review. Travel Med Infect Dis. 2013;11(2):134-7.
46. Batelli G. Zoonoses as occupational diseases. Veterinaria Italiana 2006;44(4):601-9.
47. Chang CC, Lin PS, Hou MY, Lin CC, Hung MN, Wu TM, et al. Identification of risk factors of *Coxiella burnetii* (Q fever) infection in veterinary-associated populations in southern Taiwan. Zoonoses and public health. 2010;57(7-8):e95-101.
48. Nett RJ, Helgerson SD, Anderson AD. Clinician assessment for *Coxiella burnetii* infection in hospitalized patients with potentially compatible illnesses during Q fever outbreaks and following a health alert, Montana, 2011. Vector borne and zoonotic diseases. 2013;13(2):128-30.
49. Thomas DR, Treweek L, Salmon RL, Kench SM, Coleman TJ, Meadows D, et al. The risk of acquiring Q fever on farms: a seroepidemiological study. Occupational and environmental medicine. 1995;52(10):644-7.
50. Tabibi R, Baccalini R, Barassi A, Bonizzi L, Brambilla G, Consonni D, et al. Occupational exposure to zoonotic agents among agricultural workers in Lombardy Region, northern Italy. Annals of agricultural and environmental medicine : AAEM. 2013;20(4):676-81.
51. Marrie TJ, Fraser J. Prevalence of Antibodies to *Coxiella burnetii* Among Veterinarians and Slaughterhouse Workers in Nova Scotia. The Canadian veterinary journal.1985;26(6):181-4.
52. Dolce P, Belanger MJ, Tumanowicz K, Gauthier CP, Jutras P, Masse R, et al. *Coxiella burnetii* seroprevalence of shepherds and their flocks in the lower Saint-Lawrence River region of Quebec, Canada. The Canadian journal of infectious diseases. 2003;14(2):97-102.
53. Bernard H, Brockmann SO, Kleinkauf N, Klinc C, Wagner-Wiening C, Stark K, et al. High seroprevalence of *Coxiella burnetii* antibodies in veterinarians associated with cattle obstetrics, Bavaria, 2009. Vector borne and zoonotic diseases. 2012;12(7):552-7.
54. Whitney EA, Massung RF, Candee AJ, Ailes EC, Myers LM, Patterson NE, et al. Seroepidemiologic and occupational risk survey for *Coxiella burnetii* antibodies among US veterinarians. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009;48(5):550-7.
55. Dorko E, Rimarova K, Kecerova A, Pilipcinec E, Dudrikova E, Lovayova V, et al. Potential association between *Coxiella burnetii* seroprevalence and selected risk factors among veterinary students in Slovakia. Annals of agricultural and environmental medicine : AAEM. 2011;18(1):47-53.
56. Valencia MC, Rodriguez CO, Punet OG, de Blas Giral I. Q fever seroprevalence and associated risk factors among students from the Veterinary School of Zaragoza, Spain. European journal of epidemiology. 2000;16(5):469-76.
57. Szymanska-Czerwinska M, Galinska EM, Niemczuk K, Zasepa M. Prevalence of *Coxiella burnetii* infection in foresters and ticks in the south-eastern Poland and comparison of diagnostic methods. Annals of agricultural and environmental medicine : AAEM. 2013;20(4):699-704.
58. Levesque B, Messier V, Bonnier-Viger Y, Couillard M, Cote S, Ward BJ, et al. Seroprevalence of zoonoses in a Cree community (Canada). Diagnostic microbiology and infectious disease. 2007;59(3):283-6.

59. Wattiau P, Boldisova E, Toman R, Van Esbroeck M, Quoilin S, Hammadi S, et al. Q fever in Woolsorters, Belgium. Emerging infectious diseases. 2011;17(12):2368-9.
60. Cilla G, Montes M, Perez-Trallero E. Q fever in the Netherlands - what matters is seriousness of disease rather than quantity. Eurosurveillance. 2008;13(37).
61. Villumsen S, Jorgensen CS, Smith B, Uldum S, Schiellerup P, Krogfelt KA. Determination of new cutoff values for indirect immunofluorescence antibody test for Q fever diagnosis in Denmark. Diagnostic microbiology and infectious disease. 2009;65(2):93-8.
62. Heymann DL e. Control of Communicable Diseases Manual. 19th edition ed. Association APH, editor. Washington, DC2008.
63. Moran GJ. Threats in bioterrorism. II: CDC category B and C agents. Emerg Med Clin North Am. 2002;20(2):311-30.
64. Hellenbrand W, Breuer T, Petersen L. Changing epidemiology of Q fever in Germany, 1947-1999. Emerging infectious diseases. 2001;7(5):789-96.
65. Georgiev M, Afonso A, Neubauer H, Needham H, Thiery R, Rodolakis A, et al. Q fever in humans and farm animals in four European countries, 1982 to 2010. Eurosurveillance. 2013;18(8).
66. Buhariwalla F, Cann B, Marrie TJ. A dog-related outbreak of Q fever. Clinical infectious diseases. 1996;23(4):753-5.
67. Chmielewski T, Tylewska-Wierzbanowska S. Q fever outbreaks in Poland during 2005-2011. Medical science monitor : international medical journal of experimental and clinical research. 2013;19:1073-9.
68. Langley JM, Marrie TJ, Covert A, Waag DM, Williams JC. Poker players' pneumonia. An urban outbreak of Q fever following exposure to a parturient cat. The New England journal of medicine. 1988;319(6):354-6.
69. Marrie TJ, Schlech WF, 3rd, Williams JC, Yates L. Q fever pneumonia associated with exposure to wild rabbits. Lancet. 1986;1(8478):427-9.
70. Stein A, Raoult D. Pigeon pneumonia in provence: a bird-borne Q fever outbreak. Clinical infectious diseases. 1999; 29(3): 617-20.
71. Carrieri MP, Tissot-Dupont H, Rey D, Brousse P, Renard H, Obadia Y, et al. Investigation of a slaughterhouse-related outbreak of Q fever in the French Alps. European journal of clinical microbiology & infectious diseases. 2002;21(1):17-21.
72. Hawker JI, Ayres JG, Blair I, Evans MR, Smith DL, Smith EG, et al. A large outbreak of Q fever in the West Midlands: windborne spread into a metropolitan area? Communicable disease and public health / PHLS. 1998;1(3):180-7.
73. Brouqui P, Badiaga S, Raoult D. Q fever outbreak in homeless shelter. Emerging infectious diseases. 2004;10(7):1297-9.
74. Porten K, Rissland J, Tigges A, Broll S, Hopp W, Lunemann M, et al. A super-spreading ewe infects hundreds with Q fever at a farmers' market in Germany. BMC infectious diseases. 2006;6:147.
75. McQuiston JH, Gibbons RV, Velic R, Nicholson WL, Castrodale L, Wainright SH, et al. Investigation of a focus of Q fever in a nonfarming population in the Federation of Bosnia and Herzegovina. Annals of the New York Academy of Sciences. 2003;990:229-32.
76. Winner SJ, Eglin RP, Moore VI, Mayon-White RT. An outbreak of Q fever affecting postal workers in Oxfordshire. The Journal of infection. 1987;14(3):255-61.
77. Tostmann A, Bousema T, Oliver I. Investigation of outbreaks complicated by universal exposure. Emerging infectious diseases. 2012;18(11):1717-22.
78. Wilson LE, Couper S, Prempeh H, Young D, Pollock KG, Stewart WC, et al. Investigation of a Q fever outbreak in a Scottish co-located slaughterhouse and cutting plant. Zoonoses and public health. 2010;57(7-8):493-8.
79. Gilroy N, Formica N, Beers M, Egan A, Conaty S, Marmion B. Abattoir-associated Q fever: a Q fever outbreak during a Q fever vaccination program. Australian and New Zealand journal of public health. 2001;25(4):362-7.
80. Rauch AM, Tanner M, Pacer RE, Barrett MJ, Brokopp CD, Schonberger LB. Sheep-associated outbreak of Q fever, Idaho. Archives of internal medicine. 1987;147(2):341-4.
81. Henning K, Hotzel H, Peters M, Welge P, Popps W, Theegarten D. [Unanticipated outbreak of Q fever during a study using sheep, and its significance for further projects]. Berliner und Munchener tierarztliche Wochenschrift. 2009;122(1-2):13-9.
82. Hall CJ, Richmond SJ, Caul EO, Pearce NH, Silver IA. Laboratory outbreak of Q fever acquired from sheep. Lancet. 1982;1(8279):1004-6.
83. George M, Reich A, Cussler K, Jehl H, Burckhardt F. Live Cell Therapy as Potential Risk Factor for Q Fever. Emerging infectious diseases. 2017;23(7).
84. van Woerden HC, Mason BW, Nehaul LK, Smith R, Salmon RL, Healy B, et al. Q fever outbreak in industrial setting. Emerging infectious diseases. 2004;10(7):1282-9.
85. Wade AJ, Cheng AC, Athan E, Molloy JL, Harris OC, Stenos J, et al. Q fever outbreak at a cosmetics supply factory. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2006;42(7):e50-2.
86. Alonso E, Lopez-Etxaniz I, Hurtado A, Liendo P, Urbaneja F, Aspiritxaga I, et al. Q Fever Outbreak among Workers at a Waste-Sorting Plant. PloS one. 2015;10(9):e0138817.
87. Kaplan MM, Bertagna P. The geographical distribution of Q fever. Bulletin of the World Health Organization. 1955;13(5):829-60.
88. Wolff JW, Kouwenaar W. [Investigation on occurrence of Q fever in Netherlands]. Nederlands tijdschrift voor geneeskunde. 1954;98(39):2726-32.
89. Dekking F, Zanen HC. Q fever in the Netherlands. Tropical and geographical medicine. 1958;10(2):157-62.

90. Jordans GH. [A case of Q fever]. Nederlands tijdschrift voor geneeskunde. 1958;102(32):1548-9.
91. Westra SA, Lopes Cardozo E, Ten Berg J. [The first cases of Q fever in the Netherlands]. Nederlands tijdschrift voor geneeskunde. 1958;102(2):69-72.
92. Schimmer B, Morroy G, Dijkstra F, Schneeberger PM, Weers-Pothoff G, Timen A, et al. Large ongoing Q fever outbreak in the south of The Netherlands, 2008. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2008;13(31).
93. Stevens H, Beeres MP, Meijer JG, Koeman J, van Kregten E, Bartelink AK. [Q fever: not just in sheep]. Nederlands tijdschrift voor geneeskunde. 2000;144(27):1297-300.
94. Dijkstra F, van der Hoek W, Wijers N, Schimmer B, Rietveld A, Wijkmans CJ, et al. The 2007-2010 Q fever epidemic in The Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. FEMS immunology and medical microbiology. 2012;64(1):3-12.
95. van Loenhout JA, Wielders CC, Morroy G, Cox MJ, van der Hoek W, Hautvast JL, et al. Severely impaired health status of non-notified Q fever patients leads to an underestimation of the true burden of disease. Epidemiology and infection. 2015;143(12):2580-7.
96. van der Hoek W, Hogema BM, Dijkstra F, Rietveld A, Wijkmans CJ, Schneeberger PM, et al. Relation between Q fever notifications and *Coxiella burnetii* infections during the 2009 outbreak in The Netherlands. Eurosurveillance. 2012;17(3):20058.
97. Wouda W, Dercksen DP. [Abortion and stillbirth among dairy goats as a consequence of *Coxiella burnetii*]. Tijdschrift voor diergeneeskunde. 2007;132(23):908-11.
98. van den Wijngaard CC, Dijkstra F, van Pelt W, van Asten L, Kretzschmar M, Schimmer B, et al. In search of hidden Q fever outbreaks: linking syndromic hospital clusters to infected goat farms. Epidemiology and infection. 2011;139(1):19-26.
99. Maassen KS, L. Wouters, I. van Duijkeren, E. Janse, I. Hagenaars, T. IJzermans, J. van der Hoek, W. Heederik, D. Livestock farming and the health of local residents (in Dutch). Bilthoven, the Netherlands: National Institute of Public Health and the Environment; 2016 05-07-2016. Contract No.: RIVM Report 2016-0058.
100. van Asseldonk MA, Prins J, Bergevoet RH. Economic assessment of Q fever in the Netherlands. Preventive veterinary medicine. 2013;112(1-2):27-34.
101. van der Hoek W, Dijkstra F, Schimmer B, Schneeberger PM, Vellema P, Wijkmans C, et al. Q fever in the Netherlands: an update on the epidemiology and control measures. Eurosurveillance. 2010;15(12).
102. Gidding HF, Wallace C, Lawrence GL, McIntyre PB. Australia's national Q fever vaccination program. Vaccine. 2009;27(14):2037-41.
103. Sellens E, Norris JM, Dhand NK, Heller J, Hayes L, Gidding HF, et al. Q Fever Knowledge, Attitudes and Vaccination Status of Australia's Veterinary Workforce in 2014. PloS one. 2016;11(1):e0146819.
104. Isken LD, Kraaij-Dirkzwager M, Vermeer-de Bondt PE, Rumke HC, Wijkmans C, Opstelten W, et al. Implementation of a Q fever vaccination program for high-risk patients in the Netherlands. Vaccine. 2013;31(23):2617-22.
105. Roest HI, Ruuls RC, Tilburg JJ, Nabuurs-Franssen MH, Klaassen CH, Vellema P, et al. Molecular epidemiology of *Coxiella burnetii* from ruminants in Q fever outbreak, the Netherlands. Emerging infectious diseases. 2011;17(4):668-75.
106. Tilburg JJ, Rossen JW, van Hannen EJ, Melchers WJ, Hermans MH, van de Bovenkamp J, et al. Genotypic diversity of *Coxiella burnetii* in the 2007-2010 Q fever outbreak episodes in The Netherlands. Journal of clinical microbiology. 2012;50(3):1076-1078.
107. Boarbi S, Mori M, Rousset E, Sidi-Boumedine K, Van Esbroeck M, Fretin D. Prevalence and molecular typing of *Coxiella burnetii* in bulk tank milk in Belgian dairy goats, 2009-2013. Veterinary microbiology. 2014;170(1-2):117-24.
108. Kuley R, Smith HE, Janse I, Harders FL, Baas F, Schijlen E, et al. First Complete Genome Sequence of the Dutch Veterinary *Coxiella burnetii* Strain NL3262, Originating from the Largest Global Q Fever Outbreak, and Draft Genome Sequence of Its Epidemiologically Linked Chronic Human Isolate NLhu3345937. Genome Announc. 2016;4(2).
109. Wielders CC, Morroy G, Wever PC, Coutinho RA, Schneeberger PM, van der Hoek W. Strategies for early detection of chronic Q fever: a systematic review. Eur J Clin Invest. 2013;43(6):616-39.
110. Wielders CC, van Loenhout JA, Morroy G, Rietveld A, Notermans DW, Wever PC, et al. Long-Term Serological Follow-Up of Acute Q Fever Patients after a Large Epidemic. PloS one. 2015;10(7):e0131848.
111. Roest HIJM, C.B.M. van de Giessen, A. van Zijderveld, F.G. The Dutch Q fever situation - lessons learned? . Planet@Risk, Special Issue on One Health (Part I/II) Davos: Global Risk Forum GRF Davos. 2014;2(3):166-8.
112. Wu CJ, Hsueh PR, Ko WC. A new health threat in Europe: Shiga toxin-producing *Escherichia coli* O104:H4 infections. J Microbiol Immunol Infect. 2011;44(5):390-3.
113. van Dijk G, van Dissel JT, Speelman P. et al. Van verwerping tot verheffing Q-Koortsbeleid in Nederland 2005-2010. [Evaluation Committee for handling of Q Fever in Netherlands 2005-2010].Report, 22 november 2010. Available: https://www.rijksoverheid.nl/documenten/rapporten/2010/11/22/van-verwerping-tot-verheffing
114. van der Giessen J, Kramer T. Fanoy E, de Rosa M, Roest HJ, Broens E; Vellema P,Notermans D, Swaan, C, Maassen K. [Early Warning Meeting Zoonoses celebrates five year anniversary]. Tijdschrift voor diergeneeskunde. 2016;6:48-9.
115. Eussen BGM, Schaveling J, Dragt MJ, Blomme RJ. Stimulating collaboration between human and veterinary health care professionals. BMC veterinary research. 2017;13(1):174.

2

Seroprevalence of *Coxiella burnetii* in the Dutch general population and time-course of antibody responses following acute Q fever



CHAPTER 2.1

Low seroprevalence of Q fever in The Netherlands prior to a series of large outbreaks

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Low seroprevalence of Q fever in The Netherlands prior to a series of large outbreaks

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SUMMARY

The Netherlands has experienced large community outbreaks of Q fever since 2007. Sera and questionnaires containing epidemiological data from 5654 individuals were obtained in a nationwide seroprevalence survey used to evaluate the National Immunization Programme in 2006–2007. We tested these sera for IgG phase-2 antibodies against *Coxiella burnetii* with an ELISA to estimate the seroprevalence and to identify determinants for seropositivity before the Q fever outbreaks occurred. Overall seroprevalence was 1·5% [95% confidence interval (CI) 1·3–1·7]. Corrected for confirmation with immunofluorescence results in a subset, the estimated seroprevalence was 2·4%. Seropositivity ranged from 0·48% (95% CI 0·00–0·96) in the 0–4 years age group to 2·30% (95% CI 1·46–3·15) in the 60–79 years age group. Keeping ruminants, increasing age and being born in Turkey were independent risk factors for seropositivity. The low seroprevalence before the start of the outbreaks supports the hypothesis that The Netherlands has been confronted with a newly emerging Q fever problem since spring 2007.

Key words: *Coxiella*, epidemiology, Q fever, risk factors, serology.

INTRODUCTION

Q fever is a worldwide zoonosis caused by *Coxiella burnetii*, a Gram-negative bacterium that can survive for a prolonged time in the environment in a spore-like stage. Until 2007, Q fever was a rarely reported notifiable disease in The Netherlands, with 5–20 cases presenting annually and without seasonal trend. However, underdiagnosis and consequently underreporting were suspected as very few laboratories consistently tested pneumonia cases for *Coxiella* infection. Q fever emerged in 2007, followed by subsequently larger epidemics in 2008 and 2009 [1, 2], which were probably related to intensive dairy goat farming [3–5]. There is some evidence for a few retrospectively identified clusters of hospital admissions for respiratory illness in 2005 and 2006 that might have been caused by Q fever [6]. A seroprevalence study in the 1980s by Richardus *et al*. found very high prevalence estimates among blood donors and certain risk groups ranging from 15% to 65% [7].

A recent community-based study from the USA showed a seroprevalence of 3·1% [8], while 4% was

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found in a blood donor study in southern France in the late 1980s [9] and 3·6% in Japanese blood donors in the late 1990s [10]. Higher seroprevalences were observed in other western countries: in Northern Ireland, 12·8% in sera collected in 1986–1987 [11], 7·9% in rural Wales in the mid-1990s [12], similar to the 7·5% found in southwestern Germany in 2008–2009 [13]. In northwestern Russia a marked increase in seroprevalence in a healthy population was observed, from 1·1% in 1993 to 11·1% in 2003 [14]. In the UK the seroprevalence was 11·2% in an occupational control cohort in the late 1980s [15]. Mediterranean countries report even higher seroprevalence levels: in Spain, 23·1% in blood donors in Albacete [16], 48·6% in Eastern Cantabria [17], and 15·3% in the Barcelona region [18], while 13·5% was found in north Turkey [19] and 52·7% in Cyprus [20]. A more detailed overview of human seroprevalence studies done in European countries was recently presented in reports by the European Food Safety Authority and the European Centre for Disease Prevention and Control [13, 21]. To understand the baseline epidemiology of Q fever in The Netherlands prior to the epidemics, available serum samples from the general population, collected between February 2006 and June 2007 for evaluation by the Dutch National Immunization Programme [22] were used to obtain a seroprevalence estimate of the general Dutch population just before the recent epidemics. In addition, risk factors for seropositivity were identified.

METHODS AND MATERIALS

Study population and questionnaire data

A large population-based seroprevalence study, called the PIENTER project, was carried out primarily for the evaluation of the Dutch National Immunization Programme. Eight municipalities within each of five geographical Dutch regions (Northeast, Central, Northwest, Southwest and Southeast) were sampled with probabilities proportional to their population sizes (Fig. 1). In addition, there was an oversampling of non-Western migrants in 12 of the above municipalities. Data collection started in February 2006 and was finalized in June 2007. The study design and details of the data collection in the PIENTER project have been published [22]. The participants donated blood and completed a questionnaire, one version for children aged $\leqslant$14 years, and another version for persons aged $\geqslant$15 years. The questionnaire covered, among others, data on demographics, health perception and diseases, and activities possibly related to infectious diseases (e.g. travelling, profession, food habits, gardening).

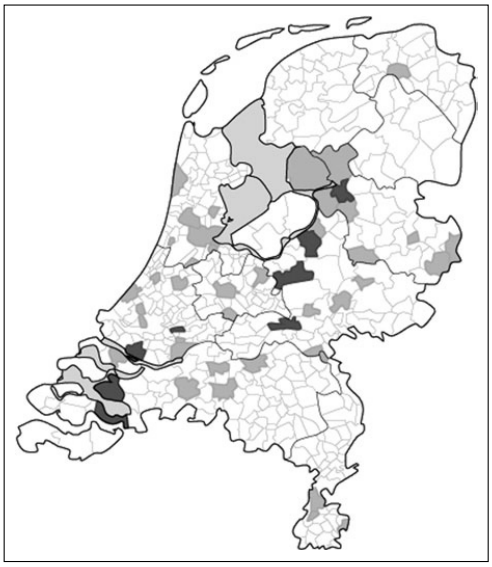

Fig. 1. Selected municipalities in the seroprevalence study. Light grey municipalities are included in the nationwide sample ($n=40$). Dark grey municipalities are low immunization municipalities that are included in the PIENTER project but not in the present study.

Laboratory analysis

Stored sera of the nationwide sample of the PIENTER project were screened for the presence of *C. burnetii* IgG phase-2-specific antibodies by a commercial enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Serion ELISA classic, Virion/Serion, Germany). A positive ELISA test was defined according to the manufacturer as a concentration of $\geqslant$30 U/ml, and a borderline-positive ELISA was defined as a concentration between 20 U/ml and 30 U/ml. We considered ELISA-borderline-positive and ELISA-positive samples as a positive test result. Immunofluorescence assay (IFA) is considered the reference method for diagnostic screening of *C. burnetii* and a lower sensitivity of the ELISA test compared to IFA was anticipated, with a similar specificity [23]. ELISA-borderline-positive and ELISA-positive samples were subsequently confirmed by IFA (Focus Diagnostics,

USA) for IgG phase-1 and phase-2 specific antibodies using a 1:32 and 1:128 dilution. In addition, a random subset of ELISA-negative samples ($n=504$) was screened for IgG phase-1 and phase-2 specific antibodies by IFA at an initial dilution of 1:32 to estimate the proportion of false-negative test results. A positive IFA sample was defined as a sample with an IFA IgG phase-2 titre of $\geqslant 32$ (either or not combined with positive IgG phase 1 of $\geqslant 1:32$). By extrapolation we additionally estimated the national seroprevalence adjusting for the proportion of false-positive and false-negative results using the IFA as the gold standard.

Statistical analysis

The national sample that was screened by ELISA was used to estimate the seroprevalence of *C. burnetii* IgG antibodies representative of the general population of The Netherlands. Oversampled migrant participants were included to allow studying differences in seroprevalence by country of origin in more detail. Participants from municipalities with low immunization coverage were excluded from the seroprevalence estimation. To produce national estimates, the weighted frequencies were averaged over the 40 participating municipalities in eight different provinces. To avoid missing possible infections, borderline laboratory results were considered positive in the statistical analysis. Weights were calculated proportional to the reference population (Dutch population, 1 January 2007) taking sex, age, ethnicity and degree of urbanization (>2500 *vs.* <2500 addresses per km² in the neighbourhood) into account. We adjusted for the two-stage cluster sampling by taking into account the strata (regions) and clusters (municipalities). The weighted overall and age-, sex- and region-specific seroprevalences were estimated for the Dutch population.

Based on the ELISA results of the complete sample we performed the further risk factor analysis. Univariate odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for selected variables possibly relevant for *C. burnetii* exposure, i.e. geographical region, degree of urbanization, country of birth, religion, educational level, household income, number of persons in the household, consumption of raw meat and unwashed vegetables, being a vegetarian, gardening, playing in a sandbox (only children aged <15 years), keeping a pet (past 5 years), keeping livestock (past 5 years), tick bites (past 5 years), and occupational contact with animals (past 5 years). Information from Statistics Netherlands was collected on goat, sheep and cattle density in the participating municipalities. Variables which reached a significance level of $P<0{\cdot}20$ in the univariate analysis were included in a multivariate logistic regression model. Multivariate analysis was done by a General Logistic Mixed Model with municipality added as a block effect. Selection of model terms was done by backwards elimination to determine independent risk factors for seropositivity using $P<0{\cdot}05$ as significance level (R, version 2.10; R Foundation, Austria).

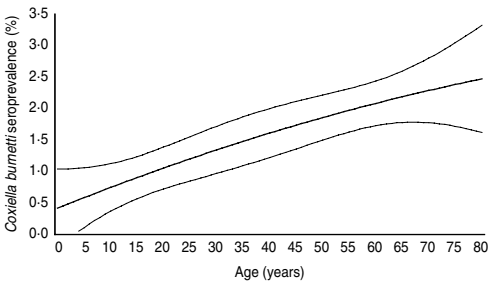

Fig. 2. Age-specific weighted seroprevalence of *C. burnetii* IgG antibodies in a representative sample of the Dutch population aged 0–79 years ($n=5654$), PIENTER project, 2006–2007. Prevalence rates per age group were estimated using a linear model with a spline function for age (i.e. second-degree polynomial).

RESULTS

Overall seroprevalence

The 5654 stored sera available from the nationwide sample of the PIENTER project were screened for the presence of *C. burnetii* IgG phase 2 by ELISA. Antibodies were detected in sera of 85 study participants, of which 47 had borderline levels, resulting in a weighted rough seroprevalence of 1·5%. Based on ELISA test results only, the weighted seroprevalence was higher in males than in females (1·65% *vs.* 1·28%). Seropositivity increased with age from 0·48% (95% CI 0·00–0·96) in the 0–4 years age group to 2·30% (95% CI 1·46–3·15) in the 60–79 years age group (Fig. 2). The seroprevalence for those born abroad was slightly higher than for persons born in The Netherlands. Persons with low educational level (i.e. no education or elementary-school level) and those living in a household with a very low monthly income ($\leqslant$€850) had the highest seroprevalence.

Table 1. *Weighted* seroprevalence of Coxiella burnetii IgG phase-2 antibodies (Serion ELISA, IgG phase 2) in the Dutch population aged 0–79 years (n = 5654), PIENTER 2 project, The Netherlands, February 2006–June 2007*

	N (*n* positive)	Seroprevalence (95% CI)
Overall	5654 (85)	1·46 (1·18–1·74)
Sex		
Male	2522 (45)	1·64 (1·21–2·07)
Female	3132 (40)	1·28 (0·90–1·65)
Country of birth		
Other	735 (15)	1·78 (0·98–2·59)
The Netherlands	4833 (68)	1·41 (1·08–1·75)
Age category (yr)		
0–4	571 (4)	0·48 (0·00–0·96)
5–19	1228 (9)	0·71 (0·24–1·19)
20–39	1321 (19)	1·56 (0·92–2·20)
40–59	1266 (23)	1·65 (0·93–2·37)
60–79	1268 (30)	2·30 (1·46–3·15)
Education		
Elementary level	1373 (30)	2·05 (1·33–2·77)
High school	2668 (30)	1·13 (0·68–1·57)
University level	1515 (23)	1·54 (0·88–2·19)
Net income (€/month)		
⩽850	354 (10)	2·94 (1·15–4·74)
>850	4126 (62)	1·44 (1·10–1·78)
Region		
Northeast	1328 (19)	1·48 (1·04–1·92)
Central	1025 (18)	1·84 (0·93–2·76)
Northwest	1332 (22)	1·49 (0·86–2·12)
Southwest	998 (13)	1·21 (0·67–1·76)
Southeast	971 (13)	1·23 (0·67–1·78)

CI, Confidence interval.
* Weighted for sex, age, ethnicity and degree of urbanization.

No regional differences in seroprevalence were observed (Table 1).

The 85 ELISA-positive samples were subsequently screened by IFA. Fifteen samples (17·6%) turned out IFA-negative. Of the remaining 70 IFA-positive sera, 13 had a low-level IgG phase-2 titre ranging between 1:32 and <1:128, while 57 sera had a titre of ⩾1:128. In the IFA-tested subset of 504 ELISA-negative samples, six samples (1·2%) had low-level IgG phase-2 titres ranging from 1:32 ($n = 5$) to 1:128 ($n = 1$). Correcting the ELISA results with the IFA results, the adjusted overall seroprevalence estimate was 2·4% (70 true positives and 66 false negatives divided by the total number of 5654 study participants).

Determinants of *C. burnetii* seropositivity

Based on ELISA test results only, risk factors significant at the $P < 0{\cdot}20$ level were increasing age, being male, marital status, not being born in The Netherlands, net income, religion, contact with cats (past 12 months), kept livestock (past 5 years), frequency of eating raw meat, and occupational contact with animals (Table 2). No significant association was found between seropositivity and goat, sheep or cattle density in the 40 municipalities. In the multivariate logistic regression model, seropositivity was found to be associated with keeping ruminants (past 5 years) with or without other farm animals [adjusted OR (aOR) 8·2, 95% CI 3·3–20·8 and aOR 3·8, 95% CI, 1·1–13·1, respectively], being born in Turkey (aOR 5·1, CI 2·1–12·5), and increasing age (aOR 5·4, 95% CI 1·4–20·4 for the 15–39 years age group; aOR 6·0, 95% CI 1·6–22·9 for the 40–59 years age group, and aOR 6·6, 95% CI 1·8–24·0 for the oldest age group of 60–79 years compared to the reference age group of 0–14 years), with borderline significance being observed for occupational contact with animals. Other factors contributing to the multivariate model were low monthly net income (⩽€850), and being male, although these did not reach statistical significance (Table 3). In the multivariate analysis, keeping pets seemed to play a protective role (aOR 0·53, 95% CI 0·32–0·87). This variable was included based on the significance of keeping rabbits as a variable in the univariate analysis ($P = 0{\cdot}18$).

DISCUSSION

The overall Q fever seroprevalence, based on this representative sample of the Dutch population from 2006 to 2007 is relatively low. The overall estimate of 1·5%, and IFA-corrected estimate of 2·4% reflect the seroprevalence in a pre-epidemic period as the tested sera were collected in 2006 and the first half 2007, just before the major Q fever epidemics in 2007–2009. The *a-priori* expected *C. burnetii* IgG phase-2 seroprevalence was estimated to be around 4% based on screening of serum samples of pregnant women outside the epidemic area carried out in 2007 by IFA [24]. For comparison, since the epidemic rise in 2007, seropositivity rates of the general population have probably increased, as shown by a 24% seropositivity rate in the population of the Q fever epicentre of 2007 [3]. In the 1980s, the seroprevalence for *C. burnetii* in blood donors was studied in different regions of

The Netherlands using an IFA developed in-house. Using a low cut-off for positivity in this IFA (screening dilution of 1:16), a high seroprevalence was found, ranging between 15% and 65%, depending on region, sex and age [7]. Unfortunately, no information was available on exact titres measured. It is unknown how current IFAs and ELISAs relate to this previously used in-house IFA, as neither the test nor the sera are available at present. The high seroprevalences found in the 1980s led to the suggestion that *C. burnetii* may have been far more prevalent for a considerable time than the number of notifications suggested, and that before 2007 clinical cases were not detected because of the aspecific presentation of most symptomatic Q fever infections as well as a large proportion of asymptomatic and subclinical infections. Few laboratories had serology for *C. burnetii* in their standard panel for pneumonia patients before national awareness was raised by the Q fever outbreaks.

Several other population studies observed seroprevalences between 2% and 4%, similar to the estimated seroprevalence found in our study [8–10]. However, comparisons of seroprevalence estimates should be made with caution as different study populations, different serological assays and criteria for positivity are used. The latter is especially important in population surveys, while comparisons of assays in The Netherlands for diagnoses of acute cases demonstrate a high concordance of test results. Increasing age, being born in Turkey, keeping ruminants and to a lesser extent occupational contact with animals were identified as independent risk factors for Q fever seropositivity in this study. The increase in seroprevalence with age is consistent with findings from other seroprevalence studies [8, 25]; however, the range of age-specific seroprevalences in our study is smaller. In accord with other studies [8, 11], we showed that young children and adolescents have a very low seroprevalence, which is supported by a very small proportion of children in the notified clinical cases in our routine Q fever surveillance. Keeping ruminants was an independent risk factor in our study as shown also in other studies [8, 11, 25]. Turkey as country of birth was an independent risk factor. In accord with this observation, recent studies in Turkey indicate that Q fever is highly prevalent: prevalence of *C. burnetii* anti-phase-2 IgG was 13·5% in healthy subjects in the west Black Sea region [19] and 32·3% by ELISA in blood donors from Ankara [26].

In southwestern Germany, the seroprevalence showed a linear increase with sheep density in different municipalities [13]. In our study we did not find an association between goat, sheep and cattle density, and seropositivity in the 40 municipalities, suggesting that the dominant role of the dairy goats in the epidemiology of Q fever in The Netherlands is a relatively recent occurrence. Occupational animal contact was not a strong independent predictor in the multivariate analysis as there was a large overlap with those keeping ruminants. The kind of occupational animal contact was not further specified in the questionnaire which also included contact with non-ruminant species. Livestock farmers, veterinarians, slaughterhouse workers and animal laboratory staff are known occupational risk groups for Q fever, as exemplified by higher seroprevalence levels than the general population [11, 25]. In order to investigate the actual risk for professionals dealing with livestock, separate seroprevalence studies in livestock farmers, veterinarians and persons actively involved in culling activities at infected dairy farms are currently performed in The Netherlands. Although poverty was a risk factor in a seroprevalence study from the USA [8], we cannot fully support this observation as the category with the lowest monthly net income ($\leqslant$€850) was not statistically significant in the multivariate model. This is possibly caused by a large proportion in this income group that chose not to disclose their monthly income. Males generally have higher seroprevalence than females, which is often explained by occupational contact. In our study, being male is a possible confounding factor, at least partially explained by occupational exposure to animals. In laboratory surveillance studies, males are more likely to be diagnosed as they more often develop symptoms, as shown in our routine laboratory surveillance. Others suggested that this is explained by sex hormones that may control the host's immune response to a *C. burnetii* infection [27], resulting in gender differences in clinical attack rates. In the case-control study performed during the first epidemic season in 2007 in the main cluster area in the south of The Netherlands we observed a higher proportion of males developing symptoms, while seroprevalence due to the airborne exposure was equally distributed among males and females [3]. It is unclear why keeping pets turned out to be a protective factor. Other studies did not find any association with keeping pets, or in contrast, found pets to be a risk factor, such as in a recent study from Germany where pet rats were surprisingly found as a risk factor (S. Brockmann, unpublished observations). Moreover, several Q fever

Table 2. *Coxiella burnetii IgG antibodies (%) in study population (n=5654) and adjusted* univariate analysis of factors associated with seropositivity to Coxiella burnetii PIENTER 2 project, The Netherlands, February 2006–June 2007*

Variable	No. (%) of respondents		OR (95% CI)	*P*
	Seropositive (*n*=85)	Seronegative (*n*=5569)		
Degree of urbanization				0·91
Very urbanized	20 (1·64)	1197 (98·36)	1·34 (0·60–3·28)	
Urbanized	37 (1·47)	2488 (98·53)	1·18 (0·57–2·75)	
Moderately urbanized	10 (1·44)	685 (98·56)	1·18 (0·46–3·13)	
Little urbanized	10 (1·83)	536 (98·17)	1·51 (0·59–4·00)	
Rural	8 (1·19)	663 (98·81)	Reference	
Age category (yr)				<0·01
0–14	7 (0·46)	1516 (99·54)	Reference	
15–39	25 (1·57)	1572 (98·43)	4·02 (1·80–10·22)	
40–59	23 (1·82)	1243 (98·18)	4·62 (2·05–11·81)	
60–79	30 (2·37)	1238 (97·63)	5·87 (2·69–14·73)	
Sex				0·12
Male	45 (1·78)	2477 (98·22)	1·41 (0·92–2·18)	
Female	40 (1·28)	3092 (98·72)	Reference	
Marital status				0·02
Married, registered partnership, living together	52 (1·87)	2733 (98·13)	0·58 (0·31–1·14)	
Not married	18 (2·13)	829 (97·87)	Reference	
Divorced, living apart, widow, widower	7 (1·64)	419 (98·36)	0·48 (0·17–1·29)	
Country of birth				0·02
The Netherlands	68 (1·41)	4765 (98·59)	Reference	
Turkey	6 (6·32)	89 (93·68)	5·23 (1·96–11·72)	
Other country	9 (1·41)	631 (98·59)	0·98 (0·45–1·90)	
Net income (€/month)				0·08
⩽850	10 (2·82)	344 (97·18)	2·16 (1·02–4·14)	
>850	62 (1·50)	4064 (98·50)	Reference	
Chose not to answer/ missing	13 (1·11)	1161 (98·89)	0·8 (0·42–1·43)	
Religion				0·08
Protestant Christian	28 (2·15)	1273 (97·85)	1·72 (0·97–3·08)	
Catholic	18 (1·11)	1601 (98·89)	0·88 (0·46–1·65)	
Muslim	11 (2·81)	381 (97·19)	3·23 (1·14–7·98)	
Other religion	5 (1·59)	310 (98·41)	1·38 (0·46–3·43)	
No religion	22 (1·12)	1941 (98·88)	Reference	
Allergies (self-reported)				0·13
No allergies	80 (1·45)	5423 (98·55)	Reference	
Dust allergy	2 (9·09)	20 (90·91)	5·87 (0·90–21·63)	
Contact with cats†				0·09
No contact	47 (1·90)	2428 (98·10)	Reference	
Yes, contact with young cats	0 (0·0)	192 (100·0)	0·00 (0·00–13·49)	
Yes, contact with older cats	25 (1·15)	2141 (98·85)	0·69 (0·42–1·12)	
Yes, both or of unknown age	11 (1·75)	619 (98·25)	1·18 (0·57–2·25)	
Kept pets (past 5 years)				0·21
No	48 (2·00)	2349 (98·00)	Reference	
Yes	35 (1·10)	3147 (98·90)	0·70 (0·44–1·11)	
Kept livestock (past 5 years)				<0·01
No	69 (1·33)	5116 (98·67)	Reference	
Yes, ruminants only	3 (4·62)	62 (95·38)	4·25 (1·00–12·31)	
Yes, ruminants and other farm animals	7 (8·86)	72 (91·14)	8·99 (3·54–19·93)	
Yes, but no ruminants	2 (1·14)	173 (98·86)	1·09 (0·18–3·56)	

Table 2 (*cont.*)

Variable	No. (%) of respondents		OR (95% CI)	*P*
	Seropositive ($n=85$)	Seronegative ($n=5569$)		
Frequency of eating raw meat				0·13
Does not eat raw meat (including vegetarians)	31 (2·23)	1357 (97·77)	Reference	
Daily/weekly	16 (2·22)	704 (97·78)	1·23 (0·64–2·28)	
Monthly	14 (1·57)	876 (98·43)	0·89 (0·45–1·70)	
Less than monthly	12 (0·96)	1243 (99·04)	0·53 (0·26–1·02)	
Occupational contact				0·01
No contact with animals	63 (1·69)	3657 (98·31)	Reference	
Contact with animals	10 (4·02)	239 (95·98)	2·75 (1·30–5·25)	

OR, Odds ratio; CI, confidence interval.
* Adjusted for sex, age and ethnicity.
† Adjusted for sex and age.

Table 3. *Final multivariable model for risk factors associated with seropositivity to Coxiella burnetii, PIENTER 2 project, The Netherlands, February 2006–June 2007*

Variable	OR (95%CI)	*P*
Age category (yr)		
0–14	Reference	
15–39	5·41 (1·43–20·40)	0·01
40–59	6·04 (1·59–22·89)	<0·01
60–79	6·62 (1·83–23·99)	<0·01
Sex		
Male	1·42 (0·92–2·21)	0·12
Female	Reference	
Country of birth		
The Netherlands	Reference	
Turkey	5·07 (2·05–12·54)	<0·01
Other country	0·92 (0·44–1·92)	0·83
Net income (€/month)		
⩽850	1·62 (0·79–3·33)	0·19
>850	Reference	
Chose not to answer/ missing	0·66 (0·35–1·24)	0·20
Kept pets (past 5 years)		
No	Reference	
Yes	0·53 (0·32–0·87)	0·01
Kept livestock (past 5 years)		
No	Reference	
Yes, ruminants only	3·83 (1·12–13·13)	0·03
Yes, ruminants and other farm animals	8·24 (3·26–20·81)	<0·01
Yes, but no ruminants	1·10 (0·26–4·65)	0·89
Occupational contact		
No contact with animals	Reference	
Contact with animals	2·04 (0·93–4·44)	0·07

OR, Odds ratio; CI, confidence interval.

outbreaks in the USA and Canada were associated with parturient cats and dogs [28, 29].

Our study has several limitations as the exposure information collected in the questionnaire was mainly focused on vaccine-preventable infections instead of zoonotic infections. Information on exposures does not necessarily relate to the relevant time period as we do not know at what moment the actual infection with *C. burnetii* occurred in those testing serologically positive. Close contact with animals other than through occupation or ownership and proximity to animal stables or farms could not be studied. Further, the questionnaire did not include information on exact occupation. Possible associations between history of miscarriage or stillbirth in female participants and seropositivity could not be studied, as no information on reproductive history was collected. Our study demonstrated a low prevalence of *C. burnetii* infection in the period 2006–2007, around 1·5% based on ELISA. The Serion IgG ELISA has shown a suboptimal sensitivity of 58% [23], which at least led to an underestimate of the national seroprevalence, which was adjusted in retrospect although based on only a subset of seronegatives. We consider it unlikely that misclassification of serological status was related to the exposure variable and has systematically biased our risk-factor analysis. However, risk factors will be flawed because of dilution of the effect by probable random misclassification. A two-step screening approach, using ELISA as a screening tool and IFA for confirmation, was used in a recent seroprevalence study from the USA [8]. We confirmed that using the same approach, with only the 70 IFA-confirmed ELISA-positive samples in the statistical analysis

instead of 85 ELISA-positive samples, the same risk factors were observed as in the current study (data not shown). A separate risk-factor analysis only based on the subset of IFA-tested samples was not possible due to low seroprevalence.

Further modelling studies are necessary to study the relationship between IFA and ELISA and improve or replace the cut-off for ELISA as a binomial outcome to a probability, which will improve the multivariate analysis of the risk factors, but confidence intervals will remain large. Municipalities in the highest-incidence Q fever areas in 2007–2010 were not part of the study sample. In 2007 a much larger part of Noord-Brabant province was already affected. Several other municipalities in this early-affected province were included in our study sample. However, adjacent municipalities did not show a higher seroprevalence nor was a relationship of seroprevalence with goat density observed.

This study will serve as a baseline for future population-based seroprevalence studies performed after the emergence of Q fever. Prior to the recent Q fever epidemics the overall seroprevalence of *C. burnetii* infection in The Netherlands was relatively low and it was not associated with goat, sheep or cattle density, but merely with keeping ruminants or occupational contact with animals. This supports the hypothesis that The Netherlands has been confronted with a newly emerging Q fever problem in the general population since the spring of 2007. Before the start of the epidemics, high-risk groups for Q fever were individuals with animal contact, including occupational exposure, and Turkish immigrants, probably infected in their home country. Further modelling studies are needed to study the relationship between IFA and ELISA in order to improve comparison between seroprevalence studies.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Schimmer B, *et al.*** Large ongoing Q fever outbreak in the south of The Netherlands, 2008. *Eurosurveillance* 2008; **13**: pii = 18939 (http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId = 18939).
2. **Schimmer B, *et al.*** Sustained intensive transmission of Q fever in the south of theNetherlands, 2009. *Eurosurveillance* 2009; **14**: pii = 19210 (http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId = 19210).
3. **Karagiannis I, *et al.*** Investigation of a Q fever outbreak in a rural area of The Netherlands. *Epidemiology and Infection* 2009; **137**: 1283–1294.
4. **Schimmer B, *et al.*** The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. *BMC Infectious Diseases* 2010; **10**: 69.
5. **van der Hoek W, *et al.*** Q fever in the Netherlands: an update on the epidemiology and control measures. *Eurosurveillance* 2010; **15**: pii = 19520 (http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId = 19520).
6. **van den Wijngaard CC, *et al.*** In search of hidden Q-fever outbreaks: linking syndromic hospital clusters to infected goat farms. *Epidemiology and Infection* 2011; **139**: 19–26.
7. **Richardus JH, *et al.*** Q fever in the Netherlands: a seroepidemiological survey among human population groups from 1968 to 1983. *Epidemiology and Infection* 1987; **98**: 211–219.
8. **Anderson AD, *et al.*** Seroprevalence of Q fever in the United States, 2003–2004. *American Journal of Tropical Medicine and Hygiene* 2009; **81**: 691–694.
9. **Tissot DH, *et al.*** Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. *American Journal of Medicine* 1992; **93**: 427–434.
10. **Abe T, *et al.*** A seroepidemiological study of the risks of Q fever infection in Japanese veterinarians. *European Journal of Epidemiology* 2001; **17**: 1029–1032.
11. **McCaughey C, *et al.*** Human seroprevalence to Coxiella burnetii (Q fever) in Northern Ireland. *Zoonoses and Public Health* 2008; **55**: 189–194.
12. **Davies TR, *et al.*** Prevalence of Q fever in a rural practice. *Journal of Public Health Medicine* 1997; **19**: 324–327.
13. **European Centre for Disease Prevention and Control.** Technical report: risk assessment on Q fever. Stockholm, Sweden: ECDC, 2010, pp. 40.
14. **Tokarevich NK, *et al.*** Anthropogenic effects on changing Q fever epidemiology in Russia. *Annals of the New York Academy of Sciences* 2006; **1078**: 120–123.
15. **Thomas DR, *et al.*** The risk of acquiring Q fever on farms: a seroepidemiological study. *Occupational and Environmental Medicine* 1995; **52**: 644–647.
16. **Bartolome J, *et al.*** Seroepidemiology of *Coxiella burnetii* infection among blood donors in Albacete. *Enfermedades Infecciosas y Microbiologia Clinica* 2007; **25**: 382–386.

17. **Pascual-Velasco F, *et al.*** High seroprevalence of Coxiella burnetii infection in Eastern Cantabria (Spain). *International Journal of Epidemiology* 1998; **27**: 142–145.
18. **Cardenosa N, *et al.*** Short report: seroprevalence of human infection by Coxiella burnetii in Barcelona (northeast of Spain). *American Journal of Tropical Medicine and Hygiene* 2006; **75**: 33–35.
19. **Gozalan A, *et al.*** Seroprevalence of Q fever in a district located in the west Black Sea region of Turkey. *European Journal of Clinical Microbiology and Infectious Diseases* 2010; **29**: 465–469.
20. **Psaroulaki A, *et al.*** Epidemiological study of Q fever in humans, ruminant animals, and ticks in Cyprus using a geographical information system. *European Journal of Clinical Microbiology & Infectious Diseases* 2006; **25**: 576–586.
21. **European Food Safety Authority.** Scientific opinion on Q fever. *EFSA Journal* 2010; **8**: 1–114.
22. **van der Klis FR, *et al.*** Second national serum bank for population-based seroprevalence studies in the Netherlands. *Netherlands Journal of Medicine* 2009; **67**: 301–308.
23. **Blaauw GJ, *et al.*** The application of an enzyme-linked immunosorbent assay or an immunofluorescent assay test leads to different estimates of seroprevalence of *Coxiella burnetii* in the population. *Epidemiology and Infection* 2011. doi:10.1017/S0950268811000021.
24. **Meekelenkamp JC, *et al.*** Seroprevalence of Coxiella burnetii in pregnant women in the province of Noord-Brabant in 2007. *Infectieziekten Bulletin* 2009; **20**: 57–61.
25. **Whitney EA, *et al.*** Seroepidemiologic and occupational risk survey for Coxiella burnetii antibodies among US veterinarians. *Clinical Infectious Diseases* 2009; **48**: 550–557.
26. **Kilic S, *et al.*** Prevalence of Coxiella burnetii antibodies in blood donors in Ankara, Central Anatolia, Turkey. *New Microbiologica* 2008; **31**: 527–534.
27. **Leone M, *et al.*** Effect of sex on Coxiella burnetii infection: protective role of 17betaestradiol. *Journal of Infectious Diseases* 2004; **189**: 339–345.
28. **Pinsky RL, *et al.*** An outbreak of cat-associated Q fever in the United States. *Journal of Infectious Diseases* 1991; **164**: 202–204.
29. **Buhariwalla F, Cann B, Marrie TJ.** A dog-related outbreak of Q fever. *Clinical Infectious Diseases* 1996; **23**: 753–755.

CHAPTER 2.2

Time-course of antibody responses against *Coxiella bunetii* following acute Q fever

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Time-course of antibody responses against *Coxiella burnetii* following acute Q fever

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SUMMARY

Large outbreaks of Q fever in The Netherlands have provided a unique opportunity for studying longitudinal serum antibody responses in patients. Results are presented of a cohort of 344 patients with acute symptoms of Q fever with three or more serum samples per patient. In all these serum samples IgM and IgG against phase 1 and 2 *Coxiella burnetii* were measured by an immunofluorescence assay. A mathematical model of the dynamic interaction of serum antibodies and pathogens was used in a mixed model framework to quantitatively analyse responses to *C. burnetii* infection. Responses show strong heterogeneity, with individual serum antibody responses widely different in magnitude and shape. Features of the response, peak titre and decay rate, are used to characterize the diversity of the observed responses. Binary mixture analysis of IgG peak levels (phases 1 and 2) reveals a class of patients with high IgG peak titres that decay slowly and may represent potential chronic cases. When combining the results of mixture analysis into an odds score, it is concluded that not only high IgG phase 1 may be predictive for chronic Q fever, but also that high IgG phase 2 may aid in detecting such putative chronic cases.

Key words: *Coxiella*, mathematical modelling, Q fever, serology, statistics.

INTRODUCTION

Since mid-2007 human cases of Q fever in The Netherlands have increased sharply, with major outbreaks in 2008 and 2009, reaching a total of about 4000 cases by 2010 [1–4]. Cases were predominantly clustered in the south of The Netherlands [3] and the majority of the laboratory samples were submitted to one regional hospital: the Jeroen Bosch Hospital in 's-Hertogenbosch (about 2000 confirmed acute Q fever cases). Follow-up of cases extended over a period of up to 24 months. In the 3 years that this outbreak persisted, laboratory results from over 2000 cases were collected in a laboratory database. This

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database provides a unique opportunity for studying the serum antibody response to infection with *Coxiella burnetii*, and in particular enough data to obtain insight into natural variation in the time-course of serum antibodies in acute cases [5, 6].

The immunofluorescence assay (IFA) is considered the reference method for serological diagnosis of acute and chronic Q fever [7, 8]. The IFA used for Q fever is a semi-quantitative assay: test sera are visually compared to a fluorescence standard and the titre is scored on a scale of twofold serial dilutions. In this study IgM and IgG against both phase 1 and phase 2 antigen [9] were analysed, yielding time-courses of four variables. The IgM response precedes that of IgG and phase 2 antibodies indicate acute infection while persistently high titres of IgG phase 1 antibodies are associated with chronic Q fever [9].

In order to improve interpretation of serological data a dynamic mathematical model was used to quantitatively describe the serum antibody response. Characteristic features such as time to peak, peak antibody level, and decay rate are estimated as (joint) probability distributions, to describe their variation in individual patients. Using these longitudinal antibody response patterns, a binary mixture approach is used to identify two distinct classes of serum antibody responses, by their peak antibody levels and decay rates.

MATERIALS AND METHODS

Collection of clinical and serum antibody data

Samples from cases diagnosed with acute Q fever (positive serum PCR [8] and/or IgM phase $2 \geqslant 1:32$) from 1 January 2007 to 20 July 2009 were included in the laboratory database of the Jeroen Bosch Hospital. As described in Morroy *et al*. [10], 870 Q fever patients who were part of the 2007 and 2008 cohorts were mailed an informed consent form and a questionnaire for the date of onset of illness.

Patient consent was obtained between February 2009 and April 2009. According to Dutch law for research involving human subjects there was no need for approval by a medical ethics committee.

From the laboratory database patients with three or more blood samples were selected. A total of 344 patients belonging to the 2007 and 2008 cohorts were included in this study after having given informed consent for linking their laboratory data with the questionnaire data including date of onset of illness.

From this database patients referred for Q fever diagnostics, with three or more blood samples, were selected, yielding a total of 344 patients with at least three blood samples over a period of about 2.5 years.

The diagnosis of chronic Q fever was made independently from the present study by a multidisciplinary team of medical specialists, based on serological profile, PCR results [8], presence of clinical data [11], radiological imaging, clinical presentation and other patient characteristics. Patients had proven chronic Q fever infection when they were PCR-positive in a blood sample obtained more than 3 months after the onset of acute Q fever, and had a clinical syndrome compatible with chronic Q fever.

Serology

In all serum samples IgM and IgG antibody titres against phase 1 and phase 2 *C. burnetii* were measured by IFA [7, 12].

The IFA used for Q fever produces semi-quantitative data. Immunofluorescence in serial dilutions of the test sera are visually compared to a standard and the titre is scored as a dilution factor (IFA, Focus Diagnostics, USA) [5, 13]. As dilutions increase twofold, any observed antibody titre is known up to a single dilution step of magnitude 2. For a quantitative interpretation, the reported measurements must be translated to antibody concentrations. Table 1 shows examples of the interpretation of IFA data. Any observed titre is always an interval-censored observation. Note that concentrations may be too low to read ($<1:32$) or sera may not have been diluted sufficiently to allow measurement to within a single dilution step.

Dynamic model for serum antibody responses

Any observed titre consists of two numbers as a pair of observations (X_1, X_2), representing an upper and a lower margin for the concentration (Table 1). In the case of a missing lower margin $X_1 = 0$, and when an upper margin is missing $X_2 = \infty$. As titres are measured on a twofold scale a lognormal error model is a natural choice [14]. If $\Phi(x|\log(\mu), \sigma)$ is a cumulative normal probability function with mean $\log(\mu)$ and standard deviation σ,

$$\text{Prob}(\log(X) \leqslant u) = \Phi(u|\log(\mu), \sigma). \quad (1)$$

By fitting a normal model with mean $\log(\mu)$ to $\log(X)$ (transforming both data and model) the measurement

Table 1. *Quantitative interpretation of immunofluorescence assay data: example of various censored observations*

Readout	Interpreted as
$<1:32$	$0 < X < 32$
$1:32$	$32 \leqslant X < 64$
$1:512$	$512 \leqslant X < 1024$
$>1:1024$	$1024 < X < \infty$

For example readout: dilution stage 32 means that the fluorescence threshold is reached at dilution 1:32. All these observations must be weighted appropriately.

error is described by a lognormal distribution. Then the likelihood of an observation with interval boundaries (X_1, X_2) is

$$\ell_{\text{obs}}(\mu, \sigma | X_1, X_2) = \Phi(\log(X_2) | \log(\mu), \sigma) - \Phi(\log(X_1) | \log(\mu), \sigma), \quad (2)$$

where the expected value of the serum antibody titre (μ) at time t post-infection is described by a longitudinal function $f(t, \theta)$, representing the serum antibody response $f(t, \theta)$, modelled by assuming that pathogens grow with a constant rate producing antigen ($y(t)$), that leads to the production of inactivating antibodies ($x(t)$), produced with a rate proportional to their chance of encountering antigen. Pathogens (antigen) and antibodies interact as a chemical reaction system with mass-action behaviour. This leads to the classic predator–prey model of Lotka and Volterra [15]

$$\begin{cases} y'(t) = +ay(t) - bx(t)y(t) \\ x'(t) = -cx(t) + dx(t)y(t) \end{cases} \quad \begin{cases} y(0) = y_0 \\ x(0) = x_0 \end{cases}. \quad (3)$$

Initial conditions are the numbers of pathogens present at the time of infection (y_0) and baseline antibody level x_0 and the time-course of serum antibody titres described by the function $f(t, \theta) = x(t|a, b, c, d, x_0, y_0)$.

If for patient n at K different times $T_n = \{T_{n,1}, T_{n,2}, \ldots, T_{n,K}\}$ sera have been sampled with titres

$$\mathbf{X}_n = \left\{ \begin{pmatrix} X_{n,1,1} \\ X_{n,1,2} \end{pmatrix}, \begin{pmatrix} X_{n,2,1} \\ X_{n,2,2} \end{pmatrix}, \ldots, \begin{pmatrix} X_{n,K,1} \\ X_{n,K,2} \end{pmatrix} \right\}, \quad (4)$$

then for that patient the contribution to longitudinal likelihood is

$$\ell_n(\theta_n, \sigma | \mathbf{T}_n, \mathbf{X}_n) = \prod_{k=1}^{K} \ell_{\text{obs}} \times (f(T_{n,k}, \theta_n), \sigma | X_{n,k,1}, X_{n,k,2}), \quad (5)$$

assuming measurement errors independent and identically distributed. Parameters were transformed: $u = \sqrt{ac}$, $w = \sqrt{bd}$, $v = \sqrt{a/c}$ and $z = \sqrt{b/d}$ and these new parameters were log-transformed. u and w were fixed (assumed not to vary between patients), w and z were random, as was the initial antibody titre x_0. The initial pathogen level was fixed at $y_0 = 1$. Uncorrelated normal priors were used for $\log(u)$ and $\log(v)$, both $\log(w)$ and $\log(z)$ were assumed to be normally distributed among patients with hyperparameters for the population means μ_w and μ_z normal, and standard deviations σ_w and σ_z gamma distributed. A full account of the longitudinal model has been published [16, 17].

Using Markov Chain Monte Carlo (MCMC) methods a Monte Carlo sample is obtained of the individual parameters θ_n, as well as a set of hyperparameters describing their joint (multivariate normal) distribution over the sampled population [18]. The posterior probability of any sample of the Markov chain can be calculated, allowing selection of the most likely (posterior) parameter set.

The four different antibodies studied (IgM and IgG, phases 1 and 2) were fitted separately.

Instead of using the parameters of the longitudinal model, the resulting estimates were used to calculate characteristics of the response: Time to peak in days, peak titre in IFA units, and decay rate in days^{-1}. These characteristic features of the serum antibody response are easy to interpret and illustrate the variability of the individual responses in patients.

Binary mixture analysis

The estimated peak levels were analysed for clustering: a suspected heterogeneous sample may be analysed as a mixture of two or more component distributions, representing two distinct subpopulations. Such binary distribution mixtures are well suited for classification in serology [19].

After log transformation the distributions of peak antibody titres and half-times (time to decrease from peak titre to half of the peak titre) obtained from the fitted longitudinal responses may be described by a mixture of two normally distributed components $g(\,)$ with different parameters. The contribution of a single peak titre u to the mixture likelihood is

$$\ell_{\text{mix}}(u | \mu_1, \sigma_1, \mu_2, \sigma_2, p) = (1-p)g(u|\mu_1, \sigma_1) + (p)g(u|\mu_2, \sigma_2), \quad (6)$$

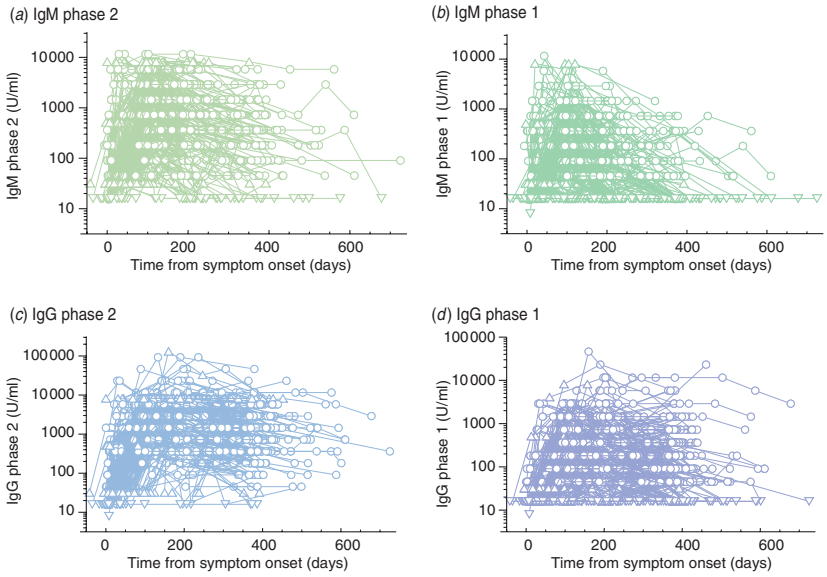

Fig. 1 [*colour online*]. Observed individual IgM and IgG titres against phase 2 and 1 *C. burnetii* against time following symptom onset in 344 patients measured by immunofluorescence assay. Data from the same patient are connected. Symbols indicate censoring: circles at geometric mean when both an upper and lower level have been observed. Triangles indicate absence of either a lower bound (downward symbol) or an upper bound (upward symbol).

where $g(u|\mu_1,\sigma_1)$ and $g(u|\mu_2,\sigma_2)$ are the distributions of 'negative' and 'positive' subpopulations and p is the proportion 'positive' samples. The two fitted components allow quantification of specificity and sensitivity [20]. For half-times a similar likelihood function can be constructed.

When described as a binary distribution mixture, any titre can be assigned a probability of belonging to either subpopulation. Using the ratio

$$r(U) = \frac{g(U|\mu_2,\sigma_2)}{g(U|\mu_1,\sigma_1)} \quad (7)$$

individual classification can be done, assigning odds $r(U)$ of a positive specimen to any set of observations for a case U.

RESULTS

Observed serum antibody titres

A total of 1624 serum samples were used in the analysis. The average age of the 344 patients (209 males, 135 females) was 51 years (range 9–87 years), 34 patients became ill in 2007 and 310 had an onset of illness in 2008. The median serological follow-up since first positive IgM phase 2 was 363 days (range 273–577 days).

Figure 1 shows graphs of the individual time-course of all serum antibody measurements in all patients. Note that there are three different types of censoring: both a lower and an upper margin are present (represented by a circle at the geometric mean of upper and lower margins); an upper margin is present but a lower margin is missing (downward pointing triangle placed at the upper margin level) and lower margin present but upper margin missing (upward pointing triangle placed at the lower margin level).

There was no clear difference between any of the antibodies measured in males or females, nor could an age pattern be established (see Appendix Fig. A3).

Characteristics of the antibody response

The modelled antibody responses showed considerable heterogeneity. Both the magnitude and the shape of the serum antibody response varied strongly in individual patients. IgM and IgG against phase 2 tended to reach higher levels than the corresponding phase 1 responses, while IgG antibodies tended to be more persistent than IgM.

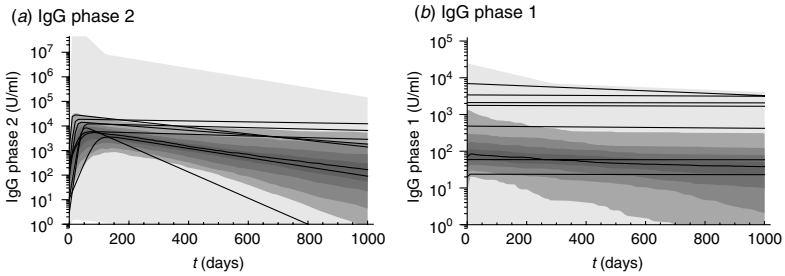

Fig. 2. The grey areas show quantile charts of the time-course of fitted responses for IgG phases 1 and 2. Quantiles shown are (from the outside inwards): 0–100 %, 10–90 %, 20–80 %, 30–70 %, 40–60 %, and 50 % (black line). The superimposed black curves are the fitted responses of seven individual confirmed chronic patients.

Peak titres of phase 2 antibodies were higher, almost by an order of magnitude compared to phase 1 antibodies. Estimated decay rates were smallest (slowest decay) in IgG phase 1, and more or less the same in all other antibody responses.

Due to the low decay rates, patients with high estimated peak titres tend to keep these high titres for a prolonged period, for more than a year after diagnosis of acute Q fever. Correlation coefficients of these characteristics are given in the Appendix. As different antibody classes have been fitted independently, correlation has not been included into the longitudinal models. However, by using the parameter estimates of the individually fitted responses any correlation in the observed data is conserved in the fitted responses. Apart from negative correlation of time to peak and peak titre (and, to a lesser extent, decay rate) there appears to be weak positive correlation between peak levels and decay rates, indicating that high antibody titres tend to decay more rapidly. Time to peak and peak titre tend to be weakly correlated in all antibody classes, correlation between decay rates is lower, especially between IgG phase 2 and IgM (phases 1 and 2) (see Tables A1 and A2).

A contour graph showing quantiles of the fitted responses to IgG phases 1 and 2 (Fig. 2) shows the difference in response patterns. Also shown in these graphs are the responses of confirmed chronic cases.

The peak titres and half-times of these chronic cases do not show a clear segregation into a distinctive subpopulation (see also Appendix Fig. A1).

Classification by binary mixture

As high serum antibody titres are considered predictive for chronic Q fever [5] we attempted to find evidence of a subclass with high peak titres and/or slow decay (long half-times) using binary distribution mixtures. By using symmetric (normally) distributed components we attempted to detect a subclass with overdispersed (large) peak titres/half-times in a homogeneous background distribution. Joint fitting of both phase 1 and phase 2 IgG (separate distribution components for peak titres and half-times, same prevalence, ignoring correlation between phase 1 and 2 IgG) resulted in a 'positive' (high peak titre) fraction of 8·8 % (95 % confidence interval 6·1–11·5 %) of the studied population (Fig. 3).

Characterization as a binary distribution mixture allows specification of the probabilities of false and true positive and negative classifications. Sensitivity [Prob(true pos.)] and specificity [Prob(true neg.)] can be calculated [receiver operating characteristic (ROC) diagrams in Appendix Fig. A2 and Table A3]. The area under the ROC curves (AUC) is 0·991 (peak titres IgG phase 1), 0·914 (half-times IgG phase 1), 0·645 (peak titres IgG phase 2) and 0·973 (half-times IgG phase 2), respectively.

Note that for peak titres of IgG phase 2 antibodies the procedure failed to produce clearly separated mixture components, although the fitted distributions pick up a right-hand tail of high peak titres. Peak titres of IgG phase 2 antibodies do not appear to allow classification with this method.

The fitted distributions may be used to translate any set of the four response variables (peak titre and half-time, IgG phases 1 and 2) into an odds score, as in equation (7). The distributions of these odds are shown in Figure 4 for the seven identified confirmed chronic cases and for the remainder of the patients. Also shown is the combined odds score, obtained by multiplying the odds for the four response variables. It can be seen that chronic patients tend to have odds >1 of falling into the positive subpopulation.

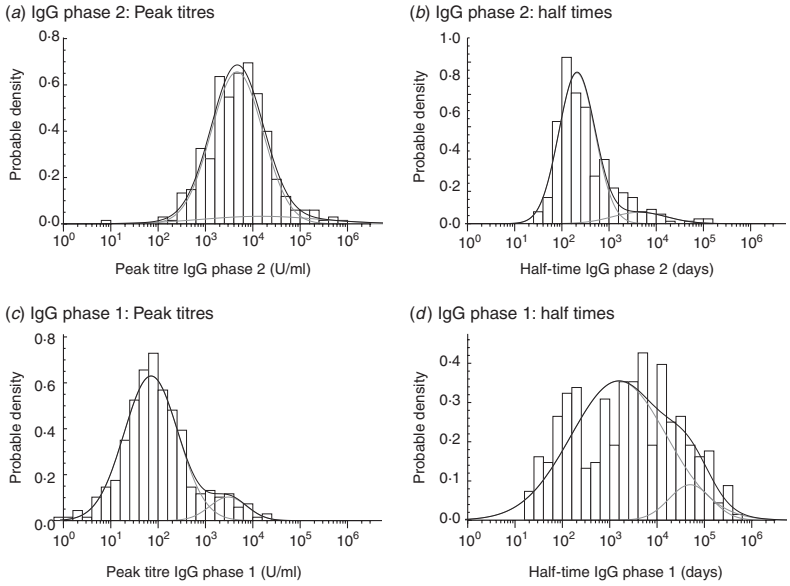

Fig. 3. Discrimination of high and low peak titres by means of a binary mixture of normal distributions. Note that antibody peak titres are shown on a logarithmic scale. The four variables (IgG phase 2 and 1, peak titres and half-times) have their own mixture components but share the same fraction positives.

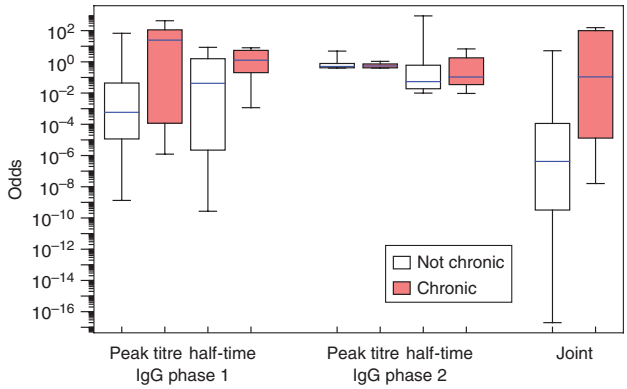

Fig. 4. [*colour online*]. Distribution of the odds for each patient of falling into the 'positive' category as defined by binary mixtures for four variables (peak titre and half-time, for IgG phases 1 and 2). Box plots (median, quartiles and 95% range) for separate variables and the product of all odds scores (joint).

Use of a single ('posterior mode') set of peak titres and half-time results in the odds are shown in Figure 4. Some uncertainty is associated with classification by this method, as is evident from the prediction intervals for the ROC curves (as shown in Appendix Fig. A2). A more comprehensive uncertainty assessment is possible, by determining peak titres and half-times for all patients in any sample of the Monte Carlo set of posterior parameter estimates, determining binary mixture components for each of these sets of peak titres and half-times, and then calculating the odds distributions for all patients over all fitted mixtures. The resulting marginal distribution (of a sample of 1000 fitted mixtures) is essentially similar to that seen in Figure 4 in that the combined odds of confirmed chronic patients are higher than those of the

Table 2. *Geometric mean and 95% confidence interval of the characteristic features of the serum antibody response: time to peak (from symptom onset), peak titre, and half-time of antibody decay after reaching peak levels*

	Time to peak (days)	Peak titre (IFA units)	Half-time (days)
IgM 2	3·09 (0·23–17·1) $\times 10^1$	1·99 (0·20–38·0) $\times 10^3$	2·07 (0·25–43·0) $\times 10^2$
IgM 1	1·07 (0·02–13·5)	1·87 (0·19–50·4) $\times 10^2$	2·67 (0·18–120) $\times 10^2$
IgG 2	5·26 (0·55–21·9) $\times 10^1$	5·23 (0·49–65·0) $\times 10^3$	3·18 (0·64–50·0) $\times 10^2$
IgG 1	0·41 (0·01–4·37)	1·01 (0·07–28·0) $\times 10^2$	2·26 (0·04–122) $\times 10^3$

IFA, Immunofluorescence assay.

remaining patients. These results are summarized in Appendix Figure A4.

DISCUSSION AND CONCLUSIONS

Of the 344 selected patients with acute Q fever, IgG antibody responses characterized by peak titres and half-times could be classified into two categories, with 8·8% of the study population of acute Q fever patients falling into the presumed category with high peak titres and slow decay. Although seven PCR and clinically confirmed chronic cases did not clearly share the characteristics of this 'positive' subpopulation (Fig. 2), their odds of belonging to this positive group (median $\log_{10}$ odds −0·96, 90% range −7·8 to 2·19) were higher than those of the remaining patients (median $\log_{10}$ odds −6·4, 90% range −16·7 to 0·71).

The number of chronic cases is small, and strong heterogeneity in seroresponses to *C. burnetii* may obscure classification attempts. As time passes more chronic cases may be found in the studied population. With higher numbers of cases the validity of serological tests to detect chronic Q fever can be evaluated [11].

This study also has shown that serum antibodies against *C. burnetii* are highly persistent, so that when a person generates high peak titres, any serum sample taken within a year after infection has occurred, is also likely to have high antibody levels (Fig. 2).

The longitudinal analysis was based on semi-quantitative IFA data. As a consequence, the quantitative results may be uncertain, as some output may be based on weak information in the observed data. In particular the estimated time-to-peak characteristics may be uncertain because the time of symptom onset may be incorrect. Clinical symptoms of Q fever may be aspecific and in areas with high incidence, previously resolved Q fever cases may have been misdiagnosed as acute Q fever, thus causing the presence of improper serological data in our study population, leading to misspecification of the responses and incorrect estimation of peak titres and decay rates.

It should also be noted that selecting for patients with multiple samples may imply selection bias: patients with more severe symptoms may have been more willing to submit follow-up samples. If this selection bias resulted in higher antibody titres in the study population, the distinction between chronic patients and the remaining patient population may in reality be clearer than found here.

Moreover, several of the early titre measurements were censored (in order to ascertain that the sample was positive, i.e. $>1:32$). With this caveat in mind, the shorter time-to-peak estimates found for phase 1 antibodies compared to their phase 2 counterparts (Table 2) may not be of note. Peak titres in IgG phase 1 do, however, seem to be more heterogeneous than those in IgG phase 2. Binary mixture analysis confirms that this heterogeneity may be due to a separate high titre class of responses that is more pronounced in IgG phase 1 than in IgG phase 2.

Estimated decay rates are very slow, generally, half-times up to several years are common, with IgG antibodies more persistent than IgM, but mostly because the former show more variation in decay rates.

Peak titres of serum antibody responses cannot be measured in clinical practice, because it is unknown when individual patients reach their maximum antibody titres. However, as shown in Figure 2, antibody decay is very slow and a high peak titre shortly after acute infection is likely to lead to high antibody titres for months to follow.

Many patients present a 'chronic serological profile' [21] during their follow-up and diagnoses of chronic Q fever based on serology alone should be treated with caution [22, 23], particularly because many patients received antibiotic treatment that may have influenced their antibody responses [22].

Notwithstanding the unclear relation between serology and chronic Q fever, the present study shows

that combined information on peak titres and half-times for phase 1 and 2 IgG improves the power of serological detection of chronic cases (Fig. 4). These conclusions can be of use for future studies of Q fever serology.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Schimmer B, *et al.*** Large ongoing Q fever outbreak in the south of The Netherlands. *Eurosurveillance* 2008; **13**(31).
2. **Schimmer B, *et al.*** Sustained intensive transmission of Q fever in the south of The Netherlands. *Eurosurveillance* 2009; **14**(9).
3. **van der Hoek W, *et al.*** Q fever in The Netherlands: an update on the epidemiology and control measures. *Eurosurveillance* 2010; **15**(12).
4. **Dijkstra F, *et al.*** The 2007–2010 Q fever epidemic in The Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. *FEMS Immunology and Medical Microbiology* 2012; **64**: 3–12.
5. **Dupont HT, Thirion X, Raoult D.** Q fever serology: cutoff determination for microimmunofluorescence. *Clinical Diagnostics and Laboratory Immunology* 1994; **1**: 189–196.
6. **Marrie TJ, de Carolis E.** Seroepidemiology of *Coxiella burnetii* infection and its frequency as a cause of community-acquired pneumonia in Canada. *Canadian Journal of Infectious Diseases* 2002; **13**: 164–166.
7. **Fournier PE, Marrie TJ, Raoult D.** Diagnosis of Q fever. *Journal of Clinical Microbiology* 1998; **36**: 1823–1834.
8. **Schneeberger PM, *et al.*** Real-time PCR with serum samples is indispensable for early diagnosis of acute Q fever. *Clinical and Vaccine Immunology* 2010; **17**: 286–290.
9. **Raoult D, Marrie TJ, Mege JL.** Natural history and pathophysiology of Q fever. *Lancet Infectious Diseases* 2005; **5**: 219–226.
10. **Morroy G, *et al.*** The health status of Q fever patients after long-term follow up. *BMC Infectious Diseases* 2011; **11**: 97.
11. **van der Hoek W, *et al.*** Follow-up of 686 patients with acute Q fever and detection of chronic infection. *Clinical Infectious Diseases* 2011; **52**: 1431–1436.
12. **Landais C, *et al.*** From acute Q fever to endocarditis: serological follow-up strategy. *Clinical Infectious Diseases* 2007; **44**: 1337–1340.
13. **Dupont HT, *et al.*** Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. *American Journal of Medicine* 1992; **93**: 427–434.
14. **Teunis PFM, *et al.*** Kinetics of IgG antibody response to pertussis toxin after infection with B. pertussis. *Epidemiology and Infection* 2002; **129**: 479–489.
15. **Edelstein-Keshet L.** *Mathematical Models in Biology* (Birkhauser Mathematics series). New York: McGraw-Hill Inc., 1988.
16. **Simonsen J, *et al.*** Estimation of incidences of infectious diseases based on antibody measurements. *Statistics in Medicine* 2009; **28**: 1882–1885.
17. **Teunis PFM, *et al.*** Biomarker dynamics: estimating infection rates from serological data. *Statistics in Medicine* (in press).
18. **Teunis PFM, Ogden ID, Strachan NJC.** Hierarchical dose response of *E. coli* O157:H7 from human outbreaks incorporating heterogeneity in exposure. *Epidemiology and Infection* 2008;**136**: 761–770.
19. **Gilks WR, Richardson S, Spiegelhalter DJ (eds).** *Markov Chain Monte Carlo in Practice*. London: Chapman & Hall, 1996.
20. **Teunis PF, *et al.*** Usefulness of sero-surveillance for *Trichinella* infections in animal populations. *Veterinary Parasitology* 2009; **159**: 345–349.
21. **Sunder S, *et al.*** Chronic Q fever: relevance of serology. *Clinical Infectious Diseases* 2011; **53**: 749–750.
22. **Limonard GJM, *et al.*** One-year follow-up of patients of the ongoing Q fever outbreak: clinical, serological and echocardiographic findings. *Infection* 2010; **38**: 471–477.
23. **Hung MN, *et al.*** Serologic assessment of the risk of developing chronic Q fever in cohorts of acutely infected individuals. *Journal of Infection* 2011; **62**: 39–44.

APPENDIX

Additional output

Table A1. *Correlations between characteristics of the same antibody*

	Time to peak	Peak titre	Decay rate	Time to peak	Peak titre	Decay rate
	(*a*) IgG phase 1			**(*b*) IgM phase 1**		
Time to peak	1	−0·911	−0·353	1	−0·895	−0·588
Peak titre	−0·911	1	0·185	−0·895	1	0·392
Decay rate	−0·353	0·185	1	−0·588	0·392	1
	(*c*) IgG phase 2			**(*d*) IgM phase 2**		
Time to peak	1	−0·816	−0·408	1	−0·790	−0·525
Peak titre	−0·816	1	0·250	−0·790	1	0·315
Decay rate	−0·408	0·250	1	−0·525	0·315	1

Table A2. *Correlations of characteristics between antibodies*

	IgG 1	IgM 1	IgG 2	IgM 2
	(*a*) Time to peak			
IgG 1	1	0·411	0·340	0·231
IgM 1	0·411	1	0·258	0·326
IgG 2	0·340	0·258	1	0·169
IgM 2	0·231	0·326	0·169	1
	(*b*) Peak titre			
IgG 1	1	0·454	0·429	0·214
IgM 1	0·454	1	0·269	0·325
IgG 2	0·429	0·269	1	0·387
IgM 2	0·214	0·325	0·387	1
	(*c*) Decay rate			
IgG 1	1	0·302	0·165	0·128
IgM 1	0·302	1	0·055	0·275
IgG 2	0·165	0·055	1	0·087
IgM 2	0·128	0·275	0·087	1

Table A3. *Binary mixture for classification of peak titres: specificity and sensitivity as a function of cut-off level*

	IgG phase 1				IgG phase 2			
	Peak titre		Half-time		Peak titre		Half-time	
Cut-off (IFA)	Spec.	Sens.	Spec.	Sens.	Spec.	Sens.	Spec.	Sens.
10	0·067	1·000	0·015	1·000	0·000	0·995	0·000	1·000
20	0·167	1·000	0·031	1·000	0·000	0·990	0·004	1·000
50	0·394	1·000	0·070	1·000	0·000	0·977	0·053	1·000
100	0·603	1·000	0·119	1·000	0·001	0·960	0·203	0·998
200	0·785	0·999	0·189	1·000	0·006	0·934	0·481	0·990
500	0·932	0·977	0·313	1·000	0·038	0·882	0·838	0·950
1000	0·978	0·890	0·425	1·000	0·111	0·827	0·962	0·870
2000	0·994	0·677	0·543	0·999	0·251	0·758	0·995	0·726
5000	0·999	0·291	0·692	0·988	0·523	0·648	1·000	0·463
10000	1·000	0·094	0·788	0·942	0·729	0·554	1·000	0·268
20000	1·000	0·019	0·864	0·815	0·877	0·458	1·000	0·126
50000	1·000	0·001	0·932	0·502	0·970	0·334	1·000	0·033
100000	1·000	0·000	0·963	0·251	0·993	0·251	1·000	0·009

IFA, Immunofluorescence assay; Spec., specificity; Sens., sensitivity.

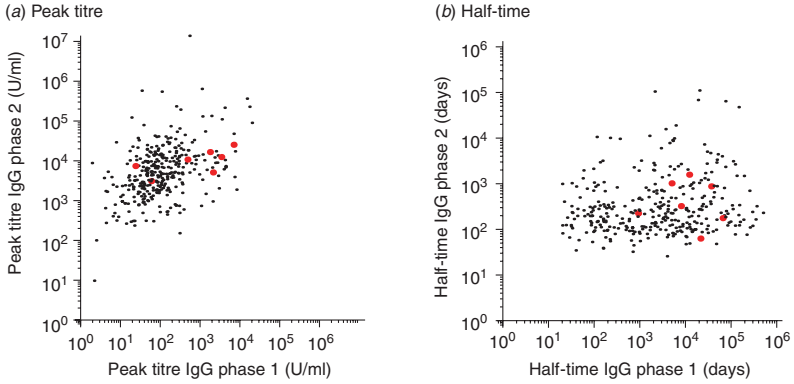


Fig. A1 [*colour online*]. Scatterplots of (*a*) peak titres and (*b*) half-times of IgG phase 1 and 2 antibodies for presumed chronic (grey) and non-chronic patients (black).

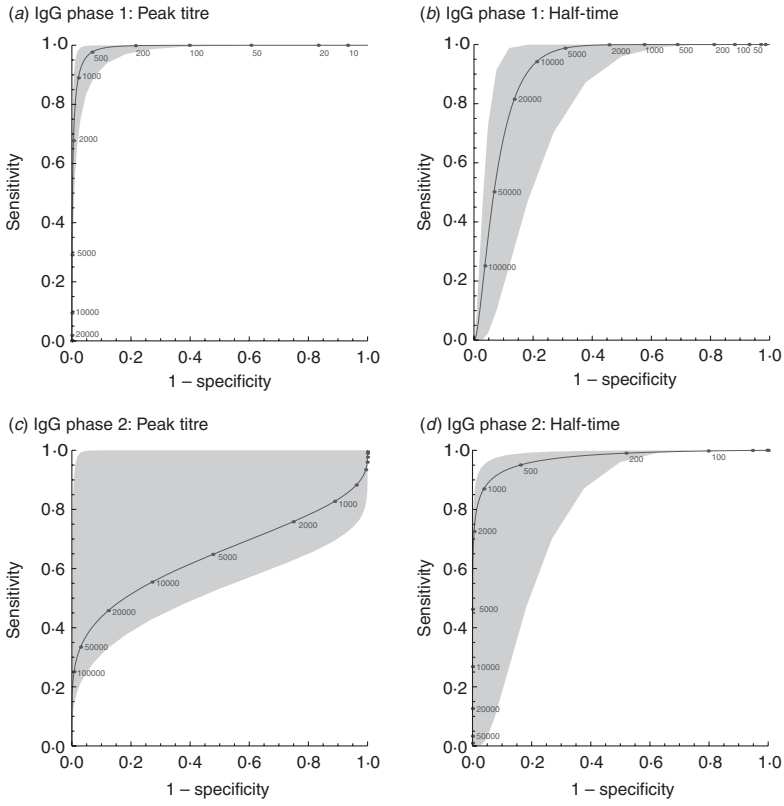


Fig. A2. Specificity and sensitivity of discrimination by peak titre by means of a binary mixture of normal distributions. Receiver operating characteristic (ROC) diagrams shown for the most likely (maximum likelihood) components, and 95% uncertainty interval (grey area). Also shown are levels for peak titres and half-times corresponding to the charted sensitivities and specificities (numbers along the graphs).

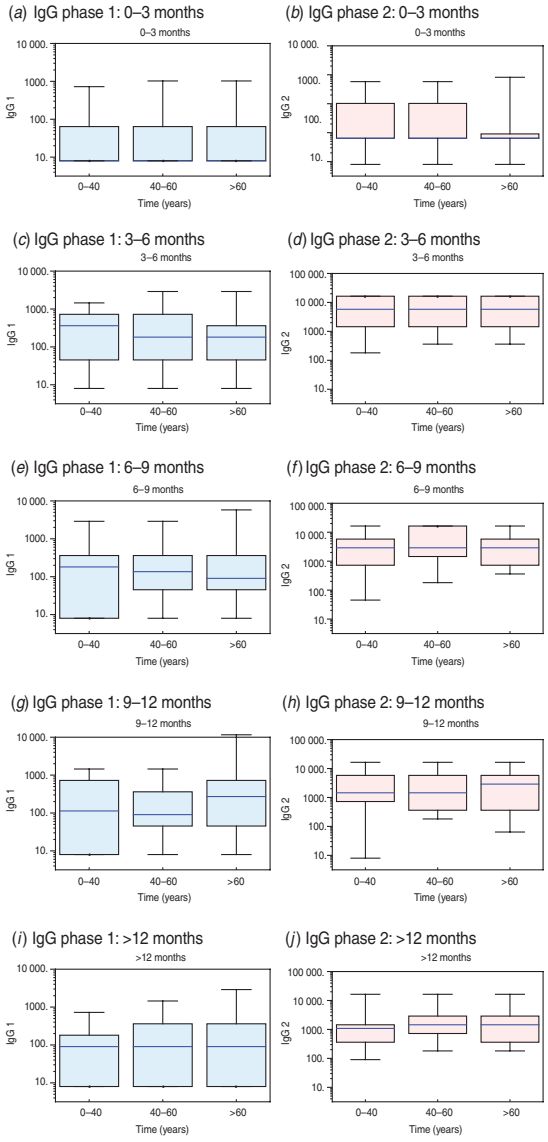


Fig. A3 [*colour online*]. IgG phase 1 and 2 titres by age (<40, 40–60, >60 years) at 0–3, 4–6, 7–9, 10–12, and >12 months following symptom onset.

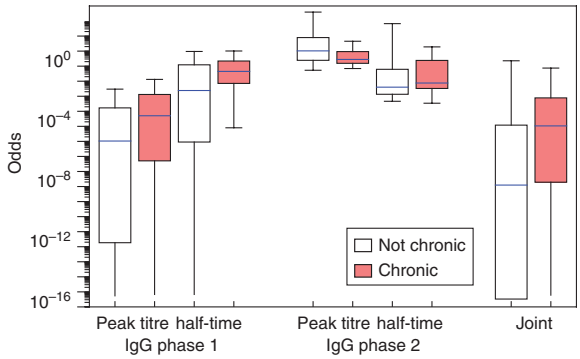
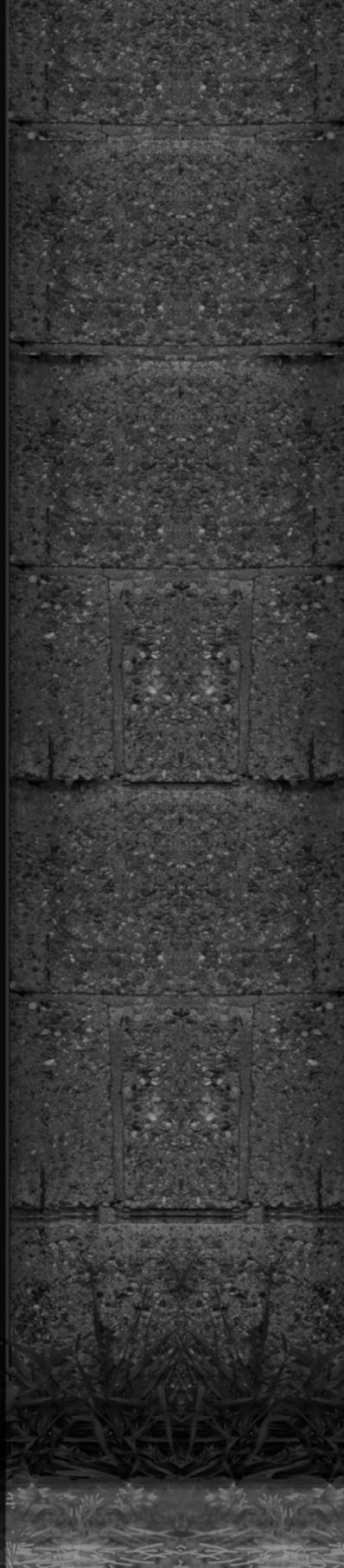


Fig. A4 [*colour online*]. Distribution of the odds for each patient of falling into the 'positive' category (cf. Fig. 4). Assessment of uncertainty in the classification by using a (Markov chain) Monte Carlo sample of fitted longitudinal responses, fitting a binary normal mixture to each individual (posterior) set of peak titres and half-times, and calculating odds over all patients in all fitted mixtures.

3

Outbreak investigations during the Dutch Q fever epidemic



CHAPTER 3.1

Investigation of a Q fever outbreak in a rural area of The Netherlands

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Investigation of a Q fever outbreak in a rural area of The Netherlands

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SUMMARY

A Q fever outbreak occurred in the southeast of The Netherlands in spring and summer 2007. Risk factors for the acquisition of a recent *Coxiella burnetii* infection were studied. In total, 696 inhabitants in the cluster area were invited to complete a questionnaire and provide a blood sample for serological testing of IgG and IgM phases I and II antibodies against *C. burnetii*, in order to recruit seronegative controls for a case-control study. Questionnaires were also sent to 35 previously identified clinical cases. Limited environmental sampling focused on two goat farms in the area. Living in the east of the cluster area, in which a positive goat farm, cattle and small ruminants were situated, smoking and contact with agricultural products were associated with a recent infection. Information leaflets were distributed on a large scale to ruminant farms, including hygiene measures to reduce the risk of spread between animals and to humans.

Key words: Aerosols, disease outbreaks, Q fever, weather.

INTRODUCTION

Q fever is a zoonosis caused by *Coxiella burnetii*, an intracellular bacterium that appears almost everywhere in the world [1]. The most common reservoirs for *C. burnetii* that cause human infections are ruminants, primarily cattle, sheep and goats, although some documented outbreaks have been associated with parturient cats or birds [2–4]. Humans typically acquire an infection from inhaling infected aerosols or dust generated by infected animals or animal products [5].

Q fever usually occurs sporadically, but common occupational exposures have been reported to cause outbreaks, mostly in abattoirs and among veterinarians or research staff using sheep as experimental animals [6–8]. An old study in The Netherlands showed significantly higher prevalence of IgG antibodies against *C. burnetii* among veterinarians, residents of dairy farms and taxidermists compared to controls from the general population [9]. Outbreaks not involving occupational exposure to *C. burnetii*

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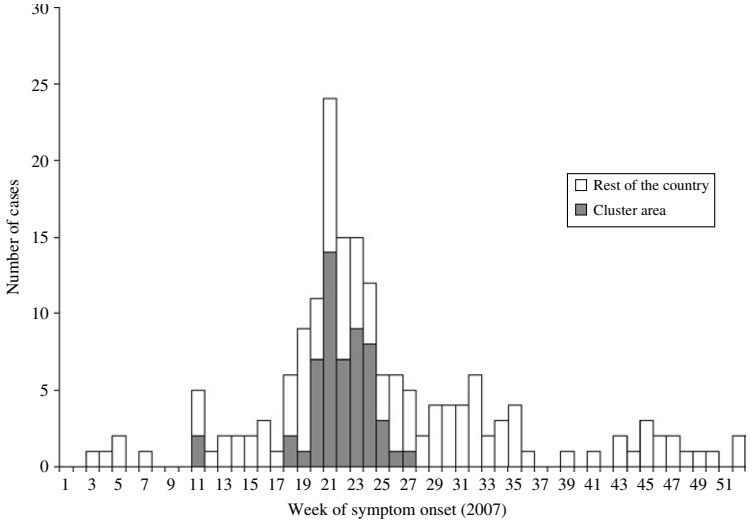

Fig. 1. Distribution of week of symptom onset for notified cases of Q fever in The Netherlands in 2007 ($n=178$). Cluster area ($n=55$).

have also been described, particularly in Europe. These are usually temporally linked to the lambing season in ruminants [10–12].

In The Netherlands Q fever is a mandatory notifiable disease in humans, but only since June 2008 in small ruminants. Five to 20 human cases are notified annually, but no community-acquired outbreak had been reported before 2007 [13].

On 29 May 2007, a general practitioner (GP) from a rural village in the south of The Netherlands alerted the municipal health service about an unusual increase in pneumonia cases in adults [14]. By the end of 2007, 178 Q fever cases with symptom onset in 2007 had been notified and appeared in the national surveillance database (Fig. 1). The peak of the outbreak was in week 21 with most cases occurring between 30 April and 30 June 2007 [15]. A substantial number of the cases ($n=55$) were notified in a well-defined cluster area in the east of the municipality of Oss (Fig. 2).

As notification of further cases was received, an outbreak investigation was launched in this cluster area to describe the outbreak, find the source and route of transmission and investigate possible links to animal reservoirs in the region in order to decide on appropriate control measures. The present study describes this outbreak investigation.

MATERIALS AND METHODS

A frequency-matched case-control study with seronegative controls was used. Both cases and controls were restricted to the adult population of the cluster area.

Thirty-five confirmed cases had been identified in the cluster area by September 2007, when the case-control study was initiated. Our statistical assumptions and criteria were: (*a*) an achievement of a statistical power of 80%, (*b*) assumed overall attack rate of 15%, (*c*) participation rate of 33% in males and 40% in females (*d*) α error equal to 5%, (*e*) 1:2 case: control (seronegative for *C. burnetii*) ratio. Hence, we calculated that 696 inhabitants aged 18–84 years should be asked to complete a questionnaire and provide a blood sample. Previously identified cases were not excluded from the sampling procedure so as to achieve a truly random sample of the population. The overall estimated attack rate of 15% was based on a small survey in pregnant women in this area. The invited participants were frequency-matched with the known cases by village of residence, sex and age category (18–19, 20–29, 30–39, …, 70–79, 80–84 years). All eligible participants were invited by mail. Moreover, all of the 35 cases diagnosed before the study began were invited to complete the same

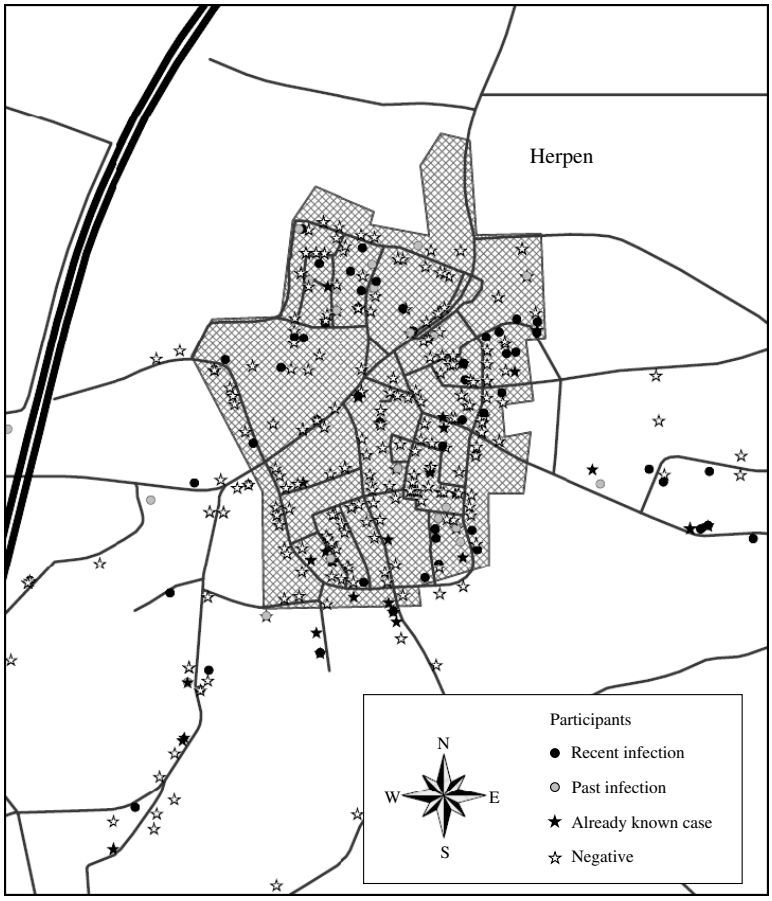

Fig. 2. Participants with laboratory findings compatible with a recent or past *C. burnetii* infection, negative findings and cases previously identified in the centre of the cluster area.

questionnaire. The questionnaire included age, sex, postcode, working situation (including working in open air), residence information, distance of house to farms and meadows with livestock, animal possession, contact with animals, contact with unhandled animal products, consumption of raw milk products, visits to specific places or events, outdoor activities, house ventilation and health conditions – predispositions. Besides self-reported distances, distances from participants' residence to farms with goats, sheep and cows in the cluster area and meadows where dung was spread were calculated using GIS software. We also calculated distance from participants' residence to all postcodes where ruminant farms were situated in the area, in order to identify neighbourhoods associated with recent infections. Participants were also asked about specific symptoms between 7 May and 8 July 2007. The recall period for the exposure variables was the end of April and the whole month of May 2007, i.e. up to 4 weeks before the peak of onset of cases (week 21 of 2007, i.e. 21–27 May 2007).

Cases included all laboratory-confirmed cases previously identified in the area at the time of the start of the study and all seropositive participants indicating a recent infection from the serological survey. The term 'cases' in the univariate and multivariable analyses of the case-control study refers to this whole group of seropositive participants. 'Controls' included participants from the serological survey with negative results for a recent *C. burnetii* infection. All participants with

laboratory evidence for a past infection were excluded from the analysis.

The case-control study was approved by the Medical Ethical Committee of the University Medical Centre Utrecht (reference number: 07-241).

Statistical analysis

Odds ratios (OR) and corresponding 95% confidence intervals (CI) were calculated through logistic regression analysis to identify potential risk factors for the acquisition of a recent *C. burnetii* infection. Variables that were statistically significant at the 20% level and could explain at least 20% of the cases in the univariate analysis were included in the multivariable analyses. In the latter analysis, sex and age were always adjusted for. All other variables were tested with the use of manual backwards-elimination techniques. We also tested for interaction terms between variables in the final multivariable analysis model. All analyses were run in Stata version 10.0 (Stata Corp, College Station, TX, USA).

Laboratory screening

Study participants were screened for Q fever infection with an immunofluorescence assay (IFA) (Focus Diagnostics, Cypress, CA, USA) for IgG and IgM antibodies with a single 1:64 serum dilution. In order to harmonize the screening method, all 35 previously confirmed cases were reconfirmed by IFA and all met the case definition of a recent infection. Screening was performed to distinguish between uninfected, recently infected and individuals who had been infected in the past. Samples with unequivocal results were further analysed using twofold dilutions. A recent infection was defined as IgM phases 1 and $2 \geqslant 1:64$ or an individual with an isolated titre of IgM phases 1 or $2 \geqslant 1:512$. Individuals with a past infection did not match the IgM criteria in the definition of a recent infection, but had IgG phases 1 and $2 \geqslant 1:64$ or an isolated titre of IgG phases 1 or $2 \geqslant 512$. The remaining samples were scored negative and were used as seronegative controls in the case-control study.

Environmental investigation

Within the cluster area, environmental and animal samples were taken from a commercial (goat population: 3794) and a hobby goat farm, both probably related to incident cases, to track possible sources of *C. burnetii*. DNA was extracted using several modified NucliSens DNA extraction protocols (bioMérieux, France), depending on the environmental matrix examined. Detection of *C. burnetii* was performed by using a newly developed multiplex Q-PCR assay. Genomic targets that are most frequently used for detection of *C. burnetii* (*icd*, *com1* [16] and IS*1111* [17]) were incorporated into one multiplex Q-PCR assay. For these three targets, primers and Taqman probes (Biolegio, The Netherlands) were designed using Visual OMP 6 for simultaneous detection. The specificity of the multiplex quantitative real-time PCR (Q-PCR) was tested on a large panel of non-target organisms to verify any cross-reaction with other closely related species. These non-target organisms included *Bacillus cereus*, *B. mycoides*, *B. thuringiensis*, *Yersinia pseudotuberculosis*, *Y. agglomerans*, *Y. enterocolitica*, *Y. frederiksenii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Legionella pneumophila*, *L. bozemonii*, *L. longbeachae*, *L. micdadei*, *L. dumoggii* and *L. anisa*. No cross-reactions were observed for these organisms. The sensitivity of the assay was tested in a probit analysis (De Bruin *et al.*, personal communication) and differed between the three targets. The minimal number of genome equivalents per reaction that could be detected with a 95% probability was found to be below five copies for the single copy targets icd and com1, and below three copies for the multicopy target IS*1111*.

Weather data for April 2007 and the same month over the last 30 years were acquired from the nearby weather station of the National Meteorological Institute (KNMI) at Eindhoven and weather conditions (temperature and wind direction) for this month were compared to mean long-term climatic values for April in the region.

RESULTS

Descriptive results

Of all 696 invited participants, 515 (74·0%) completed a questionnaire and 443 (63·6%) provided a blood sample. All participants who provided a blood sample also completed a questionnaire. Of the 35 previously identified cases, 30 (85·7%) completed the questionnaire. All the cases were eventually confirmed by IFA. Full respondents, i.e. participants who provided both a questionnaire and a blood sample, did not differ in age or sex distribution from the rest of the invited population.

Table 1. *Laboratory results for recent* C. burnetii *infection in participants in the most affected village of the cluster area* (*Herpen*)

Age category (years) ($n=381$)	Males			Females			Total		
	Total	No. pos.*	P(T^+)†	Total	No. pos.*	P(T^+)†	Total	No. pos.	P(T^+)‡
18–19 ($n=11$)	2	1	50·0	9	3	44·4	11	4	45·5
20–29 ($n=7$)	4	2	50·0	3	2	66·7	7	4	57·1
30–39 ($n=17$)	6	1	16·7	11	3	27·3	17	4	23·5
40–49 ($n=137$)	98	22	22·4	39	8	20·5	137	28	21·9
50–59 ($n=83$)	62	11	17·7	21	3	19·0	83	13	18·1
60–69 ($n=91$)	63	9	14·3	28	3	10·7	91	12	13·2
70–79 ($n=27$)	23	2	8·7	4	0	0·0	27	1	7·4
80–84 ($n=8$)	5	1	20·0	3	0	0·0	8	1	12·5
Total (adjusted)‡	263	49	23·0	118	22	22·3	381	67	22·8

* Numbers include cases identified at the time of the study set-up.
† P(T^+) refers to the percentage of participants with a laboratory-confirmed recent infection, including cases identified at the time of the study set-up. The crude P(T^+) in Herpen, not standardized for age and sex distribution of the population, was 19·2% (18·6% for males and 20·3% for females). Excluding past infections from the denominator, the overall attack rate in Herpen was estimated to be 23·9% (24·8% for males and 23·0% for females).
‡ P(T^+) adjusted percentages for the overall age and sex distribution of the village in inhabitants aged 18–84 years.

Table 2. *Self-reported symptoms in patients with a serologically confirmed* C. burnetii *infection in the cluster area in spring/summer 2007*

Symptoms	Participants with a recent infection ($n=73$)			Previously identified cases ($n=30$)*		
	Total	Yes	%	Total	Yes	%
Fever (>38 °C)	63	16	25·4	28	28	100
Malaise	67	26	38·8	26	24	92·3
Headache	64	26	40·6	27	23	85·2
Cough	67	25	37·3	26	18	69·2
Severe fatigue	68	29	42·7	27	24	88·9
Shortness of breath or respiratory difficulties	65	14	21·5	24	16	66·7
Pain or pressure on the pain	64	6	9·4	24	14	58·3
Diarrhoea	67	15	22·4	26	14	53·8
Joint pain	68	18	26·5	27	22	81·5
Night sweating	65	20	30·8	27	25	92·6
Jaundice, hepatitis (as a clinical diagnosis)	64	1	1·6	17	3	17·6
Loss of weight	65	7	10·8	26	14	53·8
Pneumonia (as a clinical diagnosis)	63	4	6·4	24	16	66·7

* Only 30 of the 35 cases identified at the time of the study set-up were supplied with a questionnaire.

Of all 443 people who provided a blood sample, 332 (74·9%) were seronegative for *C. burnetii*, 38 (8·6%) had a past infection and the remaining 73 (16·5%) had a recent infection. Of these recently infected participants, 67 (91·8%) lived in Herpen, the most affected village. The highest percentage of recent infections was observed in participants aged <30 years (median age of cases 49 years, for seronegative controls 54 years), while these percentages did not differ between the two sexes (Table 1). The median age in participants with a past infection was 54·2 years (36–80 years) and the male : female ratio was 2·2.

The most frequent symptoms in the 30 previously confirmed cases that provided a questionnaire were fever (100·0%), night sweating (92·6%) and general malaise (92·3%). Of these cases, 11 (36·7%) were

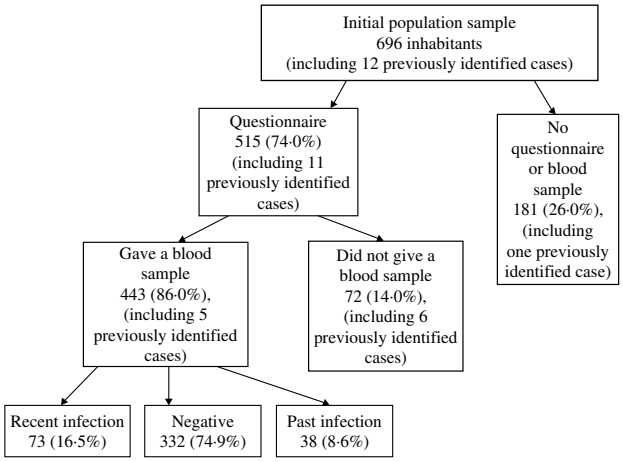

Fig. 3. Participation of the invited inhabitants and results of the serological study for *C. burnetti*, 2007.

hospitalized (Table 2). In the 73 seropositive participants in the serological study, severe fatigue (42·7%), headache (40·6%) and general malaise (38·8%) were the most common symptoms. Fever was only reported by a minority (25·4%). In this group, 25 (34·3%) individuals reported none of the symptoms they were asked about. Consultation with a physician and hospitalization were reported by 17 (23·3%) and one (1·4%) of these seropositive individuals, respectively (Table 2).

Case-control study

The final group of cases for the analyses consisted of the 35 previously diagnosed cases at the time of the study's initiation and the 73 randomly selected participants with a recent infection, the latter including five of the confirmed cases. Therefore, the total number of cases in the case-control study was 103 (98 for risk-factor analyses; no questionnaire was available for five of the confirmed cases) and the number of seronegative controls was 332. The 38 participants with a past *C. burnetii* infection were excluded from the analysis (Fig. 3). The latter participants did not differ from the group of 103 cases in sex distribution, but were on average 5·2 years older.

Several variables were shown to be potential risk factors for the acquisition of a recent *C. burnetii* infection in the univariate analysis. An increase in age by 1 year resulted in a decrease in risk for infection by 3·1% (OR 0·97, 95% CI 0·95–0·99). People aged $\leqslant$44 years had the highest risk of infection. Several risk factors that were associated with exposure to the open air and animals or animal/agricultural products, as well as distance to farms and meadows with goats, sheep and cows or where dung was spread were also significant (Table 3). Household size, type of residence and work in meat treatment, the agricultural sector and wool or leather treatment, as well as having spent nights in a different area during the incubation period, were not found to be related to the acquisition of a recent *C. burnetii* infection. The same applied to doing household-related activities outside the house as proxies for exposure to the open air.

In the multivariable analysis, smoking, contact with agricultural products such as manure, hay and straw and distance to a farm or one of four neighbourhood postal codes to the east of Herpen ('area A') remained statistically significant, also adjusted for sex and age (Table 3). Adding interaction terms between distance to area A and contact with agricultural products was not statistically significant. Smoking was not clearly associated with symptom acquisition, given an infection; in 60 seropositive participants for whom smoking habits were known, 15/24 (62·5%) non-current smokers developed symptoms compared to 27/36 current smokers (75%).

Environmental investigation

Seventy-nine environmental and animal samples were collected from two farms (10 and 69 samples from the hobby and commercial farm respectively), of which 75 were screened for *C. burnetii*. Twenty-five (33%) were

positive for all three targets and six (8%) were positive for the IS*1111* target only. Animal samples screened included: urine, milk and vaginal swabs from individual animals, a swab of a dead animal in a cadaver bin and manure from stable floor. Only urine and milk samples showed no positive results for the presence of *C. burnetii*. Environmental samples screened included straw (from stable floor), surface swabs (floors and walls), insects (collected from a UV lamp), and water (drinking buckets). *C. burnetii* was found to be present in all types of samples, apart from water. All positive samples originated from the same large commercial goat farm, where an abortion wave had occurred in April 2007.

April 2007 was a record warm and dry month in The Netherlands, with an average temperature of 13·4 °C (4·4 °C higher than the 1977–2006 average). Easterlies (wind direction from 45° to 135°) were the most prominent wind direction (11 out of the 30 days in April) and had never been so prominent during April for at least 30 years. This may have contributed to the spread of *C. burnetii* from area A to Herpen.

Control measures and other actions taken

In early 2008, while the lambing season was ongoing, an information leaflet was distributed by regular mail to all goat farms in Noord-Brabant, the province with the highest goat density in the country. This included background information about the disease in both animals and humans and recommended preventive and control measures to reduce spread of *Coxiella*, especially during and after the lambing season. This information was also put on several websites to reach ruminant farmers, including sheep farmers nationwide. Furthermore, it was agreed between the National Public Health Institute and the Animal Health Service (GD), which diagnose the majority of *Coxiella* problems in ruminants, to communicate all postal codes in a radius of 5 km from a newly diagnosed farm. This was done to help to alert physicians in high-risk areas to consider *C. burnetii* for patients with compatible symptoms. On 12 June, reporting of Q fever symptoms in small ruminants held in deep litter houses became notifiable for farmers and veterinarians, and a ban was introduced on the spreading of manure in the 90 days following Q fever-positive status at the farm. Finally, the outbreak triggered several studies to assess the prevalence of *C. burnetii* infections in small ruminants, their milk, the farmers, their families and the general human population, as well as studies on risk factors for infections in ruminants and (sporadic) human cases to generate more evidence-based control measures.

DISCUSSION

In 2007, the first community-acquired Q fever outbreak was identified in The Netherlands. The results of the outbreak investigation suggested that the source of the infections in the cluster area was situated in a rural zone with eight hobby and commercial ruminant farms. These include three dairy cattle farms (about 60–100 cows), one large dairy goat farm (at least 3700 goats), one small sheep breeding farm and three hobby farms with small numbers (<10) of goats or sheep. Although a specific farm or meadow could not be pinpointed, at least one large commercial goat farm in this area was known to suffer from abortion waves due to *C. burnetii* in the spring of 2007. Ideally, more environmental samples are needed to fine-tune the highest-risk area, which was not feasible during the present investigation.

While all previously identified cases at the inception of the study reported having had fever, this symptom was reported by only a quarter of the recently infected individuals obtained from the random population sample. Similarly, all symptoms shown in Table 2 were systematically more frequent in the initial cases that had been reported through the mandatory notification system. The name 'Q fever' may better correspond to clinical cases and should not be considered a prerequisite when considering a *C. burnetii* infection.

Compared to other outbreak reports, full screening of IgG and IgM phases 1 and 2 was performed in order to improve diagnostic accuracy and detect past infections. As the study was a population survey in an outbreak setting, we used a single 1:64 dilution for screening of the population sample in contrast to diagnostic testing of individual clinical cases. The cut-off is a balance between increasing/decreasing sensitivity *vs.* decreasing/increasing specificity, which will thereby influence the positive and negative predictive value, which is further dependent on the prevalence in the study population; the latter is expected to be relatively high in an outbreak setting. There is a lack of standardization in interpretation of serology results [18]. The choice of 1:64 dilution is further justified by our current experience with the 2008 outbreak, which is still under investigation. Finally, IFA results of, among others, outbreak sera were

Table 3. *Results of the univariate and multivariable analyses of the case-control study for acquisition of* C. burneti*i infection in the cluster area, 2007*

		Cases ($N=103$)* *n* (%)	Controls ($N=332$) *n* (%)	OR	95% CI
Univariate analysis					
Age category (years)† (ref.: >62 years)	⩽45	41 (40)	78 (23)	2·57	1·35–4·91
	46–61	42 (41)	158 (48)	1·24	0·67–2·35
Sex† (ref.: female)	Male	68 (66)	215 (65)	1·06	0·66–1·68
Working condition (ref.: not working)	>32 h/week†	49 (52)	142 (43)	1·77	1·01–3·12
	20–31 h/week	8 (8)	28 (9)	1·47	0·59–3·66
	12–19 h/week	8 (8)	24 (7)	1·71	0·68–4·33
	<12 h/week	8 (8)	21 (6)	1·96	0·76–5·02
Work industry (ref.: other than food preparation, animal care, meat treatment, agricultural sector, wool and leather treatment)	Food preparation	5 (7)	5 (2)	3·18	0·88–11·5
	Animal care	3 (5)	1 (1)	9·55	0·95–96·3
Possession of animals (ref.: no possession of a specific animal)	Dogs†	45 (46)	115 (35)	1·60	0·99–2·59
	Pigs	4 (4)	3 (1)	4·67	0·77–32·3
Possession of animals (ref: possession of any animal)	No†	28 (29)	127 (38)	0·65	0·38–1·08
Contact with animals (ref: no contact with any animal)	With other people's animals†	49 (50)	128 (39)	1·59	0·99–2·57
Contact with animals (ref: contact with any animal)	No contact with any animal†	21 (21)	102 (31)	0·61	0·34–1·07
Seen animals (<5 m) (ref: not having seen a specific animal)	(Wild) birds†	30 (31)	76 (23)	1·49	0·87–2·51
	Sheep†	23 (23)	59 (18)	1·42	0·78–2·51
	Goats†	33 (34)	73 (22)	1·80	1·06–3·02
	Horses, ponies†	34 (35)	84 (25)	1·57	0·93–2·61
	Poultry†	32 (33)	74 (22)	1·69	0·99–2·84
	Rodents, rabbits†	35 (36)	76 (23)	1·87	1·11–3·12
	Reptiles	5 (5)	2 (1)	8·87	1·42–94·0
Touched animals (ref: not having touched the specific animal)	Horses, ponies†	21 (21)	39 (12)	2·05	1·13–3·7
	Goats	13 (13)	29 (9)	1·60	0·79–3·22
	Dogs†	57 (58)	157 (47)	1·55	0·96–2·51
	Poultry	15 (15)	30 (9)	1·82	0·93–3·55
	Rodents	20 (20)	39 (12)	1·93	1·06–3·5
	Reptiles	3 (3)	0 (0)	n.a.	2·69–∞
Contact with animal products [ref.: not having had contact with specific (group of) product(s)]	Dung, excreta	20 (20)	48 (14)	1·52	0·80–2·78
	Hay or straw	31 (32)	76 (23)	1·56	0·91–2·62
	Hay, straw or dung†	35 (34)	87 (26)	1·45	0·87–2·37
Consumption (ref.: no consumption)	Raw milk products†	25 (24)	48 (14)	1·90	1·05–3·36
Visits to… (ref.: not attended a specific event)	Children's farm	9 (9)	12 (4)	2·70	0·97–7·21
	Party or BBQ in the neighbourhood	9 (9)	18 (5)	1·76	0·67–4·31
	Herps Mertje†‡	52 (53)	146 (44)	1·44	0·89–2·32
Activities: horse-riding (ref.: seldom or never)	Almost daily	5 (6)	2 (1)	9·93	1·83–53·8
	1–3 times/week	4 (5)	11 (4)	1·44	0·44–4·69
	2–3 times/ month	2 (2)	4 (1)	1·99	0·35–11·11
	<Once/month	2 (2)	1 (0)	7·94	0·70–90·5
Smoking† (ref.: never)	Current smoker†	76 (74)	206 (62)	1·72	1·03–2·93
Prior clinical conditions, medical history (ref.: no consumption of antibiotics)	Antibiotics consumption (end of April and month of May)	14 (14)	22 (7)	2·22	1·00–4·74

Table 3 (*cont.*)

		Cases ($N=103$)* *n* (%)	Controls ($N=332$) *n* (%)	OR	95% CI
Distance from residence to goat farm (m)	Distance to farm No. 4†			0·999	$P=0·033$
	Distance to farm No. 13†			0·999	$P=0·013$
	Distance to farm No. 14†			0·999	$P=0·002$
	Distance to farm No. 15†			0·999	$P=0·017$
Multivariable analysis ($n=430$: 98 cases, 332 controls)					
Smoking (ref.: never smoked)	Current smoker			2·14	1·17–3·90
Contact with hay, straw, dung (ref.: no)	Yes			1·69	1·03–2·80
Distance to farm (m)§	No. 14			0·999	$P=0·001$

OR, Odds ratio; CI, confidence interval; n.a., not available.

* Ninety-eight for analysis of risk factors obtained from the questionnaire. Because distance to farm No. 14 was calculated with GIS software, this information was available for all 103 cases as residence address was also known for the five non-respondents.

† Variables included in the multivariable model before backward elimination.

‡ Large annual open-air market with among others small ruminants as part of a mobile pet farm.

§ Distance to farm No. 14 could be replaced by either one of four adjacent postal codes, with almost equal results for the model. Therefore, it was assumed that farm 14 was not uniquely associated with recent infections. It should be noted that odds ratios should be interpreted as living further away from the farm is protective.

compared with results of two commercially available ELISAs, and showed that the ELISAs had a lower sensitivity compared to IFA and that the IFA showed no substantial cross-reactivity (P. Schneeberger, personal communication).

Rodolakis *et al.* [19] showed that goats excreted *C. burnetii* primarily in milk, and that sheep shed the bacteria primarily in faeces and vaginal mucus. Our results showed that goats also shed *C. burnetii* in faeces and vaginal mucus, which is in agreement with their observations that human Q fever cases are more often related to ovine (in our case caprine) than bovine flocks affected by Q fever. We found no *C. burnetii* presence in the milk samples, but the number of milk samples screened (four) was too small for a valid comparison with other studies.

Weather conditions in April 2007 were favourable for the spread of *C. burnetii*. The unseasonably warm and dry weather conditions, in addition to an unusually easterly component in wind direction, probably contributed to a wide spread of aerosols from contaminated farms to nearby residential areas. Daily maximum temperatures were up to 15 °C higher than the average for April and hardly any precipitation had fallen during that month. The predominant wind direction was also in agreement with the location of area A.

The role of windborne spread is also indirectly supported by the lack of a gender difference in cases in the case-control study. Usually, more males are infected, mainly through occupational exposure. A windborne spread would not give a preference for either females or males; an even sex distribution was indeed observed in the outbreak discussed here.

Only 39/98 cases (39·8%) reported direct contact with, possibly contaminated, agricultural products such as manure, hay and straw. However, contact with hay or straw can cause aerosols and by that contaminate a wide environment. This may explain why no particular common exposures were found by the previously performed hypothesis-generating interviews targeted at the cases diagnosed in the regular medical circuit, which preceded this epidemiological outbreak investigation.

As stated in the first brief outbreak report [14], the initial hypothesis was that the increase in pneumonia cases was caused by *Mycoplasma pneumoniae*. As Q fever is a relatively rarely notified disease in The Netherlands, clinicians usually do not test for *C. burnetii* infection in patients with an atypical pneumonia. Consequently, small or diffuse clusters of cases might easily be missed. This outbreak clearly stresses the added value of early warning by physicians based on the clinical picture only. Therefore, reporting of

unusual numbers of cases with common symptoms to the local health authorities is strongly encouraged. The higher numbers of Q fever cases notified in the second half of 2007 (Fig. 1) and first quarter of 2008 may be partly attributable to the raised awareness among clinicians and increased diagnostic testing at laboratories following the outbreak.

Contact with animals and consumption of raw milk products were not significant risk factors in the multivariable analysis of our study. In theory, this could have been because inhabitants residing closer to area A tended to have more contact with animals. However, a closer inspection of our data showed no interaction between distance of residence to area A and contact with animals. Thomas and colleagues showed that exposure to cattle (but not sheep), cats, raw milk and hay, all reported sources of Q fever, are associated with *C. burnetii* IgG by univariate analysis, but this association was not independent from animal contact [20].

No information on possible general exposures in the past was available from the present study. Individuals with a past infection were older than recent cases, which could represent the cumulative risk for acquiring a *C. burnetii* infection. This group worked more frequently in the agricultural (11·1% *vs.* 2·8%) and meat-handling (7·4% *vs.* 1·4%) sector than recent cases, but these differences were not statistically significant.

Smoking was found to be an important risk factor for the acquisition of a *C. burnetii* infection. A possible explanation could be outdoor smoking habits, resulting in smokers being more exposed to outdoor contaminated aerosols than non-smokers. No information on smoking habits was collected to confirm this. Alternatively, smoking might represent an increased risk by more hand–mouth contact or an increased risk for respiratory infections in general because of alterations in structural and immune defences, as suggested by others [21]. Smoking was not associated with development of symptoms in seropositive participants in the present study, although a slight tendency towards more symptoms for smokers was observed. In contrast, McCaughey and colleagues in a study in Northern Ireland concluded that smoking was not a significant risk factor for the acquisition of infection, but suggested that it was only associated with developing symptomatic disease [22]. No clear explanation can be given for this discrepant result, but different laboratory tests were used and sera in the study in Northern Ireland were 20 years old. Moreover, that study [22] refers to general population sera, where the infection risk is multifactoral and scattered in place and time, while the present study is targeted at an outbreak setting with an overall more homogeneous risk of exposure.

The present study shows that small ruminants were most likely responsible for the outbreak. This group of animals has been implicated in Q fever outbreaks previously [5, 7, 23–25]. It should also be noted that contact with one's own animals was not found to be a significant risk factor in the present study, which might assume partial immunity. The percentage of a past Q fever infection was 10·6% in those who had animals and 4·5% in those who did not. The goat density in the south of the country is the highest (about 38·1 goats/km^2 while the national average is 9·2 goats/km^2) and the goat population is still increasing. Public health education and control measures are, hence, of great importance to avoid future similar outbreaks.

Although not included in the final model, some of the significant univariate associations deserve further attention, as they might have played an intermediate or minor role in the outbreak. Having touched horses and ponies and frequent horse-riding are among these possible risk factors, associated only univariately. This may either indicate Q fever being transmitted from an infected horse, as *Coxiella* infection in horses has been documented previously [26], or the activities related to horse-riding such as cleaning stables, handling straw or hay and brushing the crest might have lead to exposure to contaminated dust or air from the environment. Second, having seen rodents, rabbits and reptiles was univariately found to be associated with Q fever, although the latter was reported by a very small group of cases only. This is of particular interest as Q fever has been shown to be rodent-associated in some cases [27]. In further analyses, having seen rodents, including rabbits, was strongly associated with having had contact with unhandled animal products, such as hay, straw or dung; of those that had contact with these animal products, 84·6% had also seen rodents in the recall period. Adjusted for age and sex, having seen rodents had a positive interaction with distance from 'area A' (OR 1·001, 95% CI 1·000–1·002). Interestingly, this seems to suggest that windborne transmission was most likely for those living in proximity to 'area A', while rodents, possibly infected through the environment near area A, might have facilitated the transmission beyond the reach of the wind.

Our study had several limitations which need attention. Participants were asked about possible exposures that had taken place 5 months before. Consequently, some participants may have answered based on their usual habits rather than their behaviour in the time period they were asked about. However, we believe that recall bias did not occur, as our analysis was restricted to a population sample which included mainly cases with milder symptoms. Moreover, self-reported symptoms might have been underreported, especially the ones that are mild and non-specific, although the proportion of asymptomatic infected individuals was quite similar to others [1].

Just before and during our study, media reports were released promoting the idea of goats being a plausible cause of the outbreak (Q fever was called 'goat flu'). We were expecting *a priori* that this would cause reporting bias for questions about goats. However, univariately, not only contact with goats but also with other ruminants were associated with infection and both subjectively calculated distances of their residence to goat farms and GIS-based distances were associated with infection. In the present study, only the latter objective distances were used in the multivariable analyses.

This first documented outbreak of Q fever in The Netherlands received plenty of attention from both the public health and the veterinary authorities. It was an excellent chance for these parties to cooperate with each other and facilitate long-term communication channels. Further, it was a unique opportunity to test and improve diagnostic assays for *C. burnetii* in humans, animals and environmental samples. Unfortunately, immediate implementation of control measures was hampered because of the failure to identify the exact source of the outbreak. In future similar outbreaks, an earlier start of the epidemiological investigation combined with more intensive environmental sampling should improve the quality of data, provide more detailed exposure and contamination data and, by that, enhance adequate control.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Raoult D, Marrie TJ, Mege JL.** Natural history and pathophysiology of Q fever. *Lancet Infectious Diseases* 2005; **5**: 219–226.
2. **Pinsky RL, *et al.*** An outbreak of cat-associated Q fever in the United States. *Journal of Infectious Diseases* 1991; **164**: 202–204.
3. **Marrie TJ, *et al.*** An outbreak of Q fever probably due to contact with a parturient cat. *Chest* 1988; **93**: 98–103.
4. **Stein A, Raoult D.** Pigeon pneumonia in Provence: a bird-borne Q fever outbreak. *Clinical Infectious Diseases* 1999; **29**: 617–620.
5. **Lyttikäinen O, *et al.*** An outbreak of sheep-associated Q fever in a rural community in Germany. *European Journal of Epidemiology* 1998; **14**:193–199.
6. **Pedogy RG, *et al.*** Epidemiological features of *Coxiella burnetii* infection in England and Wales: 1984 to 1994. *Communicable Disease Report* 1995; **6**: 128–132.
7. **Sawyer LA, Fishbein DB, McDade JE.** Q fever: Current concepts. *Reviews of Infectious Diseases* 1987; **5**: 935–946.
8. **Grilc E, *et al.*** Outbreak of Q fever among a group of high school students in Slovenia, March–April 2007. *Eurosurveillance* 2007; **12**(7): E070719.1 (http://www.eurosurveillance.org/ew/2007/070719.asp#1).
9. **Richardus JH, *et al.*** Q fever in the Netherlands: a sero-epidemiological survey among human population groups from 1968 to 1983. *Epidemiology and Infection* 1987; **98**: 211–219.
10. **Robert Koch Institut.** Large Q fever outbreak in Jena, June 2005 [in German]. *Epidemiologisches Bulletin* 2006; **45**: 391–398 (http://www.gpk.de/downloadp/STIKO_Bulletin45_061110_Q_Fieber2005.pdf).

11. **Porten K, *et al.*** A super-spreading ewe infects hundreds with Q fever at a farmers' market in Germany. *BMC Infectious Diseases* 2006; **6**: 147.
12. **Orr HJ, *et al.*** Case-control study for risk factors for Q Fever in southwest England and Northern Ireland. *Eurosurveillance* 2006; **11**(10) (http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId = 655).
13. **van Lier EA, Rahamat-Langendoen JC, van Vliet JA.** State of infectious diseases in The Netherlands 2006 [in Dutch]. RIVM Rapport 210211002/2007.
14. **Karagiannis I, *et al.*** A Q fever outbreak in the Netherlands: a preliminary report. *Eurosurveillance* 2007; **12**: 3247 (http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId = 3247).
15. **van Steenbergen JE, *et al.*** An outbreak of Q fever in the Netherlands – possible link to goats [in Dutch]. *Nederlandse Tijdschrift voor Geneeskunde* 2007; **151**: 1998–2003.
16. **Klee SR, *et al.*** Highly sensitive real-time PCR for specific detection and quantification of *Coxiella burnetii*. *BMC Microbiology* 2006; **19**: 2.
17. **Zhang GQ, *et al.*** Clinical evaluation of a new PCR assay for detection of *Coxiella burnetii* in human serum samples. *Journal of Clinical Microbiology* 1998; **36**: 77–80.
18. **Schimmer B, *et al.*** Authors reply: Q fever in the Netherlands – what matters is seriousness of disease rather than quantity. *Eurosurveillance* 2008; **13**: 18976 (http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18976).
19. **Rodolakis A, *et al.*** Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine and ovine herds. *Journal of Dairy Sciences* 2007; **90**: 5352–5360.
20. **Thomas DR, *et al.*** The risk of acquiring Q fever on farms: a seroepidemiological study. *Occupational and Environmental Medicine* 1995; **52**: 644–647.
21. **Carolan BJ, *et al.*** Decreased expression of intelectin 1 in the human airway epithelium of smokers compared to nonsmokers. *Journal of Immunology* 2008; **181**: 5760–5767.
22. **McCaughey C, *et al.*** Human seroprevalence to *C. burnetii* (Q fever) in Northern Ireland. *Zoonoses and Public Health* 2008; **55**: 189–194.
23. **Australian Broadcasting Company.** Q fever – Australia (South Australia): Abattoir. ProMed. 13 July, 2007 (http://www.promedmail.org), archive number: 20070713.2244.
24. **Hatchette TF, *et al.*** Goat-associated Q fever: a new disease in Newfoundland. *Emerging Infectious Diseases* 2001; **7**: 413–419.
25. **Bamberg WM, *et al.*** Outbreak of Q fever associated with a horse-boarding ranch, Colorado, 2005. *Vector Borne Zoonotic Diseases* 2007; **7**: 394–402.
26. **George J, Marrie TJ.** Serological evidence of *Coxiella burnetii* infection in horses in Atlantic Canada. *Canadian Veterinary Journal* 1987; **28**: 425–426.
27. **Gardon J, *et al.*** Suburban transmission of Q fever in French Guiana: evidence of a wild reservoir. *Journal of Infectious Diseases* 2001; **184**: 278–284.

CHAPTER 3.2

The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q fever outbreak

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The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q fever outbreak

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Abstract

Background: A Q-fever outbreak occurred in an urban area in the south of the Netherlands in May 2008. The distribution and timing of cases suggested a common source. We studied the spatial relationship between the residence locations of human cases and nearby small ruminant farms, of which one dairy goat farm had experienced abortions due to Q-fever since mid April 2008. A generic geographic information system (GIS) was used to develop a method for source detection in the still evolving major epidemic of Q-fever in the Netherlands.

Methods: All notified Q-fever cases in the area were interviewed. Postal codes of cases and of small ruminant farms (size >40 animals) located within 5 kilometres of the cluster area were geo-referenced as point locations in a GIS-model. For each farm, attack rates and relative risks were calculated for 5 concentric zones adding 1 kilometre at a time, using the 5-10 kilometres zone as reference. These data were linked to the results of veterinary investigations.

Results: Persons living within 2 kilometres of an affected dairy goat farm (>400 animals) had a much higher risk for Q-fever than those living more than 5 kilometres away (Relative risk 31.1 [95% CI 16.4-59.1]).

Conclusions: The study supported the hypothesis that a single dairy goat farm was the source of the human outbreak. GIS-based attack rate analysis is a promising tool for source detection in outbreaks of human Q-fever.

Background

Q-fever, a zoonosis caused by the bacterium *Coxiella burnetii*, is an emerging public health problem in the Netherlands. In May 2007, the first community Q-fever outbreak was documented around a single village in the province of Noord Brabant. A case control study carried out subsequently suggested a role of warm, dry weather conditions in addition to a residence location close to ruminant farms [1].

The unprecedented magnitude (>3000 notified human cases) and geographical spread within the province during the following years (2008-2009) suggested multiple sources of infection [2]. The affected area matches with the part of the Netherlands that has the highest concentration of dairy goat farms, some of which are very large (>5000 goats) [3]. From 2005 to 2008, clinical Q-fever represented by abortions or stillbirths in small ruminants, was diagnosed at 22 large (>50 goats) commercial dairy goat farms and 2 dairy sheep farms, mainly in the affected province [4]. Although large dairy goat farms were implicated, questions about exact transmission routes and risk factors remained unanswered.

In May 2008 the municipal health service (MHS) in the southeast region of the affected province was alerted by a cluster of human Q-fever cases in an urban area (88,000 inhabitants). Based on the distribution of cases in time and place, a common source was suspected. The MHS had been informed by the Animal Health Service that a dairy goat farm was diagnosed with Q-fever in their region. Some patients that were interviewed by the MHS indicated a pet farm in their neighbourhood as a potential source.

The objective of the study was to assess whether the cluster of human Q-fever cases in this urban area could be linked to the suspected source on the basis of the distribution of illness onset and residence location relative to small ruminant farms. Furthermore, the study explored the usefulness of a generic geographic information system (GIS) for source detection in the still evolving major epidemic of Q-fever in the Netherlands.

Methods

Human Q-fever cases

Q-fever is a notifiable disease in the Netherlands and the regional MHS was responsible for the outbreak investigation. A regional laboratory provided the majority of microbiological diagnostic services for the inhabitants of the MHS region. Submitted serum samples were screened with a *C. burnetii* complement fixation test (CFT, Siemens, the Netherlands). Notification criteria for acute Q-fever were a clinical presentation with at least fever, or pneumonia, or hepatitis and a fourfold rise in titre in the CFT or a single high titre or a high titre in two samples without a fourfold increase (CFT titre ≥1:4). All persons notified to the Dutch national surveillance system for infectious diseases (OSIRIS) who were residing or had visited the MHS region of Brabant Southeast and who had an illness onset from week 16 (14 April 2008) to week 32 (10 August 2008) were included.

A hypothesis generating questionnaire was used to get insight into possible sources of *C. burnetii* infection. Questions pertained to age, sex, day of illness onset, self-reported symptoms and complications, and a range of potential risk factors, such as travel history and occupational exposure. A recall period of four weeks before illness onset was used to investigate if cases had had relevant exposures immediately before the incubation period. Questionnaires to all notified cases were administered by trained public health employees from the MHS during house visits, or self-administered by mail.

Data on human cases was collected as part of the routine system of infectious disease surveillance and outbreak control. Because no additional information or laboratory materials were collected from patients, a medical ethical review was not needed.

Meteorological data

We determined the predominant wind direction by day and calculated the number of days that the wind had blown in every wind direction during the estimated exposure period. These data were available for weather station Volkel (station 375 of the Royal Netherlands Meteorological Institute), located within 15 kilometres from the centre of the cluster area.

Veterinary data

Veterinary data were obtained from the Animal Health Service. A mandatory veterinary notification system was only introduced on 12 June 2008 (week 24). If a veterinary source was suspected based on the exposure information from patients, the MHS notified the Food and Consumer Product Safety Authority.

Data analysis

Residence locations of all Q-fever cases were plotted as point data on a digital map of the area. The Animal Health Service provided the locations and specifications of goat and sheep farms that were located within 5 kilometres of the average longitudinal and lateral coordinate of the cases, thus defining the centre of the cluster. The residential addresses of the cases was considered a proxy for level of exposure to small ruminant farms. We hypothesized that the attack rate would be higher for persons living close to the source and would decrease with increasing distance. Around each farm with more than 40 small ruminants we made concentric rings each expanding with a 1 kilometre radius (range 0-5000 meters) and a reference ring with a range from 5000 - 10,000 meters). Attack rates for residents living within these zones were calculated using a digital map of population distribution. Relative risks for 5 zones were estimated using the 5000-10,000 meters zone as reference. Mapping and spatial analyses were done with ArcGis 9 software (ESRI, Redlands, CA, USA).

Results

Between week 16 and 33 (14 April-15 August 2008) the MHS Brabant Southeast notified 96 Q-fever cases. In 55 patients positive CFT titres were found in two sequential (≥14 days interval) samples (of which 42 cases had a fourfold increase in titre) and 41 patients had a single positive CFT titre. The median age of cases was 53 years (interquartile range 42-61) and the male to female ratio was 2.3:1. Day of onset of illness was known for 95 cases (median 4 June 2008). Nineteen individuals (20%) were hospitalised.

Eighty one (84%) of the 96 cases completed a questionnaire. Most commonly reported symptoms were fever >38°C (95%), fatigue (83%), headache (70%), night sweats (62%), dyspnoea (53%), malaise (52%), and myalgia (51%). The majority of cases (79%) presented clinically with a pneumonia. Five patients (6%) presented with hepatitis. Seventy eight (81%) of the 96 cases that were notified in the MHS region Brabant Southeast resided in one city with 87,752 inhabitants and a surface area of 54.56 km^2 (1608/km^2). Eight cases were from adjacent municipalities and 10 from more distant municipalities and none of these reported to have a work address within the city.

We estimated from the epidemic curve (Figure 1) that the likely period of infection was from week 15 (mid-April) to week 24 (mid-June). This encompassed the known two to four week incubation period for *C. burnetii* and was based on date of symptom onset for 95 of the 96 cases.

Veterinary investigations

There were 60 locations with small ruminants within the 5 kilometre zone around the centre of the human Q-fever cluster. Seven of these locations were large farms (A-G) with more than 40 small ruminants, including one large dairy goat farm (A, >400 goats), one mixed sheep/goat farm (E, 126 goats and 90 sheep) and 5 sheep farms (B, C, D, F, and G with between 57 and 118 sheep). These farms are mapped in figure 2. Farm A reported an abortion wave on 21 April 2008 (week 17) to the Animal Health Service and submitted two aborted lambs for post mortem examination. *C. burnetii* infection was confirmed in placental tissue from aborted goats by immunohistochemistry. The first abortions were noticed in week 15 (mid April) 2008. No other farms in the area reported abortions in the weeks prior to the outbreak. At the time of the visit by the Animal Health Service on 15 May (week 20), 40 out of 120 pregnant goats had aborted (33%), 20 (17%) had an uncomplicated pregnancy and got healthy offspring, while another 60 animals still had to deliver. No veterinary problems were reported by the other farms. A pet farm in the part of the city where most of the cases resided was visited by the Food and Consumer Product Safety Authority. Three tested goats and one sheep had a weak positive PCR (only one of three *Coxiella* specific genomic targets positive). However these animals had not been pregnant recently. Hygiene standards at the pet farm were good and none of the employees of the pet farm developed symptoms or had been diagnosed with acute Q-fever. It was concluded that these animals were not the main source of the large cluster of human cases.

Meteorological data

With an east to north-eastern wind most of the city lies downwind of the dairy goat farm and could potentially have been exposed to contaminated dust particles. During the study period these wind conditions were common in week 16 (14-20 April) with 4 out of 7 days and in week 20 (12-18 May) with 6 out of 7 days (Figure 1).

GIS analysis

Distance-related attack rates and relative risks for increasingly larger sequential ring buffers from each of

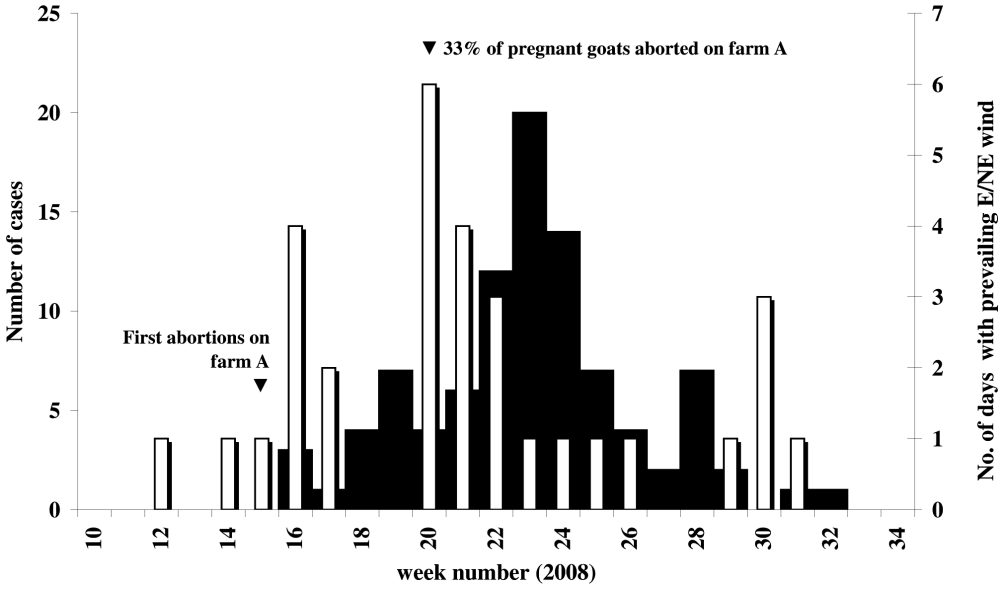

Figure 1 Epidemic curve of Q-fever and wind direction. Number of Q-fever cases in the Municipal Health Service region Brabant Southeast by week of illness onset (n = 95, black bars); and number of days in the week with prevailing eastern or north-eastern wind (white bars). Information on date of illness onset is missing for one case.

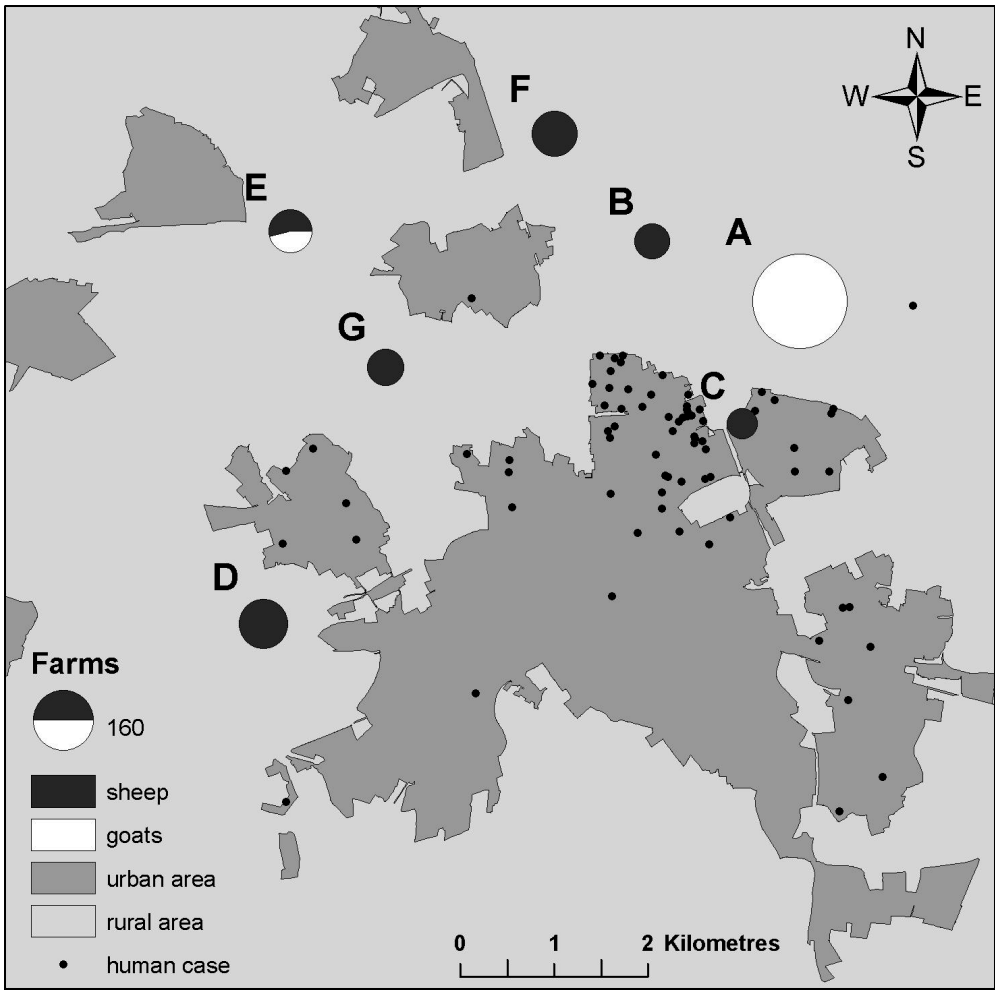

Figure 2 Map of the study area. Locations of goat and sheep farms with >40 animals (farm A-G) and residential addresses of Q-fever cases in urban and rural areas, 14 April to 10 August 2008.

the 7 large farms (A-G) are shown in Table 1. A gradual diminishing relative risk with increasing distance was observed for farm A, the large dairy goat farm with the reported abortion wave, and two small sheep farms B and C.

Farm A and B both showed a gradual diminishing relative risk with increasing radius, with the exception of the first 1-kilometer zone, possibly due to the small denominator. There were no significant differences in attack rates between farm A and B. The attack rates within 2 and within 3 kilometres were significantly higher for farm A than for farm C ($p = 0.004$ and $p = 0.001$ respectively). The highest attack rate for farm A was for inhabitants residing in the south to south-western direction within 2 kilometres of the farm. Persons living in this zone had a much higher risk for Q-fever than those living more than 5 kilometres away (Relative risk 31.1 [95% CI 16.4-59.1]). In the 4-5 kilometre zone from farm A, only 1 case was added and the risk of Q-fever for people living in this zone was very low. The

Table 1 Attack rates of acute Q-fever among residents within circular distance rings around each potential source (A-G) expressed per 100,000 persons over week 16-32 (14 April-10 August 2008), with relative risks (RR) and 95% confidence intervals (95% CI)

Distance from source	A	B	C	D	E	F	G
0-1 kilometre							
Attack rate[1]	0	0	413	178	0	0	0
RR[2] (95% CI)	0	0	53 (24-114)	8 (1-61)	0	0	0
Q-fever cases	0	0	35	1	0	0	0
Population	92	136	8482	562	119	281	1444
0-2 kilometre							
Attack rate	376	352	203	46	0	13	76
RR (95% CI)	31 (16-59)	25 (13-49)	26 (12-54)	2 (1-5)	0	1 (0-5)	5 (2-13)
Q-fever cases	33	27	59	6	0	1	8
Population	8788	7671	29,098	13,022	9852	7632	10,582
0-3 kilometre							
Attack rate	241	228	137	28	19	69	102
RR (95% CI)	20 (11-36)	16 (9-30)	18 (8-36)	1 (1-3)	1 (0-2)	4 (2-8)	7 (4-13)
Q-fever cases	59	58	69	9	4	12	28
Population	24,461	25,487	50,349	32,709	20,521	17,506	27,329
0-4 kilometre							
Attack rate	124	133	101	26	60	147	98
RR (95% CI)	10 (6-19)	10 (5-17)	13 (6-27)	1 (1-2)	2 (1-4)	8 (5-13)	7 (4-12)
Q-fever cases	70	66	71	15	19	49	62
Population	56,585	49,570	70,151	58,002	31,425	33,270	63,547
0-5 kilometre							
Attack rate	92	88	86	53	94	100	79
RR (95% CI)	8 (4-14)	6 (3-11)	11 (5-23)	3 (2-4)	4 (3-6)	5 (3-9)	6 (3-10)
Q-fever cases	71	74	78	51	54	71	72
Population	77,558	84,374	90,779	96,402	57,329	70,680	91,123
>5-10 kilometre							
Attack rate	12	14	8	21	25	18	14
RR	1	1	1	1	1	1	1
Q-fever cases	13	13	8	39	40	21	17
Population	107,680	93,469	102,192	185,269	162,141	113,966	121,978

[1] Attack rate: number of Q-fever cases per 100,000 population during the outbreak

[2] RR = relative risk, attack rate divided by the attack rate of the reference category 5-10 km, with 95% confidence interval (CI)

other farms D, E, F, G had low attack rates close to the farms compared to farms A-C and they did not show a gradual diminishing relative risk with increasing distance and were considered unlikely infection sources.

Discussion

Within an on-going large epidemic of Q-fever in a province in the south of the Netherlands we identified a distinct urban cluster of notified Q-fever cases. We combined epidemiological data on notified cases, veterinary and meteorological data in a generic geographic information system to analyse this cluster. The study showed that living within 2 kilometres of a dairy goat farm with abortion problems posed a high risk for Q-fever. Furthermore, the time period between the duration of the abortion wave on this dairy goat farm and illness onset in the human cases suggests that airborne transmission of *C. burnetii* from the dairy goat farm could have been the cause of this outbreak. This was further supported by predominant easterly winds on a number of days that could have taken contaminated dust particles to the people living southwest from the farm.

Findings from this urban outbreak are consistent with those from the first community Q-fever outbreak in the Netherlands that occurred in a rural area in 2007. In the 2007 outbreak, airborne transmission from nearby small ruminant farms located to the northeast of the village was suspected as the main route, facilitated by a predominant wind direction from the east during a period of extreme dry weather [1].

There are reports from the international literature that *C. burnetii* particles can spread from farmland over long

distances, often facilitated by strong winds towards urban areas [5]. In a community outbreak in the United Kingdom cases were identified up to 18 kilometres from the source [6]. Our results suggest a low risk beyond 5 kilometres from the infection source. The role of wind speed and direction is difficult to analyse when a farm is infectious for a longer period of time.

Source detection

In the high incidence area in the south of the Netherlands there are thousands of locations with goats and sheep in addition to the many cattle, pig and poultry farms. In this environment the investigation and sampling of all potential animal infection sources to explain human clusters is impossible. Goats, sheep and cattle on many farms throughout the country show serological evidence of previous infection with *C. burnetii*. However, based on the experience since 2007, the prevailing opinion among the human and veterinary public health community is that abortion waves at large dairy goat farms play the predominant role in transmission [4]. Abortion rates of more than 5% on large dairy goat and sheep farms have to be notified since June 2008. However, abortions in deep litter houses with many hundreds of animals might easily go unnoticed. Attack rate analysis as proposed in the present paper could be an additional tool for source detection.

In order to apply this method real-time, detailed geographic information with residence locations of cases and farm locations, and early notification of human clusters by health professionals are required. The complement fixation test is known to be specific but definitive diagnosis may be delayed because often paired samples are required [7]. Increased awareness among patients and physicians, as well as introduction of rapid laboratory assays, such as PCR, may further reduce the delay between onset of illness and diagnosis and notification [8]. Moreover, in June 2008 Q-fever became a veterinary notifiable disease. This legal framework has facilitated communication between the human and veterinary public health sectors. Clearly, it is essential that the MHS can alert general practitioners in a region where there is a small ruminant farm with clinical Q-fever. Veterinary information on Q-fever status of farms in the near proximity, for example clinical Q-fever or positive bulk milk status, could facilitate to pinpoint the most likely sources in an exposed area.

The analysis of the 2008 urban cluster was facilitated by the relatively small number of farms in the area. Even so, the attack rate analysis showed similar results for three farms (A-C) that were located very close to each other. The tool can provide only a rough indication but could facilitate efficient source detection by placing animal locations in classes of 'possible' and 'unlikely'. This would have to be followed by a veterinary risk assessment and possible sampling at suspected farms. Generating the attack rates requires experience in the use of GIS software. When adequate tools for GIS analyses are available, the workload is limited and the procedure can be automated.

Limitations of the study

This retrospective cohort study was performed in a new endemic area for Q-fever in the Netherlands where dairy goat farming started recently. Q-fever was laboratory confirmed on the newly established dairy goat farm but no systematic environmental sampling took place in the cluster area. Therefore we cannot exclude that other sources might have been present in the same time period in the region.

Most Q-fever infections remain asymptomatic or give aspecific signs and symptoms. Differences in diagnostic testing and alertness of general practitioners might have led to ascertainment bias. To provide a reliable estimate of the attack rate all patients with symptoms compatible with Q-fever would have to be tested or population surveys would have to be done to include asymptomatic cases.

Research priorities

Conclusive evidence of a link between certain animals and human Q-fever cases can only be provided by genetic analysis of human, animal and environmental samples. Current typing techniques provide insufficient contrast and methods for culturing and sequencing carry bio safety concerns and are still under development. The presented attack rate analysis assumes homogeneity in the distribution of *Coxiella* in each concentric ring. In future analyses landscape features should be taken into account to make a more informative analysis possible. This should be combined with information on movement patterns of human cases, recreational activities and other behavioural factors that might change the risk of exposure.

Conclusions

We found a clear epidemiological link between a cluster of human Q-fever cases and a Q-fever positive dairy goat farm. The present study suggests an effective range of airborne *C. burnetii* spread of <5 kilometres. GIS-based automated attack rate analysis is a promising tool that could classify animal locations as possible or unlikely infection sources. This methodology should be transformed from a retrospective analysis to a real-time pinpointing tool to guide environmental sampling of potential sources. This requires close collaboration between the human and veterinary public health sectors to ensure timely detection of cases, identification of plausible sources and standardised environmental sampling, and application of public health and veterinary preventive measures.

References

1. Karagiannis I, Schimmer B, van Lier A, Timen A, Schneeberger P, van Rotterdam B, de Bruin A, Wijkmans C, Rietveld A, van Duynhoven Y: **Investigation of a Q fever outbreak in a rural area of The Netherlands.** *Epidemiol Infect* 2009, **137**:1283-1294.
2. Schimmer B, Morroy G, Dijkstra F, Schneeberger PM, Weers-Pothoff G, Timen A, Wijkmans C, Hoek van der W: **Large ongoing Q fever outbreak in the south of The Netherlands, 2008.** *Euro Surveill* 2008, **13, pii: 18939.**
3. Schimmer B, Dijkstra F, Vellema P, Schneeberger PM, Hackert V, ter Schegget R, Wijkmans C, van Duynhoven Y, Hoek van der W: **Sustained intensive transmission of Q fever in the south of the Netherlands, 2009.** *Euro Surveill* 2009, **14, pii: 19210.**
4. Brom Van den R, Vellema P: **Q fever outbreaks in small ruminants and people in the Netherlands.** *Small Ruminant Res* 2009, **86**:74-79.
5. Tissot-Dupont H, Amadei MA, Nezri M, Raoult D: **Wind in November, Q fever in December.** *Emerg Infect Dis* 2004, **10**:1264-1269.
6. Hawker JI, Ayres JG, Blair I, Evans MR, Smith DL, Smith EG, Burge PS, Carpenter MJ, Caul EO, Coupland B, Desselberger U, Farrell ID, Saunders PJ, Wood MJ: **A large outbreak of Q fever in the West Midlands: windborne spread into a metropolitan area?** *Commun Dis Public Health* 1998, **1**:180-187.
7. Péter O, Dupuis G, Peacock MG, Burgdorfer W: **Comparison of enzyme-linked immunosorbent assay and complement fixation and indirect fluorescent-antibody tests for detection of Coxiella burnetii antibody.** *J Clin Microbiol* 1987, **25**:1063-1067.
8. Schneeberger PM, Hermans MHA, van Hannen EJ, Schellekens JJA, Leenders ACAP, Wever PC: **Real-time PCR on serum samples is indispensable for early diagnosis of acute Q-fever.** *Clin Vaccine Immunol* 2010, **17**:286-290.

CHAPTER 3.3

A Q fever outbreak in a psychiatric care institution in the Netherlands

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A Q fever outbreak in a psychiatric care institution in The Netherlands

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SUMMARY

In May 2008 the Nijmegen Municipal Health Service (MHS) was informed about an outbreak of atypical pneumonia in three in-patients of a long-term psychiatric institution. The patients had been hospitalized and had laboratory confirmation of acute Q fever infection. The MHS started active case finding among in-patients, employees of and visitors to the institution. In a small meadow on the institution premises a flock of sheep was present. One of the lambs in the flock had been abandoned by its mother and cuddled by the in-patients. Samples were taken of the flock. Forty-five clinical cases were identified in employees, in-patients and visitors; 28 were laboratory confirmed as Q fever. Laboratory screening of pregnant women and persons with valvular heart disease resulted in one confirmed Q fever case in a pregnant woman. Of 27 samples from animals, seven were positive and 15 suspect for *Coxiella burnetii* infection. This outbreak of Q fever in a unique psychiatric setting pointed to a small flock of sheep with newborn lambs as the most likely source of exposure. Care institutions that have vulnerable residents and keep flocks of sheep should be careful to take adequate hygienic measures during delivery of lambs and handling of birth products.

Key words: Community outbreaks, coxiellae, zoonoses.

INTRODUCTION

Q fever is a zoonosis caused by *Coxiella burnetii*, a small, pleomorphic Gram-negative obligate intracellular bacterium. Ruminants, mainly sheep, goat and cattle are the most common reservoir. *C. burnetii* infections in animals are usually asymptomatic, but may cause abortions in sheep and goats. High

concentrations of *C. burnetii* can be found in birth products of infected mammals [1]. Humans can acquire infection by inhaling contaminated dust and aerosols. The incubation period varies from 1 to 6 weeks depending on the number of inhaled organisms, with most patients becoming ill within 3 weeks of exposure [2]. About half of those infected with *C. burnetii* show signs of clinical illness, and 20% develop a more severe infection complicated with pneumonia, hepatitis or another clinical diagnosis. Fatal infections are rare [1, 2]. About 5% of cases are hospitalized but, in a previous large outbreak in The Netherlands, this reached more than 20% [3]. Certain conditions such as pregnancy, heart valve and other vascular abnormalities predispose individuals to chronic Q fever [4].

In The Netherlands, acute Q fever is notifiable. Between 2001 and 2006 the annual number of cases varied from 5 to 19 per year. In 2007 and 2008, 191 and 1000 cases were notified, respectively [3]. In other countries, such as Switzerland, Germany and the UK several community outbreaks of Q fever in rural areas due to aerosolized spread have been described [5–10]. In most cases these outbreaks were attributed to sheep, although sometimes no source was detected. Other outbreaks have been associated with goats, cattle, pigeons, cats and rabbits [11–15]. Outbreaks have also been described in abattoirs [16, 17].

Boschini and colleagues described an outbreak in an Italian residential ('closed') facility for the rehabilitation of drug users [18]. No other Q fever outbreaks in a healthcare setting have been described.

OUTBREAK INVESTIGATION

Outbreak alert

On 9 May 2008, a physician in a long-term psychiatric institution located south-east of the city of Nijmegen reported to the regional Municipal Health Service (MHS) that three residents of the institution had been hospitalized with atypical pneumonia. (For CXR of one of the residents see Supplementary Fig. S1, available online.) Symptoms included high fever, headache, cough and chills. The physician suspected an outbreak. In all three patients the diagnosis of Q fever was confirmed by polymerase chain reaction (PCR) on throat swabs or sputum samples. No other infections, including *Legionella* and influenza were diagnosed. Because of their presence on the premises of the institution, sheep and birds were considered as likely sources. Upon the alert day (day 0) MHS Nijmegen started an outbreak investigation to determine the source and extent of the outbreak.

Outbreak setting

A total of 127 in-patients were resident in this long-term psychiatric care institution; in addition, there were 1285 ambulatory patients. Most patients suffered from chronic psychiatric disorders such as schizophrenia and bipolar disorders. The institution had 350 employees. The institution was openly accessible to visitors.

Case finding

The MHS immediately started active case finding among residents and employees of the institution. The nursing staff were asked to be on the alert for clinical symptoms in their in-patients. All staff of the psychiatric institution and nearby surrounding institutes were also asked to be alert for possible cases. In addition, local and regional general practitioners and appropriate hospital clinicians were warned to be aware of possible cases, both in patients residing at the institution as well as those in the local community. Case finding of ambulatory patients and visitors was not actively performed.

Case definitions

A suspected case was defined as a person who had been living or working at the psychiatric institution or in one of the neighbouring organizations on the same premises; or had visited the premises; or lived within 500 m of the area in the 6 weeks prior to the alert and who, in addition, had fever ($>38{\cdot}5\ ^{\circ}C$) and three or more of the following symptoms: severe headache, pneumonia (clinical or radiological), chills, sweats, coughing, aching muscles, diarrhoea, fatigue or malaise. A case was confirmed if *C. burnetii* was detected in throat swab and/or blood samples using PCR, and/or a fourfold rise in serum antibody titres to *C. burnetii* complement fixation test (CFT), and/or detection of IgM using an immunofluorescence assay (IFA).

PCR tests of clinical specimen (both blood samples and throat swabs) of suspected cases, CFTs for IgG, and phase 1 (IgM) and phase 2 antibody IFAs were performed.

From each confirmed case the following information was obtained: general characteristics (age,

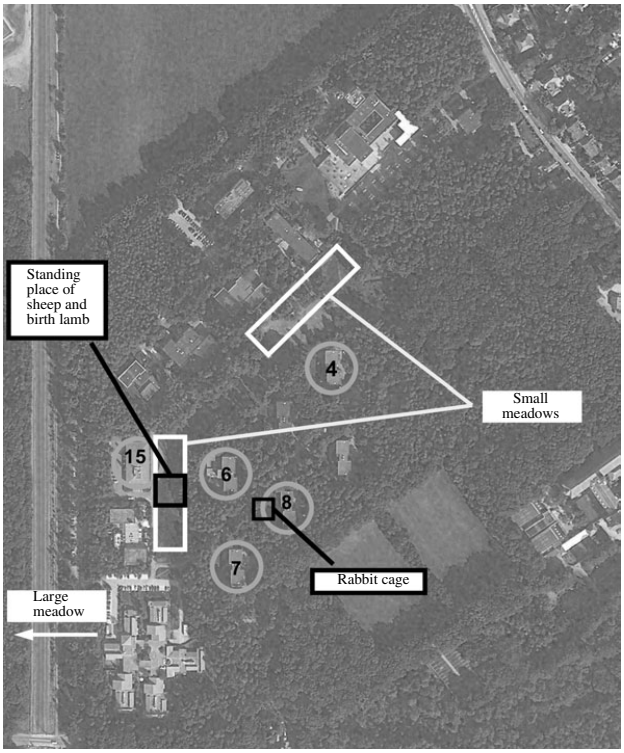

Fig. 1. Map of the psychiatric institution, Nijmegen. Location of the meadows where the flock of sheep was grazing (white), location of the birth of the abandoned lamb, rabbit cage (black) and client residences with confirmed Q fever clients (grey circles) are indicated. Attack rate (AR) per building: building 4, AR 4/25 (16%); building 6, AR 1/26 (4%); building 7, AR 2/26 (8%); building 8, AR 1/21 (5%); building 15 AR 1/68 (1%).

gender, place of residence), medical status (medical history), and exposure information (contact with animals at or around the premises of the institution). At 10 weeks after Q fever diagnosis, 17 cases were followed up and asked whether they still had symptoms. In addition to the active case finding, active screening of pregnant women and persons with valvular heart disease linked to the institution was performed.

Epidemiological investigation

During the epidemiological investigation two main hypotheses were explored. First, a flock of six sheep present in a small meadow on the institution premises for 5–6 years was considered a possible source of the outbreak. Since this flock produced five lambs in the weeks prior to the outbreak alert [the first born in the beginning of April (day 38), the last on 8 May (day 1)] it was the most likely source. In-patients and outpatients could have been infected by inhaling contaminated aerosols after close animal contact, particularly with pregnant or newborn animals. One of the lambs, born on 14 April (day 25), was abandoned by its mother and was adopted by one of the in-patients, who took it into her bedroom and living room (building 4, Fig. 1) and bottle-fed it six times a day. Several in-patients cuddled this lamb. Three days after its birth, it was placed in a rabbit cage on the premises of the institution, and cared for by in-patients and employees.

The other hypothesis was that cases were infected by a large flock of sheep in a large meadow directly opposite the main entrance of the institution. A flock of about 200 sheep had grazed there until 1 April (day 38). Two shepherds and the wife of one of the shepherds had been confirmed as Q fever cases with onset in March 2008. The hypothesis was that infection could have occurred by windborne spread from

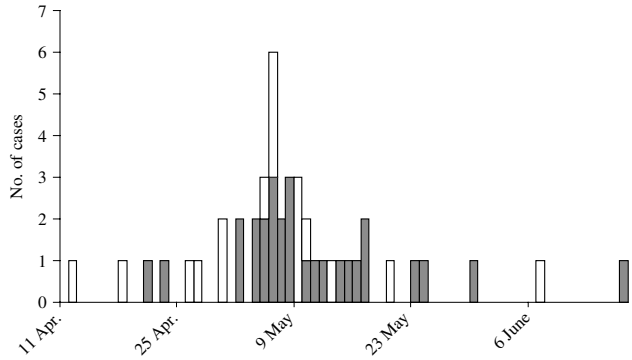

Fig. 2. Confirmed (■) and possible (□) Q fever cases in a psychiatric care institution by day of illness onset, April–June 2008 ($n=45$).

the meadow, or by introduction of contaminated animal products from this flock (such as straw, hay, or compost), or by wild animals. Considering windborne spread, the MHS assumed human Q fever cases could have also occurred in the neighbouring community.

A detailed map of the institution premises was used to plot cases, calculate attack rates (ARs) for each building and indicate environmental information such as the location of the meadow, the rabbit cage and the predominant wind direction (Fig. 1).

Veterinary and environmental investigation

In order to identify the source of the outbreak an environmental and veterinary investigation was undertaken by the Food & Consumer Safety Authority and by scientists of the Laboratory of Zoonoses and Environmental Microbiology of the National Institute for Public Health and the Environment (RIVM). Animal samples were collected from: (i) rabbits (anal and oral swabs) at the psychiatric institution, (ii) the sheep and lambs (vaginal and udder swabs) who had been residing at the institution, but had now moved, and (iii) the flock of sheep opposite the entrance of the institution. Environmental samples taken included faecal samples from the rabbit cage and meadow (sheep) at the institution, and faecal samples and wool from the flock of sheep opposite the institution. All samples were sent for PCR testing at the RIVM using a newly developed multiplex Q-PCR [19]. MLVA (multiple locus VNTR analysis) typing was used for some of the human and animal specimens.

RESULTS

Q fever cases

Through active case finding 45 persons were identified as suspected cases. Of those 28 (62%) were confirmed by laboratory tests, as per the case definition. Of the other 17 persons screened, all laboratory results (PCR, CFT, IFA) were negative. Of the confirmed cases there were 16 employees (one from a neighbouring organization), 10 in-patients, one friend of an employee who had visited the institution and walked in the meadow, and one person living nearby (< 500 m) (Fig. 2). The friend of the employee and the person living nearby did not have close contact with the lamb or sheep. The average mean age of the confirmed cases was 42 years (range 15–63 years). Fifteen of the 28 cases were male.

The dates of onset of illness extended from 21 April (day 18) to 17 June (day 39). Follow-up of 17 cases showed that the median duration of illness was 2 weeks (range 0·5–3·5 weeks). At 10 weeks after infection, 7/17 cases reported to be still suffering from fatigue (a common sequela of Q fever). In all, 29% (8/28) of cases were hospitalized, six of whom were in-patients and two employees of the institution.

The overall clinical AR was 7·9% (10/127) in in-patients residing at the institution and 4·6% (16/350) in employees. The ARs appeared to be highest (16%) in building 4 (Fig. 1). This is probably due to the fact that the lamb was placed in building 4 directly after delivery. Nine of 10 confirmed cases resided in five of the 15 buildings on the premises. These five buildings were close to the small meadows where the sheep were grazing. Denominators for the number of employees

per building were unavailable, and ARs for employees per building could not be calculated. The buildings with confirmed Q fever residents were in general closer to one of the small meadows or the rabbit cage (or harboured the abandoned lamb, building 4) compared to the other buildings.

Screening of employees pregnant or with valvular lesions

Screening of 24 asymptomatic pregnant employees of the institution resulted in one positive Q fever infection in a woman who was 38 weeks pregnant. This woman was hospitalized and labour was induced, because of the risk of placentitis. A healthy infant was born and PCR on birth products was negative. None of the six screened persons with valvular heart disease became infected.

Results of the veterinary and environmental investigation

Of 27 animal samples analysed, five were negative, 15 were suspect for Q fever, with one or two out of three genomic targets positive in Q-PCR, but concluded to be negative, and seven were found to be positive (all three genomic targets positive in Q-PCR). The positive samples were obtained from three ewes and the abandoned lamb from the small meadow. Vaginal and udder swabs were found to be positive in two ewes. A vaginal swab and a wool sample were found to be positive in a third ewe. The abandoned lamb was found to be positive from a throat swab. No other potential wild or domestic animal sources were identified. Of the flock of sheep that had been grazing outside the premises 10 were tested; all were negative.

DISCUSSION

To our knowledge, this is the first reported outbreak of Q fever related to lambing, in an open healthcare institution. Our investigation pointed to a small flock of sheep with newborn lambs on the premises of the institution as the most probable source of exposure. Due to the unique and restricted setting of this outbreak in a psychiatric care institution, exhaustive screening of risk groups was feasible. However, an analytical study in this type of setting was not possible. Urgent outbreak control was also paramount.

This outbreak investigation was thus limited to descriptive analyses on active case finding and active screening of risk groups, and it was not possible to investigate exposure of asymptomatic cases or involve controls.

Our outbreak investigation nevertheless strongly suggested that the flock of sheep and its newborn lambs were the most likely sources of the outbreak, in accord with other studies [5, 8, 18, 20]. During the Q fever outbreak in The Netherlands, which has been ongoing since 2007, MLVA typing of samples from patients, ewes and lambs showed similar relationships between each other. In this study a swab specimen from a newborn lamb showed an identical MLVA genotype to that from a patient who developed severe pneumonia after close cuddling with a lamb in another incident [21].

It is possible that other, untested lambs of the small flock, that were born before or after the birth of the abandoned lamb, were carriers of *C. burnetii* and caused illness. Presuming the abandoned lamb was the main source of this outbreak, the infectious potential of this one lamb was comparable to that of entire flocks in different settings as described in the study of a superspreading ewe [20]. If the abandoned lamb at birth on 14 April 2008 was the main source of this Q fever outbreak, the mean incubation period of the cases was 27 days (range 7–64 days), which is higher than the incubation period of 3 weeks found in comparable studies [9, 20]. This suggests that transmission could have taken place during delivery, but in addition also after delivery. The rejection by the mother offered an opportunity for the psychiatric patients to have frequent and in some instances, intensive contact with the abandoned lamb. In addition, the possibility of infection by inhalation of contaminated dust (from manure or birth products) by in-patients and employees should be considered, taking into account that the first day of illness of some cases exceeds the maximal incubation time of 6 weeks after the birth of the lamb.

The observed overall ARs in in-patients (7·9%) and employees (4·6%) are comparable with those described in a recent study of an outbreak in a German village, which showed an AR of 4·3% in citizens living in the area within 400 m of a suspected meadow [8].

The proportion of clinical cases hospitalized was 29%, which is similar to a 25% hospitalization rate in the outbreak caused by a superspreading ewe in a market in Germany [20], but substantially

higher than the average of 9% found in other studies [6, 9, 18].

Markedly, 60% (6/10) of Q fever cases in in-patients were hospitalized, compared to 13% (2/16) of the employees. Possibly, case finding in employees was more complete and included milder cases.

Future preventive measures should focus on reducing contact with lambing sheep and increase awareness in personnel and occupational health professionals of the potential health risks posed in similar settings. After a study of a large outbreak of Q fever at a farmers' market in Germany [20] the authors recommended that pregnant sheep should not be displayed in public during the third trimester, and that animals in petting zoos should be tested regularly for *C. burnetii* [20]. Another study recommend not keeping sheep within 500 m of residential areas and that lambing of sheep should not occur outdoors [8]. Care institutions with vulnerable residents maintaining flocks of sheep should be made to take adequate hygienic measures during delivery of the sheep and handling of birth products.

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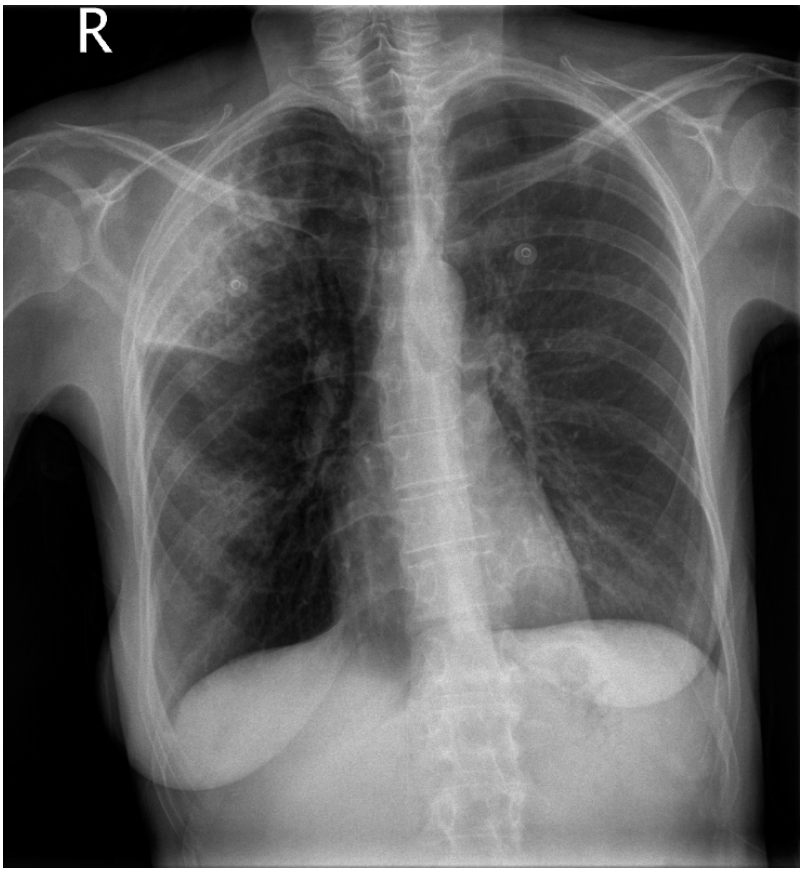
DECLARATION OF INTEREST

None.

REFERENCES

1. **Raoult D, Marrie T.** Q fever. *Clinical Infectious Diseases* 1995; **20**: 489–495.
2. **Maurin M, Raoult D.** Q fever. *Clinical Microbiology Reviews* 1999; **12**: 518–553.
3. **Schimmer B, *et al.*** Large ongoing Q fever outbreak in the south of the Netherlands, 2008. *Eurosurveillance* 2008; **13**(31).
4. **Fenollar R, *et al.*** Chronic endocarditis following acute Q fever. *Clinical Infectious Diseases* 2000; **33**: 312–316.
5. **Salmon MM, *et al.*** Q fever in an urban area. *Lancet* 1982; **1**: 1002–1004.
6. **Dupuis G, *et al.*** An important outbreak of human Q-fever in a Swiss Alpine valley. *International Journal of Epidemiology* 1987; **16**: 282–287.
7. **Hawker JI, *et al.*** A large outbreak of Q fever in the West Midlands: windborne spread into a metropolitan area? *Communicable Disease and Public Health* 1998; **1**: 180–187.
8. **Gilsdorf A, *et al.*** Large Q fever outbreak due to sheep farming near residential areas, Germany, 2005. *Epidemiology and Infection* 2008; **136**: 1084–1087.
9. **Lyytikïnen O, *et al.*** An outbreak of sheep-associated Q fever in a rural community in Germany. *European Journal of Epidemiology* 1997; **14**: 193–199.
10. **Robert Koch Institut.** Large Q fever outbreak in Jena, June 2005 [in German]. *Epidemiologisches Bulletin* 2006; **45**: 391–395.
11. **Hatchette TF, *et al.*** Goat-associated Q fever: a new disease in Newfoundland. *Emerging Infectious Diseases* 2001; **7**: 413–419.
12. **Tylewska-Wierzbanowska S, *et al.*** Epidemics of Q fever in Poland in 1992-1994. *Roczniki Akademii Medycznej w Bialymstoku* 1996; **41**: 123–128.
13. **Stein A.** Pigeon pneumonia in provence: a bird-borne Q fever outbreak. *Clinical Infectious Diseases* 1999; **29**: 617–620.
14. **Pinsky RL.** An outbreak of cat-associated Q fever in the United States. *Journal of Infectious Diseases* 1991; **164**: 202–204.
15. **Marrie TJ, *et al.*** Q fever pneumonia associated with exposure to wild rabbits. *Lancet* 1986; **1**: 427–429.
16. **Carriere MP, *et al.*** Investigation of a slaughterhouse-related outbreak of Q fever in the French Alps. *European Journal of Clinical Microbiology & Infectious Diseases* 2002; **21**: 17–21.
17. **Donaghy M, *et al.*** Outbreak of Q fever in workers at a meat processing plant in Scotland, July 2006. *Eurosurveillance* (http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3031).
18. **Boschini A, *et al.*** Consecutive epidemics of Q fever in a residential facility for drug abusers: impact on persons with human immunodeficiency virus infection. *Clinical Infectious Diseases* 1999; **28**: 866–872.
19. **De Bruin A, *et al.*** A Query for Q-fever using a new developed multiplex Q-PCR. 4th Annual meeting MedVetNet Network of Excellence EU 2008, St. Malo, France. Poster DC27 (http://www.medvetnet.org/pdf/Conferences/abstract_book_website.pdf). Accessed June 2008.
20. **Porten K, *et al.*** A super-spreading ewe infects hundreds with Q fever at a farmers' market in Germany. *BMC Infectious Diseases* 2006; **6**: 147.
21. **Klaassen CHW, *et al.*** Multigenotype Q Fever outbreak, the Netherlands [Letter]. *Emerging Infectious Diseases* 2009; **15**: 613.

Supplementary Figure S1. Posterior-anterior chest X-ray of a female patient showing infiltrates in the right upper and lower lobes.



CHAPTER 3.4

Visits on ‘lamb-viewing days’ at a sheep farm open to the public was a risk factor for Q fever in 2009

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Visits on 'lamb-viewing days' at a sheep farm open to the public was a risk factor for Q fever in 2009

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SUMMARY

Between February and May 2009, 347 laboratory-confirmed cases of acute Q fever were reported in a southern municipal health service region in The Netherlands. Commercial dairy-goat farms were implicated and control measures were initially targeted there. A preliminary investigation also implicated a non-dairy sheep farm, open to the public on 'lamb-viewing days'. This study tested the association between visiting the non-dairy sheep farm and developing Q fever in residents of the region between February and May 2009. A case-control study of 146 cases and 431 address-matched controls was conducted. Multivariable logistic regression analysis confirmed the association between visiting to the sheep farm and Q fever disease (matched odds ratio 43, 95% confidence interval 9–200). Other risk factors were being a smoker, having a past medical history and being aged >40 years. Vaccination of sheep and goats on farms open to the public should help to reduce the number of future human cases.

Key words: Case-control study, *Coxiella*, Q fever, The Netherlands.

INTRODUCTION

Q fever is a zoonosis caused by the bacterium *Coxiella burnetii*. The bacterium is found in the milk, urine, faeces and wool of infected animals (particularly sheep, goats and cattle) but birth products are known to be highly contagious [1–3]. It is hypothesized that human infection occurs through inhalation of contaminated aerosols [4]. Since 2007, over 3000 cases have been reported in The Netherlands (2354 cases in 2009 alone), peaking annually in spring–early summer. Commercial dairy-goat farms and some dairy-sheep farms have been implicated in the spread of disease. Control measures in 2008 and 2009 were centred on these farms, as the risk associated with non-milk-producing farms was thought to be low [5].

Limited data are available in relation to what other agricultural sectors might have contributed to the spread of disease.

In 2009, the municipal health service in the south-east region of Brabant province (MHS Brabant Southeast) received over 400 notifications of Q fever

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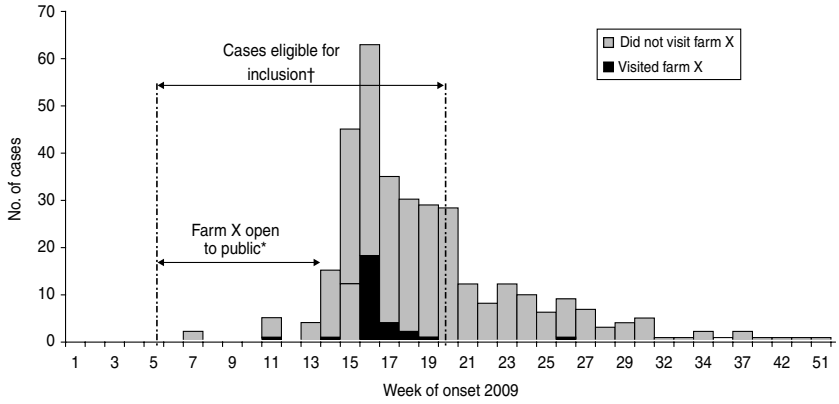

Fig. 1. Q fever cases by week of onset of symptoms and visits to farm X. Brabant Southeast, The Netherlands, 2009. * Farm X was open on weekends only from 1 February (week 5) to 31 March (week 14). † 246 cases had a date of onset of illness between 1 February 2009 and 15 May 2009 and were eligible for inclusion in the study.

of which 379 were laboratory-confirmed cases (Fig. 1). The majority of these cases were probably explained by residence near an infected dairy-goat farm located northeast of Helmond city [6]. Following a trawling questionnaire conducted with 343 confirmed cases, a second cluster, possibly associated with a non-dairy sheep farm, was identified in the region. Forty-six cases spontaneously indicated in an open-text question that they had visited the sheep farm (farm X) during the lambing season in February–March 2009. In the trawling questionnaires other veterinary sources were listed, such as a visit to a pet goat farm and a zoo, but these locations were common for only two and three cases, respectively.

Farm X has been open to the public during the lambing season since 2004. In 2009, it was open each Saturday from 1 February until 31 March and there were an estimated 12 000 visitors to the farm, most of whom lived locally within 25 km of the farm. More than 1200 lambs were born that season (the majority in January and February), and visitors could occasionally witness the birth of a lamb and were encouraged to watch the lambs play. Only three abortions were reported on the farm during this season, which is less than might be expected in a typical season, unaffected by Q fever.

The aim of this study was to test whether there was an association between visiting farm X and developing Q fever in residents of Southeast Brabant in February–May 2009. A secondary objective was to identify risk factors for acquiring Q fever (or a *C. burnetii* infection) in people who visited the farm.

METHODS

Epidemiological investigation

Southeast Brabant is predominantly an agricultural region in the south of The Netherlands bordering Belgium. A case-control study was conducted in the region in June 2010. All adults aged $\geqslant 18$ years who were normally resident in Southeast Brabant between 1 February and 31 of March 2009 were eligible for inclusion ($n = 732\,731$). The exposure of interest was a visit to farm X between 1 February (end of week 5) and 31 March (beginning week 14) in 2009 (the period when the farm was open to the public). Given a minimum incubation period of 3 days and a maximum incubation period of about 6 weeks [4, 7], cases were defined as adult inhabitants of the region who were notified with Q fever and for whom illness onset was between 1 February (end of week 5) and 15 May (end of week 20), 2009 (Fig. 1). In The Netherlands, criteria for notification of acute illness are presence of at least fever, pneumonia, or hepatitis plus laboratory confirmation of *C. burnetii* infection with (*a*) polymerase chain reaction (PCR), or (*b*) detection of a fourfold rise in serum antibody titres to *C. burnetii* or a high titre in two samples without a fourfold increase, or (*c*) a single high titre of IgM to phase II antigen. Controls were householders randomly selected based on having an address in the same six-digit postcode area (living on the same street) as each case.

Data were collected by means of a postal questionnaire addressed individually to named cases, and

to 'the householder' at the control address. Questions related to demography (age, gender, occupation), exposure (number of visits to farm X and two additional agricultural sites Y and Z offering viewing/petting of sheep or goats, and which were open to the public in the area), month of visit, duration of direct contact with animals (time spent on the premises, presence at birth of lambs on the farm), outcome (symptoms, hospitalizations for Q fever-like symptoms), behavioural factors (travel history, smoking) and medical history.

Sample size calculation and data analysis

Of all notified adult cases in the region in 2009, 248 reported onset of illness between the start of week 5 and the end of week 20, and 42 of these had visited the farm between the first week of February and the last week of March (Fig. 1). The exposure among the cases was therefore 17% (42/248). For the detection of a minimum odds ratio (OR) of 3, two controls per case were required at a precision (alpha) of 5% [two-sided 95% confidence interval (CI)] to achieve power of 80%. Assuming 50% of cases responded, the minimum number of case respondents required was therefore 134 and for controls it was 268. Eight controls per case were invited to participate, with the aim of achieving a 25% response rate from controls.

Data were entered using Access and analysed using Stata v. 10.1 (StataCorp., USA). Baseline characteristics of cases and controls were compared using χ^2 test. Univariable and multivariable analyses of the distribution of exposures in cases and controls were examined using matched ORs (conditional logistic regression) with 95% exact CIs. Risk factors which were statistically significantly associated with being a case at the 0·25 univariable level were selected for a conditional backward stepwise multivariable model. Significance level was set to 0·05 for the latter model.

Environmental investigation

Vaginal swabs were collected from 20 sheep on farm X on 19 May 2009, and were tested by multiplex quantitative real-time PCR (qPCR) at the laboratory in the National Institute of Public Health and the Environment in The Netherlands. Eight environmental aerosol samples were also obtained on 20 May 2009 in the vicinity of the farm, at a distance of 500 m and 1000 m in all four wind directions (north, east, south, west). A summary description of laboratory methods is given here, but these will be discussed in detail more appropriately elsewhere (A. De Bruin *et al*., unpublished data).

The qPCR detects two *C. burnetii* targets (*com1* and IS*1111*), and one *Bacillus thuringiensis* internal control target (*cry1b*). *B. thuringiensis* spores were added to samples to control both DNA extraction and PCR amplification. Each DNA extract obtained was tested in triplicate for *C. burnetii* presence. Three μl of DNA extract per reaction were used in qPCR assays performed on a Lightcycler 480 Instrument (Roche Diagnostics Nederland BV, The Netherlands). In addition, 3 μl DNA from the *C. burnetii* Nine Mile RSA phase I strain were included as positive control, or 3 μl H_2O as negative control. Analysis was performed on the instruments software: Lightcycler 480 software release 1.5.0. SP3.

Cq values were calculated using the second derivative method. Samples were scored as positive when at least one of the *C. burnetii* targets (*com1, or* IS*1111*) showed a positive signal. Samples were scored as negative when (*a*) no *C. burnetii* targets showed positive signals, and (*b*) there was a positive result for the internal control.

RESULTS

Epidemiological investigation

Sixty-five percent (162/248) of cases responded as did 35% (686/1985) of controls. As the sample size requirements had been fulfilled, no reminder letter was issued. Cases were individually matched on address and the matched analysis was performed on 146 cases matched to between one and six controls per case ($1:M_k$ matching; total matched sample, $n=579$). Cases were of a similar age distribution to controls (mean age of cases 54·9 years, range 18–89; mean age of controls 54·8, range 22–79). Fifty-seven percent ($n=85$) of cases and 47% ($n=202$) of controls were male (Table 1). Of the cases, 62% ($n=86$) reported having pneumonia, two reported having hepatitis and three reported endocarditis; 38% of cases reported a variety of other symptoms including exhaustion, headache, high fever and cough. Overall, 35 (25%) cases were hospitalized. Mean duration of illness for cases was 21 days (median 10 days, range 0–365). No cases were pregnant at the time.

Based on trawling questionnaire data received from the first 32 cases (where precise date of farm visit and date of onset of illness were recorded), and assuming a

Table 1. *Matched univariable and multivariable odds ratios of factors associated with the Q fever outbreak in Southeast Brabant region, The Netherlands, February–May, 2009*

	Cases		Controls		Univariable matched† OR			Multivariable matched OR		
	n	*n*/*N* %*	*n*	*n*/*N* %	OR	95% CI	*P*	OR	95% CI	*P*
Demographics										
Age group										
<40 yr	17	11·6	69	16·4	Ref.	Ref.		Ref.	Ref.	
40–59 yr	74	50·7	178	42·3	1·7	(0·9–3·2)	0·094	5·4	(1·9–15·3)	0·001
⩾60 yr	55	37·7	174	41·3	1·2	(0·6–2·3)	0·594	3·8	(1·3–10·6)	0·012
Gender (male)	85	57·4	202	47·4	1·8	(1·1–2·9)	0·013			
Visits to agricultural sites and events										
Visited farm X	31	21·1	6	1·4	24·2	(8·4–69·2)	0·000	43·3	(9·4–200·1)	0·000
Visited site Y	5	3·5	14	3·3	1·2	(0·4–3·5)	0·773			
Visited site Z	1	0·7	0	0·0	—	—	—			
Other agricultural site for recreational visit (unspecified)	17	12·1	23	5·6	1·9	(0·9–3·7)	0·076			
Other public event with animals	8	5·7	15	3·6	1·4	(0·5–3·4)	0·520			
Visited other sheep or goat farm, not otherwise named	6	4·2	9	2·1	2·1	(0·7–6·2)	0·186			
Other contact with animals										
Work in industry related to agriculture	1	0·7	6	1·4	0·6	(0·1–4·9)	0·620			
Pets at home	58	39·2	176	40·9	0·9	(0·6–1·4)	0·671			
Farm animals at the home-place	3	2·1	7	1·7	1·8	(0·4–8·1)	0·473			
Sheep at home	3	2·0	1	0·2	—	—	—			
Goats at home	0	0·0	1	0·2	—	—	—			
General health										
History of medical problems	80	60·0	193	47·3	1·5	(1·0–2·3)	0·054	1·6	(0·9–2·8)	0·084
History of Q fever prior to February 2009	0	0·0	1·0	0·3	—	—	—			
Current smoker	52	35·6	85	20·0	2·0	(1·3–3·0)	0·002	2·2	(1·3–3·8)	0·006
Occurrence of disease in households										
Another person in the household with Q fever in 2009	9	6·1	2	0·5	10·3	(2·2–48·7)	0·003	4·8	(0·6–36·1)	0·126

OR, Odds ratio; CI, confidence interval.

* *N* is the total number of respondents to the question. Where there is missing data, this may not total 146 for cases and 486 for controls.

† 1:M_k matching: cases matched to between 1 and 6 controls.

Table 2. *Distribution of exposures on the farm among cases and controls who visited the farm between 1 February and 31 March 2009*

	Cases		Controls		Univariable matched OR		
	n	*n*/*N* %*	*n*	*n*/*N* %	OR	95% CI	*P*
Held or cuddled a lamb during visit	23	85	3	60	3·8	(0·2–44·6)	0·185
Witnessed the birth of a lamb during visit	3	12	0	0	—	—	—

OR, Odds ratio; CI, confidence interval.
* *N* is the total number of respondents to the question. Missing data is not included.

point-source exposure, the average incubation time, defined as the time between day of illness onset and day of visit farm X, was 20·7 days (range 9–43 days).

At univariable level, 21% ($n=31$) of cases reported visiting farm X compared to 1% ($n=6$) of controls resulting in an OR of exposure between cases and controls of 24 (95% CI 8·4–69·2). When adjusted for other risk factors based on univariable findings (age group, gender, recreational visits to other agricultural sites or events, visits to other sheep/goat farms otherwise unspecified, medical history, smoking status and having a family member who had Q fever in 2009) the multivariable adjusted OR for a visit to farm X was 43·3 (95% CI 9·4–200·1). Other significant independent risk factors (Table 1) included being a current smoker (OR 2·2, 95% CI 1·3–2·8), and being aged >40 years (40–59 years: OR 5·4, 95% CI 1·9–15·3; ⩾60 years: OR 3·8, 95% CI 1·3–10·6).

Subgroup analysis: risk of Q fever for those visiting farm X

Thirty-seven respondents reported visiting farm X during the specified time period. No significant association was found between different behaviours on the farm (Table 2) in cases and controls.

Environmental investigation

Seventeen out of 20 vaginal swabs taken from sheep on the farm were positive for *C. burnetii* multicopy target IS*1111* only, indicating a relatively low level of *C. burnetii* DNA present in these samples. One sample was found positive for both *C. burnetii* targets (*com1* and IS*1111*).

Seven out of the total of eight aerosol samples taken 500 m and 1000 m from farm X were positive for *C. burnetii* target IS*1111* in 2009; the only negative aerosol sample was located 500 m north of the farm.

DISCUSSION

This study confirms the association between a visit to 'lamb-viewing days' on sheep farm X between 1 February and 31 March 2009, and Q fever in cases. Other risk factors included increasing age, smoking and positive medical history (consistent with findings elsewhere [9]). The reported mean incubation period of 21 days is also consistent with findings from other research [3]. Increased risk associated with handling or petting sheep and lambs, or witnessing the birth of a lamb was not demonstrated here (possibly related to the small number of cases and controls who reported such behaviours).

This study had a number of limitations. A cohort study of farm visitors could not be performed as no visitor list was available, therefore no attack rate or risk ratio could be calculated. The outbreak occurred in early 2009 and given the time lapse between the outbreak and this study and the degree of media coverage of outbreaks nationwide, there is potential recall bias. A farm visit is a distinct event however, and as the farm of interest was open for only a limited period it is likely that visitors would recall attending – in fact less than 1% reported not remembering whether they visited – although some could not remember the precise date.

This cluster occurred in the context of a much larger outbreak in the region. Farm X is situated in a region with other infected farms in proximity [6, 10], and given the potential role of the wind and other forms of indirect spread of C. burnetii [3, 11, 12], it would in any case prove difficult to establish a causal link to farm X. In the absence of trawling questionnaires indicating the farm as a possible source, it is likely that this cluster would have remained unrecognized, and other unidentified sources may also be implicated in this outbreak. In this study, we matched controls to cases by street address in an attempt to control for some of these unknown factors.

We tested the association with visits to other farm sites Y and Z, and public agricultural events in the area, but none was found. Q fever cases associated with flocks of non-dairy sheep and newborn lambs have been reported previously [13] and in one study, hundreds of infections were attributed to a single ewe at a farmer's market [3]. Given these findings, the positive vaginal swabs from sheep on the farm, and the fact that DNA was isolated from three aerosol sampling locations proximal to the farm, it is plausible that of those who visited farm X between 1 February and 31 March 2009, 95% of the cases that occurred were attributable to the visit (the attributable fraction among the exposed).

It is estimated that up to 60% of Q fever cases are asymptomatic, and therefore there were potentially cases in the control group. No serological testing of controls was conducted in this study; however, recent analysis of blood donor samples in the region confirmed a prevalence of anti-IgG antibodies of 12% in 2009 [14]. If this prevalence were applied to our data, the impact on the ORs reported here is uncertain. If cases and controls were correctly classified, the reported ORs may be an overestimate of the association. If, however, a greater proportion of farm visitors than expected were reclassified as cases, the OR of association would not necessarily be reduced. In either case, a visit to farm X would still be a strong independent risk factor for Q fever.

In the Brabant region there are 35 petting farms and 14 zoos open to the public and in 2008, there were 1·6 million recreational visits to farms and farmland in the area. 'Lamb-viewing days' during lambing season were particularly popular [15]. 'Agri-tourism' is therefore an important recreational and revenue-generating activity in the region. In January 2010, the Ministry of Health issued a hygiene protocol to all farms with a public function including petting farms and those offering 'lamb-viewing days' [16]. Control measures implemented throughout the country included isolation of pregnant sheep and goats (away from public areas), mandatory animal vaccination, and cessation of 'lamb-viewing days' until vaccination was complete. As a result, farm X was closed to the public in February–March 2010, pending vaccination of the herd which has since been completed. The farm reopened to the public in spring 2011. As a result of the vaccination campaign in sheep and goats nationally, it is expected that the number of human cases will fall in the coming years [17], but farm visitors should continue to be vigilant. Vulnerable groups such as pregnant women, people with cardiovascular anomalies, and those with reduced immunity, should be aware of their elevated risk with regard to Q fever. For all farm visitors, hygiene and preventive measures should continue to be practised according to recommendations [18].

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Abinanti FR, *et al.*** Q fever studies XVIII. Presence of Coxiella burnetii in the birth fluids of naturally infected sheep. *American Journal of Hygiene* 1953; **58**: 385–388.
2. **Aitken ID, *et al.*** Q fever in Europe: current aspects of aetiology, epidemiology, human infection, diagnosis and therapy. *Infection* 1987; **15**: 323–327.
3. **Porten K, *et al.*** A super-spreading ewe infects hundreds with Q fever at a farmers' market in Germany. *BMC Infectious Diseases* 2006; **6**: 147.
4. **Maurin M, Raoult D.** Q fever. *Clinical Microbiological Reviews* 1999; **12**: 518–553.
5. **Q fever Evaluation Commission.** Q fever policy in the Netherlands 2005–2010 [in Dutch], 2010 (http://www.rijksoverheid.nl/documenten-en-publicaties/rapporten/2010/11/22/van-verwerping-tot-verheffing.html). Accessed 5 December 2010.
6. **Schimmer B, *et al.*** The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. *BMC Infectious Diseases* 2010; **10**: 69.
7. **Heymann D (ed.)**. *Control of Communicable Diseases Manual*, 19th edn. Washington, DC.: American Public Health Association, 2008.
8. **Bustin SA, *et al.*** The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry* 2009; **55**: 611–22.
9. **Karagiannis I, *et al.*** Investigation of a Q fever outbreak in a rural area of The Netherlands. *Epidemiology and Infection* 2009; **137**: 1283–1294.

10. **Food and Consumer Product Safety Authority, Ministry of Agriculture Nature and Food Quality.** Q Fever: map with overview of infected dairy-goat and dairy-sheep farms, 2010 (http://www.vwa.nl/onderwerpen/dierziekten/dossier/q-koorts/kaart-met-overzicht-van-besmette-bedrijven). Accessed 3 December 2010.
11. **Hawker JI, *et al.*** A large outbreak of Q fever in the West Midlands: windborne spread into a metropolitan area? *Communicable Disease and Public Health* 1998; **1**: 180–187.
12. **Lyytikainen O, *et al.*** An outbreak of sheep-associated Q fever in a rural community in Germany. *European Journal of Epidemiology* 1998; **14**: 193–199.
13. **Koene RP, *et al.*** A Q fever outbreak in a psychiatric care institution in The Netherlands. *Epidemiology and Infection* 2011; **139**: 13–18.
14. **Hogema BM, *et al.*** Coxiella burnetii infection among blood donors during the 2009 Q-fever outbreak in the Netherlands. *Transfusion*. Published online: 14 July 2011. doi:10.1111/j.1537-2995.2011.03250.x.
15. **Province of North Brabant.** Brabant in figures. Leisure Industry Trend Report, 2010 (http://www.vrijetijdshuis.nl/wp-content/uploads/2010/05/Totaal-Trendrapport2.pdf). Accessed 10 December 2010.
16. **Ministry of Agriculture Nature and Food Quality.** Hygiene protocol for goat and sheep farms with a public function, 2010 (http://www.rijksoverheid.nl/documenten-en-publicaties/rapporten/2010/01/27/hygieneprotocol-voor-geiten-en-schapenbedrijven-met-een-publieke-functie.html). Accessed 3 December 2010.
17. **van der Hoek W, *et al.*** Q fever in the Netherlands: an update on the epidemiology and control measures. *Eurosurveillance* 2010; **15**: pii = 19520.
18. **Netherlands Foundation for Petting Zoos (SKBN).** Hygiene Code, 2010 (http://www.skbn.net/de-kinderboerderij/hygiene-code). Accessed 3 December 2010.

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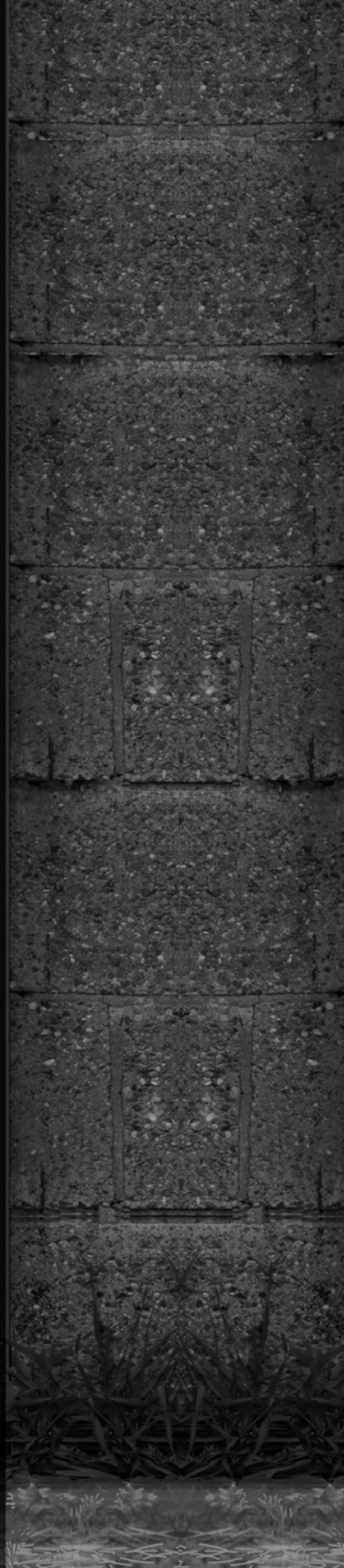
Seroprevalence and risk factors of *Coxiella burnetii* infection in residents and ruminants on commercial farms in a 'One Health' context



CHAPTER 4.1

Seroprevalence and risk factors for *Coxiella burnetii* (Q fever) seropositivity in dairy goat farmers' households in The Netherlands, 2009-2010

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Seroprevalence and Risk Factors for *Coxiella burnetii* (Q Fever) Seropositivity in Dairy Goat Farmers' Households in The Netherlands, 2009-2010

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Abstract

Community Q fever epidemics occurred in the Netherlands in 2007–2009, with dairy goat and dairy sheep farms as the implicated source. The aim of the study was to determine the seroprevalence and risk factors for seropositivity in dairy goat farmers and their household members living or working on these farms. Sera of 268 people living or working on 111 dairy goat farms were tested for *Coxiella burnetii* IgG and IgM antibodies using immunofluorescence assay. Seroprevalences in farmers, spouses and children (12–17 years) were 73.5%, 66.7%, and 57.1%, respectively. Risk factors for seropositivity were: performing three or more daily goat-related tasks, farm location in the two southern provinces of the country, proximity to bulk milk-positive farms, distance from the nearest stable to residence of 10 meters or less, presence of cats and multiple goat breeds in the stable, covering stable air spaces and staff not wearing farm boots. Goat farmers have a high risk to acquire this occupational infection. Clinicians should consider Q fever in this population presenting with compatible symptoms to allow timely diagnosis and treatment to prevent severe sequelae. Based on the risk factors identified, strengthening general biosecurity measures is recommended such as consistently wearing boots and protective clothing by farm staff to avoid indirect transmission and avoiding access of companion animals in the goat stable. Furthermore, it provides an evidence base for continuation of the current vaccination policy for small ruminants, preventing spread from contaminated farms to other farms in the vicinity. Finally, vaccination of seronegative farmers and household members could be considered.

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Introduction

Q fever is a ubiquitous zoonosis caused by the bacterium *Coxiella burnetii (C. burnetii)*. The primary animal reservoirs for human infection are cattle, sheep and goats. Bacteria are shed in especially high concentrations in placentas and birth fluids of infected animals, which may subsequently contaminate the stable environment [1]. Human infection results from inhalation of contaminated aerosols, generated by infected animals or animal products or through direct contact with milk, urine, faeces, or semen of infected animals [2,3,4]. Human Q fever may range from subclinical infection to endocarditis and ruptured aneurysms, and long-term sequelae such as chronic fatigue syndrome *(1–4)*. Ruminant farmers are considered one of the main occupational risk populations for acquiring *C. burnetii* infections [5]. In 2009–2010, our integrated human-animal-environmental Q-VIVE study among Dutch dairy goat farms showed a farm prevalence of 43.1% and a goat seroprevalence of 21.4% [6]. The aim of the present study was to determine within the same farm study population, the seroprevalence in farmers and household members living and/or working on dairy goat farms and to assess the farm-related and individual risk factors for seropositivity in order to update control measures and to provide targeted advice for this occupational group and the Dutch dairy goat industry.

Methods

All dairy goat farms in the Netherlands with at least 100 adult goats that were not vaccinated for Q-fever were selected from a national database of the Animal Health Service. On eligible farms, we approached dairy goat farmers and one or two of their household members aged 12 years and older, and in some instances, other persons working or living on the farm such as farm employees. A maximum of three participants were included per farm. Non-responders received a reminder three weeks after the initial invitation. After providing informed consent on farm and individual level, all participating farms were visited by professional laboratory assistants, who collected sera from October 2009 through March 2010. Each participant received an individual questionnaire by e-mail or post containing questions on person-

based exposures, for instance living and/or working on the farm, contact with goats, other livestock, pets and the farm environment, consumption of raw dairy products, use of protective clothing, pregnancy, smoking and underlying health conditions. A farm questionnaire was sent to the farm manager/owner containing questions on herd size, presence of other livestock and pets, farm management, stable environment, lambing season and hygiene measures. We obtained data on the Q fever bulk milk status for the period 1 October 2009–30 September 2010 from the Dutch Ministry of Agriculture. The Medical Ethical Commission of the University Medical Center Utrecht approved the study protocol (nr. 09–189/K).

Serology

Serum samples were tested for *C. burnetii* IgM and IgG antibodies, both phase I and II, using indirect immunofluorescence assay (IFA) with a screening dilution of 1:32. Study participants without any positive antibody result and participants with a solitary IgM phase I or solitary IgM phase II were defined as seronegative. All other outcomes were classified as seropositive. Those with among others IgM phase II antibodies were designated as 'relatively recent infections' and include possible current infections. The term 'relatively recent' was chosen as IgM phase II is found positive (≥1:32) in the majority of cases one year post-infection and may even persist up to three years post-infection [7], (personal communication C. Wielders). Seropositives without IgM phase II antibodies were designated as 'past infections'. As the latter also includes possible chronic infections, within the past infections a distinction was made between serological profiles which had IgG phase I <1:32 or negative and therefore not consistent with chronic infection, and serological profiles which could indicate chronic infection. IgG phase I and II end point titers were determined.

Data analysis

To study participation bias, participating and non-participating farms were compared with regard to herd size, urbanization degree, region and bulk milk status. For the risk factor analyses, first frequency tables of variables were analysed and distributions of continuous variables were studied. If the latter were not linearly related to the outcome variable, variables were divided into classes based on biological arguments and if these were lacking based on quartiles or medians and/or chosen similarly to classes used in a previous analyses of risk factors for the goats of these same farms [6]. Using SAS software version 9.2, univariate logistic regression analysis was performed to assess the main factors associated with *C. burnetii* seropositivity at the individual level ($p<0.20$ in the likelihood ratio test (−2LL)). Variables with less than 10% of data in one risk category were excluded. Age was always kept in the model because of the frequent relationship with *C. burnetii* seropositivity observed in other studies. Proxy outcomes such as bulk milk Q fever status were not included in multivariable analyses. All identified individual variables were analyzed with a manual backward elimination procedure starting with a full multivariable logistic regression model. Variables were kept in de model if the −2LL ratio test of the model with and without the variable was significant ($p<0.05$). The final individual model was tested with the Hosmer-Lemeshow-Goodness-of-fit test. Subsequently, multilevel univariate model analyses were performed to identify risk factors derived from the farm questionnaire, taking into account clustered farm-based data for all persons within the same farm, using a unique farm number as cluster variable for each farm. All univariately significant farm variables ($p<0.20$) were analyzed with a manual backward elimination procedure starting with a full multilevel model. Finally, both the individual and farm-based characteristics from the final two submodels were combined in a multivariate multilevel analysis, to examine the independent relationship between risk determinants and *C. burnetii* seropositivity. The final model fit was assessed by the QIC (Quasi-Likelihood under the Independence Model Criterion) goodness-of-fit statistic for GEE (generalized estimation equation) models.

Results

Descriptive characteristics

Of all 334 invited eligible dairy goat farms, 111 (33.2%) farms participated in this study. In total, 24.3% of the participating farms tested bulk milk-positive from October 2009 through March 2010, similar to 22.9% positive for the non-participating farms. The mean herd size was 869 goats (range 121–3805) in participating farms, not statistically different from the mean of 809 goats (105–4733) in non-participating farms. In addition, no differences between participating and non-participating farms were observed with regard to urbanization degree and region. From the 111 participating farms, 268 persons provided a blood sample (mean age 42.0 years (12–81), 53.7% male). Of these, 184 (68.7%) were seropositive; 154 (57.5%) participants had experienced a past infection and 30 (11.2%) had experienced a relatively recent infection, as demonstrated by presence of IgM phase II antibodies. IgG phase II end titres were known for the 75 participants with a past infection with IgG phase I <1:32: 1:32 (n = 20), 1:64 (n = 14), 1:128 (n = 16), 1:256 (n = 12), 1:512 (n = 9) and ≥1:1024 (n = 4). For the 79 participants with a past infection with IgG phase I ≥1:32, 11 persons had 'possible chronic Q fever' with IgG phase I titers ≥1:1024 according to diagnostic criteria used in the Netherlands [8]. Clinical information was lacking to confirm that these were truly chronic cases. Based on questions regarding clinical history in the individual questionnaire, none of them reported a history of pneumonia, hepatitis or endocarditis during the past 5 years or were diagnosed with acute Q fever by their general practitioner. Also none of them indicated a history of an immune disorder, chronic pulmonary disease or cardiovascular problem except for one case with a high blood pressure and one case with a breast malignancy in 1999. Seroprevalences in males increased by age reaching a plateau around 80% in the 35–55 years age group, while in females the highest seroprevalence (75.9%) was observed in those below 35 years. The seroprevalence was highest among farmers (73.5%) and in the small group of non-household members such as farm employees or servants (83.3%). Seroprevalences in spouses and children (12–17 years) were 66.7% and 57.1%, respectively (Table 1). Among those living or working on a bulk milk-positive farm, 95.5% were seropositive. The median duration of residence on a dairy goat farm was 10 years (0–29 years). A Q fever episode was confirmed by a general practitioner in 10 participants (4.1%) during the period 2008–2010. Based on their serum sample, 5 had a serological profile matching relatively recent infection and 5 a profile indicating past infection.

Univariate analyses on individual and farm level

Individual farm exposures, such as milking and feeding goats, supply and removal of dairy goats into and outside the stable, giving general health care and birth assistance, cleaning the stables, removal and spread of manure, contact with raw goat milk and daily contact with goat manure, dead-born animals during the lambing season, daily contact with roughage or animal feed, direct contact with cattle on own farm and residence as a child on a ruminant farm were all associated with human seropositivity

Table 1. Descriptive characteristics and seroprevalence of dairy goat farmers and family members (n = 268), September 2009–April 2010, The Netherlands.

Variable	Frequency (N)	Seroprevalence	
		% (N)	95% CI (exact)
Participant	268	68.7 (184)	62.7–74.2
Sex			
Male	144	70.1 (101)	62.0–77.5
Female	124	66.9 (83)	57.9–75.1
Age (years) in males			
<35	36	55.6 (20)	38.1–72.1
35-<45	43	79.1 (34)	64.0–90.0
45-<55	35	80.0 (28)	63.1–91.6
≥55	30	63.3 (19)	43.9–80.1
Age (years) in females			
<35	29	75.9 (22)	56.5–89.7
35-<45	47	61.7 (29)	46.4–75.5
45-<55	31	67.7 (21)	48.6–83.3
≥55	17	64.7 (11)	38.3–85.8
Function			
Farmer	132	73.5 (97)	65.1–80.8
Spouse	69	66.7 (46)	54.3–77.6
Farmers' son/daughter ≥18 years	21	66.7 (14)	43.0–85.4
Child (12–17 years)	14	57.1 (8)	28.9–82.3
Other family members	26	53.9 (14)	33.4–73.4
Other persons, such as farm employees	6	83.3 (5)	35.9–99.6

CI, Confidence Interval.

($p<0.05$). Potential risk factors with $p\geq0.05$ to $p<0.20$ were having lived and/or worked before 1997 on a dairy goat farm, being a farmer, fulltime work week, having a dog, direct contact with horses on own farm, daily contact with birth material during the lambing season, contact with cattle manure and contact with goats, dogs and cats at other farms and work experience in the cattle sector (Table 2).

The following farm-based characteristics were potential risk factors in univariate analyses: farm location (south), short distance to the nearest bulk milk-positive farm, bulk milk status from 1 October 2009 to 30 September 2010, herd size ≥800 goats, distance between residence and nearest goat stable ≤10 meters, presence of other goat breeds besides the Dutch White Goat, use of artificial insemination, extended lactation, presence of cats in the stable, use of silage and/or maize feed, using a fodder mixer or automatic feeding method, use of screen/gauze in the stable, covering air spaces in the stable for combating nuisance animals such as wild birds, ≥3 lambing periods per year, an abortion percentage of ≥4% in 2007–2009 or a reported Q fever abortion wave. Biosecurity factors such as having a closed farm tenure, goat supply from southern provinces, receiving farm visitors for school tours or organised groups, and not wearing farm boots among staff and other employees were also potential risk factors. Two spatial variables were potential risk factors: municipal cattle density of 100 ruminants per km^2 and a net goat density of ≥15 goats per km^2 within five kilometre radius. Besides risk factors, potential protective factors were found, such as presence of laying hens, stable air ventilation through a flap, membership of an organic dairy goat cooperative, and presence of rats or mice in the stable in 2008 before implementation of the hygiene protocol (Table 3).

Multivariate and multilevel analyses

Three individual and eight farm-based variables were significant in the two final multivariate submodels ($p<0.05$, $-2LL$) (Tables 4 and 5, respectively) and together with age group used as the full multilevel model. The final combined individual-farm multilevel model showed that the number of daily performed goat-related tasks (including milking, feeding, supply and removal of goats, general health care, birth assistance of goats) was an independent significant risk factor as well as farm location in the two southern provinces, distance to nearest bulk milk-positive farm, presence of a cat in the goat stable, distance between residence and goat stable ≤10 meters, presence of other goat breeds besides the Dutch White Goat and not wearing farm boots among staff and other employees (Table 6). A borderline significant risk factor and a protective factor were found, i.e. living as child on a ruminant farm and no use of extended lactation, respectively.

Discussion

This is the first study addressing the seroprevalence in dairy goat households in the Netherlands, and one of few risk factor studies on human *C. burnetii* infections in farm populations worldwide. It confirms that living and or working on a dairy goat farm poses a high lifetime risk for acquisition of a *C. burnetii* infection. Farmers and other household members are generally in closest proximity to infected goats and contaminated stables on farms, and therefore at

Table 2. Univariate logistic model of individual factors associated with human Q-fever (P<0.20) with corresponding frequency (N), seroprevalence (%), odds ratio (OR) and 95% confidence interval (CI).

Variable	Category	Freq. (N)	Seropre-valence (%)	OR [95% CI]	p-value
Age group	<35 years	65	64.6	Reference	0.57
	35-<45 years	90	70.0	1.28 [0.65–2.52	
	45-<55 years	66	74.2	1.58 [0.75–3.34]	
	≥55 years	47	63.8	0.97 [0.44–2.11]	
Start working on goat farm	1997 or after	141	66.0	Reference	0.07
	before 1997	107	76.6	1.69 [0.96–2.99]	
Function	Farmer	122	74.6	1.54 [0.89–2.68]	0.13
	Other	122	65.6	Reference	
Hours working on farm	Fulltime	124	76.6	2.46 [1.25–4.82]	0.05
	Halftime	35	74.3	2.17 [0.86–5.46]	
	Quarter week	29	62.1	1.23 [0.49–3.07]	
	Sometimes/never	56	57.1	Reference	
Number of daily goat-related tasks (milking, feeding, supply and removal, general animal health care, birth assistance)	≥3 tasks	167	79.6	3.69 [2.14–6.34]	<0.01
	≤2 tasks	101	50.5	Reference	
Milking goats	Yes	183	75.4	2.60 [1.42–4.77]	<0.01
	No	61	54.1	Reference	
Feeding goats	Yes	186	76.3	3.23 [1.74–5.97]	<0.01
	No	58	50.0	Reference	
Supply and removal of dairy goats or bucks	Yes	120	82.5	3.41 [1.89–6.15]	<0.01
	No	124	58.1	Reference	
Care for general animal health	Yes	151	78.8	2.93 [1.67–5.16]	<0.01
	No	93	55.9	Reference	
Birth assistance	Yes	151	78.2	2.70 [1.54–4.74]	<0.01
	No	93	57.0	Reference	
Remove manure	Yes	130	76.9	2.02 [1.16–3.52]	0.01
	No	114	62.3	Reference	
Spread manure	Yes	69	82.6	2.54 [1.27–5.10]	<0.01
	No	175	65.1	Reference	
Clean the stables	Yes	118	81.4	2.97 [1.66–5.32]	<0.01
	No	126	59.5	Reference	
Administration	Yes	148	74.3	1.66 [0.95–2.90]	0.07
	No	96	63.5	Reference	
Having a dog	Yes	209	71.8	1.70 [0.81–3.55]	0.16
	No	35	60.0	Reference	
Direct contact cattle own farm	Yes	34	85.3	2.78 [1.03–7.55]	0.04
	No	210	67.6	Reference	
Direct contact horses own farm	Yes	90	76.7	1.68 [0.93–3.03]	0.09
	No	154	66.2	Reference	
Direct contact goats other farm	Yes	28	82.1	2.11 [0.77–5.80]	0.15
	No	216	68.5	Reference	
Direct contact horses other farm	Yes	24	83.3	2.29 [0.75–6.94]	0.14
	No	250	68.6	Reference	
Direct contact dogs other farm	Yes	81	77.8	1.78 [0.96–3.30]	0.07
	No	163	66.3	Reference	
Direct contact cats other farm	Yes	29	82.8	2.22 [0.81–6.07]	0.12
	No	215	68.4	Reference	
Daily contact roughage/animal feed	Yes	174	74.1	1.91 [1.06–3.44]	0.03
	No	70	60.0	Reference	

Table 2. Cont.

Variable	Category	Freq. (N)	Seropre-valence (%)	OR [95% CI]	p-value
Contact raw milk	Yes	182	75.3	2.51 [1.37–4.58]	<0.01
	No	62	54.8	Reference	
Daily contact goat manure	Yes	163	75.5	2.11 [1.20–3.74]	0.01
	No/no respons	81	59.3	Reference	
Contact cattle manure	Yes	32	84.4	2.55 [0.94–6.91]	0.07
	No	212	67.9	Reference	
Daily contact dead-born animals	Yes	100	80.0	2.33 [0.94–6.91]	<0.01
	No	143	63.2	Reference	
Daily contact with placenta/birth material	Yes	156	73.1	1.48 [0.84–2.59]	0.17
	No/no respons	88	64.8	Reference	
Work in agriculture (swine)	Yes	79	77.2	1.69 [0.91–3.14]	0.09
	No	165	66.7	Reference	
Work in agriculture (cattle)	Yes	134\	74.6\	1.62 [0.92–2.80]	0.09
	No	110	64.5	Reference	
Work in agriculture (crops)	Yes	53	77.4	1.60 [0.79–3.27]	0.19
	No	191	68.1	Reference	
Work in transport (agricultural products, such as hay, straw)	Yes	31	83.9	2.44 [0.90–6.63]	0.08
	No	213	68.1	Reference	
Lived as child on a ruminant farm	Yes	172	74.4	1.79 [0.96–3.32]	0.02
	No	72	59.7	Reference	

highest risk for inhaling *C. burnetii*. We investigated *C. burnetii* presence in vaginal swabs, manure, surface area swabs, milk unit filters, and aerosols at 19 Dutch dairy goat farms in the environmental study component. Farms with an abortion history and positive bulk milk status displayed the highest level of *C. burnetii* DNA in, among others, aerosols and surface area swabs, indicating an elevated risk to farm households and visitors in acquiring Q fever [9]. This is supported by our finding that one quarter of all study participants resided on a bulk milk-positive farm, of which all were seropositive except for three persons on one farm which tested bulk milk-positive two months after human sera were taken. This indicates a highly effective transmission from infected animals to humans, as was already shown in workers involved in culling goats of whom 17.5% seroconverted post-cull [10].

The observed seroprevalence was not only high for the farmers (74.2%), as expected, but also among spouses (66.7%) and children of 12–17 years (57.1%), who lived and often also worked at the farm. The seroprevalence clearly exceeds the estimates of 2.4% found in the Dutch general population preceding the first epidemic season in 2006–2007 [11], the 24% in a rural area in the epicentre of the epidemic in September 2007 [12] and the 12.2% in blood donors living in the high-endemic regions in 2009–2010 [13]. The seroprevalence was also higher compared to those in other studies focusing on, non-further specified, farm populations, such as 49% among farmers from Northern Ireland [14], and 27% in a farm cohort in the United Kingdom [5,14], but was comparable to the seroprevalences ranging from 68% through 84% among professionals intensively working with ruminants in several other studies [15,16,17,18]. In general, comparison of seroprevalences is complicated because of the different study populations, diagnostic tests and cut-off values used. The study from Northern Ireland suggested that infection is mainly acquired in adolescence and early adulthood with slight further acquisition of infection in older age groups above 35 years [14]. We observed a different seroprevalence pattern in our study, with a simialr seroprevalence among males in the age group 12 to 25 years and 25 to 34 years (55.0% and 56.3%, respectively) and observed an increase to 79.1% in males of 35 to 44 years, while females below 25 years had already a higher seroprevalence (81.3%) compared to females in the 25–34 years age group (69.2%).

This study shows Q fever in dairy goat farm households is an actual occupational disease as one out of 9 participants had an indication for a relatively recent infection. Partially these were also diagnosed in routine medical practice in the past few years and thus with symptomatic manifestations. Eleven participants (4.1%) had a serological profile indicative for a chronic infection (IgG phase I titers ≥1:1024), classified as 'possible chronic Q-fever' according to the recent Dutch criteria [8]. Clinical information was lacking to confirm that these were truly chronic cases: we neither had date of onset of symptoms compatible with Q-fever in the acute stage of these participants, if any, to know that these high level antibodies were found more than six months following infection, nor had we any clinical follow-up information for these patients after the test result from the study was communicated to them through their general practitioner, who took care of the regular follow-up protocol.

Several independent individual and farm-based risk factors for *C. burnetii* seropositivity were found such as performing ≥3 daily goat-related tasks, farm location in the two southern provinces, distance to the nearest bulk milk-positive farm, presence of other goat breeds besides the Dutch White Goat, covering air spaces in the goat stable to combat nuisance animals such as wild birds, ≤10 meters distance between residence and nearest stable,

Table 3. Univariate multilevel model of farm-based factors associated with human Q-fever ($p<0.20$) with corresponding frequency (N), seroprevalence (%), odds ratio (OR) and 95% confidence interval (CI).

Variable	Category	Freq. (N)	Seropre-valence (%)	OR [95% CI]	p-value
Herd size	≥800 goats	124	75.8	1.82 [1.07–3.11]	0.03
	<800 goats	144	63.2	Reference	
Farm region	North (Dr, Fr, Gro)	39	51.3	Reference	<0.01
	East (Ge, Ov, Fle)	92	67.4	1.96 [0.91–4.22]	
	West (Ut, Ze, Nh, Zh)	37	56.8	1.25 [0.51–3.08]	
	South (Nb, Li)	100	82.0	4.33 [1.93–9.72]	
Q fever mandatory vaccination area 2009	Yes	124	83.1	3.71 [2.09–6.58]	<0.01
	No	144	56.9	Reference	
Bulk milk farm status	Positive	67	95.5	14.1 [4.28–46.45]	<0.01
	Negative	201	60.2	Reference	
Serological farm status (≥1 goat seropositive)	Positive	100	93.0	12.1 [5.21–28.10]	<0.01
	Negative	128	52.3	Reference	
Net goat density per km^2 within 5 km radius	≥15	110	79.1	2.32 [1.32–4.06]	<0.01
	<15	158	62.0	Reference	
Cattle density (incl meat calves)	≥100	183	72.1	1.42 [0.81–2.47]	0.13
	<100	82	64.6	Reference	
Bulk milk-positive farm within 5 km radius	Yes	81	81.5	2.51 [1.33–4.74]	<0.01
	No	187	63.6	Reference	
Distance to nearest positive farm	0- <4 km	74	83.8	4.74 [2.18–10.31]	<0.01
	4- <8 km	50	76.0	2.90 [1.30–6.48]	
	8- <16 km	75	65.3	1.73 [0.88–3.38]	
	≥16 km	69	52.2	Reference	
Farm part of organic goat farming cooperation	Yes	38	57.9	0.57[0.28–1.14]	0.12
	No	220	70.9	Reference	
Distance residence to nearest stable	≤10 m	118	77.1	2.06 [1.17–3.61]	0.01
	>10 m	124	62.1	Reference	
Other goat breeds next to white dairy goat	Present	125	78.4	2.22 [1.27–3.88]	<0.01
	Absent	124	62.1	Reference	
Number of stables	>3 stables	55	78.2	1.68[0.83–3.41]	0.15
	≤3 stables	194	68.0	Reference	
Use of artificial insemination	Yes	62	80.7	2.05[1.02–4.13]	0.04
	No	185	67.0	Reference	
Extended lactation	Yes	212	72.6	Reference	0.03
	No	32	53.1	0.43 [0.20–0.91]	
Laying hens on farm	Yes	40	55.0	0.45 [0.22–0.90]	0.03
	No	209	73.2	Reference	
Presence of cat(s) in goat stable	Present	91	75.8	1.54 [0.86–2.76]	0.15
	Absent	158	67.1	Reference	
Use of silage	Yes	165	75.8	2.13 [1.21–3.73]	<0.01
	No	84	59.5	Reference	
Use of maize	Yes	95	81.1	2.44[1.33–4.50]	<0.01
	No	154	63.6	Reference	
Feeding method	Fodder mixer or automatic	159	74.8	1.81[1.04–3.15]	0.04
	Hand/wheelbarrow	90	62.2	Reference	
Use of screen/gauze	Screen/gauze	91	78.0	1.86 [0.91–3.80]	0.12
	Windstoppers only	94	66.0	1.01 [0.52–1.98]	
	None of the above	64	65.6	Reference	
Air ventilation through flap	Yes	33	57.6	0.52 [0.25–1.11]	0.10

Table 3. Cont.

Variable	Category	Freq. (N)	Seropre-valence (%)	OR [95% CI]	p-value
	No	216	72.2	Reference	
Presence of Mice and/or rats in the stable in 2008	Present	171	67.3	Reference	0.09
	Absent	76	77.6	1.69 [0.90–3.16]	
Combat other nuisance animals in 2008	Via covering airspaces	32	84.4	2.52[0.93–6.82]	0.05
	Not via covering airspaces or no combat	217	68.2	Reference	
Lambing periods	≥3 or more periods	21	90.5	4.39 [1.00–19.33]	0.05
	≤2 lambing periods	228	68.4	Reference	
Abortion percentage 2007–2009 including abortion history due to Q fever	>4 percent	36	86.1	2.97 [1.11–7.97]	0.02
	0–4 percent	213	67.6	Reference	
Farm tenure	Closed farm for male and female goats	63	77.8	1.68 [0.86–3.28]	0.12
	Not completely closed	185	67.6	Reference	
Goat supply region	South	77	83.1	1.68 [1.87–7.69]	<0.01
	Other regions	108	56.5	Reference	
	No supply	64	78.1	2.75[1.36–5.56]	
Farm visitors	School tours	98	75.5	1.53 [0.86–2.70]	0.14
	No tours	151	66.9	Reference	
Farm boots for staff	Yes	164	66.5	Reference	0.03
	No	82	79.3	1.93 [1.03–3.60]	

presence of cats in the stable and not wearing farm boots by staff. The individual risk factor of performing ≥3 daily activities involving direct contact with goats or dust-producing activities in the goat stable, such as milking, feeding, supply and removal of goats, general health care and cleaning the stable, reflects the intensity of goat and stable environment contact. Under these circumstances the risk of inhalation of contaminated aerosols is high, with a plausible increased risk for acquiring an infection. A study among British farm workers suggested that the extent of total farm animal contact seemed more important than specific animal exposure, indicating that risk of *C. burnetii* exposure is mainly related to farm environment contact [5]. Manure-related tasks did not add to the cumulative risk of performing goat-related tasks indicating transmission through manure is probably less important as shown in a *C. burnetii* survival study showing short decimal reduction times to establish effective killing of viable bacteria in goat manure piles [19]. Persons who lived as child on a ruminant farm were more often seropositive. This is a plausible risk factor for identified past infections and in agreement with a study among Dutch veterinary students where those that grew up on a ruminant

Table 4. Results of the multivariate logistic regression with individual characteristics ($p<0.05$, $-2LL$), as independent factors related to human Q-fever status.

Individual Variables	Category	OR [95% CI]	p-value
Age group	<35 years	Reference	0.867
	35-<45 years	1.04 [0.46–2.34]	
	45-<55 years	1.31 [0.54–3.19]	
	≥55 years	0.88 [0.36–2.14]	
Number of daily goat-related tasks (milking, feeding, supply and removal, general animal health care, birth assistance)	≥3 tasks	3.73 [2.00–6.94]	<.0001
	≤2 tasks	Reference	
Lived as child on a ruminant farm	Yes	2.24 [1.17–4.28]	0.015
	No	Reference	
Direct contact with cattle	Yes	2.65 [0.93–7.53]	0.068
	No/no respons	Reference	

Multivariate logistic regression model with individual characteristics * Number of observations used: 244.

Table 5. Results of the multilevel analyses with farm-based characteristics ($p<0.05$, −2LL), as independent factors related to human Q-fever status.

Farm Variables	Category	OR [95% CI]	p-value
Farm region	North (provinces Dr, Fr, Gro)	Reference	0.011
	West (provinces Ut, Ze, Nh, Zh)	0.85 [0.26–2.71]	
	East (provinces Ge, Ov, Fle)	2.26 [0.88–5.84]	
	South (provinces Nb, Li)	5.95 [2.08–16.99]	
Distance to nearest positive farm	0- <4 km	3.38 [1.25–9.11]	0.050
	4- <8 km	2.64 [1.03–6.75]	
	8- <16 km	3.46 [1.44–8.30]	
	≥16 km	Reference	
Presence of cat(s) in goat stable	Present	2.54 [1.24–5.21]	0.011
	Absent	Reference	
Distance residence to nearest stable	≤10 m	2.44 [1.27–4.67]	0.011
	>10 m	Reference	
Other goat breeds next to white dairy goat	Present	3.38 [1.61–7.09]	0.003
	Absent	Reference	
Extended lactation	Yes	Reference	0.036
	No	0.37 [0.15–0.86]	
Combat other nuisance animals in 2008	Via covering airspaces	6.03 [1.77–20.61]	0.007
	Not via covering airspaces or no combat	Reference	
Farm boots for staff	Yes	Reference	0.025
	No	2.66 [1.12–6.32]	

Multivariate multilevel model with farm characteristics * Number of observations used: 234, number of levels used: 103 (7 with missing values).

farm had a higher risk of being seropositive and a dose-response relationship between seropositivity and years of farm residence was identified [20].

Two risk factors indicate the importance of airborne transmission between farms and to humans in high-incidence areas for Q fever: a farm location in two southern provinces of the Netherlands and a closer distance to the nearest bulk milk-positive farm. The concentration of intensive goat farming in the south of the country probably facilitated transmission between farms following the introduction of Q fever. Next to the farm families, the general population in these regions was severely affected with annual incidences up to 500–1500 notifications per 100,000 inhabitants. Of 2,421 notified cases in 2007–2009, 3.2% worked in the agricultural sector including stockbreeding, arable and dairy farming [21]. Effective airborne spread between farms and to farm families from infected farms in the vicinity is also supported by the observation that 38% of the participating farms within eight kilometers proximity from a bulk milk-positive farm were bulk milk-positive themselves. The farms's own bulk milk status became a strong significant risk factor if we added it to the final multilevel model, while the significance of distance to the nearest bulk milk-positive farm decreased (data not shown), which indicated that a positive bulk milk status of the own farm is the strongest predictor for human seropositivity. A previous study showed that the risk of acute Q fever gradually decreased with increasing distance from a dairy goat farm that experienced an abortion wave [22]. The same distance-response relationship was observed for goat seropositivity in the veterinary study component [6]. A distance of ≤10 meters between stable and residence as risk factor could be a sign of more intensive transmission through aerosol spread but could also be a proxy for more intense direct human-animal contact. The presence of other goat breeds, such as Toggenburg, Anglo-Nubian, Dutch Pied or Alpine Goat, besides the omnipresent Dutch White Goat was identified as risk factor. Compared to other goats, the Dutch White goat is specifically bred on their milk-producing quality. Animal-to-human transmission of *C. burnetii* may be influenced by breed diversity [23] and in cattle the Friesian breed has higher *C. burnetii* seroprevalences than other breeds [24]. However, presence of other goat breeds as a risk factor for human seropositivity was not previously described and therefore this finding cannot be satisfactorily explained. Possible mechanisms behind this risk factor need further investigation, for example through seroprevalence studies in different goat breeds. The study further indicates that not performing extended lactation may protect against human infections which could point to a selection on dairy goats for its qualities as a high-productive dairy goat, rather than on disease resistance, which can cause undesirable side-effects in for example immunological traits, as seen in other livestock [25]. Covering air spaces in a stable to combat nuisance animals such as wild birds could point at a more air-locked stable, facilitating *C. burnetii* accumulation inside the stable, which may increase human and goat exposure [26]. The observed risk for farms where staff did not wear farm boots may indicate the need for more stringent routine biosecurity procedures for household members, farm employees and visitors as indirect transmission through contaminated clothing has been described for Q fever [26]. The presence of cats in the stable was also observed as risk factor for both human and goat seropositivity [6], suggesting *C. burnetii* introduction or facilitation of spread by infected companion animals. In the veterinary study component, additionally the presence of dogs in the stable was a risk factor.

Table 6. Results of the multilevel analyses with all individual and farm-based variables which were associated with human Q fever status ($p<0.10$, $-2LL$) taking in account clustered data of persons within a farm.

Variable	Category	OR [95% CI]	p-value
Age group	<35 years	Reference	0.804
	35-<45 years	1.52 [0.58–3.97]	
	45-<55 years	1.22 [0.43–3.45]	
	≥55 years	1.10 [0.32–3.76]	
Number of farm tasks	≥3 tasks	3.87 [1.84–8.14]	0.001
	≤2 tasks	Reference	
Farm region	North (provinces Dr, Fr, Gro)	Reference	0.035
	West (provinces Ut, Ze, Nh, Zh)	1.14 [0.29–4.55]	
	East (provinces Ge, Ov, Fle)	2.64 [0.98–7.07]	
	South (provinces Nb, Li)	4.88 [1.67–14.26]	
Distance to nearest positive farm	0- <4 km	4.17 [1.56–11.14]	0.020
	4- <8 km	4.12 [1.53–11.13]	
	8- <16 km	5.10 [1.85–14.09]	
	≥16 km	Reference	
Presence of cat(s) in goat stable	Present	2.21 [1.01–4.84]	0.039
	Absent	Reference	
Distance residence to nearest stable	≤10 m	2.06 [1.27–5.29]	0.014
	>10 m	Reference	
Other goat breeds next to white dairy goat	Present	3.30 [1.54–7.07]	0.003
	Absent	Reference	
Extended lactation	Yes	Reference	0.071
	No	0.42 [0.18–0.98]	
Lived as child on a ruminant farm	Yes	2.01 [0.92–4.40]	0.098
	No	Reference	
Combat other nuisance animals in 2008	Via covering airspaces	6.00 [1.70–21.18]	0.006
	Not via covering airspaces or no combat	Reference	
Farm boots for staff	Yes	Reference	0.043
	No	2.51 [1.07–5.85]	

UBN used as cluster variable. Number of observations used: 227. Number of levels used: 103 (8 with missing values).

This study has some limitations. First, the response rate of 33.2% among eligible dairy goat farms was relatively low. This was probably mainly due to the stringent measures carried out during the Q fever epidemics in the Netherlands, especially the culling of pregnant goats on bulk milk-positive farms implemented late 2009, and the media attention during the period that the farms were invited for this study. As the proportion of bulk milk-positive farms was similar for participating farms and non-participating farms, and comparable with regard to herd size, urbanization degree and regional representation, we consider the observed seroprevalence and risk factors representative for all dairy goat farmers and household members in the Netherlands. Second, the exposure information collected in the farm and individual questionnaires is not necessarily related to the relevant time period for seroconversion as we do not know when the actual *C. burnetii* infection occurred in seropositive participants. This also complicated the assessment of the clinical relevance of the high seroprevalences observed. However, a relatively high percentage of relatively recent infections occurred, indicating that seroconversion in this group most likely occurred during the periods covered in the questionnaire.

To conclude, high *C. burnetii* seroprevalences indicate dairy goat farmers and household members have a substantial lifetime risk to acquire this zoonotic infection. Our study demonstrates the importance of daily goat and stable environment contact and increased risk of living on or in proximity of a bulk milk-positive farm. We recommend strengthening general biosecurity measures such as consistently wearing boots and protective clothing by farm staff to avoid indirect transmission, avoiding access of companion animals to the stable and get advice on controlling nuisance animals in the goat stables as covering air spaces seem to harbour an increased risk. Awareness among clinicians should be increased to consider Q fever in this occupational group presenting with compatible symptoms or related sequelae to allow timely diagnosis and treatment. As preventive strategies, dairy goat farmers and household members could be screened at start of goat farming or at adolescent age for children being raised at such farms and if seronegative, offered a human Q fever vaccine. This, in addition to the earlier mentioned biosecurity measures and continuation of small ruminant vaccination, both for decreasing the exposure risk for young children at the farm not yet suitable for vaccination and for inhabitants in the vicinity of the farms.

References

1. Rousset E, Berri M, Durand B, Dufour P, Prigent M, et al. (2009) Coxiella burnetii shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds. Appl Environ Microbiol 75: 428–433.
2. Maurin M, Raoult D (1999) Q fever. Clin Microbiol Rev 12: 518–553.
3. Rodolakis A (2009) Q Fever in dairy animals. Ann N Y Acad Sci 1166: 90–93.
4. Tissot-Dupont H, Amadei MA, Nezri M, Raoult D (2004) Wind in November, Q fever in December. Emerg Infect Dis 10: 1264–1269.
5. Thomas DR, Treweek L, Salmon RL, Kench SM, Coleman TJ, et al. (1995) The risk of acquiring Q fever on farms: a seroepidemiological study. Occup Environ Med 52: 644–647.
6. Schimmer B LS, Hautvast JLA, Graat EAM, Vellema P, van Duynhoven YTHP. (2011) Seroprevalence and risk factors of Q fever in goats on commercial dairy goat farms in the Netherlands, 2009–2010. BMC Veterinary Research Dec 30 7:81 doi:10.1186/1746-6148-7-81. [Epub ahead of print]
7. Wegdam-Blans MC, Wielders CC, Meekelenkamp J, Korbeeck JM, Herremans T, Tjhie HT, Bijlmer HA, Koopmans MP, Schneeberger PM. (2012) Evaluation of commonly used serological tests for the detection of Coxiella burnetii antibodies in well-defined acute and follow-up sera. Clin Vaccine Immunol 23 May 2012, doi: 10.1128/CVI.05581-11. [Epub ahead of print]
8. Wegdam-Blans MC, Kampschreur LM, Delsing CE, Bleeker-Rovers CP, Sprong T, et al. (2012) Chronic Q fever: Review of the literature and a proposal of new diagnostic criteria. J Infect 64: 247–259.
9. de Bruin RQJ, van der Plaats L, Paauwe R, Schimmer B, Vellema P, et al. (2012) Detection of Coxiella burnetii DNA on small ruminant farms during a Q fever outbreak in the Netherlands. Appl Environ Microbiol 78: 1652–1657.
10. Whelan J, Schimmer B, Schneeberger P, Meekelenkamp J, Ijff A, et al. (2011) Q fever among culling workers, the Netherlands, 2009–2010. Emerg Infect Dis 17: 1719–1723.
11. Schimmer B, Notermans DW, Harms MG, Reimerink JH, Bakker J, et al. (2012) Low seroprevalence of Q fever in The Netherlands prior to a series of large outbreaks. Epidemiol Infect: 140: 27–35.
12. Karagiannis I, Schimmer B, Van Lier A, Timen A, Schneeberger P, et al. (2009) Investigation of a Q fever outbreak in a rural area of The Netherlands. Epidemiol Infect 137: 1283–1294.
13. Hogema BM, Slot E, Molier M, Schneeberger PM, Hermans MH, et al. (2012) Coxiella burnetii infection among blood donors during the 2009 Q-fever outbreak in the Netherlands. Transfusion 52: 144–150.
14. McCaughey C, McKenna J, McKenna C, Coyle PV, O'Neill HJ, et al. (2008) Human seroprevalence to Coxiella burnetii (Q fever) in Northern Ireland. Zoonoses Public Health 55: 189–194.
15. Chang CC, Lin PS, Hou MY, Lin CC, Hung MN, et al. (2010) Identification of risk factors of Coxiella burnetii (Q fever) infection in veterinary-associated populations in southern Taiwan. Zoonoses Public Health 57: e95–101.
16. Monno R, Fumarola L, Trerotoli P, Cavone D, Giannelli G, et al. (2009) Seroprevalence of Q fever, brucellosis and leptospirosis in farmers and agricultural workers in Bari, Southern Italy. Ann Agric Environ Med 16: 205–209.
17. Richardus JH, Donkers A, Dumas AM, Schaap GJ, Akkermans JP, et al. (1987) Q fever in the Netherlands: a sero-epidemiological survey among human population groups from 1968 to 1983. Epidemiol Infect 98: 211–219.
18. Thibon M, Villiers V, Souque P, Dautry-Varsat A, Duquesnel R, et al. (1996) High incidence of Coxiella burnetii markers in a rural population in France. Eur J Epidemiol 12: 509–513.
19. Roest HI, Dinkla A, Van Rotterdam B, De Bruin A, Dercksen D, et al. (2011) Survival of Coxiella burnetii in goat manure- final report. 11/CVI0212 11/CVI0212. 1–23 p. [in Dutch] http://www.rijksoverheid.nl/documenten-en-publicaties/rapporten/2011/07/14/overleving-van-coxiella-burnetii-in-geitenmest-eindrapportage.html (accessed 14 June 2012)
20. de Rooij MT, Schimmer B, Versteeg B, Schneeberger P, Berends BR, et al. (2012) Risk factors of Coxiella burnetii (Q fever) seropositivity in Veterinary Medicine Students. PLoS One 7:e32108.
21. Dijkstra F, Hoek W, Wijers N, Schimmer B, Rietveld A, et al. (2012) The 2007–2010 Q fever epidemic in the Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. FEMS Immunol Med Microbiol. 64: 3–12.
22. Schimmer B, Ter Schegget R, Wegdam M, Zuchner L, de Bruin A, et al. (2010) The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. BMC Infect Dis 10: 69.
23. Marrie T (1990) Epidemiology of Q fever. In: Marrie T, editor. Q fever, the disease Boca Raton: CRC Press. pp. 49–65.
24. McCaughey C, Murray LJ, McKenna JP, Menzies FD, McCullough SJ, et al. (2010) Coxiella burnetii (Q fever) seroprevalence in cattle. Epidemiol Infect 138: 21–27.
25. Rauw WM, Kanis E, Noordhuizen-Stassen EN, Grommers FJ (1998) Undesirable side effects of selection for high production efficiency in farm animals: a review. Livest Prod Sci 56: 15–33.
26. Marrie TJ, Langille D, Papukna V, Yates L (1989) 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 102: 119–127.

CHAPTER 4.2

Seroprevalence and risk factors of Q fever in goats on commercial dairy goat farms in The Netherlands, 2009-2010

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Seroprevalence and risk factors of Q fever in goats on commercial dairy goat farms in the Netherlands, 2009-2010

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Abstract

Background: The aim of this study was to estimate the seroprevalence of *Coxiella burnetii* in dairy goat farms in the Netherlands and to identify risk factors for farm and goat seropositivity before mandatory vaccination started. We approached 334 eligible farms with more than 100 goats for serum sampling and a farm questionnaire. Per farm, median 21 goats were sampled. A farm was considered positive when at least one goat tested ELISA positive.

Results: In total, 2,828 goat serum samples from 123 farms were available. Farm prevalence was 43.1% (95%CI: 34.3%-51.8%). Overall goat seroprevalence was 21.4% (95%CI: 19.9%-22.9%) and among the 53 positive farms 46.6% (95%CI: 43.8%-49.3%). Multivariable logistic regression analysis included 96 farms and showed that farm location within 8 kilometres proximity from a bulk milk PCR positive farm, location in a municipality with high cattle density (≥ 100 cattle per square kilometre), controlling nuisance animals through covering airspaces, presence of cats or dogs in the goat stable, straw imported from abroad or unknown origin and a herd size above 800 goats were independent risk factors associated with Q fever on farm level. At animal level almost identical risk factors were found, with use of windbreak curtain and artificial insemination as additional risk factors.

Conclusion: In 2009-2010, the seroprevalence in dairy goats in the Netherlands increased on animal and farm level compared to a previous study in 2008. Risk factors suggest spread from relatively closely located bulk milk-infected small ruminant farms, next to introduction and spread from companion animals, imported straw and use of artificial insemination. In-depth studies investigating the role of artificial insemination and bedding material are needed, while simultaneously general biosecurity measures should be updated, such as avoiding companion animals and vermin entering the stables, next to advice on farm stable constructions on how to prevent introduction and minimize airborne transmission from affected dairy goat farms to prevent further spread to the near environment.

Keywords: *Coxiella burnetii*, small ruminants, seroprevalence, risk factors, zoonosis, goat

Background

Q fever is a zoonosis caused by *Coxiella burnetii*, an intracellular Gram-negative bacterium. From spring 2007 until the end of 2009, large community outbreaks of Q fever with over 3500 notified cases occurred in the Dutch population, mainly in the south-eastern provinces of the Netherlands [1,2]. The main transmission route is through inhalation of contaminated aerosols. Climatic conditions play a role as dry and windy conditions are favourable for transmission of the bacterium [3]. *C. burnetii* is very resistant to heat, drought and disinfectants [4]. Domestic ruminants are the primary animal reservoirs for *C. burnetii* for human infections. In addition, outbreaks due to parturient cats and dogs are described [5,6]. When infected animals give birth, large numbers of *C. burnetii* can be shed, but shedding of the bacterium can also occur via urine, faeces and milk, and is different between ruminant species in duration and

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importance of shedding routes [7]. An infection is usually asymptomatic in cattle, while in dairy goats and dairy sheep an infection may result in abortion or stillbirth [4], often without preceding symptoms. Q fever affected goat herds can show abortion rates up to 90% [8,9].

Dairy goats are considered the predominant source of the community Q fever epidemics in the Netherlands since 2007 [2,10]. The overall goat density in the Netherlands is 38 goats per square kilometre and the total number of goats has increased six-fold from 61.000 in 1990 up to 374.000 in 2009. In the period 2000 until 2009, dairy goat farming has increased almost 3-fold from 98.000 up to 274.000 dairy goats and is especially concentrated in the southern parts of the Netherlands [11]. In the Netherlands, dairy goats are mainly kept year-round in deep litter houses, with partially open walls or roofs. During 2005-2009, Q fever abortion waves were reported on 28 dairy goat farms and 2 dairy sheep farms with abortion percentages varying between 10 and 60% [12]. Human incidence of acute Q fever was highest each spring (April-June), following the main lambing season (December-April) [2]. In order to reduce the risk of exposure from *C. burnetii*-infected small ruminants to humans, mandatory vaccination started in the epicentre of the human outbreak in the southeast of the Netherlands from April 2009 onwards following a voluntary small ruminant vaccination campaign in a more restricted area in the fall of 2008. The 2009 vaccination campaign targeted all dairy goat and dairy sheep farms with at least 50 animals, all open farms (petting zoos, care farms) and all known clinically infected farms since 2005. Studies evaluating the effect of vaccination are promising, especially in nulliparous animals [13]. In October 2009, mandatory bulk milk monitoring using PCR was implemented on all dairy goat and dairy sheep farms with more than 50 animals, to actively detect *C. burnetii*-positive farms, next to the mandatory notification of abortion waves [2]. As of 25 April 2011, 96 dairy goat farms (about 25% of the about 360 large dairy goats farms in 2010) and 2 dairy sheep farms (5%) were found to be bulk milk-positive [14]. In European countries where studies have been done, the seroprevalence in goats in general varies between 6.5% and 48.2%, but is reported up to 75% if sampling is done in shedder goats such as reported in France [15,16]. Farm prevalences were 42.9% in Northern Ireland, 43.0% in a study from Italy and 47.0% in northern Spain [17-19]. Seroprevalences may vary widely within countries as demonstrated in the south-east of France, where at 39 farms without Q fever abortions during the last five years within-herd rates ranged from 0-98% [20]. In 2008, in the Netherlands, the overall Q fever seroprevalence in a convenience sample of 3,134 samples from 442 goat farms submitted for the *Brucella melitensis* monitoring program was 7.8% (95%CI 6.9-8.8%) [12]. The seroprevalence was 11.4% in the southeastern part of the Netherlands compared to 5.3% in the rest of the country. Seroprevalence was higher among the 1,290 dairy goats compared to the 1,844 non-dairy goats (14.7%, 95% CI 2.8%-16.6% versus 3.0%, 95%CI 2.2%-3.8%). The farm prevalence (at least one goat testing positive), was 17.9% (95%CI 14.2-21.5). The average within-herd prevalence on a positive dairy goat farm was 32.1% (95%CI 28.4-35.9%) (van den Brom R, Moll L, Vellema P: Q fever seroprevalence in sheep and goats in the Netherlands in 2008, submitted). Risk factor studies for Q fever in dairy goat farms in the Netherlands have not been done. The aim of this study was to assess the actual magnitude of the spread of *C. burnetii* among small ruminants following the (what turned out to be the peak-) epidemic season in humans in 2009, through testing of sera from a nationwide representative sample of dairy goat farms prior to the start of mandatory vaccination. We identified risk factors for Q fever seropositivity on farm and animal level in order to update control measures and to provide targeted advice for the Dutch dairy goat sector.

Methods

Study design and sampling strategy

The study was designed as a cross-sectional study. In March 2009, 357 dairy goat farms with more than 100 dairy goats were present in the Netherlands and approached for participation. These farms are considered commercial farms and include the size of all known clinically infected goat farms with Q fever. The wide range in herd size allows studying the influence of this size on the infection risk. For all farms, results from bulk milk monitoring using PCR were available from October 2009 until October 2010. Farms with less than 100 goats and farms with a goat population completely vaccinated during the voluntary vaccination campaign in 2008 (approximately 36,000 goats) were excluded. To estimate farm prevalence, 110 farms should be included based on an expected prevalence of 50%, with 95% confidence, 10% accuracy and 90% sensitivity of the serological test used [21]. Based on an assumed within-herd prevalence of 16% [16,19] and a herd size varying between 100 and 4000 goats, 21 goats per farm were to be screened for *C. burnetii* infection. On participating farms, the private veterinary practitioner collected serological samples from the jugular vein of goats before the vaccination campaign in 2009 started. Written informed consent was obtained from each participating farm. As the investigation by the Animal Health Service of goat serum samples taken by the private veterinary practitioner could be considered as part of regular and routine clinical-diagnostic care, no official review and

approval of the Animal Welfare Commission was needed.

Samples were collected in the mandatory vaccination area of 2009 between May and September 2009 (91% of samples were taken in May and June 2009) while samples outside this area were collected between July 2009 and May 2010 (81% of samples were taken from October 2009 until January 2010). A farm questionnaire was sent by e-mail or regular mail to all participating farms between October 2009 and May 2010 and completed by the farm owner or farm manager. The questionnaire addressed the general farm situation, number of lambs and goats, housing characteristics, vermin control and manure handling in 2008 (the year before the mandatory hygiene protocol was implemented), general health status and reproductive problems (including abortion rates) of the herd, breeding information, annual milk production and farm management, including biosecurity and hygiene measures for own staff and farm visitors.

Laboratory analysis

Individual goat serum samples were tested with an ELISA test (Ruminant serum Q Fever ELISA kit, Laboratoire Service International, Lissieu, France) on *C. burnetii* specific antibodies with a single 1:400 serum dilution. All steps were carried out according to the instruction of the manufacturer. A goat was considered ELISA-positive if the optical density percent was 40 or higher, otherwise negative. A farm was considered positive if at least one goat on the farm was classified positive.

Data analyses

Non-response analysis

Participating and non-participating farms were compared with respect to bulk milk PCR results (LSI TaqVet *Coxiella burnetii*, LSI, Lissieu, France), herd size, goat, sheep and cattle density, degree of urbanization and region where farms were located to study the representativeness of participating farms. Categorical variables among participating and non-participating farms were compared with a chi-square or Fisher exact test while numerical variables were compared using the Wilcoxon rank sum test.

Descriptive statistics and risk factor analysis

Animal and farm prevalence of *C. burnetii* with corresponding exact 95 percent confidence intervals were calculated. First, frequency tables of categorical variables were analysed and distributions of continuous variables were studied, and if not linearly related to the outcome variable divided into classes based on biological arguments, and if these were lacking based on medians. Potential risk factors on farm level were analysed by logistic regression (PROC LOGISTIC, SAS Institute Inc., 2004), and on animal level by generalized linear regression analysis accounting for farm effect (PROC GENMOD, SAS Institute Inc., 2004). For the latter, an exchangeable correlation covariance structure fitted best and was used to account for within-herd variation. First, univariable analyses were performed. In multivariable analysis, all variables with a p-value below 0.20 in the univariable analyses were included. For multivariable analysis on farm level, we excluded variables with less than 10% of data in one risk category. Proxy outcomes such as bulk milk status and mandatory vaccination area were not included in multivariable analyses. A backwards elimination procedure was performed until all variables were significant at 10% significance level in the likelihood ratio test. Two-way interactions between biological plausible and significant variables in the multivariate model were investigated. For the final model on farm level, model fit was assessed with the Hosmer-Lemeshow-Goodness-of-fit test (Hosmer and Lemeshow, 1989). Model fit on animal level was assessed by the QIC (Quasilikelihood under the Independence Model Criterion) goodness of fit statistic for GEE models.

Results

Non-response analysis

Of the 357 approached farms, 23 farms were excluded as they were not eligible for participation due to complete vaccination in 2008 or a herd size < 100 goats. In total, 123 dairy goat farms (36.8%) out of 334 eligible farms were willing to cooperate (Figure 1). Three additional dairy goat farms (0.9%) with only three goat sera were excluded from analysis and considered as a non-participant. Farms in the mandatory vaccination area participated more often than farms outside this area, 46.1% versus 30.1% ($p < 0.05$). The median number of goats among participating farms was 782 goats compared to 689 goats in the non-participating farms ($p < 0.05$). Bulk milk PCR-positivity did not differ between participating and non-participating farms, 24.4% and 22.8%, respectively. Participating and non-participating farms were also comparable with regard to location in rural areas (95.1% versus 97.2%), municipal cattle density (median: 121 versus 117), sheep density within 10 kilometres from farm (median: 21 versus 16) and affiliation to an organic goat farming cooperative (11.4% versus 13.4%).

Descriptive results

From the 123 participating farms, 51 farms (41.5%) were located in the two southern provinces, 44 (35.8%) in the eastern part, 16 (13.0%) in the western part and 12 (9.7%) in the northern part of the country. The majority of farms (95.8%) were located in rural areas (< 500 addresses/km^2). A farm questionnaire was available for

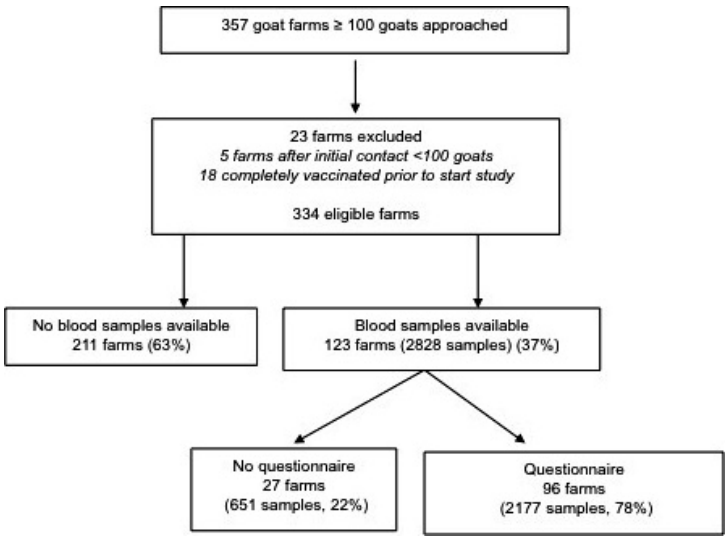

Figure 1 Study participation of invited commercial dairy goat farms (> 100 goats), The Netherlands, 2009-2010.

96 (78.0%) of the 123 dairy goat farms. Consequently, the investigation of risk factors was conducted in this subsample of 96 farms. Participating goat farms started between 1975 and 2009 (median: 1997, inter-quartile range (IQR) 1995-2000). The Dutch White goat was present on all farms. Additionally, other goat species, such as Toggenburger, Dutch pied original, Anglo-Nubian, alpine or mixed breeds were present at 50 farms (52.1%). The median annual milk production per goat was 1000 litres (IQR 900-1150 litres). Fourteen farms (14.6%) reported abortion percentages of 4% or higher in the period 2007-2009 (n = 11) and/or experienced an abortion wave due to Q fever since 2005 (n = 7).

Seroprevalence

A total of 2,828 serum samples were taken at the 123 participating farms. At 101 farms (82.1%), 21 samples per farm were taken as planned, while at the other 22 farms the median number of samples was 22 (range 13-116). Of these 2,828 samples, 21.4% were seropositive (exact 95% CI: 19.9-23.0) (Figure 2). At least one positive goat serum sample was found on 53 out of the 123 farms (43.1%; exact 95% CI: 34.2-52.3). On these 53 positive farms 46.6% of tested animals were seropositive (exact 95% CI: 43.9-49.3%). The prevalence of seropositive goats per farm varied between 4.8% and 95.2% (mean 46.0%, median 45.8% positive goats per farm, inter-quartile range 23.8%-63.6%). The average herd size on positive farms was 1,116 goats (median 890 goats, range 121-4,146) while the average herd size of negative farms was 793 goats (median 729 goats, range 120-2,970). Within the mandatory vaccination area, 58.5% of farms were classified positive compared to 25.9% of goat farms outside this area. Samples within the mandatory vaccination area were almost all taken during the end of the lambing season in 2009 (May-June) while 82.9% of samples outside this area were taken during the next lambing season in 2010 defined as December 2009 until May 2010. Median age, known for 1,474 goats, was 2.3 years. Seroprevalence increased with age: 5.8% for goats younger than 1 year, 15.7% between 1-3 years and 26.1% older than 3 years. Prevalence of the group that had lambed at least once was significantly higher than in the nulliparous group (19.4% (n = 1,251) vs. 11.9% (n = 236); for 1,341 goats lambing status was unknown.

Comparison of bulk milk and serological status

From the 123 participating farms, 30 were bulk milk PCR positive (24.4%; exact 95% CI: 17.1-33.0). Among these 30 farms, 28 (93.3%) were also classified positive based on individual animal sera, with an average animal prevalence of 54.4% per positive farm. One farm was serologically negative in May 2009, but tested bulk milk-positive in November 2009, while the other farm was serologically negative in January 2010, and tested bulk milk-positive end March 2010. At 25 (26.9%) of the 93 bulk milk-negative farms at least one goat was positive,

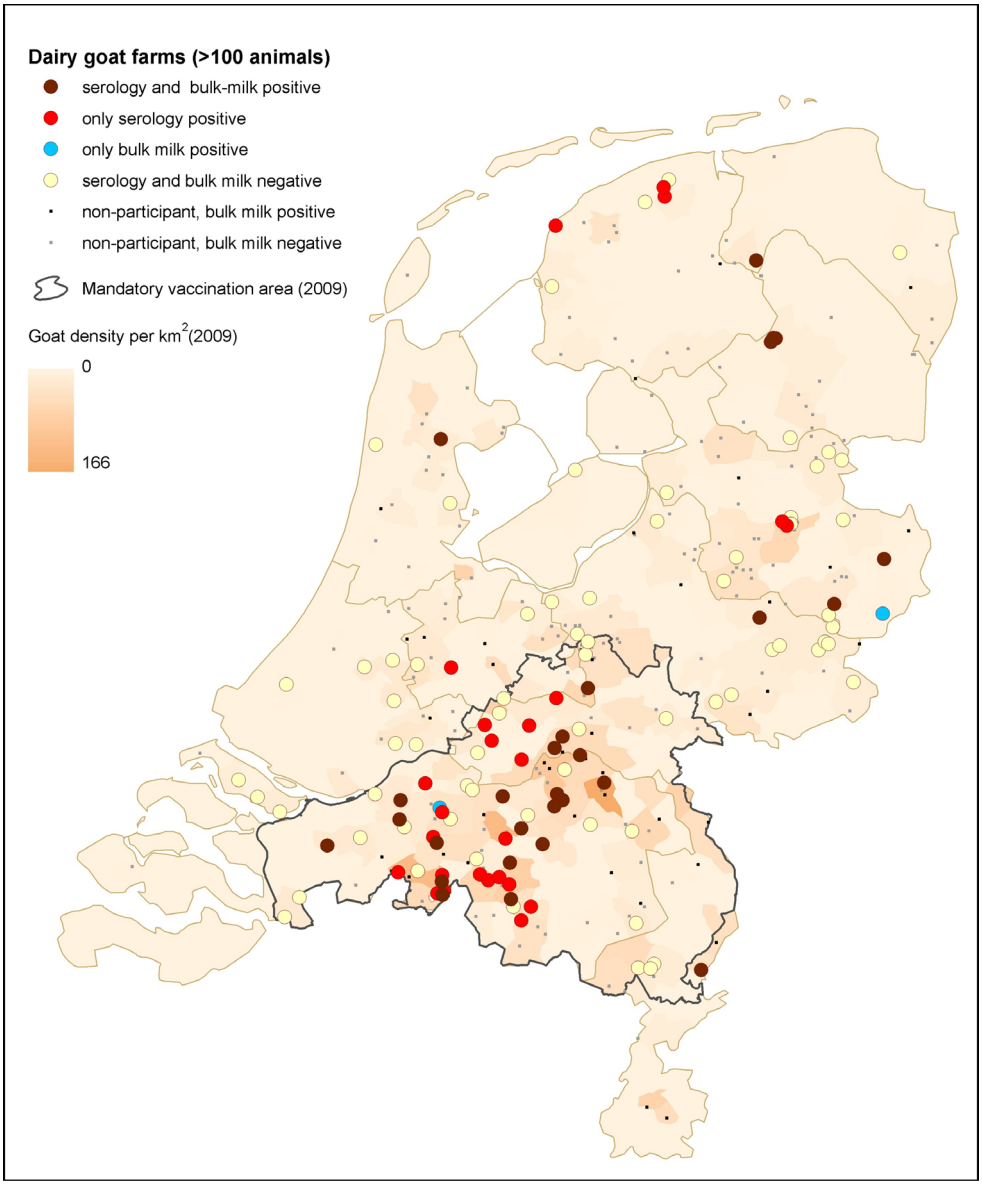

Figure 2 Serological status of participating farms and bulk milk PCR status of eligible dairy goat farms. Map of the Netherlands showing the 12 provinces, the mandatory vaccination area 2009 and the geographic locations of 123 participating dairy goat farms (median 782 goats, range 120-4146) and 211 non-participating farms (median 689 goats, range 105-4733), the serological and bulk milk PCR status of participating farms and bulk milk PCR status only of non-participating farms

with an average animal prevalence of 36.5% per positive farm.

Univariable analyses on farm level

Risk factors associated ($p < 0.20$) with farm seropositivity were herd size larger than 800 goats, location in mandatory vaccination area, farm location within 8 km from nearest bulk milk PCR-positive farm, high goat and cattle density, 3 or more stables, use of artificial insemination, having a dog at the farm, having a dog or a cat in the goat stable, unknown status of signs of vermin in roughage or litter, use of silage feed, maize and other feed such as lucerne or pulp, straw imported from abroad or unknown origin, use of a fodder mixer, controlling nuisance animals (e.g. wild birds) by covering air spaces, abortion percentage ≥ 4% during 2007-2009 or known history of abortion wave due to Q fever since 2005, spread of manure to other places than own farm or direct environment, two or more lambing seasons annually and having a closed or open tenure versus a semi-closed tenure (e.g. only bucks supplied). Protective factors were keeping rabbit(s) or pet birds, sidewall ventilation, and goat supply from the provinces of Friesland and Overijssel (Table 1).

Multivariable analysis on farm level

A total of 21 variables were included in the initial multivariable model. Artificial insemination strongly correlated with large herd size and was excluded from the model. Supply from the provinces of Friesland and Overijssel provinces was not included in the model as possible protective factor because overall goat supply or supply from other provinces were not significant risk factors in the univariable analysis. Having a rabbit or a pet bird was also not included as it correlated inversely with having a dog. The following nine variables remained independently associated with seropositivity in the final model (Table 2): farm located within 8 km proximity to a bulk milk PCR-positive dairy goat farm, high cattle density, controlling nuisance animals by covering air spaces, presence of cats and/or dogs in the goat stable, straw imported from abroad or unknown origin, a herd size of 800 goats or more and unknown status of signs of vermin in roughage or litter. Interaction terms were not statisticially significant and did not improve the model. The Hosmer and Lemeshow Goodness-of-Fit test showed no lack of fit of the model ($P = 0.60$).

Univariable risk factor analyses on animal level

The same variables as in the analysis on farm level were identified on animal level, except for having a dog in the stable and straw imported from abroad or unknown origin. Additionally the following univariable risk factors were identified on animal level: mechanic ventilation in the stable, use of windbreak curtain only or in combination with wind shields, presence of few nuisance animals (e.g. wild birds), while additional univariable protective factors on animal level were presence of the Anglo-Nubian goat and keeping laying hens (Table 3).

Multivariable risk factors analysis on animal level

The variable presence of a dog on the farm was not included on animal level, as the reference category was too small as almost all farms (88%) had a dog. Seven variables remained in the multivariable model on animal level of which five also were present in the final model on farm level i.e. farm located within 8 km proximity from a bulk milk PCR-positive dairy goat farm, high cattle density, presence of a cat in the goat stable, controlling nuisance animals by covering air spaces and unknown status of vermin in roughage or litter. Additional risk factors on animal level were use of artificial insemination and use of windbreak curtain only or in combination with wind shields compared to none of these (Table 4). Within-farm variation accounted for 34.6% of all non-explained variance of the model.

Discussion

The overall animal and farm seroprevalence of *C. burnetii* in dairy goats farms with ≥ 100 dairy goats observed in this study was 21.4% and 43.1% respectively. These seroprevalence estimates increased compared to the seroprevalence measured in 2008, when 14.7% of individual dairy goats were serologically positive and 17.9% of farms tested positive. The within-herd prevalence on positive dairy goat farms in our study was 46.6% compared to 32.1% (95%CI 28.4%-35.9%) in 2008 (van den Brom R, Moll L, Vellema P: Q fever seroprevalence in sheep and goats in the Netherlands in 2008, submitted). This study demonstrates substantial transmission of *C. burnetii* within and between dairy goat farms in recent years prior to the mandatory vaccination campaign in the Netherlands.

The relatively low overall participation rate of 37% probably reflects the reluctance to take part in the study at the same time as control measures increased, including finally the culling of pregnant goats at bulk milk PCR-positive farms. The overrepresentation of farms located in the mandatory vaccination area probably reflects that the risk perception of the farmers played a role. Because of the higher participation rate in this area, we might have overestimated the overall seroprevalence in eligible dairy goat farms in the Netherlands. As expected, dairy goat farms located in the mandatory vaccination area were more often seropositive in our study, as was previously observed in 2008 (van den Brom R, Moll L, Vellema P: Q fever seroprevalence in sheep and

Table 1 Univariable logistic regression of farm-based factors associated with serological Q fever infection on farm level

Variable	Category	N (%)	Prev(%)	OR	95% CI	P-value
Herd size (number of goats according to UBN registry)	≥ 800	47 (49.0)	59.6	3.7	1.6-8.6	0.0020
	< 800	49 (51.0)	28.6	Ref		
Mandatory vaccination area 2009	Inside	53 (55.2)	58.5	4.1	1.7-9.9	0.0010
	Outside	43 (44.8)	25.6	Ref		
Goat density per km^2 within 5 km radius from farm	≥ 25	48 (50.0)	56.3	2.8	1.2-6.5	0.0130
	< 25	48 (50.0)	31.3	Ref		
Cattle density per km^2 in farm municipality (excl. meat calves)	≥ 100	67 (69.8)	50.8	2.7	1.1-7.0	0.0329
	< 100	29 (30.2)	27.6	Ref		
Distance to nearest bulk milk PCR-positive farm (km)	0- < 4	29 (30.2)	72.4	10.0	3.4-29.1	< .0001
	4- < 8	19 (19.8)	57.9	5.2	1.7-16.5	
	≥ 8	48 (50.0)	20.8	Ref		
Number of stables	1-2 stables	40 (41.7)	35.0	Ref		0.1424
	≥ 3 stables	56 (58.3)	50.0	1.9	0.8-4.3	
Use of artificial insemination	Yes	26 (27.1)	61.5	2.7	1.1-6.7	0.0370
	No	70 (72.9)	37.7	Ref		
Having at least one dog at the farm	Yes	84 (87.5)	47.6	4.6	0.9-22.0	0.0337
	No	12 (12.5)	16.7	Ref		
Having at least one rabbit at the farm	Yes	26 (27.1)	30.8	0.5	0.2-1.2	0.1138
	No	70 (72.9)	48.6	Ref		
Having at least one pet bird at the farm	Yes	25 (25.0)	28.0	0.4	0.2-1.1	0.0606
	No	71 (75.0)	49.3	Ref		
Dog(s) in goat stable	Yes	60 (62.5)	51.7	2.4	1.0-5.8	0.0415
	No/unknown	36 (37.5)	30.6	Ref		
Cat(s) in goat stable	Yes	34 (35.4)	58.8	2.6	1.1-6.1	0.0275
	No/unknown	62 (64.6)	35.5	Ref		
Signs of vermin (mice, rats, birds) in roughage or litter during past 12 months	Unknown	14 (14.6)	64.3	2.7	0.8-8.7	0.0944
	Known (yes or no)	82 (85.4)	40.2	Ref		
Feeding silage	Yes	64 (66.7)	51.6	2.7	1.1-6.8	0.0269
	No	32 (33.3)	28.1	Ref		
Feeding maize	Yes	40 (41.7)	55.0	2.2	1.0-5.0	0.0602
	No	56 (58.3)	35.7	Ref		
Use of lucerne, pulp feed or other roughage/litter	Yes	24 (25.0)	58.3	2.2	0.9-5.6	0.0972
	No	72 (75.0)	38.9	Ref		
Origin of straw	Abroad/unknown	59 (61.5)	49.2	1.8	0.8-4.2	0.1757
	No straw or domestic straw	37 (38.5)	35.1	Ref		
Feeding method	Hand/wheelbarrow	31 (32.3)	22.6	Ref		0.0202
	Fodder mixer	56 (58.3)	57.1	4.6	1.7-12.4	
	Automatic	9 (9.4)	33.3	1.7	0.3-8.7	
Sidewall ventilation	Yes	38 (39.6)	34.2	0.5	0.2-1.2	0.1252
	No	58 (60.4)	50.0	Ref		
Control nuisance animals (e.g. wild birds) in 2008	Yes, by covering air spaces	15 (15.6)	73.3	2.1	1.3-15.4	0.0409
	Yes, only via another ways (a.o. capture cage)	13 (13.5)	38.5	1.0	0.3-3.4	
	Not applicable	68 (70.8)	38.2	Ref		

Table 1 Univariable logistic regression of farm-based factors associated with serological Q fever infection on farm level *(Continued)*

Percentage of aborting goats or goats with stillbirth in 2007-2009 or known history of abortion wave due to Q fever since 2005	< 4%	82 (85.4)	39.0	Ref		0.0234
	≥ 4% and/or abortion wave	14 (14.6)	71.4	3.9	1.1-13.5	
Spread of manure	On or near own farmland	79 (82.3)	39.2	Ref		0.0497
	To other places	15 (15.6)	66.7	3.1	1.0-9.9	
Lambing periods in 2009	≤ 1	57 (59.4)	31.6	Ref		0.0035
	≥ 2	39 (40.6)	61.5	3.5	1.5-8.1	
Type of tenure	Completely closed	27 (28.1)	55.6	2.4	0.9-6.1	0.1158
	Closed for female goats only	52 (54.2)	34.6	Ref		
	No, not closed	16 (16.7)	56.3	2.4	0.8-7.6	
Goat supply from the province of Friesland	Yes	19 (19.6)	26.3	0.4	0.1-1.2	0.0806
	No	77 (80.2)	48.1	Ref		
Goat supply from the province of Overijssel	Yes	10 (10.4)	20.0	0.3	0.1-1.4	0.0961
	No	86 (89.6)	46.5	Ref		

Frequency (N), prevalence (Prev), odds ratio (OR), and 95% confidence interval (95%CI) of variables with Likelihood ratio test P values < 0.20 (96 farms, 43.1% positive farms based on total sample of 123 farms and 2,828 goats)

goats in the Netherlands in 2008, submitted). In contrast, the estimated seroprevalence might have been underestimated as the non-eligible farms probably over-represented positive farms, such as farms with a clinical history of Q fever. These were prioritized for vaccination early 2009, and probably positive and suspected farms relatively more often volunteered for vaccination in the 2008 campaign. Nevertheless, the net effect of these biases are thought to be limited, as bulk milk-positive farms were equally represented among participating and non-participating farms. As the diversity in farms, also outside the vaccination area, was still large, effect on the risk factor analyses is considered limited and results are considered generalizable to all commercial dairy goat farms in the Netherlands.

Small ruminant studies have shown that goats test significantly more often serologically positive during pregnancy and in the periparturient period compared to early pregnancy or non-pregnant period (van den Brom R, Moll L, Vellema P: Q fever seroprevalance in sheep and goats in the Netherlands in 2008, submitted), [21]. Different sampling periods in our study, mainly at the

Table 2 Multivariable logistic regression of farm-based factors associated with serological Q fever infection on farm level

Variable	Category	N (%)	Prev (%)	aOR	95% CI
Distance to the nearest bulk milk PCR-positive farm (km)	< 8	48 50.0	66.7	12.9	3.0-54.8
	≥ 8	48 50.0	20.8	Ref	
Cattle density per km^2 in farm municipality (excl. meat calves)	≥ 100	67 69.8	50.8	14.4	2.7-78.4
	< 100	29 30.2	27.6	Ref	
Herd size (number of goats according to UBN registry)	≥ 800	47 49.0	59.6	2.8	0.8-9.4
	< 800	49 51.0	28.6	Ref	
Control nuisance animals (e.g. wild birds) in 2008	Yes, by covering air spaces	15 84.4	73.3	48.8	4.0-591.2
	By other ways or not applicable	81 15.6	38.3	Ref	
Dogs(s) in goat stable	Yes	60 62.5	51.7	3.8	1.0-14.2
	No/unknown	36 37.5	30.6	Ref	
Cat(s) in goat stable	Yes	34 35.4	58.8	6.3	1.5-25.8
	No/unknown	62 64.6	35.5	Ref	
Origin of straw	Abroad/unknown	59 61.5	49.2	5.0	1.3-19.6
	No straw or domestic straw	37 38.5	35.1	Ref	
Signs of vermin (mice, rats, birds) in roughage or litter during past 12 months	Unknown	14 14.6	64.3	4.3	0.8-22.3
	Known (yes or no)	82 85.4	40.2	Ref	

Factors associated with Q fever on farm level with their frequency (N), prevalence (Prev), adjusted odds ratio (aOR) with corresponding 95% confidence interval (95% CI) in the final multivariable logistic model (96 farms; 43.1% positive farms based on total sample of 123 farms and 2,828 goats)

Table 3 Univariable logistic regression of farm-based factors associated with serological Q fever infection on animal level

Variable	Category	N	Prev. (%)	OR	95% CI	P-value
Herd size (according to UBN registry)	< 800	1164	13.8	Ref		0.0017
	≥ 800	1013	30.2	3.3	1.6-7.0	
Mandatory vaccination area	Inside	1116	28.2	3.3	1.5-7.3	0.0042
	Outside	1061	14.3	Ref		
Goat density per km^2 in 5 km radius from the farm	< 25	1167	18.2	Ref		0.0805
	≥ 25	1010	25.3	1.9	0.9-3.9	
Cattle density per km^2 in farm municipality (excl. meat calves)	< 100	643	11.2	Ref		0.0084
	≥ 100	1534	25.8	3.5	1.4-8.9	
Distance to nearest bulk milk PCR-positive farm (km)	0- < 4	688	37.8	5.0	2.2-11.6	< .0001
	4- < 8	400	25.3	3.2	1.1-8.9	
	≥ 8	1089	9.7	Ref		
Anglo-Nubian goat	Present	402	11.9	0.5	0.2-1.1	0.0694
	Absent	1775	23.6	Ref		
Number of stables	1-2 stables	983	14.2	Ref		0.0046
	≥ 3 stables	1194	27.4	3.0	1.4-6.5	
Use of artificial insemination	Yes	572	34.6	2.7	1.3-5.6	0.0181
	No	1585	17.0	Ref		
Presence of laying hens at the farm	Yes	359	8.9	0.3	0.1-0.9	0.0184
	No	1818	23.9	Ref		
Having at least one dog at the farm	Yes	1927	23.5	Ref		0.0530
	No	250	5.6	0.2	0.0-1.0	
Having at least one rabbit at the farm	Yes	608	6.4	0.2	0.1-0.6	0.0008
	No	1569	27.3	Ref		
Having at least one pet bird at the farm	Yes	501	9.4	0.3	0.1-0.9	0.0132
	No	1676	25.1	Ref		
Cat(s) in goat stable	Yes	786	24.2	1.7	0.8-3.4	0.1718
	No/unknown	1391	19.9	Ref		
Signs of vermin (mice, rats, birds) in roughage or litter during past 12 months	Unknown	364	41.8	2.8	1.2-6.2	0.0471
	Yes or No	1813	17.4	Ref		
Feeding silage	Yes	1498	26.1	Ref		0.0218
	No	679	11.2	0.4	0.2-0.9	
Feeding maize	Yes	872	28.8	Ref		0.0333
	No	1305	16.6	0.5	0.2-0.9	
Use of lucerne, pulp feed or other roughage/litter	Yes	505	28.1	1.8	0.9-3.7	0.1445
	No	1672	19.4	Ref		
Feeding method	With hand/wheelbarrow	772	13.5	0.3	0.1-0.9	0.0299
	With fodder mixer	1191	27.0	Ref		
	Automatic	214	19.6	0.5	0.1-1.9	
Type of ventilation system of stables	Mechanic ventilation	593	30.5	2.0	1.0-4.3	0.0899
	No mechanic ventilation	1584	18.1	Ref		
Use of windbreak curtain and/or windshields	Windbreak curtain	799	30.5	3.7	1.5-9.3	0.0198
	Only wind shields	937	18.8	1.6	0.6-4.3	
	None	441	10.7	Ref		
Presence of nuisance animals (e.g. wild birds) in the stable	Yes, many	388	12.6	0.6	0.2-2.0	0.1206
	Yes, few	880	27.3	1.7	0.8-3.7	
	No	889	20.0	Ref		
Combat of nuisance animals (e.g. wild birds) in 2008	Via covering air spaces	349	38.7	3.0	1.4-6.3	0.0721

Table 3 Univariable logistic regression of farm-based factors associated with serological Q fever infection on animal level *(Continued)*

	Yes, only via other ways (e.g. capture cage)	274	21.2	1.3	0.4-4.3	
	Not applicable	1554	17.6	Ref		
Spread of manure	To other places/not applicable	352	30.7	2.1	1.0-4.7	0.1076
	On farmland or near environment	1825	19.7	Ref		
Lambing periods in 2009	≤ 1	1351	15.6	Ref		0.0039
	> 1	826	31.0	3.0	1.5-6.1	
Percentage of aborting goats or goats with stillbirth in 2007-2009 or known history of abortion wave due to Q fever since 2005	< 4%	1882	18.4	Ref		0.0251
	≥ 4%	295	40.7	3.4	1.5-7.6	
Type of tenure	Completely closed	590	29.5	2.3	1.0-5.0	0.0898
	Only closed for female goats	1217	16.1	Ref		
	Not closed	349	27.8	2.2	0.8-5.6	
Provinces where supplied animals originated	Provinces of Friesland and/or Overijssel	639	8.0	0.3	0.1-0.7	0.0028
	Supply from other provinces or no supply	1538	27.1	Ref		

Factors associated with Q fever with their frequency (N), animal prevalence (Prev), odds ratio (OR), and 95% confidence intervals (95% CI) with P value of the univariable generalized linear regression < 0.20 on animal level accounting for random herd effect (2,177 animals from 96 farms; 21.4% seropositive animals and 43.1% positive farms based on total sample of 123 farms and 2,828 goats)

end of the lambing season in 2009 inside the vaccination area and in the beginning of the lambing season 2010 outside the vaccination area, make it difficult to disentangle the possible effect of seasonal sampling on the observed significant regional differences. We think this study shows a true higher seroprevalence in the mandatory vaccination area as it (1) confirms the significant difference already observed in 2008 in the south-eastern part of the country and (2) reflects the major burden of human and veterinary clinical Q fever cases that occurred in the south-eastern part of the country [1,2,10]. A distinction with ELISA between IgG phase 1

Table 4 Multivariable logistic regression of farm-based factors associated with serological Q fever infection on animal level

Variable	Category	N (%)		Prev (%)	aOR	95% CI
Distance to the nearest bulk milk PCR-positive farm (km)	< 8 km	1088	50.0	33.2	3.2	1.4-7.3
	≥ 8 km	1089	50.0	9.73	Ref	
Cattle density per km^2 in farm municipality (excl meat calves)	< 100	643	29.5	11.2	Ref	
	≥ 100	1534	70.5	25.8	4.5	2.0-9.9
Combat of nuisance animals (e.g. wild birds) in 2008	Yes, by covering air spaces	349	16.0	38.7	3.7	1.8-7.9
	By other ways or not applicable	1828	84.0	18.2	Ref	
Signs of vermin (mice, rats, birds) in roughage or litter during past 12 months	Unknown	364	16.7	41.8	3.3	1.4-7.9
	Known (yes or no)	1813	83.3	17.4	Ref	
Cat(s) in goat stable	Yes	786	36.1	24.2	2.6	1.2-5.6
	No/unknown	1391	63.9	19.9	Ref	
Use of windbreak curtain and/or windshields	Windbreak curtain	799	36.7	30.5	2.8	1.2-6.7
	Only wind shields	937	43.0	18.8	1.7	0.7-4.1
	None	441	4.9	10.6	Ref	
Artificial insemination	Yes	572	26.3	34.6	2.3	1.2-4.7
	No	1585	72.8	17.0		

Factors associated with Q fever with their frequency (N), animal prevalence (Prev), adjusted odds ratio (aOR) with corresponding 95% confidence interval (95% CI) in the final multivariable model on animal level with random herd effect (2,177 animals from 96 farms; 21.4% positive animals; 43.1% positive farms based on total sample of 123 farms and 2,828 goats)

**within-farm variation accounts for 34.6% of non-explained variance

and 2 antibodies might have helped to distinguish more recent infection and older infections in animals to assess such a sampling effect [22].

Seroprevalence of *C. burnetii* in goats has been studied in several countries [16]. Comparison should be done with caution as study populations and study years vary and different serological assays with different performance are used [23,24]. The goat seroprevalence was 8.8% in Albania [25] and 6.5% in Northern Greece [26]. In Spain, the goat and farm prevalence was 8.7% and 45%, respectively, based on ELISA [19] similar to results of a smaller study from Northern Ireland (goat seroprevalence 9.3%, farm prevalence 42.9%) [17], and in Sardinia in 1999-2002: goat prevalence 13% and farm prevalence 47% using an alternative criterion of two or more seropositive animals per farm [18]. In our study, the farm seroprevalence was 38.2% (95%CI 29.6%-46.8%) using the same criterion, so only slightly lower than with at least one positive goat as criterion for a positive farm. Clearly different goat prevalences were observed in Poland, with the absence of *C. burnetii* IgG phase 2 antibodies in 918 goats from 48 herds [27] while high estimates were observed in Cyprus (48.2% in 420 random goats) [28] and in Gran Canaria island, Spain (60.4% in 733 goats) [29]. Ignoring these last exceptions, the overall goat prevalence of 21.4% observed in our study was relatively high compared to other European seroprevalence studies (6.5%-13%), while the farm prevalence falls within the range of farm prevalences (43-47%) in other European countries. The within-herd prevalence of 46.6% among the positive farms indicates strong circulation of the bacterium within the herds, suggesting farm conditions or practices favoring spread, such as a relatively large number of goats per farm, year-round housing in deep litter stables or reflects circulation of a unique efficiently spreading strain. In France, goat herds with a within-herd prevalence over 40% had the highest proportion of shedder goats and highest averages of shedding quantities as determined by real-time qPCR on vaginal swabs, representing a high risk level for environmental contamination and by that transmission within farms [20]. At about one quarter of the bulk milk PCR-negative farms, on average 37% of the goats tested seropositive. This might be explained by the fact that not all seropositive goats shed the bacterium in milk and that excretion of the bacterium is intermittently [7,15]. Besides, antibodies persist in goats [9], finding still positive serology but no actual excretion of DNA which is measured in the bulk milk monitoring program.

Considering the risk factors analyses, the exposure information collected in the farm questionnaire is not necessarily related to the relevant time period for seroconversion as we do not know at what moment the actual infection with *C. burnetii* occurred in serologically positive goats. However, since the median age of tested goats was 2.3 years, and infections especially occur during the first pregnancy of nulliparous goats (between 1-2 years of age) it is plausible that infection in the majority of goats occurred during the periods covered in the questionnaire. It is most likely that goats on these farms get infected the same way as humans, i.e. by inhalation of *C. burnetii* infected aerosols [4], as indicated by the increased risk of a farm location within 8 km of a bulk milk-positive small ruminant farm. From literature we know that herd size and high farm and animal densities can augment the risk for acquisition of (respiratory) zoonoses, for example in swine diseases and avian influenza in poultry [30,31]. In our study, we found that farms with more than 800 goats had a higher risk to be positive than smaller farms. This corresponds with Rupanner *et al.* who observed in the 1970s an increased infection risk of goats with *C. burnetii* with increasing herd size [32]. Similar associations with herd size were found for Q fever in dairy cattle [17,33]. This can be explained by a larger population at risk, an increased risk of introduction and transmission of pathogens within and between herds for instance by larger amounts of feed, animal supply and more professionals working at or visiting the farm. In addition, farm management practices or environmental characteristics related to large farms but not covered in our questionnaire might play a role in the observed increased risk. As about 35% of the unexplained variance in the model was explained by the farm-effect, relevant underlying factors might have been missed. Therefore, an advice to limit the herd size without further changes in farm management does not necessarily guarantee a reduction in infection risk. Artificial insemination was an independent risk factor at animal level and found to be related to farms with a herd size over 800 goats. Artificial insemination can therefore be an indirect marker of farm management practices in larger farms that were not covered in the questionnaire. From cattle studies, it is known that viable C. *burnetii* is detected in semen of seropositive bulls indicating the possibility of sexual transmission [34]. Between 3000 and 4000 inseminations each year are carried out by the main goat artificial insemination (AI) cooperative using fresh semen from the Netherlands and frozen semen from French or Dutch origin. Since end of 2008, AI bucks are routinely screened for presence of *C. burnetii*. In a targeted survey, so far, goat semen samples from 300 bucks present on bulk milk-positive farms were all negative (personal communication, P. Vellema, Animal Health Service). High cattle density in the municipality where the farm was located was also an independent risk factor, indicating the presence of one or several cattle farms in the same

municipality as the goat farm. A recent review on *C. burnetii* infection in domestic ruminants suggested a higher seroprevalence in cattle compared to goats and sheep [16]. In the Netherlands, a prevalence of bacterial DNA of 56.6% in cattle bulk tank milk was found as compared to 24.4% bacterial DNA in goat bulk tank milk among participating farms in our study, confirming widespread circulation of the bacterium among cattle [35]. However, an association with cattle density was not observed when the outcome variable 'bulk milk PCR-positivity' was used instead of ELISA-seropositivity (data not shown). Therefore, it is hypothesized that cattle especially played a role in the more historical infections in goats, while spread between dairy goat herds is responsible for the more recent infections and a large part of the epidemic observed since 2007. The serological status of cattle and foremost comparison of *C. burnetii* isolates by subtyping in different ruminant species might help to elucidate the transmission pathways between different species of ruminants and to humans. So far, one unique genotype predominated in dairy goats herds, although at 50% of the farms at least one additional genotype was observed [36]. Very sparse data on cattle isolates in the Netherlands suggest different subtypes from those found in goats, sheep and humans [37]. More and nationwide representative data are urgently needed to confirm these distinct types for cattle, and to study if some cattle types match with the non-dominant genotypes regularly observed at dairy goat farms.

Previous ruminant studies have shown that farm management practices can influence the seroprevalence of *C. burnetii* [17,38]. Straw, used widely as bedding in deep litter stables, could be a way in which Q fever was introduced in the Dutch dairy goat farms as import of straw from abroad or unknown origin was an independent risk factor. Farmers indicated straw was most often imported from Germany and France, which are endemic countries for Q fever. Microbiological examination from straw originating from France showed presence of *C. burnetii* by PCR, although the method of sampling does not exclude contamination at the farm [39]. Contact with straw and other farm products was also a risk factor for humans in the first documented outbreak in 2007 and in international outbreak studies [40-42]. The presence of dogs and cats in the goat stable was related to a seropositive Q fever status of dairy goat farms. Furthermore, the seven farms without companion animals were all seronegative. This suggests introduction of *C. burnetii* or facilitation of within farm-spread by infected companion animals. In a study in Cyprus, risk factors for Q fever abortions compared to abortions of other causes were studied in a convenience sample of ruminant farms including only two goat farms; among others presence of dogs and cats were on farm risk factors [38]. Pets, especially during kidding, have been associated with outbreaks in the past [5,6]. In a Dutch study in the early 1990s, 13.2% of dogs and 10.4% of cats tested positive for *C. burnetii* by ELISA [43]. To study the role of companion animals in current transmission, an update of this study, ideally also looking at shedding by PCR, is needed.

Covering airspaces in the stable to control nuisance animals, such as wild birds, unexpectedly was an independent risk factor. As wild birds may play a role in the transmission within and between farms and were the cause of a familial Q fever outbreak [44] a protective effect was expected if any. However, presence of wild birds in the stable was not a risk factor in the multivariable analysis but mainly indicated the farmer actively controlled nuisance animals such as by covering airspaces. In addition, we found at animal level an increased risk for farms that use windbreak curtain, sometimes in combination with windshields. These two risk factors could point at a more air-locked stable, facilitating accumulation of *C. burnetii* inside the stable, which may promote spread within the herd. This accumulation risk was indirectly shown in the study from Cyprus where a high frequency of litter cleaning was found to be a protective factor [38]. Although a less confined farm might limit the within-herd spread, such open constructions can be a risk for aerosol spread to other farms and persons in the near environment. Presence of mice and rats in the stable was not found to be a risk factor in our study, although a recent study showed presence of *C. burnetii* in rats at livestock farms in the Netherlands [45]. Whether vermin are able to maintain the transmission cycle and are able to (re) introduce Q fever at farms is currently under investigation.

Conclusions

This study shows that before the start of mandatory vaccination of small ruminants in 2009-2010, the seroprevalence of *C. burnetii* antibodies in goats at commercial dairy goat farms has increased compared to a study carried out in 2008 in the Netherlands. The overall goat prevalence of 21.4% was considerably high, but the farm prevalence of 43.1% was comparable to generally observed seroprevalences in other European countries. On positive dairy goat farms, the within-herd prevalence was 46.6%, reflecting high circulation of *C. burnetii* within a farm and a risk for environmental contamination and spread. In general, the risk for farms and dairy goats to acquire a *C. burnetii* infection seems to be multifactorial. The two strongest associated risk factors, proximity of bulk milk-positive small ruminant farms and a high cattle density, suggest aerosol spread as an

important route of infection of the dairy goat farms. Furthermore, other risk factors identified possible vehicles for introduction, spreading and/or persistence within farms, such as import of straw from abroad, access to the goat stable of cats, dogs and use of artificial insemination, and covering airspaces of the stable. Besides, larger farms with 800 or more goats seem to have an increased risk for infection, although it can not be concluded that this is entirely due to the size itself by the larger population at risk, combined with a general increased chance of introduction of pathogens in larger farms or is due to unmeasured farm characteristics strongly related to a large herd size. Based on our results, it is recommended to further prove the role in the current transmission of bedding material, goat semen and excreta from companion animals by microbiological testing. Simultaneously, as a precautionary measure general biosecurity measures should be taken next to advice on farm stable constructions targeted at avoiding access of companion animals and how to control nuisance animals in the goat stables to prevent introduction and minimizing airborne transmission from affected farms to prevent spread to humans and other farms.

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Authors' contributions

The study idea was conceived by BS and YD together with the Q-VIVE research group. BS, YD and PV participated in the design of the study. JH and YD participated in the acquisition of the data and coordinated logistics. PV provided previously acquired reference data. BS, SL, EG and YD carried out the statistical analysis. Data interpretation was done by all authors. BS and SL drafted the manuscript. All authors contributed to the critical revision of the manuscript for important intellectual content and have seen and approved the final draft.

Competing interests

The authors declare that they have no competing interests.

References

1. Schimmer B, Dijkstra F, Vellema P, Schneeberger PM, Hackert V, ter Schegget R, Wijkmans C, van Duynhoven Y, van der Hoek W: **Sustained intensive transmission of Q fever in the south of the Netherlands, 2009.** *Euro Surveill* 2009, **14(19)**:pii = 19210.
2. van der Hoek W, Dijkstra F, Schimmer B, Schneeberger PM, Vellema P, Wijkmans C, ter Schegget R, Hackert V, van Duynhoven Y: **Q fever in the Netherlands: an update on the epidemiology and control measures.** *Euro Surveill* 2010, **15(12)**:pii = 19520.
3. Tissot-Dupont H, Amadei MA, Nezri M, Raoult D: **Wind in November, Q fever in December.** *Emerg Infect Dis* 2004, **10**:1264-1269.
4. Maurin M, Raoult D: **Q fever.** *Clin Microbiol Rev* 1999, **12**:518-553.
5. Buhariwalla F, Cann B, Marrie TJ: **A dog-related outbreak of Q fever.** *Clin Infect Dis* 1996, **23**:753-755.
6. Marrie TJ, Durant H, Williams JC, Mintz E, Waag DM: **Exposure to parturient cats: a risk factor for acquisition of Q fever in Maritime Canada.** *J Infect Dis* 1988, **158**:101-108.
7. Rodolakis A, Berri M, Hechard C, Caudron C, Souriau A, Bodier CC, Blanchard B, Camuset P, Devillechaise P, Natorp JC, *et al*: **Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine, and ovine herds.** *J Dairy Sci* 2007, **90**:5352-5360.
8. Wouda W, Dercksen DP: **[Abortion and stillbirth among dairy goats as a consequence of *Coxiella burnetii*].** *Tijdschr Diergeneeskd* 2007, **132**:908-911.
9. Hatchette T, Campbell N, Hudson R, Raoult D, Marrie TJ: **Natural history of Q fever in goats.** *Vector Borne Zoonotic Dis* 2003, **3**:11-15.
10. Roest HI, Tilburg JJ, van der Hoek W, Vellema P, van Zijderveld FG, Klaassen CH, Raoult D: **The Q fever epidemic in The Netherlands: history, onset, response and reflection.** *Epidemiol Infect* 2011, **139**:1-12.
11. **StatLine database.** [http://statline.cbs.nl].
12. van den Brom R, Vellema P: **Q fever outbreaks in small ruminants and people in the Netherlands.** *Small Rum Res* 2009, **86**:74-79.
13. Hogerwerf L, van den Brom R, Roest HI, Bouma A, Vellema P, Pieterse M, Dercksen D, Nielen M: **Reduction of *Coxiella burnetii* prevalence by vaccination of goats and sheep, The Netherlands.** *Emerg Infect Dis* 2011, **17**:379-386.
14. **Food and Consumer Product Safety Authority: Address locations of Q fever infected small ruminant farms.** [http://www.vwa.nl/onderwerpen/dierziekten/dossier/q-koorts/kaart-met-overzicht-van-besmette-bedrijven].
15. Rousset E, Berri M, Durand B, Dufour P, Prigent M, Delcroix T, Touratier A, Rodolakis A: ***Coxiella burnetii* shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds.** *Appl Environ Microbiol* 2009, **75**:428-433.
16. Guatteo R, Seegers H, Taurel AF, Joly A, Beaudeau F: **Prevalence of *Coxiella burnetii* infection in domestic ruminants: a critical review.** *Vet Microbiol* 2011, **149**:1-16.
17. 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.** *Epidemiol Infect* 2010, **138**:21-27.
18. Masala G, Porcu R, Sanna G, Chessa G, Cillara G, Chisu V, Tola S: **Occurrence, distribution, and role in abortion of *Coxiella burnetii* in sheep and goats in Sardinia, Italy.** *Vet Microbiol* 2004, **99**:301-305.
19. Ruiz-Fons F, Astobiza I, Barandika JF, Hurtado A, Atxaerandio R, Juste RA, Garcia-Perez AL: **Seroepidemiological study of Q fever in domestic ruminants in semi-extensive grazing systems.** *BMC Vet Res* 2010, **6**:3.
20. Dubuc-Forfait C, Prigent M, Delcont A, Champion JL, Marois M, Kouji M, Dufour P, Sidi-Boumedine K, Rousset E: **Evaluation of *Coxiella burnetii* transmission risk levels in goat herds without apparent Q fever (P067) [abstract].** *Proceedings of the 6th International Meeting on Rickettsia and Rickettsial Diseases* 2011.

21. Ryan ED, Kirby M, Collins DM, Sayers R, Mee JF, Clegg T: **Prevalence of *Coxiella burnetii* (Q fever) antibodies in bovine serum and bulk-milk samples.** *Epidemiol Infect* 2010, 1-5.
22. Hatchette T, Campbell N, Whitney H, Hudson R, Marrie TJ: **Seroprevalence of *Coxiella burnetii* in selected populations of domestic ruminants in Newfoundland.** *Can Vet J* 2002, **43**:363-364.
23. Kittelberger R, Mars J, Wibberley G, Sting R, Henning K, Horner GW, Garnett KM, Hannah MJ, Jenner JA, Piggott CJ, *et al*: **Comparison of the Q-fever complement fixation test and two commercial enzyme-linked immunosorbent assays for the detection of serum antibodies against *Coxiella burnetti* (Q-fever) in ruminants: recommendations for use of serological tests on imported animals in New Zealand.** *N Z Vet J* 2009, **57**:262-268.
24. Rousset E, Durand B, Berri M, Dufour P, Prigent M, Russo P, Delcroix T, Touratier A, Rodolakis A, Aubert M: **Comparative diagnostic potential of three serological tests for abortive Q fever in goat herds.** *Vet Microbiol* 2007, **124**:286-297.
25. Cekani M, Papa A, Kota M, Velo E, Berxholi K: **Report of a serological study of *Coxiella burnetii* in domestic animals in Albania.** *Vet J* 2008, **175**:276-278.
26. Pape M, Bouzalas EG, Koptopoulos GS, Mandraveli K, Arvanitidou-Vagiona M, Nikolaidis P, Alexiou-Daniel S: **The serological prevalence of *Coxiella burnetii* antibodies in sheep and goats in northern Greece.** *Clin Microbiol Infect* 2009, **15(Suppl 2)**:146-147.
27. Czopowicz M, Kaba J, Szalus-Jordanow O, Nowicki M, Witkowski L, Nowicka D, Frymus T: **Prevalence of antibodies against *Chlamydophila abortus* and *Coxiella burnetii* in goat herds in Poland.** *Pol J Vet Sci* 2010, **13**:175-179.
28. Psaroulaki A, Hadjichristodoulou C, Loukaides F, Soteriades E, Konstantinidis A, Papastergiou P, Ioannidou MC, Tselentis Y: **Epidemiological study of Q fever in humans, ruminant animals, and ticks in Cyprus using a geographical information system.** *Eur J Clin Microbiol Infect Dis* 2006, **25**:576-586.
29. Rodriquez NF, Carranza C, Bolanos M, Perez-Arellano JL, Gutierrez C: **Seroprevalence of *Coxiella burnetii* in domestic ruminants in Gran Canaria island, Spain.** *Transbound Emerg Dis* 2010, **57**:66-67.
30. Gardner IA, Willeberg P, Mousing J: **Empirical and theoretical evidence for herd size as a risk factor for swine diseases.** *Anim Health Res Rev* 2002, **3**:43-55.
31. Boender GJ, Meester R, Gies E, de Jong MCM: **The local threshold for geographical spread of infectious diseases between farms.** *Prev Vet Med* 2007, **82**:90-101.
32. Ruppanner R, Riemann HP, Farver TB, West G, Behymer DE, Wijayasinghe C: **Prevalence of *Coxiella burnetii* (Q fever) and *Toxoplasma gondii* among dairy goats in California.** *Am J Vet Res* 1978, **39**:867-870.
33. Agger JF, Christoffersen AB, Rattenborg E, Nielsen J, Agerholm JS: **Prevalence of *Coxiella burnetii* antibodies in Danish dairy herds.** *Acta Vet Scand* 2010, **52**:5.
34. Kruszewska D, Tylewska-Wierzbanowska S: **Isolation of *Coxiella burnetii* from bull semen.** *Res Vet Sci* 1997, **62**:299-300.
35. Muskens J, van Engelen E, van Maanen C, Bartels C, Lam TJ: **Prevalence of *Coxiella burnetii* infection in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA.** *Vet Rec* 2011, **168**:79.
36. Roest HI, Ruuls RC, Tilburg JJ, Nabuurs-Franssen MH, Klaassen CH, Vellema P, van den Brom R, Dercksen D, Wouda W, Spierenburg MA, *et al*: **Molecular epidemiology of *Coxiella burnetii* from ruminants in Q fever outbreak, the Netherlands.** *Emerg Infect Dis* 2011, **17**:668-675.
37. Huijsmans CJ, Schellekens JJ, Wever PC, Toman R, Savelkoul PH, Janse I, Hermans MH: **Single-nucleotide-polymorphism genotyping of *Coxiella burnetii* during a Q fever outbreak in The Netherlands.** *Appl Environ Microbiol* 2011, **77**:2051-2057.
38. Cantas H, Muwonge A, Sareyyupoglu B, Yardimci H, Skjerve E: **Q fever abortions in ruminants and associated on-farm risk factors in northern Cyprus.** *BMC Vet Res* 2011, **7**:13.
39. Wever PC, van Hannen EJ, Schellekens JJ, van Lokven JW, Schneeberger P, Zaaijer HL, Hermans MH: **Detection of *Coxiella burnetii* DNA in veterinary samples and supermarket dairy products using routine hospital developed real-time PCR techniques. [abstract].** *Ned Tijdschr Med Microbiol* 2010, **18(Suppl)**:s74.
40. Karagiannis I, Schimmer B, van Lier A, Timen A, Schneeberger P, van Rotterdam B, de Bruin A, Wijkmans C, Rietveld A, van Duynhoven Y: **Investigation of a Q fever outbreak in a rural area of The Netherlands.** *Epidemiol Infect* 2009, **137**:1283-1294.
41. Salmon MM, Howells B, Glencross EJ, Evans AD, Palmer SR: **Q fever in an urban area.** *Lancet* 1982, **1**:1002-1004.
42. Van Woerden HC, Mason BW, Nehaul LK, Smith R, Salmon RL, Healy B, Valappil M, Westmoreland D, de Martin S, Evans MR, *et al*: **Q fever outbreak in industrial setting.** *Emerg Infect Dis* 2004, **10**:1282-1289.
43. Houwers DJ, van der Meer M, van Dijk AA, Ossewaarde JM: *Prevalence of infections with C. burnetii in dogs and cats in the Netherlands and the central region of the Netherlands, 1991-1992. Research report 91-102. Utrecht* 1992.
44. Stein A, Raoult D: **Pigeon pneumonia in provence: a bird-borne Q fever outbreak.** *Clin Infect Dis* 1999, **29**:617-620.
45. Reusken C, van der Plaats R, Opsteegh M, de Bruin A, Swart A: ***Coxiella burnetii* (Q fever) in *Rattus norvegicus* and *Rattus rattus* at livestock farms and urban locations in the Netherlands; could *Rattus* spp. represent reservoirs for (re)introduction?** *Prev Vet Med* 2011, **101**:124-130.

CHAPTER 4.3

Coxiella burnetii seroprevalence and risk factors in sheep farmers and farm residents in The Netherlands

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Coxiella burnetii seroprevalence and risk factors in sheep farmers and farm residents in The Netherlands

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SUMMARY

In this study, *Coxiella burnetii* seroprevalence was assessed for dairy and non-dairy sheep farm residents in The Netherlands for 2009–2010. Risk factors for seropositivity were identified for non-dairy sheep farm residents. Participants completed farm-based and individual questionnaires. In addition, participants were tested for IgG and IgM *C. burnetii* antibodies using immunofluorescent assay. Risk factors were identified by univariate, multivariate logistic regression, and multivariate multilevel analyses. In dairy and non-dairy sheep farm residents, seroprevalence was 66·7% and 51·3%, respectively. Significant risk factors were cattle contact, high goat density near the farm, sheep supplied from two provinces, high frequency of refreshing stable bedding, farm started before 1990 and presence of the Blessumer breed. Most risk factors indicate current or past goat and cattle exposure, with limited factors involving sheep. Subtyping human, cattle, goat, and sheep *C. burnetii* strains might elucidate their role in the infection risk of sheep farm residents.

Key words: Coxiellae, Q fever, risk assessment, serology, zoonoses.

INTRODUCTION

Q fever, caused by *Coxiella burnetii*, is a worldwide zoonosis with goats, sheep, and cattle as primary sources for human infections [1]. Humans are usually infected by inhalation of contaminated aerosols originating from parturient animals and their birth products [1–3]. Acute Q fever presents itself as a self-limiting febrile illness, pneumonia or hepatitis, with a small proportion developing chronic infections (mainly endocarditis and vascular infections) [4, 5].

From 2007 until 2009, large Q fever outbreaks occurred in The Netherlands, with over 3500 human cases notified [6]. Abortion waves at dairy goat farms were the primary source of these infections [7–9]. Between 2006 and 2008, *C. burnetii* abortion waves occurred on two dairy sheep farms [9]. Infected non-dairy sheep farms were not associated with an increased number of human cases living near these farms [10], although cases occurred in

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individuals living a small distance from or having direct contact with non-dairy sheep in The Netherlands [11, 12]. Internationally, several sheep-related Q fever outbreaks have been reported [13–19].

In The Netherlands, sheep farms can be distinguished from dairy farms and fat lamb-producing farms. There is a small dairy sheep industry with <50 farms, in which sheep are usually milked twice a day during several months each year. The number of sheep per farm differs from <50 to almost 1000 with most kept outdoors for part of the year. On the fat lamb-producing sheep farms the sheep are kept outside, except for a few weeks around lambing, which usually occurs inside. Except for meat production, non-dairy sheep are also kept for breeding purposes or nature management.

So far, no international studies have addressed the seroprevalence and risk factors for acquisition of *C. burnetii* infection in sheep farmers and their household members. Therefore, our aim was to determine the *C. burnetii* seroprevalence in both dairy and non-dairy sheep farmers and their household members, and for the large non-dairy sector, to identify individual and farm-related risk factors for seropositivity.

MATERIAL AND METHODS

All dairy sheep and non-dairy sheep farms in The Netherlands with at least 100 breeding ewes in November 2008, according to the national identification and registration database, were eligible. A minimum of 100 ewes, considered to be a professional farm, was chosen because in the early stage of the Dutch epidemic it was clear that only (relatively large) commercial (dairy goat) farms were incriminated as a potential source; no obvious role for small farms was observed [9]. Besides, smaller hobby farms have different management and farm residents of those farms are assumed to have a more limited exposure to sheep-related pathogens compared to commercial farms. Between September and December 2009, 32 dairy sheep farmers were approached for the study. In addition, in March and April 2010, 1344 non-dairy sheep farmers were approached for participation. At the time of inclusion in 2010, those farms with at least 60 unvaccinated breeding animals were kept in the study. Farms with vaccinated sheep were excluded because in this integrated human-veterinary study the sheep at these farms were likely to be seropositive due to vaccination; vaccine-induced and naturally induced seroresponses cannot be distinguished to assess the true seroprevalence from natural infection. Second, we assumed that the infection rate for farm residents could be different for farms with vaccinated sheep (leading to reduced exposure) compared to farms with unvaccinated sheep. About 3 weeks after the initial invitation, all non-responding farmers were sent a written reminder. Because of the small number, dairy sheep farmers who did not respond to this second invitation were contacted by telephone.

After written informed consent, a maximum of three persons were selected from each farm, i.e. the farmer and a maximum of two family members aged $\geqslant 12$ years residing at the farm; in some instances other persons working or living on the farm were selected. Each participant received a questionnaire addressing individual-based risk factors like age, gender, profession, ownership or contact with ruminants and pets, consumption of unpasteurized milk, medical history, and contact with agricultural products. In addition, the farm owner or farm manager completed a farm-based questionnaire addressing characteristics like farm hygiene and management, herd size, presence of other livestock and pets, stable environment, and lambing season characteristics. Separate farm-based questionnaires were developed for dairy farms and non-dairy farms because of clear differences in farm management. A professional laboratory assistant visited the farms to collect blood samples from all participating individuals for serology. All data of the dairy sheep farms were collected between September 2009 and September 2010, for the non-dairy sheep farms data were collected between April and September 2010. The Medical Ethical Commission of the University Medical Center Utrecht approved the study protocol (no. 09–189/K).

Serological analysis

Serum samples were tested for *C. burnetii* IgM and IgG antibodies, both phases I and II, using an indirect immunofluorescence assay (IFA) with a screening dilution of 1:32. Participants without any positive antibody result and participants with a solitary IgM phase I or solitary IgM phase II result were classified as seronegative. All other outcomes were classified as seropositive. Those with IgM phase II antibodies were designated as 'relatively recent infections' and included possible current infections. The term 'relatively recent' was chosen as IgM phase II is found

to persist in the majority of cases for 1 year post-infection and may even persist up to 4 years post-infection [20, 21] (C. C. H. Wielders, personal communication). Seropositives without IgM phase II antibodies were designated as 'past infections'. As the latter group also includes possible chronic infections, a further distinction was made between serological profiles that had IgG phase I $\geqslant$1:1024 indicative for a chronic infection according to the new Dutch consensus guidelines [22].

Statistical analyses

Dairy sheep farms

All data were analysed with SAS, version 9.2 (SAS Institute Inc., USA). For the dairy sheep farms in The Netherlands, participation bias was investigated by comparing participating and non-participating farms with regard to herd size, urbanization degree and region. The seroprevalence of *C. burnetii* in residents and the corresponding 95% confidence interval (CI) were calculated. Descriptive statistics were performed by analysing frequency tables and studying distributions of continuous variables. No risk factor analysis was performed because of the small number of participants.

Non-dairy sheep farms

To study participation bias, participating and non-participating farms were compared with regard to herd size, cattle, sheep, and goat density in the surroundings, urbanization degree, region, situated inside or outside a compulsory Q fever vaccination area, number of bulk-milk-positive dairy goat or dairy sheep farms in a radius of 5 and 10 km, and distance in metres to the closest bulk-milk-positive small ruminant farm.

The seroprevalence of *C. burnetii* and the corresponding 95% CI were calculated. For descriptive statistics, frequency tables were analysed. In addition, distributions of continuous variables were studied, and if not linearly related to the outcome variable, continuous variables were recoded into classes.

Univariate logistic regression analysis was performed to assess the main factors associated with *C. burnetii* seropositivity at the individual level [$P<0{\cdot}20$ in the likelihood ratio test (-2LL)]. Variables with <20 participants in one risk category were excluded. Age was always kept in the model because of the frequent association with Q fever seropositivity in the literature. Proxy outcomes, such as sheep seropositivity, were not included in the multivariate analysis. If several variables, which were associated in the univariate analysis, were interrelated, a preferred variable was chosen and related variables were excluded. The preferred variable was chosen based on the most informative value, the strongest association or most relevant exposure (exposure at own farm instead of comparable exposure at other farms). All identified individual variables were analysed with a manual backwards elimination procedure until all variables were significant at the 10% significance level in the likelihood ratio test, starting with a full multivariate logistic regression model.

Subsequently, potential risk factors derived from the farm-based questionnaire were analysed by univariate multilevel analyses considering clustered farm-based data for all persons within the same farm, using a unique farm number as cluster variable. All farm variables which were significant in the univariate analysis ($P<0{\cdot}20$), were analysed with a manual backward elimination procedure starting with a full multilevel model.

Finally, both the individual and farm-based characteristics from the two final submodels were combined in a multivariate multilevel analysis to identify the independent risk determinants for *C. burnetii* seropositivity. The final model fit was assessed by the quasi-likelihood under the independence model criterion (QIC) goodness-of-fit statistic for generalized estimation equation (GEE) models.

RESULTS

Dairy sheep farms

Out of the 32 invited farms, 12 participated (response rate 37·5%). The participating farms were all situated in a rural area (<500 addresses/km^2). Participating and non-participating farms were comparable with regard to urbanization degree and province distribution. However, participating farms had a median number of 529 sheep (range 143–1163) *vs.* the significantly lower median of 353 sheep (range 96–730) for non-participating farms ($P=0{\cdot}03$).

Twenty-seven study participants (mean age 38·7 years, range 14–61, 63% male), provided a blood sample. Overall, 18 (66·7%) participants were seropositive: 80·0% for the 15 farmers (12 males), and 50·0% for the 12 household members (five children, five female spouses, one male spouse, one

seasonal worker). Three (11·1%) participants had a relatively recent *C. burnetii* infection (IgM phase II antibodies). None consulted their general practitioner or were hospitalized because of influenza-like illness or fever. One participant had an IgG phase I titre of $\geqslant$1:1024, indicating a possible chronic case [22].

Non-dairy sheep farms

Non-response analyses

Out of the 1344 approached farms, at least 32 appeared to be no longer eligible because they had <60 animals at inclusion or had vaccinated all their sheep. Of the remaining 1312 farms, 119 participated in the study (response rate 9·1%).

A significant difference was found for sheep density in the 5-km radius of participating and non-participating farms, 34·5 (range 1·8–143·6) and 47·5 (range 1·0–162·9) sheep/km^2 in the 5-km radius (excluding own sheep), respectively ($P = 0{\cdot}01$). In addition, the number of sheep was borderline significantly higher at the participating farms (median 191 sheep, range 102–1310), compared to the non-participating farms (median 167 sheep, range 100–2857). For the other variables, no significant differences were found between participating and non-participating farms (Table 1).

Descriptive characteristics

The 119 participating farms were mainly situated in the provinces of Noord-Holland and Friesland, commonly (90·8%) situated in rural areas (<500 addresses/km^2) and the most common breeds at the farms were Texel (57·0%) and Swifter (46·5%). The farms were mainly started after 1950 (9·6% 1875–1950, 39·4% 1951–1980, 51·0% after 1980). Out of the 114 farms with a farm-based questionnaire, 23 (20·2%) kept one or more goats, 45 (39·5%) kept dairy cattle and/or beef cattle, and 13 (11·4%) other farms reported that cattle were present on their pastures. The farms could have one or more function; 95 (83·3%) farms kept sheep for meat production, 53 (46·5%) farms for rearing, and 20 (17·5%) farms for nature management. Of those 20 farms, 12 farms kept their sheep exclusively for nature management.

From the 119 farms, 271 persons provided a blood sample (mean age 47, range 12–93 years, 55% male). Of those, 266 completed the individual self-administered questionnaire and from 261 individuals information was available from the farm-based questionnaire.

C. burnetii seroprevalence was 51·3% (95% CI 45·5–57·4). In the univariate analysis, seroprevalence was significantly higher for farmers (58·8% *vs.* 36·3% for spouses) and for males (57·7% *vs.* 43·4% for females). Out of the 271 participants, seven (2·6%) had a relatively recent infection (IgM phase II antibodies). No participant had an IgG phase I titre suggestive for chronic infection.

Although the seroprevalence of the farm residents was higher for those living on a dairy sheep farm, the difference was not statistically significant [odds ratio (OR) 1·9, 95% CI 0·8–4·4] for dairy sheep farmers *vs.* non-dairy sheep farmers).

Univariate analyses at individual and farm level

All individual and farm-based variables, which were tested in the univariate analysis for relationship with human *C. burnetii* seropositivity, are displayed in Tables 2 and 3.

Multivariate and multilevel analyses

In the multivariate analyses, from 23 individual variables which were associated in the univariate analysis, four were independently associated with *C. burnetii* seropositivity (Table 4). In addition, 10/23 farm-based variables included in the multilevel analyses were significantly independent risk or protective factors and together were used as the full multilevel start model (Table 5).

Combined multilevel analyses of individual and farm-based factors

In the final combined multilevel model, significant risk factors were contact with cattle at own or other farm, past employment in the cattle sector, high goat density in the vicinity of the farm, living or working at a farm that was started in 1990 or later, the presence of Blessumer breed on the farm, cattle on the same pastures used by sheep, although not simultaneously with the sheep, high frequency of refreshing the bedding in the sheep stables, and sheep supplied from the provinces of Groningen or Noord-Holland (Table 6). Borderline significant risk factors were age 40–49 years, and presence of dairy cattle during the stabling period of the sheep. In addition, sheep lambing outside was a significant protective factor, and air entering the stable through the door was a borderline significant protective factor.

Table 1. *Non-response analyses of non-dairy sheep farms, comparison of participating and non-participating farms*

Numerical variables	Participating farms ($N=119$) Median	Non-participating farms ($N=1193$) Median	*P* value
Number of sheep	191	167	0·05
Cattle density (number of cattle/km^2 in the municipality)*	134·7	135·5	0·16
Cattle density without veal calves (number of cattle/km^2 in the municipality)*	114·7	119·5	0·10
Goat density (number of goats/km^2 excluding own animals in a 5-km radius)*	2·6	3·5	0·17
Sheep density (number of sheep/km^2 excluding own animals in a 5-km radius)*	34·5	47·5	0·01
Closest Q fever bulk-milk-positive dairy goat or dairy sheep farm (metres)*	13 960	13 806	0·70
Number Q fever bulk-milk-positive dairy goat or dairy sheep farms in a 5-km radius*	0 (min = 0, max = 2)	0 (min = 0, max = 4)	0·62
Number Q fever bulk-milk-positive dairy goat or dairy sheep farms in a 10-km radius*	0 (min = 0, max = 4)	0 (min = 0, max = 9)	0·71
Categorical variables	*n* (%)	*n* (%)	*P* value
Inside vaccination area	20 (16·8)	181 (15·2)	0·64
Outside vaccination area	99 (83·2)	1012 (84·8)	
Urbanization			
Very high urban area*†	0 (0·0)	2 (0·2)	0·37
High urban area	0 (0·0)	3 (0·3)	
Moderate urban area	4 (3·3)	14 (1·2)	
Minor urban area	7 (5·9)	84 (7·0)	
Rural area	108 (90·8)	1086 (91·3)	
Province			
Drenthe*	4 (3·4)	57 (4·8)	0·52
Flevoland	1 (0·8)	9 (0·8)	
Friesland	18 (15·1)	213 (17·9)	
Gelderland	14 (11·8)	170 (14·3)	
Groningen	11 (9·2)	93 (7·8)	
Limburg	4 (3·4)	23 (1·9)	
Noord-Brabant	12 (10·1)	74 (6·2)	
Noord-Holland	29 (24·4)	241 (20·3)	
Overijsel	11 (9·2)	86 (7·2)	
Utrecht	2 (1·7)	48 (4·1)	
Zeeland	2 (1·7)	49 (4·1)	
Zuid-Holland	11 (9·2)	126 (10·6)	

N, Total number of individuals.
* Four missing values at non-participating farms.
† Urbanization degree: very high urban area >2500 addresses/km^2; high urban area = 1500–2500 addresses/km^2; moderate urban area = 1000–1500 addresses/km^2; minor urban area = 500–1000 addresses/km^2; rural area <500 addresses/km^2.

DISCUSSION

Seroprevalence

The seroprevalence of non-dairy (51·3%) and dairy sheep farm residents (66·7%) is clearly higher compared to the seroprevalence estimate of 2·4% in the general population before the outbreak occurred in The Netherlands in 2006–2007. It is even higher compared to the seroprevalence found in a small community in the epicentre of the Q fever outbreak in 2007 (25·1%), and in blood donors in the most Q fever-affected areas in 2009 (12·2%), indicating that sheep farm residents have an increased life-time risk of acquiring a *C. burnetii* infection compared to the general Dutch population [7, 23, 24].

The observed seroprevalence in Dutch sheep farm households is also high compared to a study of sheep farmers in Sweden (28·5%) [25], and of

Table 2. *Univariate logistic model of individual factors related to* C. burnetii *seropositivity in non-dairy sheep farm residents (P < 0·20, −2LL)*

Variable	Category	Frequency (*N*) (*N* = 266)	Sero-prevalence (%)	OR (95% CI)
Gender*	Male	144	57·6	1·77 (1·09–2·88)
	Female	122	43·4	Reference
Age (years)*	12–19	21	57·1	2·04 (0·72–5·76)
	20–39	45	51·1	1·60 (0·70–3·63)
	40–49	68	58·8	2·18 (1·03–4·63)
	50–59	84	50·0	1·53 (0·74–3·13)
	>60	48	39·6	Reference
Work and/or live on farm	Work and live	188	53·7	1·61 (0·83–3·15)
	Work, but not live	35	48·6	1·31 (0·53–3·22)
	Not work, but live	43	41·9	Reference
Function	Farmer	136	58·8	2·51 (1·42–4·44)
	Spouse	80	36·3	Reference
	Child†	39	53·9	2·05 (0·94–4·46)
	Other‡	11	54·6	2·11 (0·59–7·53)
How often in stable	Every day	185	55·7	Reference
	Every week	56	41·1	0·56 (0·30–1·02)
	Every month	10	50·0	0·80 (0·22–2·84)
	Less than once a month/never	15	33·3	0·40 (0·13–1·21)
Amount of work at farm*	Full working week	61	63·9	2·39 (1·25–4·56)
	Up to half a working week	97	52·9	1·49 (0·86–2·59)
	Never/occasionally	108	42·6	Reference
Feed sheep*	Yes	225	55·6	3·41 (1·63–7·14)
	No	41	26·8	Reference
Load and unload sheep	Yes	194	56·2	2·14 (1·23–3·72)
	No	72	37·5	Reference
General healthcare of sheep	Yes	201	55·7	2·15 (1·21–3·82)
	No	65	36·9	Reference
Remove manure	Yes	180	57·8	2·31 (1·36–3·92)
	No	86	37·2	Reference
Spread manure*	Yes	124	58·9	1·80 (1·10–2·92)
	No	142	44·4	Reference
Clean stables	Yes	167	56·3	1·75 (1·06–2·89)
	No	99	42·4	Reference
Administrative work	Yes	193	54·4	1·62 (0·94–2·78)
	No	73	42·5	Reference
Wear overalls or boots*	Yes	234	54·3	3·03 (1·35–6·84)
	No	32	28·1	Reference
Contact with cattle at own or other farm*§	Yes	172	63·4	4·29 (2·49–7·40)
	No	94	28·7	Reference
Contact with horses at own or other farm*§	Yes	145	59·3	2·07 (1·27–3·38)
	No	121	41·3	Reference
Contact with pigs at own farm*§	Yes	24	37·5	0·54 (0·23–1·29)
	No	242	52·5	Reference
Indirect contact with poultry at own farm*‖	Yes	93	57·0	1·44 (0·87–2·39)
	No	173	48·0	Reference
Indirect contact with rats at own farm*‖	Yes	45	64·4	1·93 (0·99–3·76)
	No	221	48·4	Reference
Contact with goats at other farm*§	Yes	32	62·5	1·70 (0·79–3·63)
	No	234	49·6	Reference
Contact with sheep at other farm*§	Yes	102	60·8	1·89 (1·14–3·12)
	No	164	45·1	Reference
Contact with dogs at other farm*§	Yes	112	58·9	1·72 (1·05–2·82)
	No	154	45·5	Reference

Table 2 (*cont.*)

Variable	Category	Frequency (*N*) (*N* = 266)	Sero-prevalence (%)	OR (95% CI)
Indirect contact with poultry at other farm‖	Yes	38	63·2	1·78 (0·87–3·61)
	No	228	49·1	Reference
Indirect contact with cats at other farm*‖	Yes	81	59·3	1·60 (0·95–2·72)
	No	185	47·6	Reference
Direct contact with wool*	Yes	113	60·2	1·89 (1·15–3·09)
	No	153	44·4	Reference
Direct contact with hay, straw or animal feed*	Yes	228	54·8	2·98 (1·41–6·29)
	No	38	29·0	Reference
Direct contact with raw milk	Yes	72	62·5	1·91 (1·10–3·32)
	No	193	46·6	Reference
Drink raw milk from cattle*	Yes	45	66·7	2·17 (1·11–4·26)
	No	221	48·0	Reference
Direct contact with cattle manure	Yes	110	68·2	3·30 (1·97–5·52)
	No	155	39·4	Reference
Direct contact with live-born animals during lambing period	Yes	246	53·3	3·42 (1·21–9·69)
	No	20	25·0	Reference
Direct contact with dead-born animals/placenta*	Yes	210	54·3	1·84 (1·01–3·35)
	No	56	39·3	Reference
Tick bite*	Yes	61	42·6	0·64 (0·36–1·14)
	No	205	53·7	Reference
Did not work in animal husbandry/agriculture in the past	Yes	114	39·5	0·44 (0·27–0·72)
	No	152	59·9	Reference
Employment in cattle sector in the past*	Yes	107	64·5	2·49 (1·50–4·14)
	No	159	42·1	Reference
Worked in animal transport/ transport of agricultural products in the past*	Yes	37	70·3	2·56 (1·21–5·42)
	No	229	48·0	Reference
As a child lived at:	Cattle farm	151	59·6	2·04 (1·18–3·53)
	Other kind of farm	34	35·3	0·75 (0·33–1·73)
	No farm	81	42·0	Reference
As a child worked in animal care/with manure/hay/in vegetation care*	Yes	178	56·2	1·85 (1·10–3·11)
	No	88	40·9	Reference

N, Total number of individuals; OR, odds ratio; CI, confidence interval, −2LL, likelihood ratio test.
* Variables included in subsequent multivariate individual analyses before manual backward elimination.
† Children aged <18 years ($n = 17$) and older children ($n = 22$) of the farmer.
‡ Employees, shepherds, other family members.
§ See animals at <5 m or touch animals.
‖ See animals at <5 m.

farmers from all types of farms: 17·8% in Poland, and 27·3% in the UK [26, 27]. Generally, it is difficult to compare international seroprevalence studies, because most studies use different tests or cut-off values. The cut-off value of the test in our study ($\geqslant$1:32) was chosen because it allowed comparison with other population surveys conducted in The Netherlands [23, 28].

Dairy sheep farm residents had a higher seroprevalence compared to non-dairy sheep farm residents. Although no statistically significant difference in seroprevalence was found between the residents of both farm types, this might be due to lack of power because of the small number of participants from dairy sheep farms. In this study it was impossible to assess which risk factors were responsible for the higher seroprevalence in dairy sheep farm residents, due to the low number of participating dairy sheep farm residents. In addition, because of the differences in farm management, the farm-based questionnaires of both farm types were not the same, therefore pooling the analysis with the other sheep farm residents to increase power was not an option. Specific research, targeting all current dairy sheep farms

in The Netherlands (n~40), might elucidate further risk factors next to the higher sheep seroprevalence, explaining the higher seroprevalence in dairy sheep farm residents. Nevertheless, it might well be that dairy farm residents were more exposed to *Coxiella*, as the seroprevalence in dairy sheep at these same farms was significantly higher compared to that of non-dairy sheep (data not shown). A higher vulnerability for infection of breeds selected for milk production rather than for disease resistance has previously been observed for dairy cattle, dairy sheep, and dairy goats [29, 30]. In addition, dairy sheep are more often housed in stables compared to non-dairy sheep which spend most of the year outside. Indoor housing might facilitate the spread of *C. burnetii* in dairy sheep and to humans. Moreover, the higher seroprevalence in dairy farm residents might be explained by more intense contact with dairy sheep.

The seroprevalence of the dairy sheep farm residents (66·7%) was comparable to the seroprevalence of dairy goat farm residents (68·7%) in The Netherlands [28]. Furthermore, the percentage of relatively recent infections (clinical status unknown as no questions addressed current Q fever compatible symptoms) in the dairy sheep farm residents (11·1%) is comparable to that of the dairy goat farm residents (11·2%) [28]. Additionally, the percentage of participants with an indication for a possible chronic infection is also similarly high for dairy sheep and dairy goat farm residents (3·7% and 4·1%, respectively) [28]. In contrast, the percentage of relatively recent infections and possible chronic infections are lower for non-dairy sheep farm residents (2·6% and 0%, respectively). Therefore, currently *C. burnetii* infection seems to be a more serious and on-going health problem in dairy goat and dairy sheep farm residents compared to non-dairy sheep farm residents, although the numbers are relatively small.

Although numbers are too low to draw any conclusion and do not allow for valid statistical testing, the 10 (three from dairy and seven from non-dairy farms) relatively recent (IgM phase II positive) cases were generally younger (median 37 years *vs.* median 50 years for the seronegatives), were more often male (80% *vs*. 48%) and more often lived on a dairy sheep farm (30% of the recently infected *vs.* 6% of the seronegatives). This may point to ongoing infections especially in male dairy sheep farm residents, in the relatively early days of their contact with sheep.

Risk and protective factors for non-dairy sheep farm residents

One of the protective factors for *C. burnetii* seropositivty was sheep lambing outside. Farm residents might be less exposed to contaminated aerosols in that situation, compared to lambing inside stables.

In addition, several risk factors for *C. burnetii* seropositivity were identified in this study. McCaughey *et al.* [31] suggested in his study in the general population (age 12–64 years) that most people acquired *C. burnetii* infection between ages 25 and 34 years and after that age seroprevalence remained stable. This age trend was not seen in our study; sheep farm residents had already a high seroprevalence at young age (12–19 years). This might be explained by exposure to infected animals at a young age. The highest seroprevalence found in humans (age 40–49 years), matches the most common age group of notified clinical Q fever cases in The Netherlands [9]. The increased risk at this age seems not to be explained by differences in specific work activities, frequency of cattle contact, or hours worked. Perhaps host factors play a role in the increased risk, or it generally reflects regular exposure to the bacterium and repeated development of antibodies (booster effect), not adequately measured by the questions in the questionnaire.

Animal movement is a known risk factor for the transfer of microorganisms and should be discouraged [32, 33]. Why specifically supply of sheep from the northern provinces of Noord-Holland and Groningen showed an independent increased risk for infection of the farm residents is not clear. The seroprevalence in sheep in these two provinces was not significantly different from prevalences in other provinces, both in the current study (B. Schimmer *et al*., unpublished data) and in a previous study in 2008 using convenience serum samples from sheep [30].

It is also unknown why the fact that a farm started before 1990 was a risk factor. No change in farm management is known around that year that could influence the risk of a *C. burnetii* infection.

Having the Blessumer sheep breed on the farm was the next significant risk factor. This breed is a crossing of the breeds of Texel (non-dairy sheep) and Flemish sheep (dairy sheep); therefore, the Blessummer breed might have a lower disease resistance [29, 30]. Differences in infection rates between sheep breeds have not yet been studied to investigate whether Blessumer sheep are more often infected.

Table 3. *Univariate multilevel analysis of farm-based factors related to* C. burnetii *seropositivity in non-dairy sheep farm residents (P<0·20)*

Variable	Category	Frequency (*N*) (*N*=261)*	Sero-prevalence (%)	OR (95% CI)
Urbanization†‡§	Moderate or minor urban area	28	67·9	2·00 (0·80–5·04)
	Rural area	242	49·2	Reference
Goat density (number of goats/km^2 excluding own animals in a 5-km radius)†§	<2·9	135	38·5	Reference
	2·9–11·3	67	68·7	3·59 (1·86–6·91)
	⩾11·4	68	58·8	2·38 (1·18–4·79)
Sheep density (number of sheep/km^2 excluding own animals in 5-km radius)†§	<33·7	133	41·4	Reference
	33·7–79·0	69	53·6	1·68 (0·87–3·25)
	⩾79·1	68	67·7	2·98 (1·54–5·78)
Cattle density (number of cattle/km^2 in the municipality)†§	< 200·0	240	47·9	Reference
	⩾200·0	30	76·7	3·20 (1·37–7·51)
Number of Q fever bulk-milk-positive dairy goat or dairy sheep farms in a 10-km radius†§	0	166	45·8	Reference
	1–4	104	59·6	1·78 (1·02–3·11)
Closest Q fever bulk-milk-positive dairy goat or dairy sheep farms (km)§	<5·0	35	62·9	Reference
	5·0–9·9	69	58·0	0·39 (0·14–1·13)
	10·0–14·9	53	41·5	0·87 (0·30–2·54)
	15·0–19·9	41	61·0	0·82 (0·32–2·14)
	⩾ 20·0	72	40·3	0·42 (0·16–1·10)
Year farm started†	Before 1990	165	44·2	Reference
	1990 or later	75	61·3	1·97 (1·12–3·48)
Distance between house and pastures	<30 m	127	40·2	Reference
	⩾30 m	103	61·1	2·20 (1·23–3·94)
Number of male sheep 2010†	<6	130	46·9	Reference
	6–20	56	60·7	1·78 (0·85–3·75)
	>20	41	51·2	1·20 (0·53–2·70)
	No	16	56·3	1·30 (0·42–4·00)
Zwartbles breed present on farm†	Yes	30	63·3	1·75 (0·89–3·42)
	No	228	48·7	Reference
Rijnlam breed present on farm	Yes	7	85·7	5·72 (0·78–42·12)
	No	251	49·4	Reference
Blessumer breed present on farm†	Yes	21	76·2	3·51 (1·25–9·81)
	No	237	48·1	Reference
Animals at same pasture simultaneously with sheep	None	160	52·5	Reference
	Cattle	66	59·1	1·30 (0·73–2·33)
	Other	27	18·5	0·21 (0·07–0·66)
Cattle at same pasture but not simultaneously with sheep†	Yes	62	74·2	3·90 (1·74–8·72)
	No	188	42·0	Reference
Straw bedding in the stables	Yes	243	50·2	0·69 (0·40–1·21)
	No	5	60·0	Reference
	No stable	10	50·0	0·31 (0·24–1·68)
How often bedding in stable is refreshed†	Every other day or more	200	53·0	1·77 (0·83–3·76)
	Once or twice a week	47	38·3	Reference
	No stable	10	50·0	1·46 (0·49–4·35)
Air enters stable through door†	Yes	163	46·6	0·64 (0·35–1·18)
	No	79	58·2	Reference
	No stable	10	50·0	0·67 (0·25–1·80)
No farm animals present on farm other than sheep	Yes	73	42·5	0·63 (0·34–1·14)
	No	183	53·6	Reference
Other farm animals present in sheep stables	Yes	164	54·9	1·71 (0·98–3·00)
	No	92	42·4	Reference
Laying hen in stable†	Yes	35	65·7	2·11 (0·88–5·04)
	No	215	47·9	Reference

Table 3 (*cont.*)

Variable	Category	Frequency (*N*) (*N* = 261)*	Sero-prevalence (%)	OR (95% CI)
Dairy cattle in stable†	Yes	66	71·2	3·37 (1·76–6·45)
	No	184	42·9	Reference
Type of feed method	By hand/ wheelbarrow	208	48·1	Reference
	Mixer	14	71·4	2·91 (0·92–9·23)
	Shovel	33	48·5	1·02 (0·53–1·97)
Lambing outside†	Yes	27	37·0	0·55 (0·26–1·20)
	No	234	51·3	Reference
Number of yearlings which lambed in 2009†	<40	208	46·6	Reference
	⩾40	50	62·0	1·79 (0·89–3·63)
Number dead-born lambs in 2009	<6	49	40·8	Reference
	6–14	93	57·0	1·88 (0·85–4·15)
	15–24	53	41·5	1·09 (0·47–2·50)
	>25	48	54·2	1·69 (0·71–4·05)
Abortion rate 2007, 2008, 2009(%)†	<4 in all three years	195	46·2	Reference
	⩾4 in at least one year	51	66·7	2·35 (1·12–4·92)
Afterbirth of normally lambed animal†	Leave in stable or pasture	50	58·0	Reference
		84	47·6	0·64 (0·30–1·36)
	Direct or once a day render bucket	100	51·0	0·72 (0·34–1·53)
	Direct or once a day manure yard	20	30·0	0·31 (0·10–0·97)
	Other			
Farm tenure †	Closed for ewes and rams or only closed for ewes	185	43·2	Reference
	Not closed for ewes and rams	72	65·3	2·37 (1·24–4·54)
Sheep supplied from Groningen†	Yes	26	69·2	2·50 (0·82–7·57)
	No	226	48·2	Reference
Sheep supplied from Noord- Brabant†	Yes	27	63·0	1·93 (0·81–4·58)
	No	225	48·9	Reference
Sheep supplied from Noord- Holland†	Yes	76	59·2	1·67 (0·89–3·15)
	No	176	46·6	Reference
Sheep supplied from Utrecht	Yes	15	73·3	2·69 (0·73–9·86)
	No	237	49·0	Reference
Presence of hygienic locker room	Yes	19	68·4	2·32 (0·81–6·62)
	No	231	48·5	Reference
Presence of disinfection bucket†	Yes	36	61·1	1·80 (0·89–3·65)
	No	214	48·1	Reference

N, Total number of individuals; OR, odds ratio; CI, confidence interval.

* Not all numbers add up to the total due to missing values.

† Variable included in later multivariate farm-based analyses before manual backward elimination.

‡ Urbanization degree: moderate urban area = 1000–1500 addresses/km^2; minor urban area = 500–1000 addresses/km^2; rural area <500 addresses/km^2.

§ For the geographical data, information was available for all 270 individuals, including the nine people without a farm-based questionnaire.

In the environment of dairy goat farms with a history of abortion waves and of farms having PCR-positive bulk milk, relatively high levels of *C. burnetii* DNA were found [34]. A high goat density in the surrounding area of a participating farm is therefore considered a plausible risk factor for people living in the vicinity at the time of data collection. This was also demonstrated in several local outbreak investigations in The Netherlands in 2008–2009 [7, 8].

Maredly, several risk factors for *C. burnetii* seropositivity in non-dairy sheep farm residents point to cattle exposure at present or in the past. This might

Table 4. *Results of the multivariate logistic regression analysis for the individual characteristics (P<0·10, −2LL) in relation to non-dairy sheep farm residents* C. burnetii *seropositivity*

Variable	Category	OR (95% CI)
Age (years)	12–19	2·81 (0·85–9·35)
	20–39	1·42 (0·57–3·54)
	40–49	2·29 (1·00–5·24)
	50–59	1·12 (0·50–2·48)
	>60	Reference
Amount of work at farm	Full working week	2·42 (1·13–5·15)
	Up to half a working week	1·23 (0·65–2·33)
	Never/occasionally	Reference
Contact with cattle at own or other farm*	Yes	3·87 (2·13–7·04)
	No	Reference
Worked in cattle sector in the past	Yes	1·79 (1·01–3·18)
	No	Reference

OR, Odds ratio; CI, confidence interval; −2LL, likelihood ratio test; AIC, Akaike's Information Criterion.
Number of observations used: 266 (AIC = 340·38).
* See animals at <5 m or touch animals.

Table 5. *Results of the multilevel analysis with farm-based characteristics (P<0·10) as independent factors in relation to non-dairy sheep farm residents* C. burnetii *seroprevalence*

Variable	Category	OR (95% CI)
Goat density (number of goats/km^2 excluding own animals in a 5-km radius)	<2·9	Reference
	2·9–11·3	1·60 (0·75–3·43)
	⩾11·4	3·80 (1·67–8·65)
Year farm started	Before 1990	Reference
	1990 or later	3·97 (1·79–8·82)
Blessumer breed present on farm	Yes	5·19 (2·36–11·41)
	No	Reference
Cattle at same pasture but not simultaneously with sheep	Yes	5·14 (2·17–12·19)
	No	Reference
How often bedding in stable is refreshed	Every other day or more	3·24 (1·49–7·07)
	Once or twice a week	Reference
	No stable	8·91 (2·17–36·68)
Air enters stable through door	Yes	0·46 (0·23–0·92)
	No	Reference
	No stable	8·91 (2·17–36·68)
Dairy cattle present during stabling period of sheep	Yes	3·33 (1·17–9·46)
	No	Reference
Lambing outside	Yes	0·34 (0·14–0·86)
	No	Reference
Sheep supplied from Groningen	Yes	4·17 (1·59–10·97)
	No	Reference
Sheep supplied from Noord-Holland	Yes	3·93 (1·74–8·90)
	No	Reference

OR, Odds ratio; CI, confidence interval; QIC, quasi-likelihood under the independence model criterion.
Number of observations used: 212. Number of levels used: 107 (QIC = 232·9560).

suggest that cattle were partially responsible for the infections observed in the sheep farm residents. In a previous study in farmers (all farm types) contact with cattle was also described as a risk [27]. A recent published review including worldwide studies, suggested a higher seroprevalence of *C. burnetii* in cattle compared to goat and sheep [35]. In The Netherlands, a prevalence of 78·6% for antibodies

Table 6. *Results of the multilevel analysis with individual and farm-based characteristics (P<0·10) as independent factors in relation to non-dairy sheep farm residents* C. burnetii *seroprevalence*

Variable	Category	OR (95% CI)
Age (years)	12–19	0·96 (0·29–3·21)
	20–39	1·96 (0·56–6·90)
	40–49	2·43 (0·98–6·04)
	50–59	1·54 (0·63–3·78)
	>60	Reference
Contact with cattle at own or other farm*	Yes	2·32 (1·02–5·29)
	No	Reference
Worked in cattle sector in the past	Yes	3·98 (1·71–9·25)
	No	Reference
Goat density (number of goats/km^2 excluding own animals in a 5-km radius)	<2·9	Reference
	2·9–11·3	1·11 (0·46–2·68)
	⩾11·4	5·86 (1·81–18·95)
Year farm started	Before 1990	Reference
	1990 or Later	3·67 (1·45–9·31)
Blessumer breed present on farm	Yes	4·49 (1·59–12·65)
	No	Reference
Cattle at same pasture but not simultaneously with sheep	Yes	5·77 (2·29–14·56)
	No	Reference
How often bedding in stable is refreshed	Every other day or more	4·58 (1·69–12·37)
	Once or twice a week	Reference
	No stable	8·34 (1·71–40·60)
Air enters stable through door	Yes	0·47 (0·21–1·01)
	No	Reference
	No stable	8·34 (1·71–40·60)
Dairy cattle present during stabling period of sheep	Yes	2·69 (0·81–8·95)
	No	Reference
Lambing outside	Yes	0·33 (0·12–0·92)
	No	Reference
Sheep supplied from Groningen	Yes	5·05 (1·73–14·69)
	No	Reference
Sheep supplied from Noord-Holland	Yes	3·63 (1·27–10·33)
	No	Reference

OR, Odds ratio; CI, confidence interval; QIC, quasi-likelihood under the independence model criterion.
Number of observations used: 208. Number of levels used: 105 (QIC = 219·1157).
* See animals at <5 m or touch animals.

in cattle bulk tank milk was found, confirming widespread circulation of the bacterium in cattle [36]. To further assess the risk for human infection from cattle, a similar study addressing the seroprevalence and risk factors in dairy cattle farm residents is being finalized in The Netherlands. A role for cattle in the human infections observed in the current sheep farm study, is also supported by the fact that the high seroprevalence in sheep farm residents does not seem to correspond with the low sheep seroprevalence at the participating farms (<2%). The role of specific activities with sheep for the infection risk was presumably relatively small, although not absent taking into account the significant association between human and sheep seroprevalence at the participating non-dairy farms. Whether sheep themselves are at increased risk for infection because of contact with cattle or nearby goat populations is currently under investigation. In The Netherlands, a dominant *C. burnetii* genotype was identified in humans, goats, and sheep throughout the entire affected area; the genotype found in cattle appeared to be different [37, 38].

Based on the results of the present study, some recommendations can be made. First, we want to elucidate the transmission cycle between different species of ruminants and farm residents; strains from goat, sheep, cattle, and sheep farm residents could be subtyped and compared. Second, more research is needed to investigate whether the Blessumer breed is more often infected compared to other breeds. Third,

in this study a high seroprevalence in spouses was found (36·3% non-dairy farm spouses, 50·0% dairy farm spouses). Therefore, we emphasize the importance of the advice that pregnant women should avoid contact with sheep during the lambing season, and that they should avoid contact with birth products of sheep. Currently, the Dutch Health Council is preparing an advice about vaccination of high-risk professionals, including several farm populations. For this advice, they also will take into account the results of this study.

Limitations

The study of non-dairy sheep farms had a low response rate of 9·1%. As reported by several farmers not willing to participate, sheep were outside when the request to participate was made, and it would be too labour-intensive to collect about 60 sheep for blood sampling. In addition, this part of the sheep industry was not affected by the implemented control measures, mainly targeted at farms with dairy sheep and dairy goats. Therefore, non-dairy sheep farmers might be less motivated to participate compared to the small dairy sheep sector, which had a response rate of 38%.

Except for differences in sheep density in the surroundings and the number of sheep on their farms, participating and non-participating non-dairy sheep farms appeared to be comparable. As both factors were not related to seropositivity, this selective response is not thought to be of influence on the study results, which are therefore considered representative for the Dutch professional non-dairy sheep sector.

At 79% of the 119 participating non-dairy farms both the farmer and partner participated in the study. Therefore, results for the farmers and partners are considered representative of the group of farmers/partners at the participating farms. It was not registered how many children aged $\geqslant$12 years lived at the participating non-dairy farms, and we cannot be absolutely sure that the participating children were representative of all children in this age category.

CONCLUSION

This study demonstrates that *C. burnetii* infection is common in individuals living and/or working at a sheep farm in The Netherlands. Except for their sheep, the risk also seems dictated by contact with cattle at present or in the past and by nearby goat populations.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Maurin M, Raoult D.** Q fever. *Clinical Microbiology Reviews* 1999; **12**: 518–553.
2. **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. *Epidemiology and Infection* 1989; **102**: 119–127.
3. **Gonder JC, *et al.*** Cynomolgus monkey model for experimental Q fever infection. *Journal of Infectious Diseases* 1979; **139**: 191–196.
4. **Raoult D, *et al.*** Q fever 1985–1998. Clinical and epidemiologic features of 1,383 infections. *Medicine (Baltimore)* 2000; **79**: 109–123.
5. **van der Hoek W, *et al.*** Shifting priorities in the aftermath of a Q fever epidemic in 2007 to 2009 in The Netherlands: from acute to chronic infection. *Eurosurveillance* 2012; **17**: pii=20059.

6. **van der Hoek W, *et al.*** Q fever in the Netherlands: an update on the epidemiology and control measures. *Eurosurveillance* 2010; **15**: pii=19520.
7. **Karagiannis I, *et al.*** Investigation of a Q fever outbreak in a rural area of The Netherlands. *Epidemiology and Infection* 2009; **137**: 1283–1294.
8. **Schimmer B, *et al.*** The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. *BMC Infectious Diseases* 2010; **10**: 69.
9. **Schimmer B, *et al.*** Sustained intensive transmission of Q fever in the south of the Netherlands, 2009. *Eurosurveillance* 2009; **14**: pii=19210.
10. **van der Hoek W, *et al.*** Human Q fever and non-dairy farm [in Dutch]. National Institute of Public Health and Environment, 2010. Report No.: 2010/02.
11. **Koene RP, *et al.*** A Q fever outbreak in a psychiatric care institution in The Netherlands. *Epidemiology and Infection* 2011; **139**: 13–18.
12. **Whelan J, *et al.*** Visits on 'lamb-viewing days' at a sheep farm open to the public was a risk factor for Q fever in 2009. *Epidemiology and Infection* 2012; **140**: 858–864.
13. **Gilsdorf A, *et al.*** Large Q fever outbreak due to sheep farming near residential areas, Germany, 2005. *Epidemiology and Infection* 2008; **136**: 1084–1087.
14. **Hellenbrand W, Breuer T, Petersen L.** Changing epidemiology of Q fever in Germany, 1947–1999. *Emerging Infectious Diseases* 2001; **7**: 789–796.
15. **Porten K, *et al.*** A super-spreading ewe infects hundreds with Q fever at a farmers' market in Germany. *BMC Infectious Diseases* 2006; **6**: 147.
16. **Medić A, *et al.*** Q fever epidemic among employees in a factory in the suburb of Zadar, Croatia. *Croatian Medical Journal* 2005; **46**: 315–319.
17. **Dupuis G, *et al.*** An important outbreak of human Q fever in a Swiss Alpine valley. *International Journal of Epidemiology* 1987; **16**: 282–287.
18. **Starnini G, *et al.*** An outbreak of Q fever in a prison in Italy. *Epidemiology and Infection* 2005; **133**: 377–380.
19. **Manfredi Selvaggi T, *et al.*** Investigation of a Q-fever outbreak in northern Italy. *European Journal of Epidemiology* 1996; **12**: 403–408.
20. **Wegdam-Blans MCA, *et al.*** Evaluation of commonly used serological tests for detection of *Coxiella burnetii* antibodies in well-defined acute and follow-up sera. *Clinical and Vaccine Immunology* 2012; **19**: 1110–1115.
21. **Hussain-Yusuf H, *et al.*** An analysis of Q fever patients 6 years after an outbreak in Newport, Wales, UK. *QJM: an International Journal of Medicine* 2012; **105**: 1067–1073.
22. **Wegdam-Blans MCA, *et al.*** Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. *Journal of Infection* 2012; **64**: 247–259.
23. **Schimmer B, *et al.*** Low seroprevalence of Q fever in The Netherlands prior to a series of large outbreaks. *Epidemiology and Infection* 2012; **140**: 27–35.
24. **Hogema BM, *et al.*** *Coxiella burnetii* infection among blood donors during the 2009 Q-fever outbreak in The Netherlands. *Transfusion* 2012; **52**: 144–150.
25. **Macellaro A, Akesson A, Norlander L.** A survey of Q-fever in Sweden. *European Journal of Epidemiology* 1993; **9**: 213–216.
26. **Cisak E, *et al.*** Prevalence of antibodies to *Coxiella burnetii* among farming population in eastern Poland. *Annals of Agricultural and Environmental Medicine* 2003; **10**: 265–267.
27. **Thomas DR, *et al.*** The risk of acquiring Q fever on farms: a seroepidemiological study. *Occupational and Environmental Medicine* 1995; **52**: 644–647.
28. **Schimmer B, *et al.*** Seroprevalence and Risk Factors for *Coxiella burnetii* (Q Fever) Seropositivity in Dairy Goat Farmers' Households in The Netherlands, 2009–2010. *PLoS One* 2012; **7**: e42364.
29. **Rauw WM, *et al.*** Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livestock Production Science* 1998; **56**: 15–33.
30. **van den Brom R, *et al.*** Demography of Q fever seroprevalence in sheep and goats in The Netherlands in 2008. *Preventive Veterinary Medicine* 2012.
31. **McCaughey C, *et al.*** Human seroprevalence to *Coxiella burnetii* (Q fever) in Northern Ireland. *Zoonoses Public Health* 2008; **55**: 189–194.
32. **Bölske G, *et al.*** Bovine tuberculosis in Swedish deer farms: epidemiological investigations and tracing using restriction fragment analysis. *Veterinary Record* 1995; **136**: 414–417.
33. **Mansley LM, *et al.*** Early dissemination of foot-and-mouth disease virus through sheep marketing in February 2001. *Veterinary Record* 2003; **153**: 43–50.
34. **de Bruin A, *et al.*** Detection of *Coxiella burnetii* DNA on small-ruminant farms during a Q fever outbreak in the Netherlands. *Applied and Environmental Microbiology* 2012; **78**: 1652–1657.
35. **Guatteo R, *et al.*** Prevalence of *Coxiella burnetii* infection in domestic ruminants: a critical review. *Veterinary Microbiology* 2011; **149**: 1–16.
36. **Muskens J, *et al.*** Prevalence of *Coxiella burnetii* infection in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA. *Veterinary Record* 2011; **168**: 79.
37. **Roest HIJ, *et al.*** Molecular epidemiology of *Coxiella burnetii* from ruminants in Q fever outbreak, the Netherlands. *Emerging Infectious Diseases* 2011; **17**: 668–675.
38. **Tilburg JJHC, *et al.*** Epidemic genotype of *Coxiella burnetii* among goats, sheep, and humans in the Netherlands. *Emerging Infectious Diseases* 2012; **18**: 887–889.

CHAPTER 4.4

Coxiella burnetii seroprevalence and risk factors on commercial sheep farms in The Netherlands

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Coxiella burnetii seroprevalence and risk factors on commercial sheep farms in The Netherlands

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Coxiella burnetii seroprevalence was assessed on Dutch dairy and non-dairy sheep farms using ELISA. Risk factors for seropositivity on non-dairy sheep farms were identified at farm and sheep level by univariate and multivariate multilevel analyses. Based on 953 dairy and 5671 non-dairy serum samples, sheep seroprevalences were 18.7 per cent and 2.0 per cent, respectively, and 78.6 per cent and 30.5 per cent at farm level. Significant risk factors for non-dairy sheep farms were farm location in the south of the country, sheep kept on marginal grounds, one or several supply addresses for ewes during 2007–2009 and wearing farm boots and/or outfit by professional visitors. On sheep level, risk factors included among others farm location in the south of the country, lamb breeding as main farm purpose, goat density within 10 km farm radius, use of windbreak curtain or windshields, and presence of ≥6 stillborn lambs in 2009. Farm location in the south of the country and goat density suggests that infected goats have played a role in the transmission to non-dairy sheep. Other risk factors suggest introduction of the bacterium through sheep supply and professional visitors. Biosecurity measures should be strengthened, including avoiding infection during handling of stillborn lambs and birth products in the lambing period.

Introduction

Coxiella burnetii infections in sheep are generally not considered a veterinary concern due to lack of significant impact on animal health. In The Netherlands, approximately 1,1 million sheep were kept in 2010. Most sheep are kept for meat production next to a relatively small dairy sheep sector for the production of dairy products. Besides, sheep can also be kept for breeding purposes or nature management. The highest sheep density is found in the coastal provinces. In The Netherlands, dairy sheep are usually milked twice a day during several months a year, and most of them are kept outdoors part of the year. Non-dairy sheep are generally kept outside, except for a few weeks around lambing that usually occurs in a stable.

During the protracted Dutch Q fever epidemic in 2007–2009, abortion waves at dairy goat and dairy sheep farms were considered the primary source of human infections (Roest and others 2011). The transmission of *C. burnetii* from non-dairy sheep farms to human beings seemed not as important as for dairy small ruminant farms (goat and sheep), although direct contact with non-dairy sheep was identified as the most probable source for several human Q fever clusters in The Netherlands during this period (Koene and others 2011, Whelan and others 2012) and internationally (Lyytikainen and others 1998, Grilc and others 2007, Porten and others 2006). While source-finding studies during the Q fever epidemic in The Netherlands identified positive non-dairy sheep farms, no elevated risk for human Q fever was observed in the vicinity of such farms (van der Hoek and others 2012). The low sheep seroprevalence of 2.4 per cent in 2008 found in a convenience sample indicated that non-dairy sheep might play a minor role in the Dutch Q fever epidemic (Van den Brom and others 2013). In other European countries, recent seroprevalences on sheep level ranged between 3.1 per cent and 18.9 per cent and ranged between 3.0 per cent and 74.0 per cent on herd level. In 2008, the *C. burnetii* seroprevalence was also studied on dairy goat farms with 7.8 per cent of individual goats testing positive (ELISA) with the highest seroprevalence in the southeastern part of the country (Van den Brom and others 2013). In the same year, the herd seroprevalence on Dutch dairy cattle farms was 78.6 per cent (ELISA) and 56.6 per cent (PCR) based on a submitted bulk tank milk (BTM) sample by each farm (Muskens and others 2011).

Representative data on seroprevalence of *C. burnetii* infections in dairy and non-dairy sheep are scarce. Moreover, risk factors for *C. burnetii* seropositivity in sheep have been rarely studied (Thomas and others 1995, Ruiz-Fons and others 2010, Van den Brom and others 2013). The aim of the current study was to estimate the *C. burnetii* seroprevalence in a representative sample of non-vaccinated dairy and non-dairy sheep at farms with over 100 sheep in The Netherlands. Furthermore, we identified risk factors for seropositivity at farm and sheep level at non-dairy sheep farms in order to update control measures and to provide targeted advice for the Dutch sheep industry.

Materials and methods

Study design and sampling strategy

In this cross-sectional study in 2009–2011, we approached 33 dairy sheep farms and 1344 non-dairy sheep farms with at least 100 breeding ewes in November 2008, according to the national identification and registration database, for serum sampling and a farm questionnaire (Figs 1 and 2). A farm with a minimum of 100 ewes was chosen as it was considered to be a commercial sheep farm. In the early stage

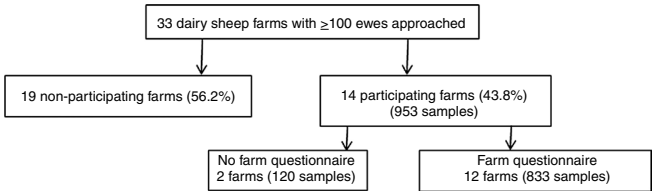

FIG 1: Study participation of invited commercial dairy sheep farms with ≥100 ewes, The Netherlands, 2009–2010

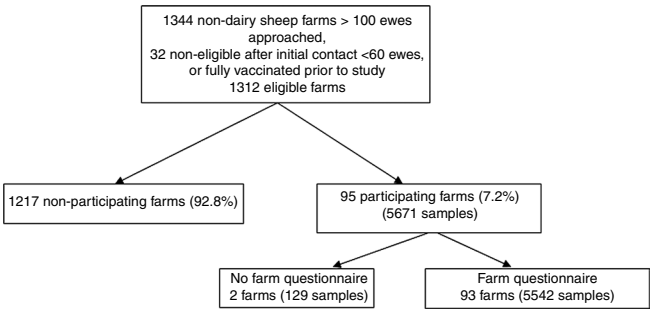

FIG 2: Study participation of invited commercial non-dairy sheep farms with ≥100 ewes, The Netherlands, 2010–2011

of the Dutch Q fever epidemic during 2007–2009, it became clear that only relatively large commercial dairy small ruminant farms (mainly dairy goats) were incriminated as potential source, and no obvious role for smaller farms was observed. Besides, small hobby farms have a different farm management system. Farms with at least 60 unvaccinated sheep at time of inclusion were kept in the study, while farms with vaccinated sheep were excluded because antibody response to natural infections cannot be distinguished from vaccine-induced antibodies. Based on preliminary results of the quick scan screening of non-dairy sheep samples by the Animal Health Service in 2008, with an assumed herd prevalence of 17 per cent and 5 per cent precision, the needed sample size was 181 sheep farms. Based on an assumed overall sheep prevalence of 3 per cent or greater with 95 per cent confidence, 10 per cent accuracy and 90 per cent sensitivity of the ELISA test used, 62 sheep needed to be screened per farm. The private veterinary practitioner collected blood samples from the jugular vein of the sheep after obtaining written informed consent from each participating farm. Serum samples were taken between September 1, 2009 and April 19, 2010 on dairy sheep farms, while samples on non-dairy sheep farms were taken between April 2, 2010 until April 4, 2011. The laboratory investigation by the Animal Health Service of serum samples taken by private veterinary practitioner is considered part of regular and routine clinical-diagnostic care. Therefore, no official review and approval of the Animal Welfare Commission is needed. The farm questionnaire was sent to all participating farms and completed by the farm owner or manager. It addressed the general farm situation, herd size, housing characteristics, vermin control and manure handling in 2008 (the year before the mandatory hygiene protocol was implemented), general health status and reproductive problems (including abortion rates), breeding information, biosecurity and hygiene measures for own staff and farm visitors.

Laboratory analysis

Individual sheep serum samples were tested with an ELISA test (Ruminant serum Q Fever ELISA kit, Laboratoire Service International, Lissieu, France) on *C. burnetii* specific antibodies with a single 1:400 serum dilution. All steps were carried out according to the instruction of the manufacturer. A sheep was considered ELISA-positive if the optical density per cent was 40 or higher. A farm was considered positive if at least one sheep on the farm was classified ELISA-positive.

Data analysis

Non-response analyses

Participating and non-participating farms were compared with respect to herd size, sheep, goat and cattle density, degree of urbanisation and region where farms were located, to study the representativeness of participating farms. Due to the low numbers of dairy sheep farms, the non-response analyses were limited to comparison of herd size, region and the degree of urbanisation. Categorical variables among participating and non-participating farms were compared with a χ^2, or Fisher exact test, while numerical variables were compared using the Wilcoxon rank sum test.

Descriptive statistics and risk factor analyses

Animal and farm prevalence with corresponding exact 95% CIs were calculated. First, we studied frequency tables of categorical variables and distributions of continuous variables, and if not linearly related to the outcome variable divided into classes based on biological arguments, and if these were lacking based on medians. Because of the small number of participating dairy sheep farms, only risk factors could be determined for the non-dairy sheep farms. Potential risk factors on farm level were analysed by logistic regression (PROC LOGISTIC, SAS Institute, 2004), and on animal level by generalised linear regression analysis accounting for farm effect (PROC GENMOD, SAS Institute, 2004). For the latter, an exchangeable correlation covariance structure fitted best and was used to account for within-herd variation. First, we performed univariate analyses. Variables with a P value below 0.20 in the univariate analyses were included in multivariable analysis. We kept herd size consistently in the model to allow studying the relationship with herd size, which is of interest for developments in the future farm industry. For multivariate analysis on farm level, we excluded variables with less than 10 per cent of data in one risk category. Proxy outcomes for seropositivity, such as BTM status, were not included in multivariate analyses. A backwards elimination procedure was performed until all variables were significant at the 10 per cent significance level in the likelihood ratio test. Two-way interactions between biological plausible and

significant variables in the multivariate model were investigated. For the final model on farm level, model fit was assessed with the Hosmer-Lemeshow Goodness-of-fit test. Model fit on animal level was assessed by the QIC (Quasilikelihood under the Independence Model Criterion) goodness-of-fit statistic for generalized estimation equation models.

Results

Non-response analyses and descriptive characteristics

Dairy sheep farms

In total, 14 of the 33 dairy sheep farms (43.8 per cent) participated in the study (Fig 1). The number of sheep on the participating dairy farms was up to 1163 sheep, with a median of 438 sheep. The participating dairy farms were all situated in a rural area (less than 500 addresses per km^2). Participating and non-participating dairy farms were comparable with regard to urbanisation degree and province distribution. However, participating farms had a median number of 470 sheep (range 143–1163) versus the significantly lower median of 346 sheep (range 96–730) for non-participating farms (P=0.05). A questionnaire was available for 12 of the 14 dairy sheep farms. In the national BTM monitoring programme, two participating dairy sheep farms and one non-participating farm had tested *C. burnetii* PCR-positive.

Non-dairy sheep farms

Of the 1344 non-dairy sheep farms, 32 were excluded from participation due to complete vaccination in 2008 or a herd size <60 sheep. Of the remaining 1312 farms, 95 non-dairy sheep farms (7.2 per cent) participated (Fig 2). The number of sheep on the participating non-dairy sheep farms was up to 1310 sheep, with a median of 190 sheep. Almost all (90.5 per cent) were located in rural areas. Participating and non-participating non-dairy farms were comparable with regard to urbanisation degree, regional and province distribution, herd size, sheep density within 10 km radius, and goat density within 5 km and 10 km farm radius and municipal cattle density. A significant difference was found for sheep density in the 5 km radius of participating and non-participating farms, 35.9 (range 3.0–143.6) and 47.2 (range 1.0–162.9) sheep per km^2 in the 5 km radius (excluding own sheep, respectively (P=0.02). A farm questionnaire was available for 93 of the 95 non-dairy sheep farms. Of these 93, 79 farms (84.9 per cent) held sheep for meat production, 45 (48.4 per cent) for breeding, and 15 farms (16.1 per cent) for nature management. The most common breeds were Texel (59.1 per cent) and Swifter (41.9 per cent). Besides non-dairy sheep, other farm animals such as horses (on 28 farms, 30.1 per cent), dairy cattle (on 24 farms, 25.8 per cent), beef cattle and goats (on 15 farms each, 16.1 per cent) were present during the stable period of the non-dairy sheep. The farms mainly started after 1950 (90.5 per cent).

Seroprevalence on dairy and non-dairy sheep farms

In total, 953 sheep serum samples were available from the 14 participating dairy sheep farms. Farm prevalence was 78.6% (95% CI 51.9% to 95.7%), the overall dairy sheep seroprevalence was 18.7% (95% CI 16.3% to 21.3%) while the within-herd seroprevalence of the 11 positive farms was 23.5% (95% CI 20.5% to 26.7%) and ranged between 3.3 per cent and 80.0 per cent (Table 1). In total, 5671 sheep serum samples from 95 non-dairy sheep farms were available. Farm prevalence among non-dairy sheep farms was 30.5% (95% CI 21.5% to 40.8%). Overall non-dairy sheep seroprevalence was 2.0% (95% CI 1.6% to 2.4%), and among the 29 positive farms the within-herd prevalence was 6.4% (95% CI 5.3% to 7.7%) and ranged between 1.6 per cent and 46.7 per cent (Table 1).

Univariate analyses on farm level

Among non-dairy sheep farms, risk factors associated with farm seropositivity in the univariate analyses (P<0.20) were farm location in the south of The Netherlands, farm location in the mandatory vaccination area of 2009, municipal cattle density of ≥135 cows per km^2, presence of one or more *C. burnetii*-positive dairy small ruminant farms based on BTM screening within 10 km radius, distance to the closest *C. burnetii* PCR-positive dairy small ruminant farm based on BTM screening, goat density of ≥5.3 goats per km^2 within 10 km radius, total number of sheep in 2010, presence of male lambs, presence of Swifter breed, sheep kept on own farmhouse pasture, on owned pasture, on a pasture owned by someone else, and on marginal grounds, feeding of pressed pulp, type of feed method (using a feed mixer), number of ewes that lambed in 2009, number of yearlings that lambed in 2009, number of stillborn lambs in 2009, number of annual sheep supply addresses in 2007–2009, sheep supply from the provinces of Groningen and Zuid-Holland and the use of farm boots and/or outfits by professional visitors as hygiene measure, while having a dog on the farm seemed to be a protective factor (Table 2).

Multivariate multilevel analysis on farm level

In the multivariate analyses, of the 14 univariately associated variables that were included in the multivariate farm-based analyses, four were independently associated with farm seropositivity. Farms were significantly more positive if they were located in the southern region of the country, had sheep grazing locations at marginal grounds, had one or more supply addresses for ewes during 2007–2009, and reported that professional farm visitors wear farm boots and/or outfit as hygiene measure (Table 3).

Univariate risk factor analyses on individual sheep level

Among non-dairy sheep farms, risk factors (P<0.20) associated with individual sheep seropositivity were farm location in the southern region, presence of one or more *C. burnetii* PCR-positive dairy small ruminant farms based on BTM screening within 10 km farm radius, sheep density within 5 km radius, goat density ≥5.3 goats within 10 km radius, breeding of lambs as main farm purpose, sheep location on own farmhouse pasture, sheep kept on heathland, use of windbreak curtain and/or windshields in the stable, not knowing or no signs of vermin in roughage during the last 12 months, lambs that only receive colostrum from the mother versus not only or no colostrum from the mother, annual replacement percentage, ≥6 stillborn lambs in 2009, lambing in the pasture and wearing farm boots or outfit by professional farm visitors, while having a pet rabbit seemed to be a protective factor (Table 4).

Multivariate multilevel analysis on individual sheep level

Of the 14 univariately associated individual variables, six were independently associated with individual sheep seropositivity: farm location in the southern region of the country, a goat density of ≥5.3 goats within 10 km farm radius, breeding of lambs as main farm purpose, not knowing, or no signs of vermin in roughage during the last 12 months and ≥6 stillborn lambs in 2009. The use of windbreak curtain and/or windshields in the stable seems also significant, although

TABLE1: Farm and sheep *Coxiella burnetii* seroprevalence among non-dairy and dairy sheep farms with ≥ 100 ewes, 2009-2010, The Netherlands

Sheep	Seropositive		Seronegative			
	n	Per cent	n	Per cent	Total	95% CI
Dairy	178	18.7	775	81.3	953	16.3 to 21.3
Non-dairy	111	2.0	5,560	98.0	5,671	1.6 to 2.4
Farm (herd) prevalence on dairy sheep farms	11	78.6	3	21.4	14	51.9 to 95.7
Farm (herd) prevalence on non-dairy sheep farms	29	30.5	66	69.5	95	21.5 to 40.8
Within-herd prevalence on positive dairy sheep farms	178	23.5	580	76.5	758	20.5 to 26.7
Within-herd prevalence on positive non-dairy sheep farms	111	6.4	1,619	93.6	1,730	5.3 to 7.7

TABLE 2: Univariate logistic regression of farm-based factors related to *Coxiella burnetii* seropositivity on non-dairy sheep farm level (p<0.20)), 2010-2011, The Netherlands

Variable	Category	Frequency (n)	Seroprevalence (%)	OR	95% CI	–2LL P value
Farm location per region*	North (Dr, Fr, Gro)	26	30.8	2.16	(0.67 to 6.92)	0.037
	East (Ge, Ov, Fle)	18	44.4	3.89	(0.13 to 13.37)	
	South (Nb, Li)	10	60.0	7.29	(1.62 to 32.79)	
	West (Ut, Ze, Nh, Zh)	41	17.1	Ref.		
Farm location in mandatory vaccination area (2009)	Yes	13	69.2	6.98	(1.94 to 25.11)	0.003
	No	82	24.4	Ref.		
Cattle density (number of cattle/km² in the municipality)*	<135.0	47	23.4	Ref.		0.139
	≥135.0	48	37.5	1.96	(0.80 to 4.80)	
Number of *C. burnetii* PCR-positive dairy small ruminant farms in 10 km radius based on BTM screening*	0	61	24.6	Ref.		0.095
	1–4	34	41.2	2.15	(0.88 to 5.27)	
Closest *C. burnetii* PCR-positive dairy small ruminant farms based on BTM screening (km)	<5.0	11	36.4	Ref.		0.159
	5.0–9.9	23	43.5	1.35	(0.31 to 5.91)	
	10.0–14.9	18	22.2	0.50	(0.10 to 2.62)	
	15.0–19.9	16	43.8	1.36	(0.28 to 6.58)	
	20.0	27	14.8	0.30	(0.06 to 1.54)	
Goat density (number of goats/km² in 10 km radius)*	<5.3	47	21.3	Ref.		0.056
	≥5.3	48	39.6	2.42	(0.98 to 6.00)	
Number of non-dairy sheep in 2010*	<225	69	24.6	Ref.		0.046
	≥225	26	46.2	2.62	(1.02 to 6.75)	
Male lambs present on farm	Yes	14	57.1	4.00	(1.22 to 13.09)	0.071
	No	72	25.0	Ref.		
	Missing	9	33.3	1.50	(0.34 to 6.62)	
Swifter breed present on farm*	Yes	39	41.0	2.38	(0.96 to 5.88)	0.173
	No	53	22.6	Ref.		
	Missing	3	33.3	1.71	(0.14 to 20.51)	
Sheep kept on farm house pasture*	Yes	73	37.0	5.87	(1.27 to 27.09)	0.023
	Not applicable/missing	22	9.1	Ref.		
Sheep kept on owned pasture*	Yes	73	34.3	2.34	(0.72 to 7.68)	0.159
	Not applicable/missing	22	18.2	Ref.		
Sheep kept on pasture owned by someone else*	Yes	48	39.6	2.42	(0.98 to 6.00)	0.056
	Not applicable/missing	47	21.3	Ref.		
Sheep kept on marginal grounds*	Yes	24	58.3	5.23	(1.94 to 14.09)	0.001
	Not applicable/missing	71	21.1	Ref.		
Dog present on farm	Yes	59	25.4	Ref.		0.197
	No	34	38.2	1.87	(0.77 to 4.55)	
	Missing	2	50.0	2.93	(0.17 to 49.85)	
Feeding of pressed pulp*	Yes	10	50.0	2.73	(0.72 to 10.34)	0.154
	No	82	26.8	Ref.		
	Missing	3	66.7	5.46	(0.47 to 63.19)	
Number of ewes which lambed in 2009	<125	52	21.2	0.46	(0.16 to 1.27)	0.134
	125–224	27	37.0	Ref.		
	≥225	11	54.6	2.04	(0.49 to 8.45)	
	Missing	5	40.0	1.13	(0.16 to 7.98)	
Number of yearlings which lambed in 2009	<40	74	25.7	Ref.		0.166
	≥40	17	47.1	2.57	(0.87 to 7.62)	
	Missing	4	50.0	2.89	(0.38 to 22.00)	
Number of stillborn lambs (in 2009)*	≤5	18	11.1	Ref.		0.130
	6–12	31	25.8	2.78	(0.52 to 14.87)	
	≥13	40	42.5	5.91	(120 to 29.23)	
	Missing	4	33.3	4.00	(0.42 to 37.78)	
Number of annual supply addresses for ewes during 2007–2009*	No supply/missing	61	19.7	Ref.		0.023
	1	18	55.6	5.10	(1.66 to 15.70)	
	2 or more	13	46.2	3.50	(0.99 to 12.34)	
	Missing	3	33.3	2.04	(0.17 to 24.43)	
Sheep supplied from Groningen Province	Yes	10	50.0	2.52	(0.67 to 9.54)	0.173
	No	81	28.4	Ref.		
	Missing	4	25.0			
Sheep supplied from Zuid-Holland Province	Yes	12	58.3	3.87	(1.11 to 13.52)	0.103
	No	79	26.6	Ref.	(0.09 to 9.35)	
	Missing	4	25.0	0.92		
Farm boots and/or outfit as hygiene measures applied for professional farm visitors*	Yes	45	42.2	3.17	(1.24 to 8.07)	0.047
	No	48	18.8	Ref.		
	Missing	2	50.0	4.33	(1.25 to 76.05)	

*Variables included in subsequent multivariate analyses before manual backward elimination.
–2LL, likelihood ratio test; BTM, bulk tank milk; Dr, Drenthe; Fle, Flevoland; Fr, Friesland; Ge, Gelderland; Gro, Groningen; Li, Limburg provinces; Nb, Noord-Brabant; Nh, Noord-Holland; Ov, Overijssel; Ut, Utrecht; Ze, Zeeland; Zh, Zuid-Holland Provinces.

this variable only applied to the subset of 83 farms which used a stable (Table 5).

Discussion

Seroprevalence in dairy and non-dairy sheep

In dairy and non-dairy sheep, the prevalence on animal level was 18.7 per cent and 2.0 per cent, while herd prevalences were 78.6 per cent and 30.5 per cent, respectively. Compared with the 2008 figures based on samples collected for the mandatory *Brucella melitensis* monitoring (Van den Brom and others 2013), the non-dairy sheep seroprevalence remained stable (2.4 per cent in 2008). The within-herd prevalence in non-dairy sheep decreased from 14.7 per cent in the 2008 study to 6.4 per cent in 2009–2010, which fits with a low perceived risk for zoonotic spread from non-dairy sheep farms to neighbouring areas.

Despite the stable individual seroprevalence in non-dairy sheep, the herd prevalence among non-dairy sheep farms was 14.2 per cent in 2008 and 30.5 per cent in 2009–2010. This could be a true increase, but because of the stable overall seroprevalence in the sheep, a more likely explanation is the difference in sample size as a median of 60 sheep per farm were tested in 2009–2010, while only 13 sheep per farm were tested in 2008. In dairy sheep, the individual seroprevalence increased from 5.7 per cent in 2008 to 18.7 per cent in 2009–2010, and at herd level from 38.5 per cent in 2008 to 78.6 per cent in 2009–2010, while the within-herd prevalence of positive farms remained stable. A similar increase in herd and animal prevalence over the same period was observed on dairy goat farms (Schimmer and others 2011).

Dairy sheep are thus more likely to be seropositive than non-dairy sheep, which is probably due to different housing conditions between both sheep types, a generally larger herd size for dairy sheep, as well as differences in susceptibility for infections as suggested earlier (Van den Brom and others 2013). Dairy sheep also have higher within-herd prevalences reflecting more circulation of *C. burnetii* within a dairy sheep farm and a risk for environmental contamination and spread (Schimmer and others 2011, Van den Brom and others 2013). Other ruminant studies also report a higher prevalence of *C. burnetii* antibodies in dairy breeds than in beef breeds, in this paper referred to as non-dairy breeds. Environmental investigations among a subset of non-dairy sheep farms included in this study showed that the percentage of positive samples and the *C. burnetii* DNA load was significantly higher for farms with seropositive sheep compared to farms with seronegative sheep, but were clearly lower in comparison to a similar study on dairy goat farms (de Bruin and others 2012). This supports their relatively limited role in the transmission of *C. burnetii* to the community in their vicinity during the Q fever epidemic in The Netherlands. It might also partially explain the relatively lower observed seroprevalence among the non-dairy sheep farm households compared to dairy sheep farm households (De Lange and others 2013) and dairy goat farm households (Schimmer and others 2012).

The observed non-dairy sheep *C. burnetii* seroprevalence in The Netherlands is generally low. The observed sheep prevalence is comparable to a study in Germany, with a prevalence of 2.7 per cent in 1714 tested sheep from 95 flocks (Runge and others 2012), a prevalence of 3.1 per cent in Canada (Hatchette and others 2002) and of 3.1 per cent in Albania (Cekani and others 2008), while higher seroprevalences in sheep, around 10 per cent or above, were reported in Portugal, Cyprus, Greece, Northern Ireland, Italy, Spain and Japan (Masala and others 2004, Psaroulaki and others 2006, Garcia-Perez and others 2009, Pape and others 2009, McCaughey and others 2010, Ruiz-Fons and others 2010, Giangaspero and others 2012, Anastacio and others 2013).

Also, our observed herd prevalence of 30.5 per cent was comparable to the 28 per cent seroprevalence in 39 randomly selected healthy sheep flocks with more than 100 ewes in Germany (Hilbert and others 2012). A somewhat higher herd prevalence was found in sheep herds in Portugal (37.5 per cent) (Anastacio and others 2013) and in Sardinia (38 per cent) (Masala and others 2004), while a somewhat lower sheep herd prevalence was found in Canada (21 per cent) (Lang and others 1991). Much higher herd prevalences were reported from Northern Spain (prevalence of 75 per cent) (Ruiz-Fons and others 2010) and Turkey (prevalence of 83.0 per cent) (Kennerman and others 2010), although direct comparison of seroprevalence estimates in different studies is complicated due to substantial differences in study design, laboratory methods and farm characteristics for the tested flocks.

Risk factors

In 2009–2010, we observed a higher seropositivity among non-dairy sheep farms located in the southern region of the country, while no difference in seroprevalence was observed per region in 2008 (Van den Brom and others 2013). This result, together with the association with goat density within 10 km radius supports the potential role of transmission from infected dairy goat farms to the non-dairy sheep in the vicinity, as the dairy goat density and epidemic spread among small ruminants was clearly higher in the south of the country (Dijkstra and others 2012).

On farm level, sheep kept on marginal grounds and one or more supply addresses for ewes were independent risk factors. These factors are most likely a proxy for movements between herds (Paul and others 2013), which is a known risk factor for introduction of zoonotic pathogens. Other explanations why sheep grazing on marginal grounds had a higher seropositivity could be the presence of other livestock on the same grounds as a proxy for increased risk of introduction of pathogens from the environment into the flock. Other factors, such as the number and different type of grazing locations used were not investigated with the questionnaire. Wearing farm boots or outfit by professional farm visitors was also an independent risk factor. This association can only be explained satisfactorily if these professional visitors took their own boots or outfits to the farm, which then can facilitate transmission between herds if not changed between farms, as observed in a Q fever outbreak with person-to-person spread by farm outfits (Marrie and others 1989). Unfortunately, the questionnaire did not specify the origin of the clothes and boots worn by the professional visitors to substantiate this with data. However, as non-dairy sheep are mainly kept outside on meadows where no dedicated farm outfits can be properly stored, this was considered a plausible explanation. All these risk factors suggest introduction of the bacterium into the farm, stressing the importance of good biosecurity. On individual sheep level, besides farm location in the southern region of the country and higher goat density, other risk factors included breeding of lambs as main farm purpose and presence of ≥6 stillborn lambs in 2009. These factors might represent conditions facilitating acquisition of infectious diseases and within farm spread. Sheep breeding being the main purpose of the farm could reflect the intensity of annual animal movements and reproduction levels, possibly leading to a higher susceptibility for infections. Half the farms (51 per cent) with breeding as the main farm purpose had annual replacement percentages of 25 per cent or higher, while only 32 per cent of the farms with other main farm purposes reported such percentages. Besides, an increasing number of stillborn lambs might indicate presence of other infectious pathogens, possibly leaving sheep more vulnerable for other infections as well. The main infectious causes of abortion in sheep in

TABLE 3: Multivariate logistic regression analysis of farm-based factors associated with *Coxiella burnetii* seropositivity on non-dairy sheep farm level (P<0.10, -2LL), 2010-2011, The Netherlands

Variable	Category	aOR (95% CI)	P value
Number of non-dairy sheep in 2010	≥225	1.31 (0.38 to 4.58)	0.67
	<225	Reference	
Sheep kept on marginal grounds	Yes	7.04 (2.04 to 24.24)	<0.01
	Not applicable/missing	Reference	
Farm region	South	5.19 (1.04 to 25.80)	0.04
	Other	Reference	
Number of annual supply addresses for ewes in 2007–2009	1	3.49 (0.94 to 13.01)	0.08
	≥2	3.89 (0.84 to 17.99)	
	No supply/missing	Reference	
Farm boots and/or outfit as hygiene measures applied for professional farm visitors	Yes	3.12 (0.96 to 10.10)	0.06
	No	Reference	

Number of observations used: 91. AIC=97.21, Hosmer and Lemeshow Goodness-of-fit Test 0.7736.
aOR, adjusted odds ratio; –2LL, likelihood ratio test; AIC, Akaike Information Criterion.

TABLE 4: Univariate logistic regression of farm-based factors related to *Coxiella burnetii* seropositivity on non-dairy sheep level, 2010-2011, The Netherlands

Variable		Frequency (n)	Prev (%)	OR	95% CI	P value
Farm location per region*	North (Dr, Fr, Gro)	1559	1.0	1.25	(0.41 to 3.84)	0.188
	East (Ge, Ov, Fle)	1072	1.9	2.33	(0.78 to 6.90)	
	South (Nb, Li)	602	9.3	12.58	(3.23 to 49.08)	
	West (Ut, Ze, Nh, Zh)	2438	0.8	Ref.		
Farm location in mandatory vaccination area (2009)	Yes	784	8.3	6.98	(3.38 to 26.31)	0.061
	No	4887	0.9	Ref.		
Number of *C. burnetii* PCR- positive dairy small ruminant farm in 10 km radius based on BTM screening*	0	3636	1.0	Ref.	(1.40 to 10.14)	0.088
	1 or more	2035	3.7	3.76		
Closest *C. burnetii* PCR-positive dairy small ruminant farm based on BTM screening (km)	<5.0	650	1.4	Ref.	(1.03 to 12.47)	0.079
	5.0–9.9	1385	4.8	3.58	(0.20 to 2.63)	
	10.0–14.9	1081	1.0	0.73	(0.51 to 4.46)	
	15.0–19.9	945	2.0	1.51	(0.07 to 0.95)	
	≥20.0	1610	0.4	0.27		
Sheep density (number of sheep per km² in 5 km radius)*	<35.9	2794	2.8	Ref.	(0.16 to 1.18)	0.191
	≥35.9	2877	1.2	0.43		
Goat density (number of goats per km² in 10 km radius)*	<5.3	2843	0.7	Ref.	(1.67 to 11.67)	0.036
	≥5.3	2828	3.2	4.42		
Total number of sheep in 2010*	<225	4094	1.8	Ref.		0.559
	≥225	1577	2.4	1.37	(0.47 to 4.00)	
Lambs present in 2010	Yes	975	2.8	1.39	(0.50 to 3.85)	0.184
	No	4203	1.9	Ref.		
	Missing	493	0.8	0.43	(0.12 to 1.51)	
Breeding lambs as main farm purpose*	Yes	2702	3.3	4.56	(1.83 to 11.37)	0.111
	No	2780	0.8	Ref.		
	Missing	189	1.1	1.50	(0.27 to 8.32)	
Sheep kept on farm house pasture*	Yes	4381	2.3	2.78	(0.43 to 17.96)	0.169
	Not applicable/missing	1290	0.9	Ref.		
Sheep kept on heathland*	Yes	571	0.7	0.32	(0.08 to 1.26)	0.100
	Not applicable/missing	5100	2.1	Ref.		
Use of windbreak curtain and/or wind-shields in the stable*	Yes	1402	3.6	2.12	(0.59 to 7.57)	0.109
	None	3536	1.6	Ref.		
	No stable/missing	733	0.4	0.25	(0.06 to 1.07)	
Rabbit present on farm*	Yes	1197	0.9	0.41	(0.14 to 1.19)	0.126
	No/missing	4474	2.2	Ref.		
Traces of vermin in roughage (past 12 months)*	Yes	2252	0.6	Ref.		0.133
	No	1882	2.5	3.82	(1.43 to 10.29)	
	Do not know	1229	3.7	5.69	(1.41 to 23.07)	
	Missing	308	1.3	2.01	(0.55 to 7.31)	
Lambs receiving colostrum from own mother*	Yes, only from mother	3541	2.7	Ref.		0.121
	Yes, but not only	1818	0.7	0.27	(0.10 to 0.68)	
	No/missing	312	1.3	0.50	(0.14 to 1.80)	
Replacement rate (in %)	<16	1076	2.0	0.62	(0.16 to 2.44)	0.132
	16–25	1743	0.5	0.16	(0.05 to 0.48)	
	>25	2295	3.2	Ref.		
	Missing	557	1.1	0.33	(0.33 to 6.20)	
Lambing outside in pasture*	Yes	546	0.4	0.17	(0.02 to 1.18)	0.141
	No	4996	2.1	Ref.		
	Missing	129	1.6	0.77	(0.16 to 3.61)	
Number of stillborn lambs (in 2009)*	≤5	1051	0.2	Ref.		0.074
	6–12	1473	1.4	8.02	(1.56 to 41.21)	
	≥13	2779	3.1	16.84	(3.77 to 75.31)	
	Missing	368	0.8	4.47	(0.74 to 27.01)	
Farm boots and/or outfit as hygiene measures applied for professional farm visitors*	Yes	2686	3.0	3.09	(1.01 to 9.39)	0.242
	No	2856	1.0	Ref.		
	Missing	129	1.6	1.67	(0.33 to 8.60)	

Factors associated with *Coxiella burnetii* seropositivity with their frequency (n), animal prevalence (Prev), odds ratio (OR); and 95% CI with P value of the univariable generalised linear regression <0.20 on animal level accounting for random herd effect (5671 non-dairy sheep from 95 farms).
*Variables included in subsequent multivariate analyses before manual backward elimination
BTM, bulk tank milk; Dr, Drenthe; Fle, Flevoland; Fr, Friesland; Ge, Gelderland; Gro, Groningen; Li, Limburg provinces; N, total number of individuals; Nb, Noord-Brabant; Nh, Noord-Holland; Ov, Overijssel; Prev, prevalence; Ut, Utrecht; Ze, Zeeland; Zh, Zuid-Holland Provinces.

The Netherlands are due to *Chlamydia abortus*, *Campylobacter* species, *Toxoplasma gondii*, *Listeria* species and *Yersinia pseudotuberculosis* (van den Brom and others 2012), although abortions due to *C. burnetii* cannot be excluded, as partially, they might more directly explain the identified risk. Not knowing, or no traces of vermin in roughage, was a risk factor on animal level for dairy goats as well (Schimmer and others 2011), although it is unclear why. The use of windbreak curtain and/or windshields in the stable was a possible risk factor in the final model applicable to farms with a stable. This same risk factor on animal level was found for dairy goats (Schimmer and others 2011), which possibly points to a more open stable, facilitating transmission of *C. burnetii* inside the stable, which may again promote spread within the herd.

Limitations

In the 2008 convenience survey, sheep tested during pregnancy and in the periparturient period, had a somewhat higher seroprevalence (3.3 per cent v 1.2 per cent in early or non-pregnant sheep), which is explained by massive *C. burnetii* multiplication in pregnant animals (Van den Brom and others 2013). As sampling of sheep is easier when they are in the stable, we assume that many of the sampled animals were in their pregnant or periparturient period, although we did not register their pregnancy status to confirm this. In theory, this might have caused an overestimation of the overall seroprevalence. However, as the observed seroprevalence among the non-dairy sheep was the same than that obtained in the year-round convenience sample, the effect seems to have been negligible.

TABLE 5: Multivariable logistic regression of farm-based factors associated with *Coxiella burnetii* seropositivity on non-dairy sheep lever, 2010-2011, The Netherlands*

Variable	Category	aOR (95% CI)	P value
Number of non-dairy sheep	≥225	0.95 (0.49 to 1.83)	0.88
	<225	Ref.	
Farm region	South	7.33 (3.99 to 13.47)	0.04
	Other	Ref.	
Goat density (number of goats per km² in 10 km farm radius)	≥5.3	2.39 (1.09 to 5.26)	0.04
	<5.3	Ref.	
Breeding lambs as main farm purpose	Yes	2.72 (1.29 to 5.72)	0.02
	No	Ref.	
Use of windbreak curtain and/or windshields in stable	Yes	2.52 (1.15 to 5.54)	0.16
	None	Ref.	
	No stable including missing category	1.50 (0.14 to 15.85)	
Traces of vermin in roughage last 12 months	Yes	Ref.	0.02
	No	5.61 (2.01 to 15.68)	
	Don't know	3.41 (1.48 to 7.89)	
Number of stillborn lambs (in 2009)	≤5	Ref.	0.06
	6-12	6.59 (1.14 to 38.08)	
	≥13	15.93 (2.71 to 93.47)	
	Missing	4.07 (0.19 to 87.91)	

Factors associated with Q fever with their frequency (N), animal prevalence (Prev), adjusted odds ratio (aOR) with corresponding 95% confidence interval (95% CI) in the final multivariable model on animal level with random herd effect.
*Clustered data is taken into account with the unique farm number used as cluster variable. Number of observations used: 5180. Number of levels used: 89. QIC=823.356
–2LL, likelihood ratio test; QIC, Quasilikelihood under the Independence Model Criterion.

Only 7.2 per cent of the approached non-dairy sheep farms participated in the study. Main reasons for this low response are probably the labour-intensive sheep sampling, as reported by several farmers not willing to participate, because sheep were outside and had to be captured for sampling. Additionally, this part of the sheep industry was not affected by the implemented control measures, which were mainly targeted at dairy small ruminant farms. Therefore, non-dairy sheep farmers a priori might have been less motivated to participate compared to the small dairy sheep sector, which had a response rate of 44 per cent. The low response rate might have caused selection bias, but except for a higher sheep density around the non-participating farms, participating and non-participating non-dairy farms appeared to be comparable. Therefore, we consider our estimates representative for the Dutch professional non-dairy sheep industry. Participating dairy sheep farms had a higher median herd size compared to non-participating dairy farms. Although we do not know if herd size is related to seropositivity in Dutch dairy sheep, associations between increased herd size to seropositive results have been shown in dairy goats (Schimmer and others 2011), cattle (McCaughey and others 2010) and mainly meat-producing sheep herds (Anastacio and others 2013). The overall seroprevalence and herd prevalence might, therefore, be overestimated on dairy sheep farms included in our study.

In conclusion, we observed a higher herd and animal prevalence among dairy sheep farms of 78.6 per cent, and 18.7 per cent, respectively, compared to herd and animal prevalence of 30.5 per cent and 2.0 per cent among non-dairy sheep farms, indicating a lower risk for infection by individual animals as well as for zoonotic spread from non-dairy sheep. Besides, comparison with 2008 figures, *C. burnetii* has further spread in the dairy sheep sector since 2008, unlike the non-dairy sheep sector where the seroprevalence remained stable around 2.0 per cent at animal level. The identified risk factors 'farm location in a southern province' and 'higher goat density' point to infected dairy goat farms that have played a role in the transmission to non-dairy sheep farms during the Dutch Q fever epidemic from 2007–2009. Other factors suggest *C. burnetii* introduction on farms through sheep supply, work boots or outfits worn by professional visitors and sheep location on marginal grounds, possibly facilitating contact between different herds. On animal level, farm-specific characteristics, such as an increasing number of stillborn lambs, breeding lambs as the primary farm purpose, and use of windbreak curtains and/or windshields in the stable, seem to represent factors facilitating acquisition of infections and further within-farm spread.

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References

ANASTACIO, S., TAVARES, N., CAROLINO, N., SIDI-BOUMEDINE, K. & DA SILVA, G. J. (2013) Serological evidence of exposure to Coxiella burnetii in sheep and goats in central Portugal. *Veterinary Microbiology* **167**, 500–505

ÇEKANI, M., PAPA, A., KOTA, M., VELO, E. & BERXHOLI, K. (2008) Report of a serological study of Coxiella burnetii in domestic animals in Albania. *Veterinary Journal* **175**, 276–278

DE BRUIN, A., VAN DER PLAATS, R. Q., DE HEER, L., PAAUWE, R., SCHIMMER, B., VELLEMA, P., VAN ROTTERDAM, B. J. & VAN DUYNHOVEN, Y. T. (2012) Detection of Coxiella burnetii DNA on small-ruminant farms during a Q fever outbreak in the Netherlands. *Applied and Environmental Microbiology* **78**, 1652–1657

DE LANGE, M. M., SCHIMMER, B., VELLEMA, P., HAUTVAST, J. L., SCHNEEBERGER, P. M. & VAN DUIJNHOVEN, Y. T. H. P. (2013) *Coxiella burnetii* seroprevalence and risk factors in sheep farmers and farm residents in The Netherlands. *Epidemiol Infect* 1–14

DIJKSTRA, F., VAN DER HOEK, W., WIJERS, N., SCHIMMER, B., RIETVELD, A., WIJKMANS, C. J., VELLEMA, P. & SCHNEEBERGER, P. M. (2012) The 2007–2010 Q fever epidemic in The Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. *FEMS Immunology and Medical Microbiology* **64**, 3–12

GARCIA-PEREZ, A. L., ASTOBIZA, I., BARANDIKA, J. F., ATXAERANDIO, R., HURTADO, A. & JUSTE, R. A. (2009) Short communication: investigation of Coxiella burnetii occurrence in dairy sheep flocks by bulk-tank milk analysis and antibody level determination. *Journal of Dairy Science* **92**, 1581–1584

GIANGASPERO, M., OSAWA, T., BONFINI, B., ORUSA, R., ROBETTO, S. & HARASAWA, R. (2012) Serological screening of Coxiella burnetii (Q fever) and Brucella spp. in sheep flocks in the northern prefectures of Japan in 2007. *Veterinary Italiano* **48**, 357–365

GRILC, E., SOCAN, M., KOREN, N., UCAKAR, V., AVSIC, T., POGACNIK, M. & KRAIGHER, A. (2007) Outbreak of Q fever among a group of high school students in Slovenia, March-April 2007. *Euro Surveillance* **12**, E070719 070711

HATCHETTE, T., CAMPBELL, N., WHITNEY, H., HUDSON, R. & MARRIE, T. J. (2002) Seroprevalence of Coxiella burnetii in selected populations of domestic ruminants in Newfoundland. *The Canadian Veterinary Journal* **43**, 363–364

HILBERT, A., SCHMOOCK, G., LENZKO, H., MOOG, U., DILLER, R., FROHLICH, A., HOFFMANN, L., HORNER, S., ELSCHNER, M., TOMASO, H., HENNING, K., NEUBAUER, H. & SPRAGUE, L. D. (2012) Prevalence of Coxiella burnetii in clinically healthy German sheep flocks. *BMC Research Notes* **5**, 152

KENNERMAN, E., ROUSSET, E., GOLCU, E. & DUFOUR, P. (2010) Seroprevalence of Q fever (coxiellosis) in sheep from the Southern Marmara Region, Turkey. *Comparative Immunology, Microbiology and Infectious Diseases* **33**, 37–45

KOENE, R. P., SCHIMMER, B., RENSEN, H., BIESHEUVEL, M., DE BRUIN, A., LOHUIS, A., HORREVORTS, A., LUNEL, F. V., DELSING, C. E. & HAUTVAST, J. L. (2011) A Q fever outbreak in a psychiatric care institution in The Netherlands. *Epidemiology and Infection* **139**, 13–18

LANG, G., WALTNER-TOEWS, D. & MENZIES, P. (1991) The seroprevalence of coxiellosis (Q fever) in Ontario sheep flocks. *Canadian Journal of Veterinary Research* **55**, 139–142

LYYTIKAINEN, O., ZIESE, T., SCHWARTLANDER, B., MATZDORFF, P., KUHNHEN, C., JAGER, C. & PETERSEN, L. (1998) An outbreak of sheep-associated Q fever in a rural community in Germany. *European Journal of Epidemiology* **14**, 193–199

MARRIE, T. J., LANGILLE, D., PAPUKNA, V. & YATES, L. (1989) Truckin' pneumonia—an outbreak of Q fever in a truck repair plant probably due to aerosols from clothing contaminated by contact with newborn kittens. *Epidemiology and Infection* **102**, 119–127

MASALA, G., PORCU, R., SANNA, G., CHESSA, G., CILLARA, G., CHISU, V. & TOLA, S. (2004) Occurrence, distribution, and role in abortion of Coxiella burnetii in sheep and goats in Sardinia, Italy. *Veterinary Microbiology* **99**, 301–305

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

MUSKENS, J., VAN ENGELEN, E., VAN MAANEN, C., BARTELS, C. & LAM, T. J. (2011) Prevalence of Coxiella burnetii infection in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA. *The Veterinary Record* **168**, 79

PAPE, M., BOUZALAS, E. G., KOPTOPOULOS, G. S., MANDRAVELI, K., ARVANITIDOU-VAGIONA, M., NIKOLAIDIS, P. & ALEXIOU-DANIEL, S. (2009) The serological prevalence of Coxiella burnetii antibodies in sheep and goats in northern Greece. *Clinical Microbiology and Infection* **15**(Suppl 2), 146–147

PAUL, S., AGGER, J. F., AGERHOLM, J. S. & MARKUSSEN, B. (2014) Prevalence and risk factors of *Coxiella burnetii* seropositivity in Danish beef and dairy cattle at slaughter adjusted for test uncertainty. *Prev Vet Med* **113**, 504–511

PORTEN, K., RISSLAND, J., TIGGES, A., BROLL, S., HOPP, W., LUNEMANN, M., VAN TREECK, U., KIMMIG, P., BROCKMANN, S. O., WAGNER-WIENING, C., HELLENBRAND, W. & BUCHHOLZ, U. (2006) A super-spreading ewe infects hundreds with Q fever at a farmers' market in Germany. *BMC Infectious Diseases* **6**, 147

PSAROULAKI, A., HADJICHRISTODOULOU, C., LOUKAIDES, F., SOTERIADES, E., KONSTANTINIDIS, A., PAPASTERGIOU, P., IOANNIDOU, M. C. & TSELENTIS, Y. (2006) Epidemiological study of Q fever in humans, ruminant animals, and ticks in Cyprus using a geographical information system. *European Journal of Clinical Microbiology & Infectious Diseases* **25**, 576–586

ROEST, H. I., TILBURG, J. J., VAN DER HOEK, W., VELLEMA, P., VAN ZIJDERVELD, F. G., KLAASSEN, C. H. & RAOULT, D. (2011) The Q fever epidemic in The Netherlands: history, onset, response and reflection. *Epidemiology and Infection* **139**, 1–12

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 Veterinary Research* **6**, 3

RUNGE, M., BINDER, A., SCHOTTE, U. & GANTER, M. (2012) Investigations concerning the prevalence of Coxiella burnetii and Chlamydia abortus in sheep in correlation with management systems and abortion rate in Lower Saxony in 2004. *Berliner und Munchener tierarztliche Wochenschrift* **125**, 138–143

SCHIMMER, B., LENFERINK, A., SCHNEEBERGER, P., AANGENEND, H., VELLEMA, P., HAUTVAST, J. & VAN DUYNHOVEN, Y. (2012) Seroprevalence and risk factors for Coxiella burnetii (Q fever) seropositivity in dairy goat farmers' households in The Netherlands, 2009–2010. *PLoS ONE* **7**, e42364

SCHIMMER, B., LUTTIKHOLT, S., HAUTVAST, J. L., GRAAT, E. A., VELLEMA, P. & DUYNHOVEN, Y. T. (2011) Seroprevalence and risk factors of Q fever in goats on commercial dairy goat farms in the Netherlands, 2009–2010. *BMC Veterinary Research* **7**, 81

THOMAS, D. R., TREWEEK, L., SALMON, R. L., KENCH, S. M., COLEMAN, T. J., MEADOWS, D., MORGAN-CAPNER, P. & CAUL, E. O. (1995) The risk of acquiring Q fever on farms: a seroepidemiological study. *Occupational and Environmental Medicine* **52**, 644–647

VAN DEN BROM, R., LIEVAART-PETERSON, K., LUTTIKHOLT, S., PEPERKAMP, K., WOUDA, W. & VELLEMA, P. (2012) Abortion in small ruminants in the Netherlands between 2006 and 2011. *Tijdschrift voor diergeneeskunde* **137**, 450–457

VAN DEN BROM, R., MOLL, L., VAN SCHAIK, G. & VELLEMA, P. (2013) Demography of Q fever seroprevalence in sheep and goats in The Netherlands in 2008. *Prev Vet Med* 109, 76–82.

VAN DER HOEK, W., VAN DE KASSTEELE, J., BOM, B., DE BRUIN, A., DIJKSTRA, F., SCHIMMER, B., VELLEMA, P., TER SCHEGGET, R. & SCHNEEBERGER, P. M. (2012) Smooth incidence maps give valuable insight into Q fever outbreaks in The Netherlands. *Geospat Health* **7**, 127–134

WHELAN, J., SCHIMMER, B., DE BRUIN, A., VAN BEEST HOLLE, M. R., VAN DER HOEK, W. & TER SCHEGGET, R. (2012) Visits on 'lamb-viewing days' at a sheep farm open to the public was a risk factor for Q fever in 2009. *Epidemiology and Infection* **140**, 858–864

CHAPTER 4.5

Coxiella burnetii seroprevalence and risk for humans on dairy cattle farms, The Netherlands, 2010-2011

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Coxiella burnetii Seroprevelance and Risk for Humans on Dairy Cattle Farms, The Netherlands, 2010-2011

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Q fever, caused by *Coxiella burnetii*, is a recognized occupational infection in persons who have regular contact with ruminants. We determined *C. burnetii* seroprevalence in residents living or working on dairy cattle farms with ≥50 adult cows and identified risk factors for seropositivity. Serum samples from farm residents, including employees, were tested for *C. burnetii* IgG and IgM; seroprevalence was 72.1% overall and 87.2%, 54.5%, and 44.2% among farmers, spouses, and children, respectively. Risk factors included farm location in southern region, larger herd size, farm employment, birds in stable, contact with pigs, and indirect contact with rats or mice. Protective factors included automatic milking of cows and fully compliant use of gloves during and around calving. We recommend strengthening general biosecurity measures, such as consistent use of personal protective equipment (e.g., boots, clothing, gloves) by farm staff and avoidance of birds and vermin in stables.

Q fever is an occupational zoonosis caused by *Coxiella burnetii*, a gram-negative bacterium (*1*). Ruminant farmers, laboratory workers, dairy workers, and veterinarians are at particular risk for infection. Humans usually acquire Q fever by inhalation of *C. burnetii* aerosolized from contaminated materials originating from infected animals. The primary animal reservoirs responsible for human infections are cattle, sheep, and goats, which can shed *C. burnetii* in urine, feces, milk, and birth products. Before 2007, the seroprevalence of *C. burnetii* antibodies within the general population of the Netherlands was 2.4%; keeping ruminants and increasing age were risk factors for seropositivity (*2*). During 2007–2009, Q fever was a major public health problem in the Netherlands; >4,000 human cases were reported (*3*). Large-scale interventions primarily targeting small ruminants were used to control the epidemic. In 2008, mandatory vaccination was conducted in a defined cluster area and later nationwide. In 2009–2010, a program was implemented to cull pregnant dairy goats and sheep on farms with *C. burnetii*–positive animals identified through a national bulk tank milk (BTM) screening (*4*). Since then, the incidence of acute Q fever cases has diminished substantially (*5*), but chronic cases still occur (*6*). No epidemiologic associations between Q fever cases in humans and dairy cattle were identified during this epidemic, nor have any been described in other Q fever outbreaks (*7*). Nevertheless, recent reports indicate that *C. burnetii* is widespread among Dutch dairy cattle herds (prevalence 78.6% [ELISA] or 56.6% [PCR] in BTM samples) (*8*). In 2008, seroprevalence was 16.0% in lactating cows and 1.0% in young animals (*8*).

C. burnetii seroprevalence estimates for dairy cattle farm residents in the Netherlands are outdated, and risk factors associated with seropositivity are seldom studied. This lack of data inhibits accurate assessment of the public health risk. To inform control measures and provide advice for persons living/working on a dairy cattle farm (DCF), we conducted a cross-sectional study to investigate the seroprevalence of *C. burnetii* antibodies in DCF residents/workers and identified participant-based and farm-based risk factors for seropositivity. The study was approved by the Medical Ethics Committee of the University Medical Centre Utrecht (no. 09–189/K).

Methods

A total of 3,000 DCFs housing ≥50 adult dairy cows were randomly selected for possible participation in the study from a national database maintained by the Animal Health Service. In September and November 2010, information and recruitment materials were sent to 1,000 and 2,000 farms, respectively. Farms were enrolled in the study after returning a completed informed-consent form. After 4 weeks, nonresponding farms from the first mailing received a written reminder. Nonresponding farms from the second mailing did not receive a reminder because the goal of enrolling 296 farms had been reached; this number was determined on the basis of power calculations assuming 50.0% prevalence and 5.5% precision. We contacted enrolled farms by telephone to confirm participation and determine the number of participants. Dairy cattle farmers and up to 2 family members or farm employees ≥12 years of age were eligible for participation in the study. Participants completed a questionnaire about personal characteristics (e.g., age, medical history, farm-related activities, contact with livestock and companion animals, consumption of unpasteurized dairy products, and use of personal protective equipment [PPE]) and provided a serum sample (collected by a laboratory assistant during a home visit). The farm owner or manager completed a questionnaire about herd size, cattle housing, presence of other livestock and companion animals, farm facilities, animal health, and hygiene measures. Participating farms were requested to provide one BTM sample for testing by ELISA and PCR, as described (*8*).

Serology

We used an immunofluorescence assay (IFA) (Focus Diagnostics, Cypress, CA, USA) to test serum samples for *C. burnetii* phase I and II IgM and IgG. All samples were screened at an initial dilution of 1:32; those with negative results were considered negative. Positive samples were further classified as indicative of relatively recent infections (IgM phase II titer ≥32) or past infections (IgG phase II titer ≥32 and IgM phase II titer <32). Samples with all other outcomes were considered negative. The term relatively recent was chosen because phase II IgM is commonly found up to 1 year after infection in acute Q fever cases, but it may persist up to 3 years (*9*). Phase I and II IgG end point titers were determined for all seropositive persons. In agreement with chronic Q fever diagnostic criteria used in the Netherlands (*10*), phase I IgG titers ≥1,024 in samples in the past infection group were considered indicative of possible chronic infection.

Data Analysis

Participating and nonparticipating farms were compared with respect to herd size; distance to nearest *C. burnetii*–positive BTM small-ruminant farm; goat, sheep, and cattle density; location by province and region; and degree of urbanization. We used the Mann-Whitney U test to determine differences in continuous variables and the χ^2 test to analyze categorical variables. We performed univariate logistic regression analyses to determine the main factors associated with *C. burnetii* seropositivity among participants ($p<0.20$, likelihood ratio test). Potential farm-based risk factors were analyzed by univariate multilevel analyses; a unique farm identifier was used as the cluster variable. Distributions of continuous variables were studied, and variables not linearly related to the outcome variable were categorized on the basis of biological arguments (e.g., nearest *C. burnetii*–positive BTM small-ruminant farm) or, if those were lacking, on medians (e.g., goat density within 5-km radius). Participant age was always kept in the model because of its frequent relation with seropositivity. Variables with <10.0% of participants in a risk category were excluded from further analysis. If several variables were found interrelated in the univariate analysis, only the most informative and relevant variable was selected for inclusion.

Risk factors determined to be significant ($p<0.20$) in univariate analyses of the participant-based and farm-based data were incorporated into multivariate logistic regression and multivariate multilevel analyses, respectively. Stratified multivariate analyses for participant risk factors were performed separately for farmers and for the remaining group. Starting with a full model, manual backward elimination was performed; all variables meeting the 10.0% significance level in the likelihood ratio test were kept in the final model. Two-way interactions between biologically plausible variables in the multivariate model were investigated. Last, variables included in the final multivariate model for participant-based factors and those included in the multilevel model for farm-based factors were combined in a multivariate multilevel analysis to identify the independent risk determinants for seropositivity. The final model fit was assessed by the quasi-likelihood under the independence model criterion goodness-of-fit statistic for generalized estimation equation models. SAS version 9.2 (SAS Institute, Cary, NC, USA) was used for all analyses.

Results

Nonresponse Analysis

Of the 3,000 invited farms, 311 provided a BTM sample, and 755 persons from 309 (10.3%) farms participated in this study by providing a serum sample. A farm-based questionnaire was available for 736 (97.5%) persons from 301 farms, and a participant-based questionnaire was completed by 729 (96.6%) persons from 308

farms. Compared with nonparticipating farms, participating farms were a median of 1.5 km closer to small ruminant farms with *C. burnetii*–positive BTM samples (Table 1). In addition, the density of sheep within a 5-km radius of participating farms was higher than that for nonparticipating farms; however, the absolute difference was very small (3 sheep/km^2).

Seroprevalence

Overall *C. burnetii* seroprevalence was 72.1% (95% CI 68.8%–75.3%), and seroprevalence among farmers, spouses, and children (12–17 years of age) was 87.2%, 54.5%, and 44.2%, respectively (Table 2). Seroprevalence was univariately significantly higher among male participants, farmers, and participants ≥35 years of age (Table 3, Appendix, wwwnc.cdc.gov/EID/article/20/3/13-1111-T3.htm). The median duration of farm residence was 28 years (range 0–56). IgG phase II end titers were known for 534 (98.9%) of 540 *C. burnetii* IgG phase II–seropositive participants: 32 (n =166), 64 (n = 92), 128 (n = 119), 256 (n = 106), 512 (n = 39), 1,024 (n = 10), 2,048 (n = 1), and 4,096 (n = 1). IgG phase I end titers were known for 283 (97.6%) of the 290 IgG phase I–seropositive participants: 32 (n = 105), 64 (n = 73), 128 (n = 61), 256 (n = 32), 512 (n = 10), 1,024 (n = 1), and 2,048 (n = 1). These last 2 participants, with phase I titers of 1,024 and 2,048, respectively, had lower IgG phase II titers (512 and 1,024, respectively), and according to chronic Q fever diagnostic criteria used in the Netherlands (*10*), these participants met the conditions for possible chronic Q fever infection. We could not confirm that these truly were chronic Q fever cases because clinical information (e.g., presence of vascular infection, endocardial involvement, or other clinical risk factors) was lacking.

Nine (1.2%) participants from 8 farms were classified as having a relatively recent infection (IgM phase II titer range 32–256). All 8 farms were within 2.5–21.2 km of the nearest *C. burnetii*–positive BTM small-ruminant farm, and 4 of the 8 were within 3 km.

Four participants reported having had Q fever diagnosed by a physician during 2008–2010. On the basis of serum samples obtained at study entry, 3 of these participants had a serologic profile indicating past infection. These 3 participants lived in the southern or eastern region of the Netherlands on farms within a 3-km radius of the nearest small-ruminant farm with *C. burnetii*–positive BTM samples. The fourth participant had no serologic evidence of a past infection and lived 14 km from the nearest small-ruminant farm with *C. burnetii*–positive BTM samples.

Table 1. Nonresponse analyses of farms in a study of *Coxiella burnetii* seroprevalence and risk for seropositivity in humans on dairy cattle farms, the Netherlands, September 2010–March 2011

Variable	Farms		p value
	Participating, n = 311	Nonparticipating, n = 2,685	
Categorical, no. (%)			
Farm located inside vaccination area	83 (26.4)	590 (21.9)	0.08
Farm region*			0.36
North	80 (25.4)	781 (29.1)	
East	104 (33.7)	911 (33.9)	
West	57 (18.7)	494 (18.3)	
South	70 (22.2)	503 (18.7)	
Degree of urbanization of the farm municipality			0.77
Moderately, strongly, or extremely (>1,000 addresses/km^2)	1 (0.3)	17 (0.6)	
Hardly (500–1,000 addresses/km^2)	10 (3.2)	94 (3.5)	
Not (<500 addresses/km^2)	300 (96.5)	2,574 (95.9)	
Numerical, median no.			
No. cows in 2008			
<1	35	35	0.44
1–2	26	26	0.65
>2	85	86	0.16
Nearest bulk tank milk positive small-ruminant farm (meters)	9,793	11,301	0.01
Goat density (animals/km^2)†			
Within 5-km radius	9.2	6.7	0.27
Within 10-km radius	9.3	9.2	0.26
Sheep density (animals/km^2)†			
Within 5-km radius	30	33	0.04
Within 10-km radius	34	35	0.11
Cattle density (animals/km^2) within 5-km radius†			
Including own animals	178	181	0.29
Excluding own animals	175	179	0.27
Cattle density (animals/km^2) within 10-km radius†			
Including own animals	170	170	0.99
Excluding own animals	169	169	0.91

*North represents Groningen, Friesland, and Drenthe Provinces; East represents Gelderland, Overijssel, and Flevoland Provinces; West represents Noord–Holland, Zuid–Holland, Utrecht, and Zeeland Provinces; and South represents Limburg and Noord–Brabant Provinces.
†Corrected for area in the Netherlands.

Table 2. Participant characteristics and *Coxiella burnetii* seroprevalence among dairy cattle farm residents, the Netherlands, September 2010– March 2011

Participant characteristic	Total no. residents/no. positive (%)	95% CI
All participants	755/544 (72.1)	68.8–75.3
Sex		
M	431/368 (85.4)	82.0–88.7
F	323/176 (54.5)	49.0–59.9
Age, y		
<35	169/107 (63.3)	56.0–70.7
35–44	176/131 (74.4)	67.9–80.9
45–54	252/185 (73.4)	67.9–78.9
≥55	132/106 (80.3)	73.4–87.2
Role		
Farmer	361/315 (87.2)	83.8–90.7
Spouse	222/121 (54.5)	47.9–61.1
Child <18 y	52/23 (44.2)	30.3–58.2
Child ≥18 y	54/40 (74.1)	62.0–86.1
Other*	40/30 (75.0)	61.0–89.0

*Represents other family members and employees.

Univariate Analyses at Participant and Farm Levels

Risk factors for seropositivity for farmers/workers and residents included age ≥35 years; farm employment; directly performing cattle-related tasks; contact with cattle, pigs, hay, cattle food, raw milk, manure, or cattle birth products; presence of rats or mice on the farm; and growing up on a farm (Table 3, Appendix). Protective factors included poultry and compost contact and fully compliant use of gloves during and around calving. Farm-based risk factors included a larger herd size, farm location in the southern region, an annual peak in calving, having beef cattle on the farm, and the presence of birds in the stable. Protective factors included automatic milking, having pet cats or rabbits, and having farm clothes and boots available for professional visitors (e.g., veterinarians and feed specialists) (Table 4). No relationship was found between PCR or ELISA status on the basis of BTM samples and participant seropositivity.

Multivariate and Multilevel Analyses

Of the 21 variables considered in the multivariate participant model, 8 were independently associated with seropositivity: age ≥55 years; working on the farm; fully compliant use of gloves during cattle birth care; contact with pigs, cattle at other farms, poultry, or compost; and indirect contact with rats or mice (Table 5). Interaction terms did not improve the model.

Of the 9 variables considered in the multilevel farm model, 6 were independently associated with seropositivity; larger herd size, farm location in the southern region, beef cattle on the farm, use of food concentrate, and presence of birds in the stable were risk factors, and automatic milking was a protective factor (Table 6). In the combined multilevel analysis, the 12 significant factors from the multivariate participant and multilevel farm models, in addition to age, were combined in 1 model. The nonstratified model had a clearly better fit than the stratified model for farmers. Farm location within 8 km of the nearest *C. burnetii*–positive BTM small-ruminant farm (odds ratio 2.3, 95% CI 1.2%–2.5%) was a risk factor in the final stratified multilevel model among farmers and was therefore included in the combined multilevel analysis. In the final overall model, independent risk factors were age ≥55 years, farm employment, pig contact, larger herd size, farm location in the southern region, beef cattle on the farm, cattle contact at other farms, and presence of birds in the stable. Indirect contact with rats or mice was borderline significant (Table 7). Protective factors were contact with poultry or compost, use of automatic milking, and fully compliant use of gloves during birth care. We ran an additional model by adding a protective variable (farm clothes and boots available for professional visitors), as described in Table 5, in the farm-based and combined multilevel models. Doing so resulted in a final model with the same factors as shown in Table 7, except that automatic milking was replaced by another protective factor (farm clothes and boots available for professional visitors) and 2 borderline significant risk factors (distance to the nearest *C. burnetii*–positive BTM small-ruminant farm and use of by-product feedstuffs) (data not shown).

Discussion

The overall seroprevalence of 72.1% among DCF residents, including employees, was high, indicating a considerable lifetime risk for acquiring *C. burnetii* infection. Seroprevalence was highest among farmers (87.2%). The observed seroprevalence was similar to that determined by a study from the 1980s that showed an estimated seroprevalence of 68.0% among 94 Dutch dairy farm residents; however, laboratory methods used in that study were different than those used by us (*11*). The 72.1% seroprevalence was also compatible with recent estimates among dairy goat farms residents (68.7%) (*12*), dairy sheep farms residents (66.7%) (*13*), and livestock veterinarians (65.1%) (*14*). Estimates for these livestock-associated groups

Table 4. Univariate logistic model of farm-based characteristics associated with *Coxiella burnetii* positivity among dairy cattle farm residents, the Netherlands, September 2010–March 2011*

Variable	No. residents total (% positive)	OR (95% CI)
No. cows on farm in 2008†‡	755 (72.1)	1.0 (1.0–1.0)
Nearest bulk tank milk positive small-ruminant farm†		
<8 km	331 (75.8)	1.4 (1.0–1.9)
≥8 km	424 (69.1)	Reference
Municipal cattle density, including beef calves§	755 (72.1)	1.0 (1.0–1.0)
Farm location		
Inside small-ruminant vaccination area	202 (78.2)	1.6 (1.0–2.3)
Outside small-ruminant vaccination area	553 (69.8)	Reference
Farm region†		
South	170 (80.6)	1.8 (1.2–2.7)
Other	585 (69.6)	Reference
Beef cattle on the farm†		
Yes	79 (82.3)	1.9 (1.1–3.4)
No	652 (70.7)	Reference
Annual peak in calving		
Yes	135 (76.3)	1.3 (0.9–2.0)
No	601 (71.1)	Reference
Automatic milking†		
Yes	154 (65.6)	0.7 (0.5–1.0)
No	580 (73.8)	Reference
Use of bedding in stables		
Yes	717 (72.4)	1.9 (1.2–2.9)
No	19 (57.9)	Reference
Pet cat		
Yes	444 (69.1)	0.6 (0. 5–0.9)
No	285 (77.9)	Reference
Pet rabbit		
Yes	202 (64.4)	0.6 (0.4–0.8)
No	527 (75.7)	Reference
Birds in stable†		
Yes	90 (82.2)	1.9 (1.0–3.6)
No	644 (70.5)	Reference
Use of by-product feedstuffs†		
Yes	229 (77.3)	1.5 (1.0–2.1)
No	507 (69.6)	Reference
No. cows that calved in 2009‡	720 (71.8)	1.0 (1.0–1.0)
No. live-born calves		
<78	335 (69.0)	Reference
≥78	344 (74.1)	1.3 (0.9–1.8)
No. twin calves		
1–2	272 (69.9)	Reference
≥3	313 (76.4)	1.4 (1.0–2.0)
Type of farm management†		
Closed herd	515 (73.4)	Reference
Purchase of cattle	213 (68.1)	0.8 (0.6–1.1)
No. cattle purchase addresses in 2007†		
0 or 1	649 (72.7)	Reference
≥2	76 (64.5)	0.7 (0.4–1.0)
Farm boots and work clothes available for professional visitors		
Yes	662 (71.3)	0.7 (0.4–1.1)
No	74 (78.4)	Reference
Work clothes available for own personnel		
Yes	556 (73.6)	1.4 (1.0–1.9)
No	180 (67.2)	Reference

*The analysis included the primary farm-based factors associated with positivity (p<0.20 in likelihood ratio test). OR, odds ratio.
†Variable included in later multivariate analysis before manual backward elimination.
‡Risk increases per cow.
§Risk decreases per cow.

exceed the seroprevalence of 2.4% for the Dutch population during the pre-epidemic period, 2006–2007 (*2*), and the seroprevalences of 12.2% and 24.0% among persons residing in the most affected outbreak areas during the epidemic in the Netherlands (*15*,*16*).

Seroprevalence studies of other farmer populations, particularly dairy cattle farmers, are scarce, and, in general, it is difficult to compare international studies because of different study populations, tests, or cutoff values used. However, published seroprevalence estimates are generally

Table 5. Multivariate logistic regression analysis of participant-based characteristics associated with *Coxiella burnetii* positivity among dairy cattle farm residents, the Netherlands, September 2010–March 2011*

Association with positivity, characteristic	OR (95% CI)
Positive association	
Age, y	
<35	Reference
35–44	1.4 (0.8–2.3)
45–54	1.0 (0.6–1.6)
≥55	1.9 (1.0–3.5)
Work on farm	
No	Reference
Part time (1–39 h/wk)	2.4 (1.1–5.2)
Full time (≥40 h/wk)	10.4 (4.2–25.7)
Contact with pigs at own or other farm	
Yes	2.6 (1.2–5.4)
No	Reference
Contact with cows at other farm	
Yes	1.6 (1.0–2.6)
No	Reference
Indirect contact with rats/mice at own farm	
Yes	1.5 (1.0–2.4)
No	Reference
Negative association	
Use of gloves during cattle birth care	
Fully compliant	0.4 (0.2–0.8)
Partly or noncompliant	Reference
No birth care	0.7 (0.4–1.1)
Contact with poultry at own farm	
Yes	0.6 (0.4–0.9)
No	Reference
Contact with compost	
Yes	0.6 (0.3–0.9)
No	Reference

*The analysis included the primary participant-based characteristics associated with positivity (p<0.10 in likelihood ratio test). The number of observations was 712. Model fit was assessed by use of the Hosmer–Lemeshow goodness-of-fit test (p = 0.91). OR, odds ratio.

lower than what we observed. A study using IFA with the same cutoff value that we used estimated a seroprevalence of 27.0% among a UK farm cohort (385 residents/workers) (*17*). Two other studies used a *C. burnetii* phase II IgG ELISA, which is somewhat less sensitive than IFA (*9*), and obtained seroprevalence estimates of 48.8% among Northern Ireland farmers from all types of farms (*18*) and 16.0% among 262 farm residents from 105 DCFs in Germany (*19*). A seroprevalence of 3.0% was observed in 163 residents from 100 farms (most likely cattle or pig) in Denmark; the study used the same IFA that we used, but cutoff values of IgG phase I and II were higher (≥512 and ≥1,024, respectively) (*20*). Using the same cutoff, we would obtain a comparable seroprevalence estimate of 2.7%.

Farm residents living in the southern part of the Netherlands were more likely to be seropositive. This was not surprising because living in the south was a risk factor for dairy goat farmers (*12*). In general, it is possible that seropositive DCF residents were partially affected by the many *C. burnetii*–positive BTM small-ruminant farms nearby. This possibility is supported by the close distance between residential addresses of persons who had a relatively recent infection and nearby *C. burnetii*–positive BTM small-ruminant farms. As determined on the basis of phase II IgM, 1.2% of DCF residents and 11.0% of small-ruminant dairy farm residents had a relatively recent *C. burnetii* infection (*12*,*13*), indicating that the infection among DCF residents was generally in the more distant past. Physicians diagnosed Q fever in 0.5% of DCF residents in our study compared with 4.1% in Dutch goat farm residents (*12*); nevertheless, to ensure a timely diagnosis and treatment, physicians should consider Q fever in patients with compatible symptoms and occupational exposure to cattle (*20*,*21*). In general, clinical illness from *C. burnetii* infection appears to be rare among DCF residents, which fits the suggestion in the literature that cattle-acquired *C. burnetii* infection has a milder clinical course (*20*). In other European countries and the United States, *C. burnetii* infection is endemic in cattle and in humans occupationally exposed to cattle, but there are few clinical cases of acute Q fever (*22*,*23*). A possible explanation is that abortion in late gestation is a key sign of infection in small dairy ruminants, but this is not the case in cattle. *C. burnetii* shedding by cattle is generally lower than that by small ruminants; concomitant and persistent shedding patterns are more frequent in clinically affected cows than healthy ones (*24*–*29*). Furthermore, sheep and goats have seasonal reproduction cycles and generally larger herd sizes, leading to huge amounts of bacteria shed during a short period. Multilocus variable-number tandem-repeat analysis genotyping has indicated that *C. burnetii* genotypes in dairy cattle herds and dairy consumer products (*30*,*31*), except for 1 placenta sample, are clearly distinct from the predominant outbreak genotype found at Dutch small ruminant dairy farms in 2007–2009 (*32*). Upcoming research should elucidate whether the cattle strains circulating in the Netherlands and other countries are less virulent.

Persons ≥55 years of age were at increased risk for seropositivity, which cannot be explained by differences in specific cattle-related tasks, frequency of cattle contact, or hours worked. It may be that host factors or continuous or regular exposure to the bacterium (booster effect) play a role that cannot adequately be assessed through a questionnaire. Full-time farm employment (≥40 h/week) was a risk factor, which corresponds with a study among Dutch livestock veterinarians in which ≥30 hours of weekly animal contact was a risk factor for infection (*14*). Full-time farm employment and working or residing on a dairy (primarily) farm were risk factors in a UK farm cohort (*17*), indicating a dose-response relationship between seropositivity and the number of working hours spent with dairy cattle or in a dairy farm environment in general.

We identified several cattle-related risk factors for seropositivity among cattle farm residents/staff: herd size, cattle

contact at other farms, and presence of beef cattle on their own farm. A larger herd size could pose a risk because of an increased chance for *C. burnetii* introduction or the presence of a larger susceptible population of cows; however, some farm-based risk factors associated with a large herd that were not assessed through the questionnaire might also have caused this effect (*19*,*33*,*34*). Cattle contact at other farms possibly reflects risk from exposure to *C. burnetii* in other infected herds. The presence of beef cattle as a risk factor for DCF residents is not easily explained, but it might reflect risk from more intense birth care and, therefore, more extensive human contact with cattle and birth products.

Protective factors included use of automatic milking and fully compliant use of gloves during birth care. Birth products of *C. burnetii*–infected ruminants are a source of human infections. A German study among veterinarians identified an association between increasing numbers of cattle obstetric procedures performed and seropositivity (*21*). Pig contact, indirect contact with rats/mice, and presence of wild or domesticated birds in the stable were indicated as risk factors in our study. Studies among veterinarians in the Netherlands and the United States identified swine contact as a risk factor (*14*,*35*); however, *C. burnetii* has not been found in pigs in the Netherlands (*30*). Rats and wild birds were identified as *C. burnetii* reservoirs in several studies (*36*–*38*) and as reservoirs on cattle farms in the Netherlands (*39*).

Fully compliant use of gloves during birth care can help farmers protect themselves against *C. burnetii* infection (*21*). Consistent use of farm boots and working clothes for professional visitors was a protective factor in our additional multilevel model. It might appear that the use of protective clothing by visitors will prevent *C. burnetii* transmission to the visitor rather than the farmer; however, providing gloves and farm clothes for visitors indicates a state of optimal awareness on the farm with regard to communicable diseases. In addition, automatic milking of cows might reflect less direct cattle exposure, especially through avoiding contact with the udders, raw milk, manure, and genital fluids, and thus might limit the chance of infection. Statistical analyses indicated lower risk for seropositivity among farm residents exposed to poultry and to compost. We have no biologically plausible explanation for this finding, and the statistical effect might have occurred by chance. Raw milk consumption was a risk factor for seropositivity in German dairy cattle farmers (*19*). Although consumption of raw milk was not an independent risk factor in our study, 21.8% of farm residents reported daily drinking of raw milk. *C. burnetii* exposure during nonautomatic milking could still implicate the risk of inhaling contaminated aerosols during pretreatment of the cow or during accidental raw milk ingestion.

Table 6. Multilevel analysis of farm-based characteristics as independent factors associated with *Coxiella burnetii* positivity among dairy cattle farm residents, the Netherlands, September 2010–March 2011*

Variable	OR (95% CI)
No. cows on farm in 2008†	1.0 (1.0–1.0)
Farm region	
South	1.8 (1.2–2.8)
Other	Reference
Beef cattle on farm	
Yes	1.7 (1.0–2.8)
No	Reference
Automatic milking	
Yes	0.7 (0.4–1.0)
No	Reference
Birds in stable	
Yes	2.0 (1.1–3.8)
No	Reference
Use of by-product feedstuffs	
Yes	1.4 (1.0–2.0)
No	Reference

*The analysis included the primary farm-based characteristics associated with positivity ($p<0.10$ in likelihood ratio test). The number of observations was 716; the number of levels used was 309 (quasi-likelihood under the independence model criterion 832.88). OR, odds ratio.
†Risk increased per cow.

The relatively low response rate of 10.4% in this study can be explained by a general lack of motivation or awareness among cattle farmers because Q fever was mainly considered a problem among small-ruminant dairy farms. A general fear of consequences resulting from possible control measures targeting the cattle sector comparable with implemented control measures for Q fever in the small-ruminant sector might also have played a role. Study results are, however, considered representative for the Dutch dairy cattle sector because participating and nonparticipating farms were generally comparable.

The overall *C. burnetii* seroprevalence of 72.1% among DCF residents is high. Multilevel analysis identified several plausible risk factors (e.g., employment on a farm, larger herd size, and cattle contact at other farms). A farm location in the southern region as risk factor suggests *C. burnetii* transmission from small-ruminant dairy farms to cattle farm residents living nearby. Use of automatic milking and fully compliant use of gloves during birth care are plausible protective factors, indicating less direct contact with cattle and, thus, a reduced chance of animal-to-human transmission. The dairy cattle sector must inform farmers about potential sources of infection. Biosecurity measures are warranted; for example, wild birds and vermin should be kept out of stables, and farmers/staff should be educated regarding the consistent use of PPE, such as wearing gloves during birth assistance and invasive procedures. Physicians should consider Q fever in the differential diagnosis for dairy cattle farmers with compatible symptoms. Future studies should more explicitly assess the clinical effect of acute and chronic Q fever in humans who live or work on DCFs.

Table 7. Combined multilevel analysis of participant- and farm-based characteristics associated with *Coxiella burnetii* seropositivity in dairy cattle farm residents, the Netherlands, September 2010–March 2011*

Variable	OR (95% CI)
Age, y	
<35	Reference
35–44	1.3 (0.8–2.3)
45–54	1.2 (0.7–2.0)
≥55	1.9 (1.1–3.5)
Work on farm	
No	Reference
Part time (1–39 h/wk)	2.5 (1.1–5.6)
Full time (≥40 h/wk)	10.7 (4.2–27.0)
Use of gloves during cattle birth care	
Fully compliant	0.4 (0.2–0.8)
Partly or noncompliant	Reference
No birth care	0.7 (0.4–1.1)
Contact with pigs at own or other farm	
Yes	2.4 (1.1–5.1)
No	Reference
Contact with poultry at own farm	
Yes	0.5 (0.3–0.8)
No	Reference
Indirect contact with rats/mice at own farm	
Yes	1.6 (1.0–2.7)
No	Reference
No. cows on farm in 2008†	1.0 (1.0–1.0)
Farm region	
South	1.9 (1.2–3.1)
Other	Reference
Automatic milking	
Yes	0.6 (0.4–1.0)
No	Reference
Birds in stable	
Yes	2.3 (1.2–4.4)
No	Reference
Contact with cows at other farm	
Yes	1.8 (1.0–3.2)
No	Reference
Contact with compost	
Yes	0.6 (0.3–0.9)
No	Reference
Beef cattle on the farm	
Yes	1.9 (1.0–3.7)
No	Reference

*The analysis included the primary participant- and farm-based characteristics associated with positivity (p<0.10 in likelihood ratio test). The number of observations was 708; the number of levels used was 309 (quasi-likelihood under the independence model criterion 695.52). OR, odds ratio.
†Risk increases per cow.

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References

1. Dorko E, Rimarova K, Pilipcinec E. Influence of the environment and occupational exposure on the occurrence of Q fever. Cent Eur J Public Health. 2012;20:208–14.
2. Schimmer B, Notermans DW, Harms MG, Reimerink JH, Bakker J, Schneeberger P, et al. Low seroprevalence of Q fever in the Netherlands prior to a series of large outbreaks. Epidemiol Infect. 2012;140:27–35. http://dx.doi.org/10.1017/S0950268811000136
3. Dijkstra F, van der Hoek W, Wijers N, Schimmer B, Rietveld A, Wijkmans CJ, et al. The 2007–2010 Q fever epidemic in the Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. FEMS Immunol Med Microbiol. 2012;64:3–12. http://dx.doi.org/10.1111/j.1574-695X.2011.00876.x
4. van der Hoek W, Dijkstra F, Schimmer B, Schneeberger PM, Vellema P, Wijkmans C, et al. Q fever in the Netherlands: an update on the epidemiology and control measures. Euro Surveill. 2010;15:19520.
5. Roest HI, Hogerwerf L, van der Brom R, Oomen T, van Steenbergen JE, Nielen M, et al. Q fever in the Netherlands: current status, results from veterinary research and expectations of the coming years [in Dutch]. Tijdschr Diergeneeskd. 2011;136:340–3.
6. van der Hoek W, Schneeberger PM, Oomen T, Wegdam-Blans MC, Dijkstra F, Notermans DW, et al. Shifting priorities in the aftermath of a Q fever epidemic in 2007 to 2009 in the Netherlands: from acute to chronic infection. Euro Surveill. 2012;17:20059.
7. Georgiev M, Afonso A, Neubauer H, Needham H, Thiery R, Rodolakis A, et al. Q fever in humans and farm animals in four European countries, 1982 to 2010. Euro Surveill. 2013;18:20407.
8. Muskens J, van Engelen E, van Maanen C, Bartels C, Lam TJ. Prevalence of *Coxiella burnetii* infection in Dutch dairy herds based on testing bulk tank milk and individual samples by PCR and ELISA. Vet Rec. 2011;168:79. http://dx.doi.org/10.1136/vr.c6106
9. Wegdam-Blans MC, Wielders CC, Meekelenkamp J, Korbeeck JM, Herremans T, Tjhie HT, et al. Evaluation of commonly used serological tests for detection of *Coxiella burnetii* antibodies in well-defined acute and follow-up sera. Clin Vaccine Immunol. 2012;19:1110–5. http://dx.doi.org/10.1128/CVI.05581-11
10. Wegdam-Blans MC, Kampschreur LM, Delsing CE, Bleeker-Rovers CP, Sprong T, van Kasteren ME, et al. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Infect. 2012;64:247–59. http://dx.doi.org/10.1016/j.jinf.2011.12.014
11. Richardus JH, Donkers A, Dumas AM, Schaap GJ, Akkermans JP, Huisman J, et al. Q fever in the Netherlands: a sero-epidemiological survey among human population groups from 1968 to 1983. Epidemiol Infect. 1987;98:211–9. http://dx.doi.org/10.1017/S0950268800061938
12. Schimmer B, Lenferink A, Schneeberger P, Aangenend H, Vellema P, Hautvast J, et al. Seroprevalence and risk factors for *Coxiella burnetii* (Q fever) seropositivity in dairy goat farmers' households in the Netherlands, 2009–2010. PLoS ONE. 2012;7:e42364. http://dx.doi.org/10.1371/journal.pone.0042364

13. De Lange MMA, Schimmer B, Vellema P, Hautvast JLA, Schneeberger PM, van Duynhoven YTHP. *Coxiella burnetii* seroprevalence and risk factors in sheep farmers and farm residents in the Netherlands. Epidemiol Infect. 2013 Aug 7:1–14. [Epub ahead of print]. http://dx.doi.org/10.1017/S0950268813001726
14. Van den Brom R, Schimmer B, Schneeberger PM, Swart WA, van der Hoek W, Vellema P. Seroepidemiological survey for *Coxiella burnetii* antibodies and associated risk factors in Dutch livestock veterinarians. PLoS ONE. 2013;8:e54021. http://dx.doi.org/10.1371/journal.pone.0054021
15. Hogema BM, Slot E, Molier M, Schneeberger PM, Hermans MH, van Hannen EJ, et al. *Coxiella burnetii* infection among blood donors during the 2009 Q-fever outbreak in the Netherlands. Transfusion. 2012;52:144–50. http://dx.doi.org/10.1111/j.1537-2995.2011.03250.x
16. Karagiannis I, Schimmer B, Van Lier A, Timen A, Schneeberger P, Van Rotterdam B, et al. Investigation of a Q fever outbreak in a rural area of the Netherlands. Epidemiol Infect. 2009;137:1283–94. http://dx.doi.org/10.1017/S0950268808001908
17. Thomas DR, Treweek L, Salmon RL, Kench SM, Coleman TJ, Meadows D, et al. The risk of acquiring Q fever on farms: a seroepidemiological study. Occup Environ Med. 1995;52:644–7. http://dx.doi.org/10.1136/oem.52.10.644
18. McCaughey C, McKenna J, McKenna C, Coyle PV, O'Neill HJ, Wyatt DE, et al. Human seroprevalence to *Coxiella burnetii* (Q fever) in Northern Ireland. Zoonoses Public Health. 2008;55:189–94. http://dx.doi.org/10.1111/j.1863-2378.2008.01109.x
19. Kopp J. Untersuchungen über Zusammenhänge von *C. burnetii*- und Chlamydien-Infektionen in Rinderbeständen und der in diesen Betrieben tätigen Personen. Berlin: Freien Universität Berlin; 2000.
20. Bosnjak E, Hvass AM, Villumsen S, Nielsen H. Emerging evidence for Q fever in humans in Denmark: role of contact with dairy cattle. Clin Microbiol Infect. 2010;16:1285–8. http://dx.doi.org/10.1111/j.1469-0691.2009.03062.x
21. Bernard H, Brockmann SO, Kleinkauf N, Klinc C, Wagner-Wiening C, Stark K, et al. High seroprevalence of *Coxiella burnetii* antibodies in veterinarians associated with cattle obstetrics, Bavaria, 2009. Vector Borne Zoonotic Dis. 2012;12:552–7. http://dx.doi.org/10.1089/vbz.2011.0879
22. Nielsen SY, Mølbak K, Nybo Andersen A, Brink Henriksen T, Kantsø B, Krogfelt K, et al. Prevalence of *Coxiella burnetii* in women exposed to livestock animals, Denmark, 1996 to 2002. Euro Surveill. 2013;18:20528.
23. Anderson AD, Kruszon-Moran D, Loftis AD, McQuillan G, Nicholson WL, Priestley RA, et al. Seroprevalence of Q fever in the United States, 2003–2004. Am J Trop Med Hyg. 2009;81:691–4. http://dx.doi.org/10.4269/ajtmh.2009.09-0168
24. Arricau-Bouvery N, Rodolakis A. Is Q fever an emerging or re-emerging zoonosis? Vet Res. 2005;36:327–49. http://dx.doi.org/10.1051/vetres:2005010
25. Guatteo R, Beaudeau F, Joly A, Seegers H. *Coxiella burnetii* shedding by dairy cows. Vet Res. 2007;38:849–60. http://dx.doi.org/10.1051/vetres:2007038
26. Guatteo R, Joly A, Beaudeau F. Shedding and serological patterns of dairy cows following abortions associated with *Coxiella burnetii* DNA detection. Vet Microbiol. 2012;155:430–3. http://dx.doi.org/10.1016/j.vetmic.2011.09.026
27. Hansen MS, Rodolakis A, Cochonneau D, Agger JF, Christoffersen AB, Jensen TK, et al. *Coxiella burnetii* associated placental lesions and infection level in parturient cows. Vet J. 2011;190:e135–9. http://dx.doi.org/10.1016/j.tvjl.2010.12.021
28. Rodolakis A, Berri M, Hechard C, Caudron C, Souriau A, Bodier CC, et al. Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine, and ovine herds. J Dairy Sci. 2007;90:5352–60. http://dx.doi.org/10.3168/jds.2006-815
29. van Moll P, Baumgartner W, Eskens U, Hanichen T. Immunocytochemical demonstration of *Coxiella burnetii* antigen in the fetal placenta of naturally infected sheep and cattle. J Comp Pathol. 1993;109:295–301. http://dx.doi.org/10.1016/S0021-9975(08)80254-X
30. Roest HIJ, van Solt CB, Tilburg JJHC, Klaassen CHW, Hovius EK, Roest TF, et al. Search for possible additional reservoirs for human Q fever, the Netherlands. Emerg Infect Dis. 2013;19:834–5. http://dx.doi.org/10.3201/eid1905.121489
31. Tilburg JJ, Roest HJ, Nabuurs-Franssen MH, Horrevorts AM, Klaassen CH. Genotyping reveals the presence of a predominant genotype of *Coxiella burnetii* in consumer milk products. J Clin Microbiol. 2012;50:2156–8. http://dx.doi.org/10.1128/JCM.06831-11
32. Roest HI, Ruuls RC, Tilburg JJ, Nabuurs-Franssen MH, Klaassen CH, Vellema P, et al. Molecular epidemiology of *Coxiella burnetii* from ruminants in Q fever outbreak, the Netherlands. Emerg Infect Dis. 2011;17:668–75. http://dx.doi.org/10.3201/eid1704.101562
33. McCaughey C, Murray LJ, McKenna JP, Menzies FD, McCullough SJ, O'Neill HJ, et al. *Coxiella burnetii* (Q fever) seroprevalence in cattle. Epidemiol Infect. 2010;138:21–7. http://dx.doi.org/10.1017/S0950268809002854
34. Ryan ED, Kirby M, Collins DM, Sayers R, Mee JF, Clegg T. Prevalence of *Coxiella burnetii* (Q fever) antibodies in bovine serum and bulk-milk samples. Epidemiol Infect. 2011;139:1413–7. http://dx.doi.org/10.1017/S0950268810002530
35. Whitney EA, Massung RF, Candee AJ, Ailes EC, Myers LM, Patterson NE, et al. Seroepidemiologic and occupational risk survey for *Coxiella burnetii* antibodies among US veterinarians. Clin Infect Dis. 2009;48:550–7. http://dx.doi.org/10.1086/596705
36. Parker NR, Barralet JH, Bell AM. Q fever. Lancet. 2006;367:679–88. http://dx.doi.org/10.1016/S0140-6736(06)68266-4
37. Ioannou I, Chochlakis D, Kasinis N, Anayiotos P, Lyssandrou A, Papadopoulos B, et al. Carriage of *Rickettsia* spp., *Coxiella burnetii* and *Anaplasma* spp. by endemic and migratory wild birds and their ectoparasites in Cyprus. Clin Microbiol Infect. 2009;15(Suppl 2):158–60. http://dx.doi.org/10.1111/j.1469-0691.2008.02207.x
38. Stein A, Raoult D. Pigeon pneumonia in Provence: a bird-borne Q fever outbreak. Clin Infect Dis. 1999;29:617–20. http://dx.doi.org/10.1086/598643
39. Reusken C, van der Plaats R, Opsteegh M, de Bruin A, Swart A. *Coxiella burnetii* (Q fever) in *Rattus norvegicus* and *Rattus rattus* at livestock farms and urban locations in the Netherlands; could *Rattus* spp. represent reservoirs for (re)introduction? Prev Vet Med. 2011;101:124–30. http://dx.doi.org/10.1016/j.prevetmed.2011.05.003

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Appendix Table 3. Univariate logistic model of participant-based characteristics associated with *Coxiella burnetii* seropositivity among dairy cattle farm residents, the Netherlands, September 2010– March 2011*

Variable	No. residents (% positive)	OR (95% CI)
Sex		
M	431 (85.4)	4.9 (3.5–6.9)
F	323 (54.5)	Reference
Age, y†		
<35	169 (63.3)	Reference
35–44	176 (74.4)	1.7 (1.1–2.7)
45–54	252 (73.4)	1.6 (1.1–2.4)
≥55	132 (80.3)	2.4 (1.4–4.0)
Role		
Farmer	361 (87.3)	5.7 (3.8–8.6)
Spouse	222 (54.5)	Reference
Child <18 y	52 (44.2)	0.7 (0.4–1.2)
Child ≥18 y	54 (74.1)	2.4 (1.2–4.6)
Other‡	40 (75.0)	2.5 (1.2–5.4)
Presence in stable		
Every day	584 (78.3)	8.4 (2.1–32.9)
Every week	117 (51.3)	2.5 (0.6–10.0)
Every month	18 (50.0)	2.3 (0.5–12.0)
<1 time/month	10 (30.0)	Reference
Work on the farm†		
No	42 (31.0)	Reference
Part time (1–39 h/wk)	333 (60.7)	3.3 (1.7–6.6)
Full time (≥40 h/wk)	354 (88.7)	17.5 (8.4–36.4)
Care for calves		
Yes	501 (78.4)	2.5 (1.8–3.5)
No	228 (59.6)	Reference
Milk cows		
Yes	442 (84.4)	4.5 (3.2–6.4)
No	287 (54.4)	Reference
Feed cattle		
Yes	515 (81.6)	4.3 (3.0–6.0)
No	214 (50.9)	Reference
Transport cattle		
Yes	414 (85.0)	4.4 (3.1–6.3)
No	315 (56.2)	Reference
Provide health care for cattle		
Yes	440 (83.0)	3.7 (2.7–5.3)
No	288 (56.6)	Reference
Provide birth care for cattle		
Yes	516 (80.8)	3.8 (2.7–5.4)
No	212 (52.4)	Reference
Remove manure		
Yes	478 (83.3)	4.6 (3.2–6.4)
No	251 (52.2)	Reference

Variable	No. residents (% positive)	OR (95% CI)
Spread manure		
Yes	254 (86.6)	3.5 (2.3–5.2)
No	474 (65.2)	Reference
Clean stables		
Yes	505 (80.2)	3.3 (2.3–4.6)
No	222 (55.4)	Reference
No. of tasks (0–9) performed on farm†§	725 (72.7)	1.3 (1.2–1.4)
Wear overalls†		
Almost every time/regularly	584 (76.7)	2.5 (1.7–3.6)
Sometimes/never	142 (57.0)	Reference
Wear boots†		
Almost every time/regularly	646 (74.6)	2.1 (1.3–3.4)
Sometimes/never	81 (58.0)	Reference
Use gloves during cattle birth care†		
Fully compliant	61 (70.5)	0.5 (0.3–0.9)
Partly or noncompliant	456 (82.2)	Reference
No birth care	211 (52.4)	0.2 (0.2–0.3)
Use disinfectant during cattle birth care		
Fully compliant	141 (82.3)	1.1 (0.7–1.9)
Partly or noncompliant	374 (80.5)	Reference
No birth care	212 (53.3)	0.3 (0.2–0.4)
Have contact with pigs at own or other farm†¶		
Yes	83 (85.5)	2.4 (1.3–4.6)
No	646 (70.9)	Reference
Have contact with sheep at own or other farm†¶		
Yes	232 (75.9)	1.3 (0.9–1.8)
No	497 (71.0)	Reference
Have contact with poultry at own farm†¶		
Yes	177 (67.8)	0.8 (0.5–1.1)
No	578 (73.4)	Reference
Have indirect contact with rats/mice at own farm†#		
Yes	213 (82.6)	2.2 (1.5–3.3)
No	516 (68.4)	Reference
Have contact with dogs at own farm**		
Yes	538 (71.0)	0.7 (0.5–1.1)
No	190 (76.8)	Reference
Have contact with dogs at other farm†**		
Yes	273 (75.8)	1.3 (0.9–1.8)
No	456 (70.6)	Reference
Have contact with cows at other farm†**		
Yes	195 (82.6)	2.2(1.4–3.3)
No	532 (68.8)	Reference

Variable	No. residents (% positive)	OR (95% CI)
Have indirect contact with cows at other farm#		
Yes	326 (77.6)	1.6 (1.1–2.3)
No	400 (68.3)	Reference
Have contact with compost†		
Yes	139 (66.2)	0.7 (0.5–1.0)
No	590 (74.1)	Reference
Have contact with hay†		
Daily (almost)	505 (80.2)	3.3 (2.3–4.6)
Weekly or less often	222 (55.4)	Reference
Have contact with cattle food†		
Daily (almost)	530 (80.9)	4.2 (3.0–6.0)
Weekly or less often	199 (50.3)	Reference
Have contact with raw milk†		
Daily (almost)	507 (78.9)	2.7 (1.9–3.8)
Weekly or less often	221 (57.9)	Reference
Have contact with sheep or goat manure†		
Yes	96 (80.2)	1.6 (1.0–2.8)
No	632 (71.4)	Reference
Have contact with cattle manure†		
Daily (almost)	477 (83.0)	4.4 (3.1–6.2)
Weekly or less often	252 (52.8)	Reference
Have contact with live–born animals		
Yes	626 (76.7)	3.7 (2.4–5.7)
No	102 (47.1)	Reference
Have contact with stillborn animals		
Yes	304 (83.9)	2.9 (2.0–4.1)
No	424 (64.5)	Reference
Have contact with placenta material or amniotic fluid†		
Yes	425 (84.2)	4.2 (2.9–5.9)
No	304 (56.3)	Reference
Consume Brie cheese†		
Yes	166 (63.9)	0.6 (0.4–0.9)
No	563 (75.1)	Reference
Lived on a farm with animals during childhood†		
Yes	604 (76.4)	2.8 (1. 9–4.2)
No	125 (53.6)	Reference

*Analysis was performed to assess the main participant-based characteristics associated with positivity (p<0.20 in likelihood ratio test)

†Variables included in later multivariate analysis before manual backward elimination.

‡Represents other family members and employees.

§No. tasks (i.e., caring for calves, milking, feeding cattle, transport of cattle, general cattle health, assistance during calving, removing manure, spreading manure and cleaning the stables) performed on the farm by 1 person (0–9 tasks). Risk increases per task.

¶See animals at <5 meters or touch animals (contact).

#See animals at <5 meters (indirect contact).

**Touch animals (direct contact).

CHAPTER 4.6

Prevalence and risk factors for *Coxiella burnetii* (Q fever) in Dutch dairy cattle herds based on bulk tank milk testing

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Prevalence and risk factors for *Coxiella burnetti* (Q Fever) in Dutch dairy cattle herds based on bulk tank milk testing

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A B S T R A C T

Despite cattle herds can harbor *Coxiella burnetii*, risk factors for *C. burnetii* presence in dairy cattle herds are largely unknown. Therefore, *C. burnetii* herd prevalence and risk factors for bulk tank milk (BTM) positivity were investigated. In this cross-sectional study, a questionnaire was filled out by the farmer and BTM from 301 farms was tested by ELISA for presence of *C. burnetii* antibodies and PCR for presence of *C. burnetii* DNA. Risk factors were identified by univariable and multivariable logistic regression analyses. Antibodies to *C. burnetii* were detected in 81.6% (CI: 77.2–85.9) and *C. burnetii* DNA in 18.8% (CI: 14.4–23.1) of the BTM samples. Herd size (OR = 1.1 per 10 cows), cleaning the bedding of the cubicles at most every other day (OR = 2.8) and purchase of cattle from at least two addresses (OR = 3.1) showed a significant and positive association with ELISA positivity and use of an automatic milking system a negative association (OR = 0.3). Risk factors for PCR positivity were purchase of cattle from at least two delivery addresses (OR = 3.2), presence of cows with ticks (OR = 2.0), use of an automatic milking system (OR = 0.2) and presence of goats or sheep on the farm (OR = 0.4). Biosecurity and general hygiene seem associated with introduction and spread of *C. burnetii* in dairy herds.

Introduction

Q fever is a human zoonosis which is caused by an obligate intracellular Gram-negative bacterium, *Coxiella burnetii* (Angelakis and Raoult, 2010). This bacterium has an almost worldwide distribution, affecting both a variety of animals and humans. In the Netherlands from 2007 to 2010, an outbreak of human cases occurred on a scale that never had been observed before (van der Hoek et al., 2010; Roest et al., 2011a). This outbreak could be related to abortion problems on dairy goat- and sheep farms. Besides sheep, goats and other animals such as cats, dogs and birds, also cattle can harbor *C. burnetii* and can function as reservoirs for human infection (Fournier et al., 1998; McQuiston et al., 2005; Parker et al., 2006; Angelakis and Raoult, 2010). While for dairy goats and sheep, an important symptom of infection is abortion, in cattle, this is rarely the case, and shedding of *C. burnetii* is of lower level (van Moll et al., 1993; Arricau-Bouvery and Rodolakis,

2005; Rodolakis et al., 2007; Hansen et al., 2011). Infected cows shed the bacterium in feces, milk and birth products (Guatteo et al., 2006, 2007). After shedding, *C. burnetii* can be transmitted to humans or to animals on the same farm or on other farms. For humans, the main infection route is by inhalation of infected aerosols (Cutler et al., 2007; Roest et al., 2011a). It has been described previously that in 2007 the seroprevalence and DNA prevalence of *C. burnetii* in bovine herds in the Netherlands were 79% and 28%, respectively (Muskens et al., 2011) which was comparable to that in other western European countries (Agger et al., 2010; Ryan et al., 2011). Because it cannot be excluded that dairy cattle may transmit *C. burnetii* to other species, it is important to be able to take preventive measures to reduce the prevalence in cattle herds. Therefore, this study presents both the herd prevalence of *C. burnetii* in dairy cattle as well as the risk factors for *C. burnetii* antibody and DNA positivity on herd level.

Materials and methods

This study is part of a larger study conducted in 2009 – 2011 that describes the relationship between the presence of *C. burnetii* in several ruminant species on farm-level and seropositivity for *C. burnetii* among household members (Schimmer et al., 2012, 2014; De Lange et al., 2014). They elaborately describe the methods of our study (Schimmer et al., 2014). In short, a total of 3000 farms with at least 50 adult dairy cows were randomly selected from the national Information and Registration database. All farms received an informative letter and an invitation to participate in 2010. Farmers could agree to participate by returning an informed consent.

The farm was visited and an electronic questionnaire was filled out by the farmer, containing questions about the herd demographics and animal health management. The questionnaire was validated by several veterinary experts, pretested by two farmers and adapted according to their comments. Ninety closed questions available for univariable analysis. Furthermore, a bulk tank milk (BTM) sample was collected for determination of the presence of *C. burnetii* DNA (by PCR) and antibodies (by ELISA).

ELISA

In this study, BTM antibodies against *C. burnetii* were measured with an indirect ELISA (LSIVet™ Ruminant Q Fever – Serum/Milk ELISA Kit, LSI, Lissieu, France). The test was used according to the manufacturer's instructions and was validated for use as bulk milk test by Muskens et al. (2011). Briefly, milk was diluted 1:20 in dilution buffer, and transferred to 96 wells ELISA plates (total volume 100 µl), coated with antigen. The samples were incubated overnight at 4 °C, washed four times and incubated with 100 µl anti-ruminant IgG peroxidase conjugate for 1 h at 37 °C. After washing four times, the wells were incubated with 100 µl tetramethylbenzidine (TMB) substrate for 10 min at 22 °C in darkness. Color development was stopped by the addition of 100 µl 0.5 M H_2SO_4. Optical density values (OD) were measured at 450 nm. Sample/Positive percentages (S/P%) were calculated using the following formula $S/P\% = (OD_{sample} - OD_{negative\ control})/(OD_{positive\ control} - OD_{negative\ control}) \times 100\%$. An S/P ratio exceeding 30% was regarded as positive (Muskens et al., 2011).

PCR

Presence of *C. burnetii* DNA in BTM samples was tested using a commercial real-time PCR assay (kit Taqvet™ *Coxiella burnetii*, LSI, Lissieu, France) which targets the repetitive transposon-like region of the bacterium. The test was used according to the manufacturer's instructions as described previously (Muskens et al., 2011). DNase RNase free water was used as negative control sample. A sample included in the kit containing 10^5 *C. burnetii*/mL was used as positive control. DNA was extracted using the Magmax Express-96 and the Magmax total nucleic acid isolation kit® according to the manufacturer's instructions. The extraction was performed directly from 175 µL of raw milk. The PCR assays were performed using ABI Prism® sequence Detection System 7500 (Applied Biosystems). Each sample was also tested with a specific primer set for ruminant Glyceraldehyde-3-phosphatedehydrogenase (GAPDH). For samples with the typical amplification curves and Ct values <40, presence of *C. burnetii*/mL was quantified, based on a reference line generated from decimal dilutions of the positive control. Since, according to the manufacturer's recommendations, numbers lower than 100 are not quantifiable, parallel to the procedure for goat BTM (van den Brom et al., 2012), presence of DNA equivalent to 100 bacteria/mL or more was regarded as positive.

Data analysis

A non-response analysis was conducted to compare participating and non-participating farms with regard to herd size (number of cows in different age categories), distance to nearest *C. burnetii* BTM positive dairy goat or sheep farm, goat density, sheep density and cattle density for the area in which the farms were situated, location in the area in which in 2009 due to the large human outbreaks all dairy goats had to be vaccinated against *C. burnetii* and province, region and urbanization degree of the region in which the farm was located. Differences between responders and non-responders were analyzed using the Mann–Whitney *U* test for continuous variables and the Chi-square test for categorical variables.

Two logistic regression models were developed, one for ELISA results and one for PCR results in BTM. Univariable logistic regression analysis was performed to select the variables for the full multivariable models based on $p < 0.20$ in the likelihood ratio test. Variables with less than 10% of the participants in the risk category were not included for further analysis. When several variables were strongly correlated in the univariable analysis or not informative, only the most informative and relevant variable was selected for inclusion. In the multivariable logistic regression analyses, manual stepwise backwards selection was used to select significant variables with a *p*-value <0.10 (likelihood ratio test). This liberal cut-off was used because the presence

Table 1
Prevalence of *C. burnetii* DNA and antibodies in Dutch dairy cattle herds ($n = 309$) in the Netherlands, tested by PCR and ELISA on bulk tank milk (number (%)).

PCR	ELISA		
	Negative	Positive	Total
Negative	57(18.4)	194(62.8)	251(81.2)
Positive	0(0.0)	58(18.8)	58(18.8)
Total	57(18.4)	252(81.6)	309(100.0)

of *C. burnetii* may have serious public health implications, therefore we did not want to miss potential risk factors even though the relationships may be weak. Confounding was monitored by calculating the change in the coefficient of a variable after removing another variable. If the change of the estimates exceeded 25%, the removed variable was considered as potential confounder. The excluded factors were added to the final model one by one to check for confounding significance. In the final model biological plausible two-way interactions between variables in the two multivariable models were investigated for significance. Goodness of fit was assessed by the Hosmer Lemeshow test statistic. All analyses were performed using SAS version 9.2.

Results

3.1. Non-response analysis

Results of the non-response analyses were published before (Schimmer et al., 2014). In short, the overall response-rate was 10.4%. Participating and non-participating farms were comparable, except that participating farms had a smaller distance to *C. burnetii* BTM positive small ruminant farms and were more frequently situated in an area with a higher sheep density.

3.2. Bulk tank milk analyses

From the 311 farmers that participated, 309 provided a BTM sample. Of those 309, an on-farm questionnaire was available from 301 farms. Of the BTM samples, 81.6% (CI: 77.2–85.9) were ELISA positive and 18.8% (CI: 14.4–23.1) were PCR positive (Table 1).

For presence of *C. burnetii* antibodies in BTM, in the univariable analysis (including 90 variables), eight variables were significant at $p < 0.20$ (Table 2). From these, four variables remained in the final model (Table 3): large herd size (increase per 10 cows; OR = 1.1; CI: 1.0–1.2), cleaning bedding of the cubicles at most every other day (OR = 2.8; CI: 1.1–7.1), having purchased cattle from at

Table 2
Univariable analysis results of farm-based factors associated with *C. burnetii* antibodies in BTM from 301 Dutch dairy cattle herds ($p < 0.20$, −2LL).

Variable	Category	Frequency (N)	Prevalence (%)	*p*-Value
Herdsize in 2008[a],[b]		309		0.067
Region[a]	Southeast	174	85.6	0.038
	Other	135	76.3	Reference
Working on farm since	Before 1990	224	77.7	0.012
	1990 or later	66	92.3	Reference
Use of automatic milking system[a]	Yes	62	67.7	0.002
	No	238	85.3	Reference
Mattress used as bedding in the cubicle[a]	Yes	182	78.6	0.122
	No	119	85.7	Reference
Frequency cleaning bedding in stable[a]	Daily or more often	234	78.6	Reference
	Every other day or less	65	90.8	0.032
Horses on farm[a]	Yes	73	75.3	0.142
	No	225	83.1	Reference
Feeding maize silage[a]	Yes	264	83.0	0.068
	No	37	70.3	Reference
Numbers of cows that calved in 2009[b]		294		0.109
Numbers of twins born in 2009	<3	113	76.1	Reference
	≥3	126	87.3	0.027
Origin of semen	Only national	111	81.1	Reference
	International	139	86.3	0.262
	Unknown	40	67.5	0.081
Numbers of cattle purchase addresses in 2009[a]	<2	260	79.6	Reference
	≥2	36	91.7	0.096
Farm boots available for professional visitors[a]	Yes	275	82.9	0.033
	No	26	65.4	Reference
Farm clothes available for professional visitors	Yes	278	82.7	0.044
	No	23	65.2	Reference

[a] Variables included in multivariable analysis before manual backward elimination.
[b] The risk increases per cow.

Table 3
Final multivariable logistic regression model for presence of *C. burnetii* antibodies in BTM of 289 Dutch dairy cattle herds ($p < 0.10$, −2LL), with corresponding Odds Ratio and 95% Confidence Interval. Hosmer–Lemeshow goodness-of-fit test $p = 0.79$.

Variable	Category	OR [95% CI]
Herd size (per 10 cows)		1.1 [1.0–1.2]
Use of automatic milking system	Yes	0.3 [0.2–0.6]
	No	Reference
Frequency cleaning bedding in stable	≥2 times/day or daily	Reference
	Every other day or less	2.8 [1.1–7.1]
Locations from which cattle are purchased in 2009	<2	Reference
	≥2	3.1 [0.9–11.2]

Table 5
Final multivariable logistic regression model for presence of *C. burnetii* DNA in BTM of 284 Dutch dairy cattle herds ($p < 0.10$, −2LL), with corresponding Odds Ratio and 95% Confidence Interval. Hosmer–Lemeshow goodness-of-fit test $p = 0.77$.

Variable	Category	OR [95% CI]
Use of automatic milking system	Yes	0.2 [0.1–0.6]
	No	Reference
Goats or sheep on farm	Yes	0.4 [0.1–1.0]
	No	Reference
Number of cattle purchase addresses in 2009	<2	Reference
	≥2	3.2 [1.4–7.5]
Ticks at dairy cattle	Present/unknown	2.0 [1.0–3.9]
	No	Reference

least two addresses in 2009 (OR = 3.1; CI: 0.9–11.2) and use of an automatic milking system (OR = 0.3; CI: 0.2–0.6). Interaction terms were not statistically significant and no confounding was present.

Ten risk factors were significantly associated with presence of *C. burnetii* DNA in BTM in the univariable analysis (Table 4). From these, four variables remained in the final model (Table 5): having purchased cattle from at least two

Table 4
Univariable analysis results of farm-based factors associated which with presence of *C. burnetii* DNA in BTM from 301 Dutch dairy cattle herds ($p < 0.20$, −2LL).

Variable	Category	Frequency (N)	Prevalence (%)	*p*-Value
Region[a]	Southeast	174	21.8	0.119
	Other	135	14.8	Reference
Distance living to pasture	<20 m	111	26.1	0.009
	≥20 m	151	13.2	Reference
Bulk tank milk cell count	<200,000	187	15.0	Reference
	≥200,000	106	22.6	0.101
Use of automatic milking system[a]	Yes	62	4.8	0.005
	No	238	21.8	Reference
Poultry on farm[a]	Yes	58	10.3	0.082
	No	240	20.4	Reference
Goats or sheep on farm[a]	Yes	53	7.5	0.016
	No	245	20.8	Reference
Cat in stable	Yes	177	20.9	0.170
	No	123	14.6	Reference
Rabbit/guinea pig in stable	Yes	10	40.0	0.087
	No	289	17.6	Reference
Use of hay as roughage[a]	Yes	218	15.6	0.054
	No	83	25.3	Reference
Ticks at dairy cattle[a]	Present/unknown	72	28.4	0.010
	No	222	14.9	Reference
Type of farm management	Closed	213	14.6	Reference
	Open	85	27.1	0.013
Number of cattle purchase addresses in 2009[a]	<2	260	15.4	Reference
	≥2	36	36.1	0.003
Pet: dog[a]	Yes	201	16.4	0.169
	No	99	23.2	
Pet: cat[a]	Yes	179	21.8	0.092
	No	122	13.9	Reference
Pet: rabbit[a]	Yes	66	24.2	0.185
	No	234	17.1	Reference

[a] Variables included in multivariable analysis before manual backward elimination.

delivery addresses in 2009 (OR = 3.2; CI: 1.4–7.5), having dairy cattle with ticks or unknown tick status (OR = 2.0; CI: 1.0–3.9), use of an automatic milking system (OR = 0.2; CI: 0.1–0.6) and having goats or sheep on the farm (OR = 0.4; CI: 0.1–1.0). Interaction terms were not statistically significant and no confounding was present.

Discussion

The aim of the study was to estimate the herd prevalence of *C. burnetii* in dairy cattle and to identify and quantify risk factors for *C. burnetii* antibody and DNA positivity on herd level.

ELISA and PCR prevalence

A considerable part of the Dutch cattle herds had *C. burnetii* antibodies (81.6%) or DNA (18.8%) in their BTM. When comparing these figures to prevalences in other countries, it must be taken into account that differences can be caused by the use of different diagnostic techniques and matrices (Guatteo et al., 2011). In the review of Guatteo et al. based on DNA and antibody tests, prevalences from 4.4% to 100% at herd level were reported worldwide, with a mean of 37.7%. In the United States, herd prevalences varied from 26.3% (McQuiston and Childs, 2002) to 94.3% (Kim et al., 2005) while in Asia, herd prevalences from 16.7% to 35.4% were determined (Guatteo et al., 2011). In Norway, no indication of *C. burnetii* infection in dairy cattle was observed (Kampen et al., 2012). However, herd prevalences of 59% in Denmark, 64.5% in Northern Ireland and 72.3% in Germany were reported (Agger et al., 2010; McCaughey et al., 2010; Bottcher et al., 2011). A large study in the Netherlands in 2007 showed a prevalence of 78.6% *C. burnetii* antibodies in BTM samples from dairy cattle herds (Muskens et al., 2011), which was comparable with the result of 81.6% seen in our study. However, in our study 18.8% of the dairy cattle herds was PCR positive, which was significantly ($p < 0.05$) lower than found by Muskens et al. (28.2%), when using the same test and cut-off value. They conducted their study in the Netherlands just in the beginning of a large human outbreak that occurred from 2007 to 2009 resulting in compulsory control measures for dairy goat herds. Therefore, the lower prevalence in 2011 in dairy cattle herds might be related to the control measures that were applied at dairy goat farms. Even though *C. burnetii* types found in dairy cattle herds were different from the type that was mainly present in dairy goats and in human cases during the 2007–2009 outbreak, the strain similar to that in goats has been found as well (Roest et al., 2011b, 2013).

Risk factors

Several factors were associated with BTM status. It cannot be excluded that there was some bias in the results due to the low response rate and the finding that participating farms had a smaller distance to *C. burnetii* BTM positive small ruminant farms and were more frequently situated in an area with a higher sheep density. However, the differences for these variables were small and participating and non-participating farms were not different for the other characteristics for which they could be compared (Schimmer et al., 2014). Therefore, the potential response bias will not to have influenced the results of the study to a large extent.

A consistent and strong correlation was found between BTM status (PCR and ELISA) and purchase of cattle from at least two addresses in 2009. In general, purchasing animals is a well-known risk factor for introduction of infectious diseases (van Schaik et al., 2002; Fevre et al., 2006). In addition, for *C. burnetii* it has been shown that use of quarantine for newly purchased animals reduced BTM ELISA positivity (Paul et al., 2012). Both findings indicate that the introduction of new animals with unknown disease status might be a substantial risk for introducing *C. burnetii* in the herd (Brennan and Christley, 2012). In our study, a larger dairy herd size turned out to be a risk factor for BTM ELISA positivity. This is in agreement with other studies (McCaughey et al., 2010; Ryan et al., 2011; Paul et al., 2012). In The Netherlands in 1211 there were 19,247 herds with on average 76 milking cows with an average annual milk production of 8.063 kg per cow (Zuivel in cijfers 2011, Dutch Dairy Board). A large herd could face an increased risk, either because of an increased chance of introduction of the pathogen or because of a decreased chance to clear the infection due to the presence of a relatively large number of susceptible animals. In addition, larger herds may differ in management practices that are favorable for a *C. burnetii* infection. Thus, farm-related risk factors that are associated with herd size may have influenced this association.

The use of automatic milking was unrelated to herd size and is a strong negatively associated factor for ELISA (OR = 0.3) and PCR positivity (OR = 0.2). To the best of our knowledge, this is the first time, that a relationship is found between milking systems and risk of infectious diseases, except mastitis (Hovinen and Pyorala, 2011). Automatic milking was introduced in the Netherlands in 1992 and its use has increased to 16% in 2012 (Stichting Kwaliteitszorg Onderhoud Melkinstallaties (KOM), The Netherlands). In our study automatic milking was performed on 20% of the farms. The management practices, cow behavior and contact structures are obviously different between herds with automatic milking and herds with conventional milking (Jacobs and Siegford, 2012). So, automatic milking could be a surrogate for other underlying risk factors not available in this study. When automatic milking is used, cows are not gathered simultaneously at pre-milking but are milked separately. It was shown previously that tie stalls have a lower risk to be *C. burnetii* BTM positive than free stalls, also probably caused by different contact structures between the cows and thus a reduced risk of transmission (Paul et al., 2012). We could not reproduce this because in our study only 3 (1%) herds were housed in tie stalls.

Cleaning the bedding in the cubicles at least once per day was negatively associated to presence of *C. burnetii* antibodies in BTM. This could be a proxy measure of hygiene practices in general. However, it could also be explained by a decreased transmission of *C. burnetii* to other cows in the herd by removing contaminated birth material or genital tract excretions deposited in the bedding when cows lie down in the cubicles. It has been shown before, that

hygiene of cubicles in free stalls influences infection rates (De Kruif, 1978). In parallel, also for endometritis, it was suggested that bedding plays a role in the transmission of endometritis related bacteria (Cheong et al., 2011).

A risk factor for PCR positivity was the presence of dairy cattle with ticks or an unknown tick status compared to not having dairy cattle with ticks. Cantas et al. (2011) also found that dairy cattle in Cyprus with ticks had an increased risk for *C. burnetii* PCR positivity (Cantas et al., 2011). Sprong et al. (2012) however, found no *C. burnetii* in ticks originating from dairy cattle in the Netherlands in 2008. However, in that study ticks from only one location were investigated. One might argue that the presence of ticks on the cows is a regional effect or is a proxy for grazing practices (Capuano et al., 2001) because in our study tick infestation was significantly more seen on cattle that grazed during summer (93.2% vs 72.2%, χ^2, $p = 0.0002$). However, the underlying factors were not statistically significant in the model.

Having sheep or goats on the farm showed a negative association with PCR positivity. This effect could partly be due to the fact that farms with sheep or goats (only a few cattle farms had only goats as small ruminants) are more often located in the northwestern part of the Netherlands, more distant from the epicenter of the Q fever outbreak in goats and humans. In addition, farms with goats and sheep may have a more extensive management system.

This study found several factors associated with *C. burnetii* BTM positivity. The results emphasize the importance of biosecurity and optimized hygiene for decreasing the risk for *C. burnetii* on farm level.

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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, 5.

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

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

Bottcher, J., Vossen, A., Janowetz, B., Alex, M., Gangl, A., Randt, A., Meier, N., 2011. Insights into the dynamics of endemic *Coxiella burnetii* infection in cattle by application of phase-specific ELISAs in an infected dairy herd. Vet. Microbiol. 151, 291–300.

Brennan, M.L., Christley, R.M., 2012. Biosecurity on cattle farms: a study in north-west England. PLoS ONE 7, e28139.

Cantas, H., Muwonge, A., Sareyyupoglu, B., Yardimci, H., Skjerve, E., 2011. Q fever abortions in ruminants and associated on-farm risk factors in northern Cyprus. BMC Vet. Res. 7, 13.

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.

Cheong, S.H., Nydam, D.V., Galvao, K.N., Crosier, B.M., Gilbert, R.O., 2011. Cow-level and herd-level risk factors for subclinical endometritis in lactating Holstein cows. J. Dairy Sci. 94, 762–770.

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

De Kruif, A., 1978. Factors influencing the fertility of a cattle population. J. Reprod. Fertil. 54, 507–518.

De Lange, M.M., Schimmer, B., Vellema, P., Hautvast, J.L., Schneeberger, P.M., Van Duijnhoven, Y.T., 2014. *Coxiella burnetii* seroprevalence and risk factors in sheep farmers and farm residents in The Netherlands. Epidemiol. Infect. 142, 1231–1244.

Fevre, E.M., Bronsvoort, B.M., Hamilton, K.A., Cleaveland, S., 2006. Animal movements and the spread of infectious diseases. Trends Microbiol. 14, 125–131.

Fournier, P.E., Marrie, T.J., Raoult, D., 1998. Diagnosis of Q fever. J. Clin. Microbiol. 36, 1823–1834.

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.

Guatteo, R., Beaudeau, F., Joly, A., Seegers, H., 2007. *Coxiella burnetii* shedding by dairy cows. Vet. Res. 38, 849–860.

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.

Hansen, M.S., Rodolakis, A., Cochonneau, D., Agger, J.F., Christoffersen, A.B., Jensen, T.K., Agerholm, J.S., 2011. *Coxiella burnetii* associated placental lesions and infection level in parturient cows. Vet. J. 190, 135–139.

Hovinen, M., Pyorala, S., 2011. Invited review: udder health of dairy cows in automatic milking. J. Dairy Sci. 94, 547–562.

Jacobs, J.A., Siegford, J.M., 2012. Invited review: the impact of automatic milking systems on dairy cow management, behavior, health, and welfare. J. Dairy Sci. 95, 2227–2247.

Kampen, A.H., Hopp, P., Groneng, G.M., Melkild, I., Urdahl, A.M., Karlsson, A.C., Tharaldsen, J., 2012. No indication of *Coxiella burnetii* infection in Norwegian farmed ruminants. BMC Vet. Res. 8, 59.

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.

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.

McQuiston, J.H., Childs, J.E., 2002. Q fever in humans and animals in the United States. Vector Borne Zoonotic Dis. 2, 179–191.

McQuiston, J.H., Nargund, V.N., Miller, J.D., Priestley, R., Shaw, E.I., Thompson, H.A., 2005. Prevalence of antibodies to *Coxiella burnetii* among veterinary school dairy herds in the United States, 2003. Vector Borne Zoonotic Dis. 5, 90–91.

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

Parker, N.R., Barralet, J.H., Bell, A.M., 2006. Q fever. Lancet 367, 679–688.

Paul, S., Agger, J.F., Markussen, B., Christoffersen, A.B., Agerholm, J.S., 2012. Factors associated with *Coxiella burnetii* antibody positivity in Danish dairy cows. Prev. Vet. Med. 107, 57–64.

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

Roest, H.I., Tilburg, J.J., van der Hoek, W., Vellema, P., van Zijderveld, F.G., Klaassen, C.H., Raoult, D., 2011a. The Q fever epidemic in The Netherlands: history, onset, response and reflection. Epidemiol. Infect. 139, 1–12.

Roest, H.I., Ruuls, R.C., Tilburg, J.J., Nabuurs-Franssen, M.H., Klaassen, C.H., Vellema, P., van den Brom, R., Dercksen, D., Wouda, W., Spierenburg, M.A., van der Spek, A.N., Buijs, R., de Boer, A.G., Willemsen, P.T., van Zijderveld, F.G., 2011b. Molecular epidemiology of *Coxiella burnetii* from ruminants in Q fever outbreak, the Netherlands. Emerg. Infect. Dis. 17, 668–675.

Roest, H.I., van Solt, C.B., Tilburg, J.J., Klaassen, C.H., Hovius, E.K., Roest, F.T., Vellema, P., van den Brom, R., van Zijderveld, F.G., 2013. Search for possible additional reservoirs for human Q fever, The Netherlands. Emerg. Infect. Dis. 19, 834–835.

Ryan, E.D., Kirby, M., Collins, D.M., Sayers, R., Mee, J.F., Clegg, T., 2011. Prevalence of *Coxiella burnetii* (Q fever) antibodies in bovine serum and bulk-milk samples. Epidemiol. Infect. 139, 1413–1417.

Schimmer, B., Notermans, D.W., Harms, M.G., Reimerink, J.H., Bakker, J., Schneeberger, P., Mollema, L., Teunis, P., van Pelt, W., van Duynhoven, Y., 2012. Low seroprevalence of Q fever in The Netherlands prior to a series of large outbreaks. Epidemiol. Infect. 140, 27–35.

Schimmer, B., Schotten, N., van Engelen, E., Hautvast, J.L., Schneeberger, P.M., van Duijnhoven, Y.T., 2014. *Coxiella burnetii* seroprevalence and risk for humans on dairy cattle farms, the Netherlands, 2010–2011. Emerg. Infect. Dis. 20, 417–425.

Sprong, H., Tijsse-Klasen, E., Langelaar, M., De Bruin, A., Fonville, M., Gassner, F., Takken, W., Van Wieren, S., Nijhof, A., Jongejan, F., Maassen, C.B., Scholte, E.J., Hovius, J.W., Emil Hovius, K., Spitalska, E., Van Duynhoven, Y.T., 2012. Prevalence of *Coxiella burnetii* in ticks after a large outbreak of Q fever. Zoonoses Public Health 59, 69–75.

van den Brom, R., van Engelen, E., Luttikholt, S., Moll, L., van Maanen, K., Vellema, P., 2012. *Coxiella burnetii* in bulk tank milk samples from dairy goat and dairy sheep farms in The Netherlands in 2008. Vet. Rec. 170, 310–316.

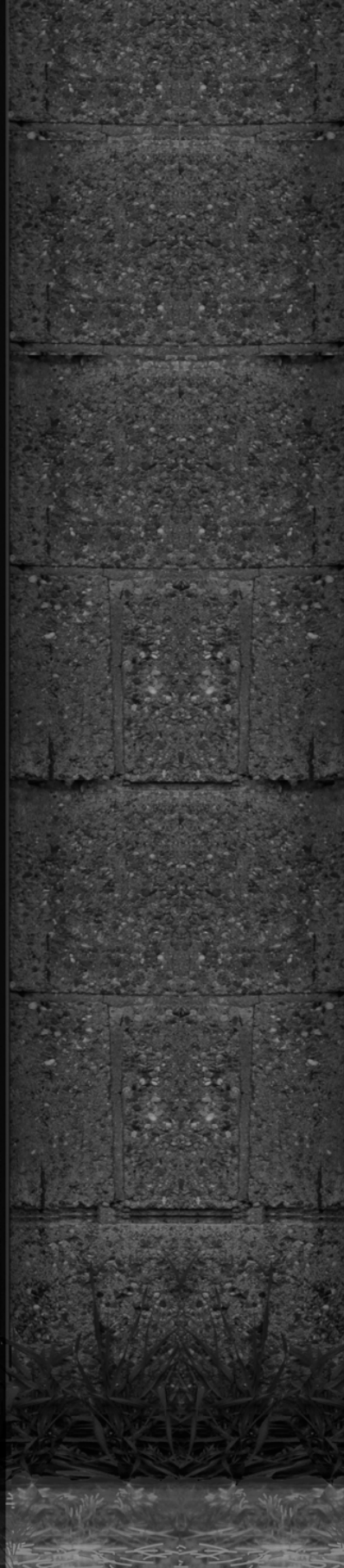
van der Hoek, W., Dijkstra, F., Schimmer, B., Schneeberger, P.M., Vellema, P., Wijkmans, C., ter Schegget, R., Hackert, V., van Duynhoven, Y., 2010. Q fever in the Netherlands: an update on the epidemiology and control measures. Euro Surveill. 15 (12), 2.

van Moll, P., Baumgartner, W., Eskens, U., Hanichen, T., 1993. Immunocytochemical demonstration of *Coxiella burnetii* antigen in the fetal placenta of naturally infected sheep and cattle. J. Comp. Pathol. 109, 295–301.

van Schaik, G., Schukken, Y.H., Nielen, M., Dijkhuizen, A.A., Barkema, H.W., Benedictus, G., 2002. Probability of and risk factors for introduction of infectious diseases into Dutch SPF dairy farms: a cohort study. Prev. Vet. Med. 54, 279–289.

5

Seroprevalence and occupational risk factors of *Coxiella burnetii* infection in veterinary-associated populations



CHAPTER 5.1

Seroepidemiological survey for *Coxiella burnetii* antibodies and associated risk factors in Dutch livestock veterinarians

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Seroepidemiological Survey for *Coxiella burnetii* Antibodies and Associated Risk Factors in Dutch Livestock Veterinarians

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Abstract

Since 2007, Q fever has become a major public health problem in the Netherlands and goats were the most likely source of the human outbreaks in 2007, 2008 and 2009. Little was known about the consequences of these outbreaks for those professional care providers directly involved. The aim of this survey was to estimate the seroprevalence of antibodies against *C. burnetii* among Dutch livestock veterinarians and to determine possible risk factors. Single blood samples from 189 veterinarians, including veterinary students in their final year, were collected at a veterinary conference and a questionnaire was filled in by each participant. The blood samples were screened for IgG antibodies against phase I and phase II antigen of *C. burnetii* using an indirect immunofluorescent assay, and for IgM antibodies using an ELISA. Antibodies against *C. burnetii* were detected in 123 (65.1%) out of 189 veterinarians. Independent risk factors associated with seropositivity were number of hours with animal contact per week, number of years graduated as veterinarian, rural or sub urban living area, being a practicing veterinarian, and occupational contact with swine. Livestock veterinarians should be aware of this risk to acquire an infection with *C. burnetii*. Physicians should consider potential infection with *C. burnetii* when treating occupational risk groups, bearing in mind that the burden of disease among veterinarians remains uncertain. Vaccination of occupational risk groups should be debated.

Introduction

Q fever is a zoonotic disease caused by the obligate intracellular bacterium, *Coxiella burnetii*, and ruminants are considered to be the primary source of infection for humans. In cattle, the disease is mainly asymptomatic [1], but in sheep and goats the main symptom is abortion, stillbirth and retention of foetal membranes [2–6]. The bacterium is shed in urine, milk, faeces and birth products of infected animals. The main route of transmission of the bacterium to humans is by aerosols [4,7,8].

Until 2007, about 20 Q fever cases were reported in the Netherlands annually [9]. In that year, Q fever became a major public health problem in the Netherlands with 168, 1000 and 2,357 human cases notified in 2007, 2008 and 2009, respectively [10]. These unprecedented annual outbreaks are largely explained by exposure of the general population living in the surroundings of infected dairy goat farms to airborne contaminated dust particles. Only 5% of the notified Q fever patients in the Netherlands report an occupation in agriculture, transporting or handling animal products, or animal care [11]. However, since its first description in abattoir workers in Australia in 1935 [12], Q fever has been considered primarily an occupational zoonotic disease for abattoir workers, sheep shearers, livestock farmers, and especially veterinarians because of their direct contact with potentially infected animals [13–19].

The aim of this survey was to estimate the seroprevalence of antibodies against *C. burnetii* among Dutch livestock veterinarians and to determine possible risk factors.

Materials and Methods

Human Population and Data Collection

In November 2009, professional laboratory assistants collected a single blood sample from Dutch livestock veterinarians and final-year veterinary students attending a veterinary conference.

Each participant filled in a self-administered questionnaire to obtain epidemiological and clinical information. The questionnaire existed of three parts, and took approximately fifteen minutes to complete. The first part focused on demographic data and included age, gender, and residence in urban, sub urban or rural area. The second part consisted of occupation-related questions regarding work location, type of veterinary occupation, years in veterinary practice, contact with livestock and livestock farms, contact with animal related products as straw, hay, soil, birth products and urine and faeces, contact with aborted animals, use of personnel protective equipment, work related wounds and

accidental vaccine exposure. The third part consisted of non-occupation related questions regarding possession of animals in the last five years, consumption of raw dairy products, outdoor activities and health conditions, including smoking, tick bites during the last five years and a known history of a clinical Q fever infection, pregnancy and abortion.

This study was approved by the Medical Ethical Committee of the University Medical Centre Utrecht, Utrecht, the Netherlands (reference number 09–322). All participants received a book to express appreciation for their cooperation.

Laboratory Methods

A serum sample from each participant was tested for the presence of IgG antibodies against C. burnetii using a Q fever indirect immunofluorescent assay (IFA; Focus Diagnostics, Cypress, CA), according to the manufacturer's protocol. Sera were screened for phase I and phase II IgG using a cut-off of 1:32. Samples with both IgG phase I and II titres of ≥1:32 were considered to be positive, while solitary IgG phase II samples were scored positive if they had a single titre of ≥1:512.

All samples were also screened for IgM using an ELISA (Focus Diagnostics), according to the manufacturer's protocol, and positive samples were confirmed with IFA. Samples with a titre of ≥1:32, both for IgM phase I and II, were considered to be positive, indicating a possibly recent infection.

Within the group of participants with a past infection, a distinction was made between serological profiles considered not likely to be compatible with a chronic infection, and serological profiles which could indicate a chronic infection. Serum samples from participants with a possibly chronic Q fever infection, having an IgG phase I titre ≥1:1024, were additionally analysed by performing a *C. burnetii* PCR.

Statistical Data Analysis

All individual laboratory results were merged with the self-administered questionnaires. Statistical analysis was carried out using STATA 11. The Chi square test and the two-sided proportion-test were used to estimate univariate associations between exposures and seropositivity. Analyses were carried out to calculate odds ratio's with 95% confidence intervals. The odds ratio (OR) was defined, in this context as the odds of a given exposure among veterinarians seropositive for *C. burnetii* divided by the odds of exposure among seronegative veterinarians. Veterinarians who did not completely fill in the questionnaire were excluded for the analysis of that particular question.

For the multivariable logistic regression, initially all variables with (2-sided) $p<0.20$ and with sufficient numbers (>10) were selected. To avoid multicollinearity, from groups of variables that had a correlation of more than 0.50 with each other, only one, the most plausible biological variable, was left in the multivariable analysis.

Stepwise backward logistic regression was carried out, starting with all data and excluding stepwise each variable that had a p-value of >0.05. All remaining variables were considered to be risk or protective factors.

Results

Descriptive Results

A total of 189 participants, being more than 90% of the attendants, completed the questionnaire and provided a blood sample during the conference. The median age of the participants was 44 years (interquartile range, 34–52 years). Of the participants, 130 (68.8%) were male and 59 (31.2%) were female (Table 1). One hundred and twelve of the participants worked as a livestock practitioner, 20 were non-practicing, 37 worked as livestock veterinarian at a veterinary institute (Utrecht University (UU) or Animal Health Service (GD)) and 20 were livestock veterinary students in their final year. A total of 108 (57.1%) of the participants had contact with livestock for more than 50% of working hours in their current job.

The overall seroprevalence was 65.1% (n = 189). In livestock veterinarians the seroprevalence was 69.2% (n = 169). The seroprevalence in livestock veterinary students was 30.0% (n = 20). Among the group of 169 livestock veterinarians the seroprevalence was 87.5% in practicing livestock veterinarians (n = 112), 45.0% in non-practicing livestock veterinarians (n = 20) and 27.0% in livestock veterinarians working at a veterinary institute (n = 37). IgG antibody titers against *C. burnetii* measured for both phase I and II ranged from 1:32 to 1:2048. Seven out of nine participants with a positive IgM ELISA result were confirmed with IFA, suggesting a recent infection. Four of those seven IFA positive study participants were livestock veterinary students. The other three were practicing livestock veterinarians. Seven participants with an IgG phase I titre ≥1:1024, a possible indication of a chronic Q fever infection, were followed up by performing a *C. burnetii* PCR on a blood sample, and in all cases PCR results were negative. Additionally, participants with an IgG phase I titre ≥1:512 are offered to participate in a follow-up study and are advised to be controlled for risk factors of a chronic Q fever infection.

Univariable Analysis

Participants who were seropositive were likely to be male over the age of 32 years (Table 1). Participants living in rural or suburban areas were significantly more often seropositive than participants living in an urban area. Occupational risk factors in univariable analysis were: graduated as a veterinarian more than two years ago; more than 10 hours of animal contact per week; practicing as livestock veterinarian; and working with cattle, horses, dogs and cats. Participants with frequent contact with animal products, like straw, hay, roughage, raw milk, birth products of ruminants as well as of pets, urine of ruminants, practicing on cattle farms with abortion, and one or more contacts on farms with abortion problems in the last five years, were significantly more often seropositive. Accidental needle injections and cutting incidents were also found to be associated with seropositivity. Non-occupational activities like cycling and shopping were associated with seronegativity. In contrast, gardening and having dogs and (pet) birds were found to be associated with seropositivity. Consumption of dairy products, health conditions like smoking behaviour, and not wearing protective clothes during work were not found to be a significant univariate risk factor. The number of participants primarily working with sheep and goats, with a history of a clinical Q fever infection, or with pregnancy and abortion was too small for statistical analysis.

Multivariable Analysis

Variables with a p-value <0.20 in the univariable analysis were used as input for the multivariable analysis. The number of years as a veterinarian was highly correlated with age and gender; the latter two were left out of the analysis. Working category and contacts with ruminants were very highly correlated to contact with hay/straw, roughage, raw milk, birth products of ruminants and with urine of ruminants; the latter 5 were left out of the analysis.

In this group of livestock veterinarians, risk factors for *C. burnetii* seropositivity in the multivariable analysis (Table 2) were: number

Table 1. Results of univariable analysis of risk factors for presence of antibodies against *Coxiella burnetii*.

	Participants							
	Seropositive[#]		Seronegative					
	No.	%	No.	%	Odds Ratio	95% confidence interval		P
Gender								
Female	24	40.7	35	59.3	1.0	.	.	.
Male	99	76.2	31	23.8	4.7	2.3	9.4	<0.001
Age								
<=32 year	19	40.4	28	59.6	1.0	.		.
33–44 year	35	71.4	14	28.6	3.7	1.6	8.6	0.003
45–52 year	37	75.5	12	24.5	4.5	1.9	10.9	0.001
53–65 year	32	72.7	12	27.3	3.9	1.6	9.5	0.002
Living region								
Urban	8	30.8	18	69.2	1.0	.	.	.
Sub-urban	21	56.8	16	43.2	3.0	1.0	8.5	0.037
Rural	94	74.6	32	25.4	6.6	2.6	16.7	<0.001
Veterinarian (years)								
Veterinarian (<=2)	13	27.7	34	72.3	1.0			
veterinarian (3–13)	36	70.6	15	29.4	6.3	2.6	15.1	<0.001
veterinarian (14–21)	33	75.0	11	25.0	7.9	3.1	20.0	<0.001
veterinarian (>=22)	40	87.0	6	13.0	17.4	6.00	50.8	<0.001
Animal contact (hours/week)								
<10 hours	9	24.3	28	75.7	1.0			
10–19 hours	25	55.6	20	44.4	3.9	1.5	10.1	0.005
20–29 hours	42	80.8	10	19.2	13.1	4.7	36.2	<0.001
>=30 hours	43	89.6	5	10.4	26.8	8.1	88.2	<0.001
Work category								
Others	23	30.7	52	69.3	1.0			
Practicing	100	87.7	14	12.3	16.2	7.7	34.0	<0.001
Contact with cows								
No	11	31.4	24	68.6	1.0	.	.	.
Yes	112	72.7	42	27.3	5.8	2.6	12.9	<0.001
Contact with swine								
No	80	61.5	50	38.5	1.0	.		.
Yes	43	72.9	16	27.1	1.7	0.9	3.3	0.131
Contact with birth products of ruminants								
No	16	33.3	32	66.7	1.0	.	.	.
Yes	107	75.9	34	24.1	6.3	3.1	12.9	<0.001
Contact with birth products of pets								
No	101	61.2	64	38.8	1.0	.	.	.
Yes	22	91.7	2	8.3	7.0	1.5	31.9	0.004
Practice on cow farm with abortion								
No	32	43.8	41	56.2	1.0	.	.	.
Yes	91	78.4	25	21.6	4.7	2.4	9.3	<0.001

[#]Sera were screened for phase I and phase II IgG using a cut-off of 1:32. Samples with both IgG phase I and II ≥1:32 were considered to be positive, while solitary IgG phase II samples were scored positive if they had a single titre of ≥1:512 (Focus Diagnostics, Cypress, CA).

of hours with animal contact per week, number of years graduated as veterinarian, living in a rural (OR, 17.9 (95% CI: 3.6–88.1)) or semi urban area (OR, 11.9 (95% CI: 2.1–68.5)), working as practicing livestock veterinarian (OR, 15.8 (95% CI: 2.9–87.2)), and occupational contact with swine (OR, 3.4 (95% CI: 1.1–10.2)).

Discussion

In this cross-sectional study, an overall *C. burnetii* seroprevalence of 65.1% among Dutch livestock veterinarians was found. The number of hours with animal contact per week, the number of

Table 2. Final multivariable model for risk factors associated with presence of antibodies against *Coxiella burnetii* in 189 veterinarians.

Variable	Category	No.	OR	[95% CI]		P
Animal contacts	<10 hours	37	1.0			
(hours/week)	10–19 hours	45	12.0	2.5	57.1	0.002
	20–29 hours	52	1.2	0.2	7.6	0.869
	>=30 hours	48	16.0	1.8	141.8	0.013
Veterinarian (years)	<=2	47	1.0			
	3–13	51	17.5	4.0	77.4	<0.001
	14–21	44	26.5	4.8	145.9	<0.001
	>=22	46	58.1	10.3	328.0	<0.001
Living region	Urban	26	1.0			
	Sub-urban	37	11.9	2.1	68.5	0.005
	Rural	126	17.9	3.6	88.1	<0.001
Work category	Others	75	1.0			
	Practicing	114	15.8	2.9	87.2	0.002
Contact with swine	No	130	1.0			
	Yes	59	3.4	1.1	10.2	0.029

years the participants were graduated and practicing as a veterinarian, were the main independent risk factors in this study. These risk factors suggest a high dose-effect relation for seropositivity in Dutch livestock veterinarians. In 1984, 84% of 222 Dutch livestock veterinarians were seropositive for IgG antibodies against *C. burnetii* [17]. The use of a different laboratory test and cut-offs, differences in study population and different infection rates of livestock over time could be possible explanations for other seroprevalence estimates.

Dutch livestock veterinarians have a high risk of getting *C. burnetii* seropositive because of intensive contact with potentially infected livestock, and the immune system can be boosted frequently because of a high prevalence in Dutch livestock [20,21]. Contact with swine was found to be an independent risk factor, but the group of veterinarians involved was also exposed to cattle. Further, the main geographical areas where pigs are kept in the Netherlands corresponds with the high-incidence areas where the human Q fever epidemic related to dairy goats was situated and where high seroprevalences were found in the rural population. On the other hand, treatment of swine has previously been described as a risk factor for seropositivity for veterinarians [19]. The natural susceptibility of swine to *C. burnetii* was demonstrated during a Q fever epidemic in Uruguay. A seroprevalence of 21.4% was measured in 391 healthy slaughter pigs [22]. No information about Q fever prevalences in swine in the Netherlands is available.

In this survey, 20 veterinary students participated, and the seroprevalence was 30%. In a survey in Spain, a seroprevalence of 11% among veterinary students was found. First course students showed a significant lower seroprevalence. Multiple risk factors were associated with *C. burnetii*: study course, contact with live animals especially ruminants and contact with persons working with animals [18]. A large serological survey (n = 674) was already carried out in the Netherlands in 2006. At that time 18.7% of the veterinary students were seropositive. Students in their final year with the livestock study direction had a seroprevalence of 37.3%. The main risk factors were a study direction focusing on large animals, advanced year of study, having had a zoonosis during study and having ever lived on a farm with ruminants [23]. To detect possible recent exposure to *C. burnetii*, testing was also performed by ELISA IgM, and it is not remarkable that four out of seven possible recent infections occurred in veterinary livestock students, indicating this group is susceptible for the infection during the practical rotations during their study. The lower prevalence in veterinary students, an indication for recent infection in seven of whom four were students, and the main risk factors we found, are another indication for a high dose-effect relation for seropositivity.

Our study clearly indicates that livestock veterinarians are an occupational risk group. The prevalence found in this study was much higher than described in several international sero-epidemical studies among livestock veterinarians [13–15,18,19,24,25], with the exception of a small survey among 12 veterinarians in southern Italy, which revealed a seroprevalence of 100% [16]. In other studies, contact with livestock is described as an important risk factor for seropositivity [14,19,24], and exposure to goats was the most important risk factor associated with *C. burnetii* infection in Southern Taiwan [14]. Treatment of cattle, swine or wildlife were main risk factors associated with *C. burnetii* seropositivity in US veterinarians [19]. In Slovakia and Nova Scotia, professional orientation and regular contact with farm animals and pets [24], and exposure to sheep placentas [15] were described as important risk factors, respectively. In contrast, in Japan, no significant correlation was found between years of occupational experience and *C. burnetii* seropositivity [13].

The final independent risk factor was living in a rural or sub-urban area. Participants living in these areas were significantly more often seropositive than participants living in an urban area. Rural and sub-urban living areas have been described before as a risk factor [26–30], although urban outbreaks also have been described, but could mostly be related to exposure to animals or animal products [31–33]. In the Netherlands, livestock farms are mainly situated in rural or sub-urban areas. The knowledge that ruminants are the main reservoir for *C. burnetii* [1,34] and the fact that *C. burnetii* can easily be spread by aerosols [4,7,8], presumably explains why living in rural or sub-urban area is a risk factor for seropositivity.

In the univariable analysis, age and gender were risk factors for seropositivity. Nevertheless, both were left out of the multivariable analysis because they were highly correlated with the number of years participants were graduated as veterinarian. The higher incidence in males than in females has been reported in several sero-epidemical studies among veterinarians, but without a clear explanation [15,17–19]. Also a Spanish study among veterinary students revealed that male students in the fifth study year had a significantly higher risk to be seropositive than female students [18]. A higher clinical incidence in males and persons aged between 40–60 years in the Dutch population has been described during the Q fever outbreaks between 2007–2010 [11]. Age above 46 years, was also previously described as a risk factor for seropositivity in veterinarians [19].

To differentiate in the group of practicing veterinarians, all analyses were repeated in the multivariable analysis for the subset of practicing veterinarians only, mainly working with cattle, swine and poultry, or individual housed animals. The analysis on the subset of practicing veterinarians did not result in additional significant results (data are not shown), and was less robust than the multivariable analysis based on the full data set.

In conclusion, Dutch livestock veterinarians are an occupational risk group with increased risk for *C. burnetii* infection presumably because of their direct contact with infected livestock. Dutch livestock veterinarians should be aware of this risk and be extra alert regarding symptoms of Q fever. Most of the infections are not notified, as they remain asymptomatic or result in only mild flu-like symptoms. Serious infections leading to pneumonia or hepatitis may occur. A *C. burnetii* infection can cause serious complications during pregnancy and in those with underlying disease, therefore these groups should be monitored properly. Vaccination of occupational groups at risk is common in Australia [35,36]. In the Netherlands, vaccination has been made available in the first half of 2011, but only for specific risk groups, as those patients with heart valve and vascular disorders. During the community Q fever outbreaks between 2007 and 2009 in the Netherlands, few patients reported occupational exposure [11]. Most veterinarians are not eligible for vaccination because the presence of antibodies is an absolute contraindication for administering the currently available Australian vaccine. However, vaccination could be considered for seronegative veterinary students at the beginning of their study [35]. Routine serological follow-up is useful as well as basic safety rules, like hygiene measures and the use of protection clothes [18,19,24,37], although in this study disregard of protective measures was not found to be an independent risk factor. Occupational exposure to several zoonotic diseases makes basic safety rules useful for protecting the livestock veterinarian.

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Author Contributions

Revised the manuscript: BS PV. Read and approved the final manuscript: RV BS PS WS Wvdh PV. Conceived and designed the experiments: RV BS WvdH PV. Performed the experiments: PS. Analyzed the data: WS RV BS. Wrote the paper: RV.

References

1. Arricau-Bouvery N, Rodolakis A (2005) Is Q fever an emerging or re-emerging zoonosis? Vet Res 36: 327–349.
2. Damoser J, Hofer E, Muller M (1993) [Abortions in a lower Austrian sheep facility caused by Coxiella burnetii]. Berl Munch Tierarztl Wochenschr 106: 361–364.
3. Hatchette TF, Hudson RC, Schlech WF, Campbell NA, Hatchette JE, et al. (2001) Goat-associated Q fever: a new disease in Newfoundland. Emerg Infect Dis 7: 413–419.
4. Maurin M, Raoult D (1999) Q fever. Clin Microbiol Rev 12: 518–553.
5. Wouda W, Dercksen DP (2007) [Abortion and stillbirth among dairy goats as a consequence of Coxiella burnetii]. Tijdschr Diergeneeskd 132: 908–911.
6. Zeman DH, Kirkbride CA, Leslie-Steen P, Duimstra JR (1989) Ovine abortion due to Coxiella burnetti infection. J Vet Diagn Invest 1: 178–180.
7. Marrie TJ (1990) Q fever - a review. Can Vet J 31: 555–563.
8. Schimmer B, Dijkstra F, Vellema P, Schneeberger PM, Hackert V, et al. (2009) Sustained intensive transmission of Q fever in the south of the Netherlands, 2009. Euro Surveill 14.
9. Van Steenbergen JE, Morroy G, Groot CA, Ruikes FG, Marcelis JH, et al. (2007) [An outbreak of Q fever in The Netherlands–possible link to goats]. Ned Tijdschr Geneeskd 151: 1998–2003.
10. van der Hoek W, Dijkstra F, Wijers N, Rietveld A, Wijkmans CJ, et al. (2010) [Three years of Q fever in the Netherlands: faster diagnosis]. Ned Tijdschr Geneeskd 154: A1845.
11. Dijkstra F, van der Hoek W, Wijers N, Schimmer B, Rietveld A, et al. (2012) The 2007–2010 Q fever epidemic in The Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. FEMS Immunol Med Microbiol 64: 3–12.
12. Derrick EH (1937) Q fever, new fever entity: clinical features, diagnosis and laboratory investigation. Med J Aus 2: 282–299.
13. Abe T, Yamaki K, Hayakawa T, Fukuda H, Ito Y, et al. (2001) A seroepidemiological study of the risks of Q fever infection in Japanese veterinarians. Eur J Epidemiol 17: 1029–1032.
14. Chang CC, Lin PS, Hou MY, Lin CC, Hung MN, et al. (2010) Identification of risk factors of Coxiella burnetii (Q fever) infection in veterinary-associated populations in southern Taiwan. Zoonoses Public Health 57: e95–101.
15. Marrie TJ, Fraser J (1985) Prevalence of Antibodies to Coxiella burnetii Among Veterinarians and Slaughterhouse Workers in Nova Scotia. Can Vet J 26: 181–184.
16. Monno R, Fumarola L, Trerotoli P, Cavone D, Giannelli G, et al. (2009) Seroprevalence of Q fever, brucellosis and leptospirosis in farmers and agricultural workers in Bari, Southern Italy. Ann Agric Environ Med 16: 205–209.
17. Richardus JH, Donkers A, Dumas AM, Schaap GJ, Akkermans JP, et al. (1987) Q fever in the Netherlands: a sero-epidemiological survey among human population groups from 1968 to 1983. Epidemiol Infect 98: 211–219.
18. Valencia MC, Rodriguez CO, Punet OG, de Blas Giral I (2000) Q fever seroprevalence and associated risk factors among students from the Veterinary School of Zaragoza, Spain. Eur J Epidemiol 16: 469–476.
19. Whitney EA, Massung RF, Candee AJ, Ailes EC, Myers LM, et al. (2009) Seroepidemiologic and occupational risk survey for Coxiella burnetii antibodies among US veterinarians. Clin Infect Dis 48: 550–557.
20. Muskens J, Mars MH, Franken P (2007) [Q fever: an overview]. Tijdschr Diergeneeskd 132: 912–917.
21. Van den Brom R, van Engelen E, Luttikholt S, Moll L, van Maanen K, et al. (2012) Coxiella burnetii in bulk tank milk samples from dairy goat and dairy sheep farms in The Netherlands in 2008. Vet Rec 170: 310.
22. Somma-Moreira RE, Caffarena RM, Somma S, Perez G, Monteiro M (1987) Analysis of Q fever in Uruguay Rev Infect Dis 9: 386–387.
23. De Rooij MM, Schimmer B, Versteeg B, Schneeberger P, Berends BR, et al. (2012) Risk factors of Coxiella burnetii (Q fever) seropositivity in veterinary medicine students. PloS One 7: e32108.
24. Dorko E, Kalinova Z, Weissova T, Pilipcinec E (2008) Seroprevalence of antibodies to Coxiella burnetii among employees of the Veterinary University in Kosice, eastern Slovakia. Ann Agric Environ Med 15: 119–124.
25. Ergonul O, Zeller H, Kilic S, Kutlu S, Kutlu M, et al. (2006) Zoonotic infections among veterinarians in Turkey: Crimean-Congo hemorrhagic fever and beyond. Int J Infect Dis 10: 465–469.
26. Gardon J, Heraud JM, Laventure S, Ladam A, Capot P, et al. (2001) Suburban transmission of Q fever in French Guiana: evidence of a wild reservoir. J Infect Dis 184: 278–284.
27. Karagiannis I, Schimmer B, Van Lier A, Timen A, Schneeberger P, et al. (2009) Investigation of a Q fever outbreak in a rural area of The Netherlands. Epidemiol Infect 137: 1283–1294.
28. Lyytikainen O, Ziese T, Schwartlander B, Matzdorff P, Kuhnhen C, et al. (1998) An outbreak of sheep-associated Q fever in a rural community in Germany. Eur J Epidemiol 14: 193–199.
29. Nebreda T, Contreras E, Jesus Merino F, Dodero E, Campos A (2001) [Outbreak of Q fever and seroprevalence in a rural population from Soria Province]. Enferm Infecc Microbiol Clin 19: 57–60.
30. Stein A, Raoult D (1999) Pigeon pneumonia in provence: a bird-borne Q fever outbreak. Clin Infect Dis 29: 617–620.
31. Langley JM, Marrie TJ, Covert A, Waag DM, Williams JC (1988) Poker players' pneumonia. An urban outbreak of Q fever following exposure to a parturient cat. N Engl J Med 319: 354–356.
32. Oren I, Kraoz Z, Hadani Y, Kassis I, Zaltzman-Bershadsky N, et al. (2005) An outbreak of Q fever in an urban area in Israel. Eur J Clin Microbiol Infect Dis 24: 338–341.
33. Schimmer B, Ter Schegget R, Wegdam M, Zuchner L, de Bruin A, et al. (2010) The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. BMC Infect Dis 10: 69.
34. Raoult D, Marrie T, Mege J (2005) Natural history and pathophysiology of Q fever. Lancet Infect Dis 5: 219–226.
35. Gidding HF, Wallace C, Lawrence GL, McIntyre PB (2009) Australia's national Q fever vaccination program. Vaccine 27: 2037–2041.
36. Marmion B (2007) Q fever: the long journey to control by vaccination. Med J Aust 186: 164–166.
37. Henning K, Hotzel H, Peters M, Welge P, Popps W, et al. (2009) [Unanticipated outbreak of Q fever during a study using sheep, and its significance for further projects]. Berl Munch Tierarztl Wochenschr 122: 13–19.

CHAPTER 5.2

Risk factors of *Coxiella burnetii* (Q fever) seropositivity in veterinary medicine students

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Risk Factors of *Coxiella burnetii* (Q Fever) Seropositivity in Veterinary Medicine Students

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Abstract

Background: Q fever is an occupational risk for veterinarians, however little is known about the risk for veterinary medicine students. This study aimed to assess the seroprevalence of *Coxiella burnetii* among veterinary medicine students and to identify associated risk factors.

Methods: A cross-sectional study with questionnaire and blood sample collection was performed among all veterinary medicine students studying in the Netherlands in 2006. Serum samples (n = 674), representative of all study years and study directions, were analyzed for *C. burnetii* IgG and IgM phase I and II antibodies with an immunofluorescence assay (IFA). Seropositivity was defined as IgG phase I and/or II titer of 1:32 and above.

Results: Of the veterinary medicine students 126 (18.7%) had IgG antibodies against *C. burnetii*. Seropositivity associated risk factors identified were the study direction 'farm animals' (Odds Ratio (OR) 3.27 [95% CI 2.14–5.02]), advanced year of study (OR year 6: 2.31 [1.22–4.39] OR year 3–5 1.83 [1.07–3.10]) having had a zoonosis during the study (OR 1.74 [1.07–2.82]) and ever lived on a ruminant farm (OR 2.73 [1.59–4.67]). Stratified analysis revealed study direction 'farm animals' to be a study-related risk factor apart from ever living on a farm. In addition we identified a clear dose-response relation for the number of years lived on a farm with *C. burnetii* seropositivity.

Conclusions: *C. burnetii* seroprevalence is considerable among veterinary medicine students and study related risk factors were identified. This indicates Q fever as an occupational risk for veterinary medicine students.

Introduction

Q fever is a zoonotic disease caused by the bacterium *Coxiella burnetii* and is, apart from community outbreaks, known as an occupational disease of veterinarians, farmers and abattoir workers [1]. Symptomatic acute Q fever mainly presents as fever and headache, hepatitis, or pneumonia [2,3]. Moreover, infection with *C. burnetii* is asymptomatic in approximately 60% of those infected [2]. Many Q fever infections are not diagnosed because of the often mild and nonspecific clinical symptoms [4]. Acute Q fever, whether or not symptomatic, can develop into chronic Q fever [3]. Chronic Q fever generally presents as a culture-negative endocarditis or vascular infection with a high case fatality [3]. Another important long-term effect is Q fever fatigue syndrome, which occurs in 10 to 20% of all acute Q fever cases [5].

C. burnetii is a pathogenic bacterium which can infect mammals, birds and arthropods [1]. Transmission of *Coxiella* to humans occurs primarily through air via bioaerosols [6]. Furthermore humans can be infected by intake of contaminated milk or food, but these routes of transmission are of minor relevance [7]. The *Coxiella* bacterium is known to have two antigenic stages: the virulent phase I variant and the avirulent phase II variant [8]. In the body, *C. burnetii* is controlled by the T-cell dependent immune system, resulting in the production of specific antibodies [2]. Immunoglobulin G (IgG) is primarily effective against phase II antigen, while Immunoglobulin M (IgM) targets both phase I and II antigens [2]. The level of IgM increases rapidly after infection, thus is considered to be a marker of recent infection, however it can persist for many months [9,10]. IgG levels increase a few weeks after infection, but remain detectable for years or even throughout life [5,9].

Before the large community outbreaks in the Netherlands starting in 2007, *C. burnetii* seroprevalence was 2.4% in a general population sample taken in 2006–2007 [11]. Furthermore the study showed that persons who kept ruminants or with occupational animal contact had a higher risk to be infected with *Coxiella* [11]. Serum samples collected in the Netherlands in November 2009 showed that more than half of the livestock veterinarians were seropositive [12]. A similarly high seroprevalence for *C. burnetii* in veterinarians has been reported in other

studies, with prevalence ranging from approximately 20 to 50% [13,14]. Hence a substantial number of veterinarians become infected during their career, or possibly during their veterinary education. Veterinary medicine students perform similar activities as veterinarians during their study and likely have an increased risk to become infected with *C. burnetii* also. Yet, little is known about seroprevalence among veterinary students and the possible risk factors.

Few serological studies have been done among veterinary students, showing prevalence figures of *Coxiella* antibodies to range from 10 to 40% [15–17]. Valencia *et al* showed that students at the beginning of their first study year had a seroprevalence of 4.0% which was significantly lower compared to the 16.8% prevalence in the fifth year, implying a gradual increase in prevalence over the study periods [16]. However, studies reporting on the seroprevalence for *C. burnetii* covering the complete educational program and study duration are thus far missing. In univariate analysis some risk factors for seropositivity were identified in these studies, i.e. male gender, contact with ruminants, and study direction, although multivariate analyses were not carried out [16,17]. We thus performed a large-scale cross sectional study to determine the seroprevalence of *C. burnetii* among all veterinary medicine students studying in the Netherlands in the year 2006. All study years and study directions were included in order to identify the pattern in seroprevalence of antibodies against *C. burnetii* and to determine the associated study-related factors and other student characteristics.

Methods

Study design and population

The cross sectional design and study population have been described before by Samadi *et al* [18]. Briefly, all 1416 students, who were registered as a student of veterinary medicine in 2006 at Utrecht University, the only faculty of Veterinary Medicine in the Netherlands, were requested to participate. Students of all study phases were asked to fill in an online questionnaire and were invited to donate a blood sample of 20 ml for serological testing. Non-responders were sent maximally two reminders. Blood collection was performed in 2006 before the start of large community outbreaks of Q fever in the Netherlands in 2007–2009.

Ethics statement

The study protocol was approved by the Ethical Committee of the Utrecht University. All participants gave written informed consent prior to blood collection.

Questionnaire

Information was collected on participants' demographic and study related characteristics and on their smoking habits and health status. Regular contact with diverse animal species was asked for during different periods of childhood and adulthood. Information was gathered about a farm childhood, the number of years lived on a farm, farm type and the activities performed on the farm. Questions about health status addressed general health, clinical symptoms and self-reported zoonotic diseases.

Study related characteristics for veterinary medicine students in the Netherlands are affected by the structure of the veterinary curriculum with its variety of directions and theoretical/practical stages. Six months after the start of the study the veterinary curriculum divides into two main directions: 'individually kept animals' and 'farm animal health'. After the second study year, the curriculum subdivides further. The direction 'individually kept animals' is split into 'companion animals' and 'equine'. The direction: 'farm animal health' is also split further in 'farm animals and veterinary public health' and 'veterinary scientific research'. The first two study years consist of theoretical courses. During the third and fourth year the content of the courses shifts gradually towards practical lessons, but the majority is still theoretical. Fifth-year students start to follow internships at all departments but with the emphasis on their own specialization. Students with the companion animal direction mostly encounter cats and dogs, students at the equine department focus on horses and students doing the farm animal health specialization encounter mainly cows, pigs, poultry, sheep and goats.

Detection of *C. burnetii* IgG and IgM

Sera were analyzed for phase I and phase II IgG antibodies against *C. burnetii* at the Regional Laboratory of Medical Microbiology and Infection Control of the Jeroen Bosch Hospital in Den Bosch, using an Immunofluorescence Assay (IFA) according to the manufacturer's protocol (Focus Diagnostics). Sera were tested in a dilution series starting from a 1:32 till a 1:4096 dilution. An antibody titer of 1:32 and above for either IgG I or II antibodies of a serum sample was defined seropositive. A positive IgG test was followed by determination of phase I and II IgM antibodies by IFA.

Statistical analysis

All statistical analyses were carried out using SPSS for Windows (version 16). Univariate regression analyses were performed to investigate the association between seropositivity and possible risk factors. Variables in univariate analysis associated with seropositivity ($p<0.20$) were selected for multivariate logistic regression analyses. These variables were tested for multicollinearity and after assumptions were met, both forward and backward regression analyses were applied. The final multivariate model was obtained with the criteria of a p-value of less than 0.05 for the model and for each variable itself. Smoothed regression analysis was performed to assess the shape of the association between seropositivity and the number of years a student had lived on a farm.

Results

Response

In total, 965 of all the 1416 veterinary medicine students responded to the questionnaire (68.2%) of which 5 were excluded in further analyses. One student was excluded because the questionnaire was not completed and four others as they represented study specializations with intrinsic low numbers. Of the 960 students providing a questionnaire, 674 students provided a blood sample as well (47.6% of the total population). The division over the different study phases and study directions of the respondents is shown in Figure 1.

Participants' characteristics

Of the participants that completed the questionnaire, 80% were women (Table 1). The mean age was 24 years with a range from 18 to 47 years. A high number (51.1%) of the students reported previous or current regular contact with farm animals outside the veterinary curriculum. Furthermore 645 students (67.2%) had regular contact with horses and 97.6% of the students had regular contact with pets. Of the students 39.5% grew up in a rural area and 13.5% had ever lived on a farm. Demographic characteristics of students who did not provide blood were generally similar to those who did, except for borderline significance for having lived on a farm or in a village (Table 1). Of the students 130 reported to have had a zoonosis during their study of which were reported

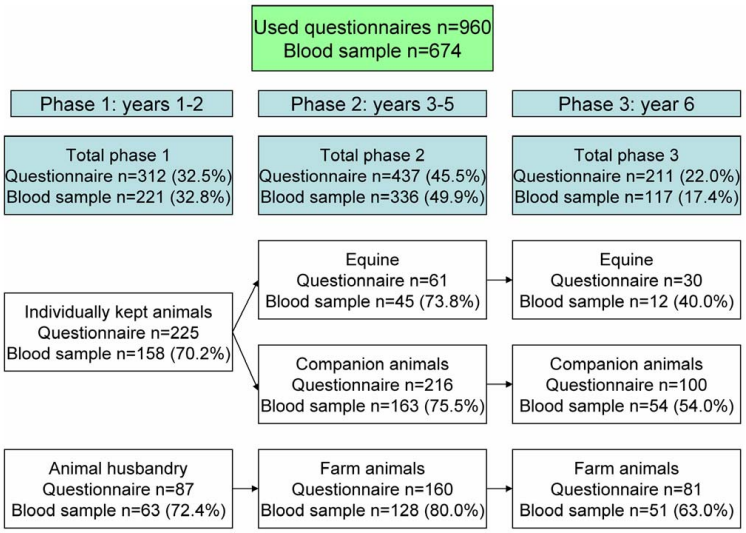

Figure 1. Numbers and percentages of participants per study direction and study phase.

most frequently: dermatophytosis (ringworm, 8.5%) and other fungal infections (5.5%, Table 2).

Serological results

Sera of 126 students (18.7%) were positive, with an IgG II titer ranging from 1:32 to 1:4096. Thirty percent (n = 38) of the students with a positive IgG II titer also had a positive IgG I titer ranging from 1:32 to 1:2084. There were no students with exclusive positive IgG I titers. Only sera with a positive IgG titer were tested for IgM antibodies. Of the IgG positives, 3% also had a positive IgM I with titers ranging from 1:32 to 1:256. While 19% of the IgG positives had also a positive IgM II indicating recent infection, with titers from 1:32 to >1:256. Seroprevalence showed an increase from study phase 1 (year 1–2) to phase 2 (year 3–5) and to phase 3 (year 6). Additionally, students mostly involved with farm animals had a much higher seroprevalence than those working with individually kept animals (Table 3).

Risk factor analyses

In the univariate analyses we identified variables associated with *C. burnetii* seropositivity as shown in Table 4. Male students were more often seropositive than females and seropositivity increased significantly with age per year. The study phase, study direction and whether or not internships were followed, were also associated. Moreover contact with cows, pigs, dogs and sheep was positively associated with seropositivity. Students who had lived on a farm were 2.9 times more likely to have *C. burnetii* antibodies. The risk was higher for having lived on a livestock breeding farm and was the highest for a ruminant farm. The risk for a positive serology significantly increased with each year the student had lived on the farm. The shape of this relationship was log-linear, implying that the risk for a positive serology significantly increased with each year the student had lived on the farm ($p = 0.028$; p-spline 2 df = 0.566; Figure 2). The following activities performed on the farm were associated with seropositivity: animal nursing and work with liquid and/or solid manure. Students reporting to have had a zoonosis during their study had a higher chance of seropositivity. However none of the students reported to have had Q fever during their study (Table 2).General health status and specific clinical symptoms like cough, headache, unusually tired feeling, flu like symptoms and shortness of breath were not associated with seropositivity.

Ten variables were included in the initial multivariate regression model. In the final model the following were identified to be associated with seropositivity: having lived on a ruminant farm (OR 2.7), study direction 'farm animals' (OR 3.3), having had a zoonotic disease during study (OR 1.7) and duration of study (phase 2 (OR 1.8) and phase 3 (OR 2.3), (Table 5)).

We performed stratified analyses for students who had lived on a farm and those who did not, to investigate whether study direction remained an independent risk factor (Table 5). Results showed that the study direction 'farm animals' remained significantly associated with seropositivity for those who grew up on a farm (OR study direction = 4.9), as well as for those who did not (OR study direction = 3.3).

Discussion

In this cross-sectional study among Dutch veterinary students, we found a *C. burnetii* seroprevalence of 18.7% and identified several associated risk factors including study related factors. Only few studies have assessed zoonotic risks for veterinary medicine students. This is the first large-scale study that examined the seroprevalence for *Coxiella* among veterinary medicine students of all study years and directions. The overall observed seroprevalence was within the range of 10 to 40% reported in other studies for veterinary students of Spain, Brazil, California and Ohio [15–17].

The found prevalence is considerably lower than the prevalence of over 80% in Dutch livestock veterinarians sampled in 2009 [12]. The prevalence among these veterinarians might be slightly

Table 1. Descriptive characteristics (n (%) or stated otherwise) of the total study population and those who did and did not provide a blood sample.

Population characteristics	total	with blood	without blood
Number of students	960	674	286
Female	771 (80.3%)	540 (80.1%)	231 (80.8%)
Age, AM[a] (SD[b])	23.7 (3.7)	23.7 (3.6)	23.9 (3.8)
Weight (kg), AM[a] (SD[b])	68.5 (11.2)	68.3 (10.7)	69.1 (12.3)
Height (cm), AM[a] (SD[b])	174.6 (8.3)	174.4 (8.2)	175.2 (8.5)
Current smoker	103 (10.7%)	69 (10.2%)	34 (11.8%)
Past Smoker	86 (8.9%)	60 (8.9%)	26 (9.0%)
Regular contact[c] with animals besides the study:			
Horses	645 (67.2%)	451 (66.9%)	194 (67.8%)
Cows	312 (32.5%)	216 (32.0%)	96 (33.6%)
Pigs	136 (14.2%)	94 (13.9%)	42 (14.7%)
Sheep	275 (28.6%)	192 (28.5%)	83 (29.0%)
Poultry	307 (32.0%)	220 (32.6%)	87 (30.4%)
Goats	232 (24.2%)	166 (24.6%)	66 (23.1%)
Dogs	717 (74.7%)	507 (75.2%)	210 (73.4%)
Cats	712 (74.2%)	496 (73.6%)	216 (75.5%)
Rodents	715 (74.5%)	505 (74.9%)	210 (73.4%)
Birds	394 (41.0%)	283 (42.0%)	111 (38.8%)
Job with previous or current regular animal contact	439 (45.7%)	307 (45.5%)	132 (46.2%)
Growing up in rural area (village)[d]	379 (39.5%)	282 (41.8%)	97 (33.9%)
Farm childhood[e]	130 (13.5%)	100 (14.8%)	30 (10.5%)
Self reported zoonosis during VM[f]	190 (19.8%)	132 (19.6%)	58 (20.3%)
Self reported Q fever	0 (0%)	0 (0%)	0 (0%)
Positive Q fever status		126 (18.7%)	

[a]AM, Arithmetic Mean.
[b]SD, Standard Deviation.
[c]Previous or current regular contact (>once a week).
[d]Chi-square between providing and not-providing blood borderline significant with $p = 0.07$.
[e]Chi-square between providing and not-providing blood borderline significant with $p = 0.08$.
[f]VM, veterinary medicine.

higher than when sampling would have taken place in 2006, due to the environmental outbreaks starting in 2007. Conversely, other studies reported high seroprevalences of 20% and more for veterinarians in countries like the United States, Canada, Slovakia and Taiwan [13,14,19–21]. Comparing seroprevalences should however be done with caution, because different study populations and diagnostic tests applied might affect the outcomes. Recently, commercial IFAs and ELISAs have become available which are now predominantly used [22]. Despite this progress, there is still a wide interlaboratory variability due to different IgG and IgM cut-off levels applied [22]. There is no general consensus of the appropriate cut-off level as it depends on the population under study and the used antigen-preparation [23]. In this study IFA was used instead of ELISA because it is considered to be the reference method to study seroprevalence of *Coxiella* [24]. We chose a cut-off level of 1:32 instead of the 1:16 cut-off recommended by the manufacturer to increase specificity thus lowering the chance of false positives.

We found that students who grew up on a farm, especially on a ruminant farm, had a higher risk of being seropositive. All kinds of animals can be affected by *Coxiella* but ruminants are the most important reservoirs [25]. Furthermore almost all students performed at least one activity on the farm on which they had lived, for example more than 80% performed animal nursing. The shedding of *Coxiella* occurs primarily during aborting or parturition, thus likely occasions whereby students were often present [26,27]. A study in Spain among veterinary students documented working with ruminants as a risk factor and in Taiwan goat exposure was a risk factor for veterinarians [16,21].

The risk for a positive serology was found to significantly increase with each year the student had lived on a farm. The biological meaning of this is not known, as profound studies concerning exposure-response relations for *Coxiella* are lacking. Our finding might just reflect the increased probability to encounter *C. burnetii* exposure, as the risk for each exposure moment is constant given that one *Coxiella* organism entering the body is enough to cause disease [1]. On the other hand, our finding might be explained by a cumulative effect of long term exposure, suggesting that a threshold exposure should be met. Lastly, the level of exposure might be of importance as well: the persons who lived longer on a farm are more likely to have performed activities like animal nursing.

Students within the 'farm animals' direction had a three times higher risk to be seropositive than students from other directions.

Table 2. Overview of self-reported zoonotic diseases reported by veterinary medicine students (n = 960) during the veterinary medicine study.

Self reported zoonoses during VM[a]	Number (%)
Brucellosis	0 (0%)
Campylobacteriosis	10 (1.5%)
Cryptosporidiosis	0 (0%)
Ecthyma	9 (1.3%)
Giardiasis	1 (0.1%)
Cat scratch	3 (0.4%)
Leptospirosis	0 (0%)
Listeriosis	2 (0.3%)
Psittacosis	0 (0%)
Q fever	0 (0%)
Salmonellosis	8 (1.2%)
Dermatophytosis (ringworm)	57 (8.5%)
Other fungal infections	37 (5.5%)
Staphylococcus	5 (0.7%)
Toxoplasmosis	0 (0%)
VTEC	2 (0.3%)
Worminfection	13 (1.9%)

[a]VM, veterinary medicine.

The 'farm animal' direction itself includes regular contact with ruminants, but 'farm animal' students also often had contact with ruminants before or beside their study (Table 3). Furthermore the percentage of students with a farm childhood in this direction is considerably higher. Stratified analyses on farm childhood however showed study direction to be a risk factor also for those with a farm childhood, suggesting two independent effects, indicating also for these students the importance of their study for the development of seropositivity.

Longer study duration was associated with an increased likelihood for seropositivity. As mentioned before, the study has an increasing amount of practical lessons from the second study phase and onwards. Furthermore the last studyphase consists solely of internships whereby largely all veterinary activities are performed by the students. Thus, towards the end of the study the number of animal contact increases as well as the number of treatments executed. The treatment of cattle, swine and wildlife were previously reported as a risk factor for veterinarians [13]. Presumably, treatment of these species by students in their last phase can partly explain studyphase being a risk factor. In addition, by default students in later study phases are older likewise their possibility of becoming infected during their lifetime is higher [9]. Age as a risk factor was also found in a study amongst a Canadian general population and among U.S. veterinarians [13,19]. It could be argued that students in higher study phases have lived longer on a farm, and therefore are more likely to become seropositive. However, the average number of years students lived on a farm in study phase 1, 2 and 3 did not differ, being respectively 15.03, 14.84 and 16.75 years.

Table 3. Characteristics of students (n (%) or stated otherwise) who provided blood for the different study phases and by study direction.

Students study phase 1 (Year 1–2)	**Farm animals**	**Individually kept animals**	
Number of students	63	158	
Contact with ruminants outside VM[a]	44 (69.8%)	43 (27.2%)	
Job with regular animal contact	29 (46.0%)	72 (45.6%)	
Growing up in rural area (village)	38 (60.3%)	52 (32.9%)	
Farm childhood	17 (27.0%)	16 (10.1%)	
Positive *C. burnetii* status	15 (23.8%)	9 (5.7%)	
Students study phase 2 (Year 3–5)	**Farm animals**	**Companion animals**	**Horse**
Number of students	128	163	45
Contact ruminants outside VM[a]	95 (74.2%)	48 (29.4%)	18 (40%)
Job with regular animal contact	57 (44.5%)	65 (39.9%)	29 (64.4%)
Growing up in rural area (village)	61 (47.7%)	59 (36.2%)	21 (46.7%)
Farm childhood	40 (31.2%)	10 (6.1%)	5 (11.1%)
Positive *C. burnetii* status	46 (35.9%)	19 (11.7%)	6 (13.3%)
Students study phase 3 (Year 6)	**Farm animals**	**Companion animals**	**Horse**
Number of students	51	54	12
Contact with ruminants outside VM[a]	27 (52.9%)	15 (27.8%)	6 (50%)
Job with regular animal contact	22 (43.1%)	27 (50.0%)	6 (50.0%)
Growing up in rural area (village)	24 (47.1%)	19 (35.2%)	8 (66.7%)
Farm childhood	7 (13.7%)	3 (5.6%)	2 (16.7%)
Positive *C. burnetii* status	19 (37.3%)	10 (18.5%)	2 (16.7%)

Note.
[a]Previous or current regular (>once a week) contact with ruminants outside the veterinary medicine curriculum.

Table 4. Univariate analysis of factors possibly associated with seropositivity for *Coxiella burnetii* among veterinary medicine students.

Variable	Odds Ratio (95% CI)	P-value
Male gender (n = 134 (19.9%))	1.74 (1.12–2.73)	0.018[b]
Age (per year)	1.10 (1.05–1.16)	0.000
Study direction farm animals (n = 242 (35.9%))	4.15 (2.76–6.22)	0.000[b]
Zoonotic disease during VM[a] (n = 132 (19.6%))	2.08 (1.34–3.24)	0.001[b]
Followed VM[a] internships (n = 171 (25.4%))	2.12 (1.41–3.21)	0.000
Regular contact with:		
Horses (n = 451 (66.9%))	1.13 (0.74–1.71)	0.601
Cows (n = 216 (32%))	2.39 (1.60–3.50)	0.000[b]
Pigs (n = 94 (13.9%))	1.72 (1.04–2.85)	0.045[b]
Sheep (n = 192 (28.5%))	1.73 (1.15–2.59)	0.009[b]
Poultry (n = 220 (32.6%))	1.29 (0.86–1.93)	0.246
Goats (n = 166 (24.6%))	1.35 (0.88–2.08)	0.207
Dogs (n = 507 (75.2%))	1.81 (1.10–3.01)	0.022[b]
Cats (n = 496 (73.6%))	0.96 (0.62–1.49)	0.911
Rodents (n = 505 (74.9%))	0.80 (0.52–1.24)	0.362
Birds (n = 283 (42.0%))	1.27 (0.86–1.88)	0.231
Former job with regular animal contact (n = 307 (45.5%))	0.91 (0.62–1.34)	0.692
Ever lived on a farm (n = 100 (14.8%))	2.86 (1.79–4.56)	0.000
Ever lived on a ruminant farm (n = 80 (11.9%))	3.78 (2.30–6.22)	0.000[b]
Ever lived on a livestock breeding farm (n = 67 (10.0%))	3.73 (2.18–6.31)	0.000
Years lived on a farm (per year)	1.07 (1.04–1.10)	0.024
Activities performed on the livestock farm:		
Animal nursing (n = 73 (82.0%))	4.40 (1.20–16.14)	0.022
Work with liquid and/or dry manure (n = 61 (68.5%))	3.23 (1.23–8.43)	0.017
Work with straw/hay (n = 75 (84.3%))	3.20 (0.86–11.94)	0.102
Plant nursing (n = 33 (37.1%))	1.61 (0.70–3.71)	0.291
Compared to currently in study phase 1		
Currently in study phase 2 (n = 336 (49.9%))	2.20 (1.34–3.62)	0.001[b]
Currently in study phase 3 (n = 117 (17.4%))	2.95 (1.64–5.34)	0.001[b]
Compared to town (15.000 to 80.000 inh) in childhood		
Grew up in a village (<15.000 inhabitants) (n = 282 (41.8%))	1.49 (0.97–2.29)	0.183
Grew up in a city (>80.000 inhabitants) (n = 110 (16.3%))	1.28 (0.72–2.27)	0.183
Compared to currently living in a student house		
Private house (n = 169 (25.1%))	1.45 (0.94–2.25))	0.218
Parental house n = 71 (10.5%))	0.95 (0.49–1.86)	0.218
Compared to a none smoker		
Past smoker (n = 60 (8.9%))	1.11 (0.57–2.17)	0.898
Current smoker (n = 69 (10.2%))	1.13 (0.61–2.12)	0.898

Note.
[a]VM, veterinary medicine.
[b]Variables included in the multivariate analysis, other variables p<0.20 were excluded because of multicollinearity.

Students reporting zoonoses since the start of their study were more likely to be seropositive, although none of the 960 students reported to have had Q fever. Of the students 20% reported a zoonosis; most prevalent were ringworm and other fungal infections. A variety of fungi are known to be commensals of the animal skin, occasionally they can also be pathogenic either for animals or humans [28]. Students with frequent animal contact are presumably more exposed to several zoonotic pathogens [29]. Good hygiene is important for the prevention of these zoonoses [30]. Presumably zoonotic diseases were found to be a risk factor for *Coxiella* seropositivity because it reflects the students' amount of animal contact and hygiene practices. Whitney *et al* examined the use of personal protective equipment by veterinarians, whereby wearing always a lab coat and always a surgical mask were protective factors [13]. These findings indicate the probable benefit of strict hygienic measures. In contrast, recent findings

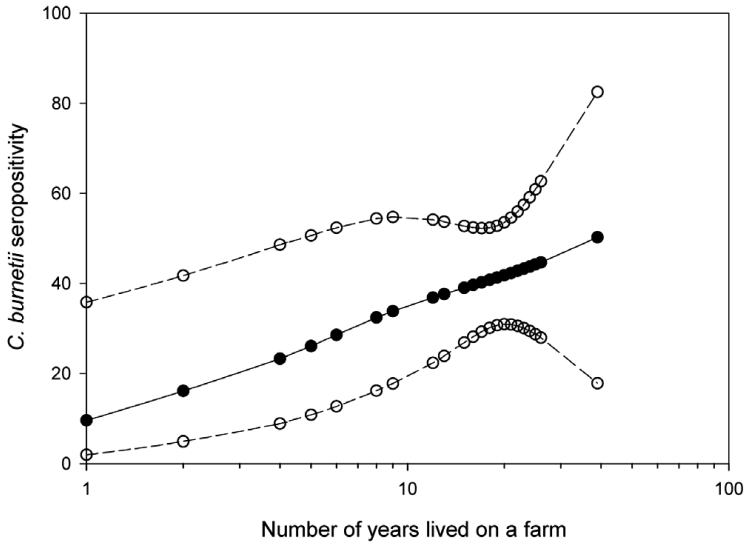

Figure 2. Association between *C. burnetii* seropositivity and number of years lived on a farm (p = 0.028, spline 2 d.f p = 0.586) for students who ever lived on a farm (n = 100). Open circles represent the 95% upper and lower confidence limits.

among culling workers showed seroconversion in around one out of five workers despite the use of personal protective equipment [31].

The seroprevalence of 18.7% for the Dutch veterinary students is high when compared to the seroprevalence of 2.4% for the general population in the Netherlands measured in the same time period, using the same methodology [11]. This indicates *C. burnetii* as a study or occupation related risk for veterinary students, as it also exists for veterinarians. It should be noted that 18.7% is the average prevalence in the study population. The risk for students in certain subgroups is considerably higher. For example the seroprevalence is 37.3% among students in the third study phase within the 'farm animals' direction. This overall prevalence of 18.7% is presumably a valid estimate for the general veterinary medicine student population, since about half of the total population provided a blood sample. The students who provided a blood sample showed to be only marginally different from the student population who did not.

The measurement series in the Netherlands revealed that the seroprevalence of students lies in between the prevalence observed in the general population and among veterinarians. However, students at the start of their study already had an increased seroprevalence of 10.9%. These students only have had theoretical courses; hence the increased seroprevalence can only be explained by other determinants, such as the frequent occurrence of a farm childhood in this population and the degree of ruminant contact prior to the start of their study. As could be expected, veterinary students have always been highly interested in animals. A large number of the students had regular contact with different animal species in childhood and around half of the students reported to have had a job with regular animal contact (Table 1). Students in the first phase within the 'farm animals' direction had a substantial higher seroprevalence (23.8%) than students in the 'individually kept animals' direction (5.7%, Table 3). This is likely a result of previous contact with ruminants, as students with a farm childhood are more likely to choose for the 'farm animals' direction.

The risk factors identified comprised most of the risk factors found by several other studies both in open population and occupational settings. However, some other risk factors have been reported before, but could not be studied as the questionnaire did not include these items. An example is contact with pond water and knowledge of Q fever [13,21].

The implications of the high occurrence rate of seropositivity on students' health are not yet known. None of the students reported to have had Q fever. Q fever has a wide variety of non-specific symptoms and is often asymptomatic, so it is difficult to collect relevant information with a questionnaire over an extended period of time [2,3]. Poor recall might also have contributed to the low reported prevalence for Q fever. Furthermore the questionnaire was primarily based on the European Community Respiratory Health Survey questionnaire, and was not specifically directed to identify acute Q fever symptoms [32]. On the other hand, a high prevalence of self reported Q fever was not expected as as approximately 60% of Q fever infections are considered to be asymptomatic [4]. Both symptomatic and asymptomatic Q fever has been described to develop into chronic Q fever, although most information is available from symptomatic acute Q fever patients [3].Therefore research is needed to explore the risk for asymptomatic seroconverters of development into chronic Q fever.

This study raises the question whether specific measures have to be taken in this population to prevent development of *C. burnetii* infection. General protective measures may not be sufficient to protect students throughout their career. Therefore offering vaccination may be considered, like in Australia for personnel with high risk occupations [33], or yearly serological screenings as suggested for wool workers [34]. Moreover, in general, awareness about study related health risks should be strengthened. Knowl-

Table 5. Factors associated with *Coxiella burnetii* seropositivity obtained by multivariate analysis for all students and stratified by ever lived on a farm.

	All	Ever lived on a farm	
	OR (95% CI)	**Yes (OR (95% CI))**	**No (OR (95% CI))**
Study direction			
Farm animal health	3.27 (2.14–5.02)	4.86 (1.54–15.29)	3.32 (2.06–5.35)
Other direction	1.00	1.00	1.00
Study phase			
Phase 3 (Year 6)	2.31 (1.22–4.39)	0.43 (0.07–2.66)	3.16 (1.55–6.46)
Phase 2 (Year 3–5)	1.83 (1.07–3.10)	1.34 (0.46–3.94)	2.03 (1.09–3.79)
Phase 1 (Year 1–2)	1.00	1.00	1.00
Zoonotic disease during VM[a]			
Yes	1.74 (1.07–2.82)	7.23 (1.74–30.09)	1.34 (0.78–2.34)
No	1.00	1.00	1.00
Ever lived on ruminant farm			
Yes	2.73 (1.59–4.67)	-	-
No	1.00	-	-
Childhood municipality			
Village	-	0.53 (0.18–1.52)	1.53 (0.89–2.62)
City	-	-	2.18 (1.15–4.14)
Town		1.00	1.00

Note. Multivariate analysis for all students obtained with Forward and Backward logistic regression.
Stratified analysis obtained with Enter.
[a]VM, veterinary medicine.

edge regarding clinical symptoms of Q fever can improve referral to the occupational physician affiliated to the university and prevent development of chronic stages of disease.

To conclude, this is the first large-scale study that examined the seroprevalence for *C. burnetii* among veterinary medicine students across all study phases. It demonstrates a considerable *C. burnetii* seroprevalence among veterinary medicine students. Besides regular contact to ruminants outside the curriculum program, also study related factors were associated with seropositivity. This suggests the importance of Q fever as an occupational risk for veterinary medicine students. Interestingly, we demonstrated a log-linear relationship between the numbers of years lived on a farm and seropositivity. Since clinical Q fever illness was not self-reported further research is recommended to study the health implications of seropositivity. Overall, this study contributes to the knowledge and the awareness of Q fever as a risk for veterinary students in order to contribute to its prevention.

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Author Contributions

Conceived and designed the experiments: DH BRB IMW. Performed the experiments: MMTR BS BV PS WH IMW. Analyzed the data: MMTR IMW. Contributed reagents/materials/analysis tools: MMTR DH BS PS WH IMW. Wrote the paper: MMTR IW. Reviewed and commented on the manuscript: MMTR BS BV PS BRB DH WH IMW.

References

1. Madariaga MG, Rezai K, Trenholme GM, Weinstein RA (2003) Q fever: A biological weapon in your backyard. Lancet Infect Dis 3: 709–721.
2. Raoult D, Marrie TJ, Mege JL (2005) Natural history and pathophysiology of Q fever. Lancet Infect Dis 5: 219–226.
3. Parker NR, Barralet JH, Bell AM (2006) Q fever. Lancet 367: 679–688.
4. Maurin M, Raoult D (1999) Q fever. Clin Microbiol Rev 12: 518–553.
5. Sukocheva OA, Marmion BP, Storm PA, Lockhart M, Turra M, et al. (2010) Long-term persistence after acute Q fever of non-infective Coxiella burnetii cell components, including antigens. QJM 103: 847–863.
6. McQuiston JH, Childs JE (2002) Q fever in humans and animals in the United States. Vector Borne Zoonotic Dis 2: 179–191.
7. Woldehiwet Z (2004) Q fever (coxiellosis): Epidemiology and pathogenesis. Res Vet Sci 77: 93–100.
8. Heinzen RA, Hackstadt T, Samuel JE (1999) Developmental biology of Coxiella burnetii. Trends Microbiol 7: 149–154.
9. Dupont HT, Thirion X, Raoult D (1994) Q fever serology: Cutoff determination for microimmunofluorescence. Clin Diagn Lab Immunol 1: 189–196.
10. van der Hoek W, Meekelenkamp JC, Leenders AC, Wijers N, Notermans DW, et al. (2011) Antibodies against Coxiella burnetii and pregnancy outcome during the 2007–2008 Q fever outbreaks in the Netherlands. BMC Infect Dis 11: 44.
11. Schimmer B, Notermans DW, Harms MG, Reimerink JH, Bakker J, et al. (2012) Low seroprevalence of Q fever in the Netherlands prior to a series of large outbreaks. Epidemiol Infect Jan;140(1): 27–35. Epub 2011 Feb 16.
12. van Duynhoven Y, Schimmer B, van Steenbergen J, van der Hoek W. The story of human Q-fever in the Netherlands [Internet]. Netherlands: Ministry of Economic Affairs, Agriculture and Innovation. Available at http://english.minlnv.nl. Accessed 1 July 2011.
13. Whitney EA, Massung RF, Candee AJ, Ailes EC, Myers LM, et al. (2009) Seroepidemiologic and occupational risk survey for Coxiella burnetii antibodies among US veterinarians. Clin Infect Dis 48: 550–557.

14. Dorko E, Kalinova Z, Weissova T, Pilipcinec E (2008) Seroprevalence of antibodies to Coxiella burnetii among employees of the veterinary university in Kosice, eastern Slovakia. Ann Agric Environ Med 15: 119–124.
15. Schnurrenberger PR, Helwig JH, Bashe WJ, Jr. (1964) The incidence of zoonotic infections in veterinary students. J Am Vet Med Assoc 144: 384–386.
16. Valencia MC, Rodriguez CO, Punet OG, de Blas Giral I (2000) Q fever seroprevalence and associated risk factors among students from the veterinary school of Zaragoza, Spain. Eur J Epidemiol 16: 469–476.
17. Riemann HP, Brant PC, Franti CE, Reis R, Buchanan AM, et al. (1974) Antibodies to Toxoplasma gondii and Coxiella burneti among students and other personnel in veterinary colleges in California and Brazil. Am J Epidemiol 100: 197–208.
18. Samadi S, Spithoven J, Jamshidifard AR, Berends BR, Lipman L, et al. (2012) Allergy among veterinary medicine students in the Netherlands. Occup Environ Med 69: 48–55. Published Online First: 31 May 2011.
19. Marrie TJ, Pollak PT (1995) Seroepidemiology of Q fever in nova scotia: Evidence for age dependent cohorts and geographical distribution. Eur J Epidemiol 11: 47–54.
20. Marrie TJ, Fraser J (1985) Prevalence of antibodies to Coxiella burnetii among veterinarians and slaughterhouse workers in Nova Scotia. Can Vet J 26: 181–184.
21. Chang CC, Lin PS, Hou MY, Lin CC, Hung MN, et al. (2010) Identification of risk factors of Coxiella burnetii (Q fever) infection in veterinary-associated populations in southern Taiwan. Zoonoses Public Health 57: e95–101.
22. Raoult D (2009) Reemergence of Q fever after 11 september 2001. Clin Infect Dis 48: 558–559.
23. Abe T, Yamaki K, Hayakawa T, Fukuda H, Ito Y, et al. (2001) A seroepidemiological study of the risks of Q fever infection in Japanese veterinarians. Eur J Epidemiol 17: 1029–1032.
24. Blaauw GJ, Notermans DW, Schimmer B, Meekelenkamp J, Reimerink JH, et al. (2012) The application of an enzyme-linked immunosorbent assay or an immunofluorescent assay test leads to different estimates of seroprevalence of Coxiella burnetii in the population. Epidemiol Infect Jan;140(1): 36–41. Epub 2011 Feb 15.
25. Tissot-Dupont H, Amadei MA, Nezri M, Raoult D (2004) Wind in November, Q fever in December. Emerg Infect Dis 10: 1264–1269.
26. Sanchez J, Souriau A, Buendia AJ, Arricau-Bouvery N, Martinez CM, et al. (2006) Experimental Coxiella burnetii infection in pregnant goats: A histopathological and immunohistochemical study. J Comp Pathol 135: 108–115.
27. Hatchette T, Campbell N, Hudson R, Raoult D, Marrie TJ (2003) Natural history of Q fever in goats. Vector Borne Zoonotic Dis 3: 11–15.
28. Bond R (2010) Superficial veterinary mycoses. Clin Dermatol 28: 226–236.
29. Cascio A, Bosilkovski M, Rodriguez-Morales AJ, Pappas G (2011) The socio-ecology of zoonotic infections. Clin Microbiol Infect 17: 336–342.
30. National Association of State Public Health Veterinarians, Inc. (NASPHV) (2011) Compendium of measures to prevent disease associated with animals in public settings, 2011: National association of state public health veterinarians, inc. MMWR Recomm Rep 60: 1–24.
31. Whelan J, Schimmer B, Schneeberger P, Meelenkamp J, Ijff A, et al. (2011) Q fever among culling workers, the Netherlands, 2009–2010. Emerg Infect Dis 17(9): 1719–1723.
32. Burney PG, Luczynska C, Chinn S, Jarvis D (1994) The European community respiratory health survey. Eur Respir J 7: 954–960.
33. Tozer SJ, Lambert SB, Sloots TP, Nissen MD (2011) Q fever seroprevalence in metropolitan samples is similar to rural/remote samples in Queensland, Australia. Eur J Clin Microbiol Infect Dis Oct;30(10): 1287–93.
34. Wattiau P, Boldisova E, Toman R, Van Esbroeck M, Quoilin S, et al. (2011) Q fever in woolsorters, Belgium [letter]. Emerg Infect Dis Dec: [Epub ahead of print].

CHAPTER 5.3

Q fever among culling workers, The Netherlands, 2009-2010

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Q Fever among Culling Workers, the Netherlands, 2009-2010

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In 2009, dairy goat farms in the Netherlands were implicated in >2,300 cases of Q fever; in response, 51,820 small ruminants were culled. Among 517 culling workers, despite use of personal protective equipment, 17.5% seroconverted for antibodies to *Coxiella burnetii*. Vaccination of culling workers could be considered.

Q fever is caused by the bacterium *Coxiella burnetii*. Since 2007 in the Netherlands, annual outbreaks originating from dairy goat and sheep farms have occurred. In 2009, a total of 2,354 cases in humans were reported, 20% of patients were hospitalized, and at least 6 died (*1*). Among acute cases, ≈2% become chronic, and fatality rates for untreated chronic patients are high (*2*). To stop spread, culling was conducted from December 19, 2009, through June 22, 2010, on 87 infected commercial dairy goat farms and 2 dairy sheep farms (Figure 1). A total of 50,355 pregnant goats and sheep and 1,465 bucks were culled (*3*). Animal pregnancies were confirmed by abdominal ultrasound; pregnant animals were sedated and euthanized, and their corpses were transported to a destruction facility. Culling workers were provided with personal protective equipment (PPE) and advised to read occupational health and hygiene regulations (*4*). To determine seropositivity of workers before culling, incidence of symptomatic and asymptomatic *C. burnetii* infection during culling, and risk factors associated with occupational exposure, we conducted a prospective cohort study.

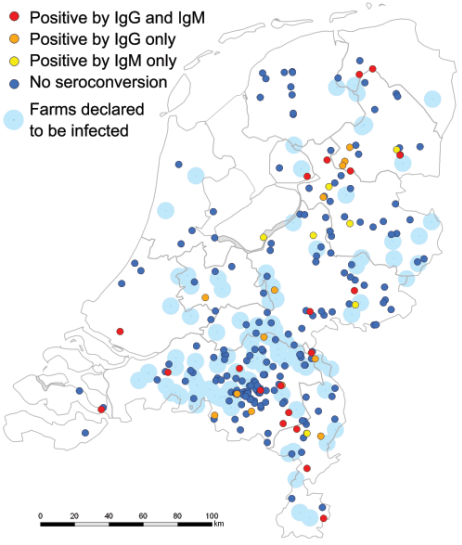

Figure 1. Residential location of 246 culling workers who were seronegative in December 2009 and their serostatus in June 2010 with location of 89 farms declared to be infected (by PCR-positive bulk-milk monitoring) in 2009 and 2010, the Netherlands. Ig, immunoglobulin. Seroconversion detected by ELISA was confirmed by immunofluorescence assay for 40 persons (38 [95%] at titers >128 and 2 [5%] at titers of 32).

The Study

Participants were 517 workers who culled goats and sheep during December 2009–June 2010. Serum samples were required from workers before employment in December 2009 (pre-cull) (*4*), and voluntary post-cull samples were requested in June 2010. In June, workers were asked to complete a questionnaire about symptoms, occupational exposure, adoption of hygiene measures and PPE use (filtering facepiece masks, gloves, overalls, hairnets), demographics, medical history, and other animal contact. Written informed consent was obtained. Information about farms (animal numbers and abortions) and workers (hours worked per person, job description) was available from occupational records.

Serum was tested for immunoglobulin (Ig) G and IgM against *C. burnetii* phase II by using ELISA (Virion/Serion, Würzburg, Germany). According to manufacturer instructions, IgG phase II seropositivity was defined as negative for titers ≤30 IU/mL and positive for titers >30 IU/mL. IgM phase II was qualitatively positive or negative. A worker was considered seronegative if a phase II sample was IgM and IgG negative and seropositive if IgM and/

or IgG positive. Positive results were confirmed by immunofluorescence assay (Focus Diagnostics, Cypress, CA, USA) titers ≥32. Symptomatic infection was defined as fever or rigors and ≥1 of the following after December 1, 2009: malaise, headache, cough, nausea, diarrhea, shortness of breath, pleuritic chest pain, or myalgia. Intensity of occupational exposure was summarized as follows: hours worked, weighted mean farm size (animal number), whether animal abortions were reported, and whether work was performed on average inside or outside the stable (proxy for direct/indirect animal contact). Months worked were dichotomized as cold (December 2009–March 2010) (*5*) and warm (April–June 2010) (*6*). Use of PPE was classified as compliant or noncompliant.

To calculate distance of workers' residence to the nearest infected farm, we used ArcGIS software (www.esri.com/software/arcgis/index.html). We used Stata version 11 (StataCorp LP, College Station, TX, USA) to examine univariable associations (Pearson χ^2 or Fisher exact test). Variables with probability $p<0.2$ and known risk factors for Q fever were selected for binomial regression analyses. Interactions between significant variables in the multivariable model were investigated. Missing values were excluded.

Of 517 participants, 453 gave pre-cull blood samples, 246 of these gave post-cull samples, and 351 completed the questionnaire. Age, gender, and residential distance from the nearest infected farm were available from occupational records. Participant median age was 47 years (range 19–67 years); 97% were male. Before culling, 14 (3.1%) were IgM II and IgG II positive, 8 (1.8%) were IgM II positive only, 36 (8%) were IgG II positive only, and 395 (87%) were IgG II and IgM II negative; i.e., any seropositivity was found for 13.0%. Pre-cull blood samples indicated more seropositivity among workers who lived within 5 km of an infected farm and had regular work contact with sheep and goats (excluding culling). Prior culling experience was more common among seronegative than seropositive workers (Table 1). Among those who were IgG seropositive before culling, none became IgM seropositive after culling.

Among the 395 workers who were seronegative before culling, 246 (62%) provided a follow-up blood

Table 1. Baseline characteristics of workers before culling small ruminants, the Netherlands, December 2009*

Characteristic	Total no. workers	No. (%) workers		p value†
		Seronegative, n = 395	Seropositive, n = 58	
Sex‡				
M	342	303 (89)	39 (11)	
F§	11	10 (91)	1 (9)	0.812
Age group, y¶				
<40	114	95 (83)	19 (17)	
40–49	157	137 (87)	20 (13)	
50–59	154	139 (90)	15 (10)	
≥60	26	22 (85)	4 (15)	0.398
Distance of residence from nearest infected farm, km¶				
≤5	116	95 (82)	21 (18)	
>5	317	282 (89)	35 (11)	0.052
Level of education¶				
Low	48	43 (90)	5 (10)	
Medium	132	117 (89)	15 (11)	
High	53	45 (85)	8 (15)	0.725
Medical history¶#				
No	159	140 (88)	19 (12)	
Yes	57	47 (83)	10 (18)	0.288
Current smoker¶				
No	189	162 (86)	27 (14)	
Yes	53	48 (91)	5 (9)	0.357
Previous culling experience¶				
No	116	94 (81)	22 (19)	
Yes	135	124 (92)	11 (8)	0.011
Regular occupational contact with sheep or goats¶				
No	202	182 (90)	20 (10)	
Yes	34	24 (71)	10 (29)	0.002

*Missing values excluded from analysis.
†Pearson χ^2.
‡Maximum 453 respondents. Data available from occupational records.
§No female respondents were pregnant.
¶Maximum 251 respondents. Data available from questionnaire responses.
#History of cardiorespiratory disease, liver disorders, diabetes, cancer, immunosuppression, allergies, skin conditions.

sample in June 2010, and 199 (80.8%) of these completed the questionnaire. Those who participated in June were more likely to be male (p = 0.015) and 40–60 years of age (p<0.001). Seroconversion among 246 seronegative respondents occurred as follows: 23 (9.4%) became IgG and IgM seropositive, 7 (2.9%) became IgM positive only, 13 (5.3%) became IgG positive only, and 203 (82.5%) remained seronegative; i.e., any seroconversion was found

Table 2. Variables associated with Q fever seroconversion among 246 workers who were seronegative before culling small ruminants, the Netherlands, 2009*

	No. (%) workers		Univariable analysis		Multivariable analysis†	
Variable	Total	Seroconversion	RR (95% CI)	p value‡	RR (95% CI)	p value
Total	246 (100)	43 (17)				
Sex						
F	6 (2)	2 (33)	Reference			
M	240 (98)	41 (17)	0.51 (0.16–1.64)	0.301		
Age, y						
≤45	96 (39)	14 (15)	Reference			
>45	150 (61)	29 (19)	1.33 (0.74–2.38)	0.339	2.0 (0.93–4.16)	0.07
Level of education						
Low	39 (21)	5 (13)	Reference			
Medium	103 (55)	18 (17)	1.36 (0.54–3.42)			
High	44 (24)	8 (18)	1.42 (0.51–3.98)	0.765		
Minimum distance of residence from nearest infected farm, km						
>5	174 (73)	32 (18)	Reference			
≤5	63 (27)	9 (14)	0.78 (0.39–1.53)	0.460		
Medical history§						
No	128 (75)	23 (18)	Reference			
Yes	42 (25)	5 (12)	0.66 (0.27–1.63)	0.358		
Current or past smoker						
No	84 (44)	16 (19)	Reference			
Yes	108 (56)	16 (15)	0.78 (0.41–1.46)	0.435		
Total hours worked inside farm perimeter¶						
0–20	81 (33)	5 (6)	Reference		Reference	
21–100	82 (34)	18 (22)	3.56 (1.39–9.12)		5.53 (0.71–42.77)	0.102
>100	80 (33)	20 (25)	4.05 (1.60–10.26)	0.003	7.75 (1.02–58.99)	0.048
Mean farm size ≥1,500 animals#						
No	167 (68)	22 (13)	Reference			
Yes	79 (32)	21 (27)	2.02 (1.18–3.44)	0.010	1.75 (0.93–3.30)	0.081
Worked mostly inside stable						
No	110 (45)	11 (10)	Reference			
Yes	133 (55)	31 (23)	2.33 (1.23–4.42)	0.006	2.58 (1.04–6.37)	0.040
Animal abortions on farm						
No	208 (85)	33 (16)	Reference			
Yes	38 (15)	10 (26)	1.66 (0.89–3.07)	0.119	0.93 (0.45–1.91)	0.844
Any previous culling experience						
No	85 (43)	15 (18)	Reference			
Yes	114 (57)	17 (15)	0.84 (0.45–1.59)	0.603		
Adherence to hygiene and preventive measures**						
Fully compliant	91 (50)	13 (14)	Reference			
Not compliant	91 (50)	17 (19)	1.31 (0.68–2.53)	0.424	0.94 (0.51–1.72)	0.829
Months spent culling						
2009 Dec–2010 Mar only (mean temperature 3.2°C)	105 (54)	18 (17)	Reference			
2010 Apr–Jun only (mean temperature 13.9°C)	2 (1)	1 (50)	2.92 (0.69–12.41)			
2009 Dec–2010 Jun	87 (45)	21 (24)	1.41 (0.80–2.47)	0.288		

*Total for each category may be <246 because of missing data. RR, risk ratio; CI, confidence interval.
†All data available for n = 180 in multivariable analysis.
‡Pearson χ^2.
§History of cardiorespiratory disease, liver disorders, diabetes, cancer, immunosuppression, allergies, skin conditions.
¶Data only available for n = 194, those who worked inside the farm perimeter.
#Weighted mean number of animals on farms worked by participants.
**Includes wearing mask, gloves, overalls, hairnet, showering after exposure.

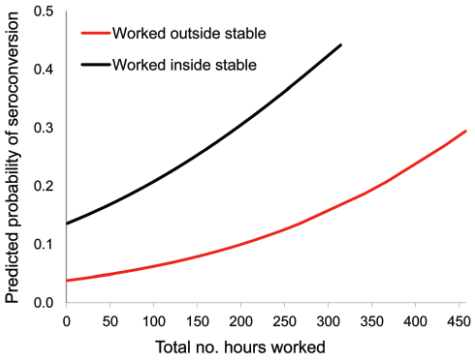

Figure 2. Predicted probabilities of seroconversion among small ruminant culling workers by total hours worked, weighted mean farm size, and location on farm while working during December 2009–June 2010, the Netherlands. Seroconversion probabilities calculated by multivariable model adjusted for age group, occurrence of animal abortions on the farms worked, and compliance with wearing personal protective equipment.

for 17.5%. Questionnaire respondents who seroconverted had more symptoms after December 1, 2009, (9 [31%] of 29) than nonseroconverters (17 [11%] of 150; relative risk 2.7, 95% confidence interval 1.4–5.5, p = 0.005). Symptomatic seroconverters reported fever and/or rigors and malaise (n = 7), headache (n = 6), cough (n = 6), or myalgia (n = 4). Mean duration of illness was 7.6 (range 1–14) days.

Univariable model indicated significance for total hours worked, farm size, and working inside the stable ($p<0.05$; Table 2). Multivariable model indicated significance for working >100 hours on the farm and working inside the stable (Table 2; Figure 2). Interaction effects were not significant.

Conclusions

Seroconversion for *C. burnetii* among 17.5% of culling workers who were seronegative before culling provides evidence of high-risk work. Before culling, seroprevalence was 13%, similar to that among blood donors in a high-incidence area in the Netherlands in 2009 (H.L. Zaaijer, pers. comm.) and in similar high-risk occupational groups (*7*). Laboratory testing by using ELISA is an accepted method in an acute setting (*8*), and positive results (including positive IgM only) were confirmed by immunofluorescence assay. Nonparticipants were in the youngest and oldest age groups; their effect on the proportion of seroconversion is uncertain. Eighteen workers (excluded for not providing a follow-up blood sample) completed the questionnaire in June. Symptom incidence for these 18 workers was the same as that for included participants.

Symptomatic infection (31% of seroconverters) was probably underestimated. A diagnosis of Q fever was self-reported (unconfirmed) to the occupational health service by 8 workers who did not participate in the study. During December–July 2010, the national infectious disease surveillance system reported 11 culling-related cases of acute Q fever; 2 of these patients were hospitalized.

A strong association was shown between risk for seroconversion and total hours worked on the farms and working inside the stable. In other settings internationally, a risk gradient has also been shown for close direct and indirect animal contact over time (*9,10*). In our study, half the participants had experience with previous animal epidemics (avian influenza, foot-and-mouth disease, classical swine fever) and using PPE. Their compliance with PPE was reportedly high; however, a key problem was not wearing PPE while taking work breaks but remaining on the farm.

Given the high risk for infection despite extensive personal protective measures during culling, additional preventive measures are needed. The Health Council of the Netherlands issued guidelines for persons in risk groups who would benefit from vaccination against Q fever (*11*). Culling workers were not included in these guidelines. The efficacy of human Q fever vaccine has been shown to be high for young and healthy persons in similar occupational groups (*12–14*). Vaccination of culling workers could be considered if further animal culling is advised.

Acknowledgments

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Dr Whelan is a fellow with the European Programme for Intervention Epidemiology Training, National Institute for Public Health and the Environment (RIVM), the Netherlands. Her research interests are Q fever in the Netherlands and other vaccine-preventable diseases.

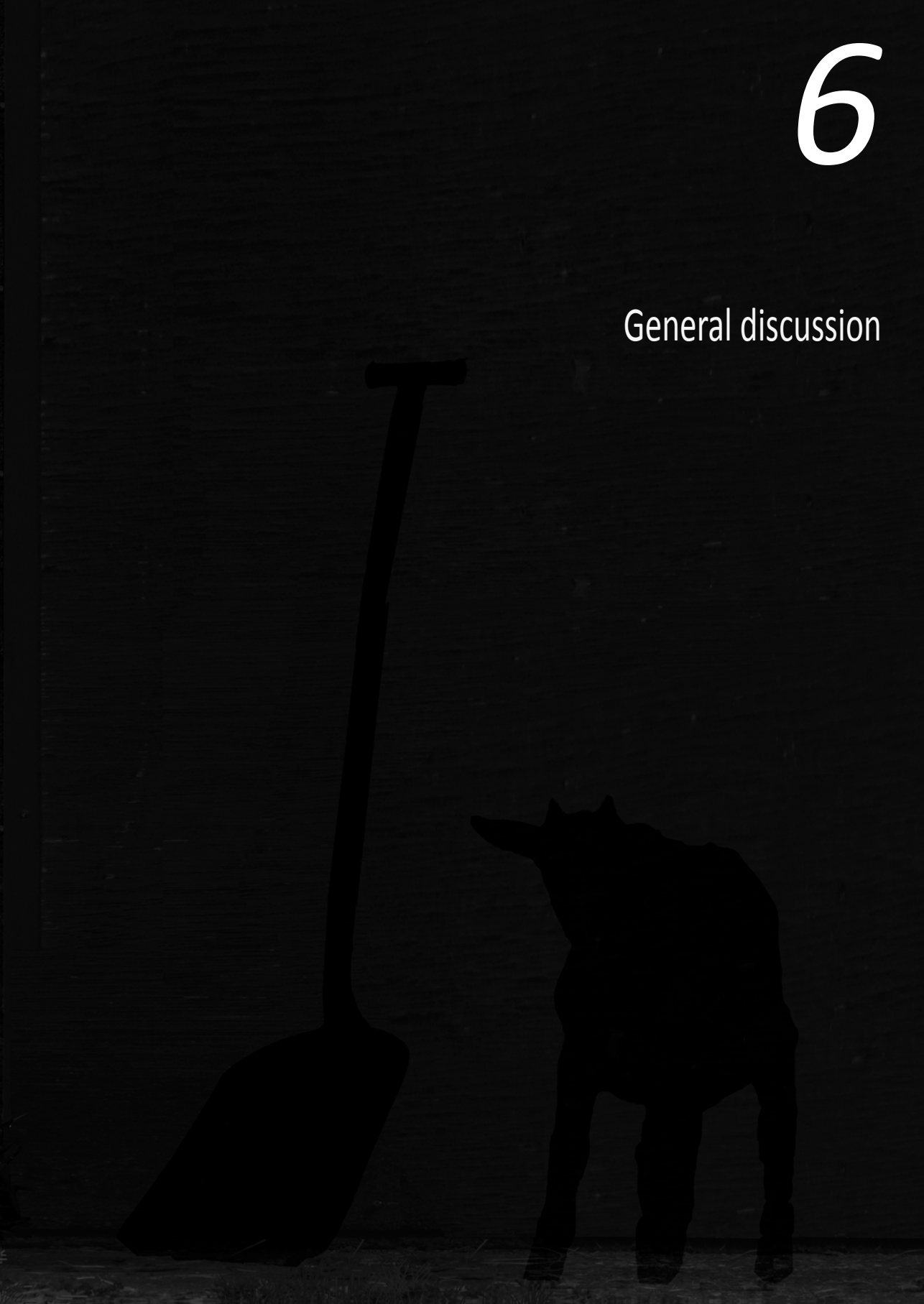
References

1. van der Hoek W, Dijkstra F, Schimmer B, Schneeberger PM, Vellema P, Wijkmans C, et al. Q fever in the Netherlands: an update on the epidemiology and control measures. Eurosurveill. 2010;15:pii=19520.

2. European Centre for Disease Prevention and Control. Risk assessment on Q fever. Stockholm: The Centre; 2010.
3. Roest HI, Tilburg JJ, van der Hoek W, Vellema P, van Zijderveld FG, Klaassen CH, et al. The Q fever epidemic in the Netherlands: history, onset, response and reflection. Epidemiol Infect. 2011;139:1–12. doi:10.1017/S0950268810002268
4. Food and Consumer Product Safety Authority, Ministry of Agriculture Nature and Food Quality. Occupational health and hygiene instructions for the management of Q-fever [in Dutch]. 2009 [cited 2010 Oct 26]. http://www.vwa.nl/txmpub/files/?p_file_id = 46449
5. Royal Netherlands Meterological Institute. Annual report. 2009 [cited 2010 Nov 12]. http://www.knmi.nl/bibliotheek/jaarverslag/annualreport2009.pdf
6. Royal Netherlands Meterological Institute. Climate data and advice: monthly overview of the weather in the Netherlands [in Dutch]. 2010 [cited 2010 Jan 6]. http://www.knmi.nl/klimatologie/mow/
7. Anderson AD, Baker TR, Littrell AC, Mott RL, Niebuhr DW, Smoak BL. Seroepidemiologic survey for *Coxiella burnetii* among hospitalized US troops deployed to Iraq. Zoonoses Public Health. 2010 Sep 29; [Epub ahead of print]. doi:10.1111/j.1863-2378.2010.01347.x
8. Boden K, Wagner-Wiening C, Seidel T, Baier M, Bischof W, Straube E, et al. Diagnosis of acute Q fever with emphasis on enzyme-linked immunosorbent assay and nested polymerase chain reaction regarding the time of serum collection. Diagn Microbiol Infect Dis. 2010:110–6. doi:10.1016/j.diagmicrobio.2010.06.001
9. Porten K, Rissland J, Tigges A, Broll S, Hopp W, Lunemann M, et al. A super-spreading ewe infects hundreds with Q fever at a farmers' market in Germany. BMC Infect Dis. 2006;6:147. doi:10.1186/1471-2334-6-147
10. Casolin A. Q fever in New South Wales Department of Agriculture workers. J Occup Environ Med. 1999;41:273–8. doi:10.1097/00043764-199904000-00009
11. Health Council of the Netherlands. Human vaccination against Q fever. Publication no. 2010/08E. The Hague: The Council; 2010 [cited 2010 Jan 6]. http://www.gezondheidsraad.nl/sites/default/files/201008E.pdf
12. Gilroy N, Formica N, Beers M, Egan A, Conaty S, Marmion B. Abattoir-associated Q fever: a Q fever outbreak during a Q fever vaccination program. Aust N Z J Public Health. 2001;25:362–7. doi:10.1111/j.1467-842X.2001.tb00595.x
13. Shapiro RA, Siskind V, Schofield FD, Stallman N, Worswick DA, Marmion BP. A randomized, controlled, double-blind, cross-over, clinical trial of Q fever vaccine in selected Queensland abattoirs. Epidemiol Infect. 1990;104:267–73. doi:10.1017/S0950268800059446
14. Ackland JR, Worswick DA, Marmion BP. Vaccine prophylaxis of Q fever. A follow-up study of the efficacy of Q-Vax (CSL) 1985–1990. Med J Aust. 1994;160:704–8.

6

General discussion



General discussion

An unprecedented epidemic of Q fever occurred in the Netherlands and resulted in the notification of more than 4000 human cases of acute Q fever during three consecutive epidemic years, from 2007 through 2009. The research included in this thesis has been carried out within a 'One Health' context and contributes to the evidence base for, and practically supports, infectious disease control for Q fever at the national level in the Netherlands and elsewhere in the world. It also, provides input for the development of occupational health guidelines for relevant livestock-associated occupational groups. This thesis includes a large population-based study that assessed the seroprevalence of *Coxiella burnetii (C. burnetii)* in the Netherlands in 2006-2007 just prior to the start of the Dutch Q fever epidemic (*Chapter 2*). Chapter 2 also covers a longitudinal study of antibody responses following acute Q fever.
This thesis comprises several in-depth field epidemiological investigations of acute Q fever outbreaks that occurred during the Dutch Q fever epidemic (*Chapter 3*). It further brings together several integrated human-veterinary studies that assessed both the *C. burnetii* seroprevalence and associated risk factors in occupational groups with intensive ruminant contact, as well as studies in samples of ruminant populations on commercial ruminant farms studying seroprevalence and risk factors on herd and animal level.
These studies pertain to:
- Farm residents of commercial dairy goat, dairy sheep, non-dairy sheep and dairy cattle farms, 2009-2011 (*Chapter 4*);
- Dairy goat, dairy sheep, non-dairy sheep and dairy cattle herds at commercial ruminant farms, 2009-2011 (*Chapter 4*);
- Veterinary students, livestock veterinarians and culling staff, 2009-2010 (*Chapter 5*).

Main findings of this thesis per chapter

Seroprevalence of *C. burnetii* in the Dutch general population and time-course of antibody responses following acute Q fever *(Chapter 2)*

Previous knowledge on this subject

Detection of past infections with *C. burnetii* in humans can be important for the estimation of the spread of Q fever in the general population and for defining risk factors for infection. Serum antibody tests can be performed on a fairly large scale using a commercial enzyme-linked immunosorbent assay (ELISA), and the serum can be stored before analysis, allowing archived samples to be analyzed. Immunofluorescence assays (IFA) can be used as a confirmative test for equivocal or positive ELISA tests (1) or as the main screening assay in order to obtain end point IgG titers against phase1 and phase2 *C. burnetii* antigens in smaller sera collections, as IFA is more labor-intensive (2). Results of seroprevalence studies can vary significantly depending on the method of antibody detection, the cut-off titer used and interlaboratory variability (3).

After infection with *C. burnetii*, the antibody response develops with a lag of seven to fifteen days after symptom onset (4, 5). Characteristic features of this antibody response are: time from symptom onset to onset seroresponse, time from onset seroresponse to peak titer, magnitude of peak titer and half time of antibody decay. Knowledge of these features is useful as the estimation of the date of symptom onset retrospectively is of relevance in seroprevalence studies, and helps to better understand infection dynamics. From previous studies it is known that there is a large variability in the magnitude of the peak titer and the antibody decay (6-8). Natural variations in antibody responses of cases with acute Q fever have been reported (9, 10). To obtain insight in these variations and long-term responses, it was necessary to study antibody responses in a large cohort of acute Q fever patients with serological follow-up.

What did these investigations add?

Screening of population-based sera collected from February 2006 to June 2007 with ELISA indicated that the general Dutch population was susceptible to *C. burnetii* exemplified by the low pre-epidemic *C. burnetii* seroprevalence of 2.4%, a relatively low figure in the European context.

This pre-epidemic seroprevalence estimate served as a national baseline for other seroprevalence studies carried out during and in the aftermath of the Dutch Q fever epidemic, such as in blood donors (11), groups of residents (12) and pregnant women (13) in Q fever high-incidence areas, in specific patient groups screened for chronic Q fever (14) and in professional groups occupationally exposed to *C. burnetii* (15-17). No recent nationwide post-epidemic figures are available (18). The national *C. burnetii* seroprevalence will probably be assessed in the Pienter-3 study based on sera taken in 2016-2017.

For early detection of chronic Q fever, the Jeroen Bosch Hospital in 's Hertogenbosch (Noord-Brabant province) offered standard serological follow-up to all acute Q fever patients at 3, 6 and 12 months after diagnosis.
The time course of antibody responses against *C. burnetii* was studied in a cohort of 344 acute Q fever patients by using their IgG and IgM phase-1 and phase-2 antibody levels in a dynamic mathematical model. It showed that *C. burnetii* antibodies are highly persistent and confirmed the large variability in peak titers and duration of antibody decay. IgG phase 2 serum antibody peaked at a mean of almost 2 months after symptom onset and then declined slowly, with the half-time of the antibody decay rate being estimated at 318 days. This study contributed, but also showed the difficulty, to determine the estimation of the latency of the *C. burnetii* infection and the presumed date of symptom onset in any individual with *C. burnetii* antibodies. Later in the epidemic, with systematic serological follow-up data available from over 2,000 acute Q fever patients, a similar modelling study showed that this variability in antibody responses is already present between individuals at the beginning of the antibody response. A more reliable estimate could be provided as sera were used from patients diagnosed with a positive *C. burnetii* PCR-test (19), instead of using samples obtained after the peak titer. In this way, the date of the seroresponse onset could be estimated based on *C. burnetii* IgG and IgM phase-1 and phase-2 antibody concentrations and be compared with the actual observed date of symptom onset. A serological follow-up strategy has not yet been implemented for asymptomatic acute Q fever cases or as part of periodical serological screening of professionals with an identified occupational risk, including clinical follow-up.

One of the main challenges would be to identify infections in an early stage especially in the absence of symptoms, and to determine the screening intervals that are needed to get robust data. These data could be collected in existing cohorts of livestock farmers (20) and veterinary students (21, 22) or as part of targeted occupational screening as has been done in slaughterhouses (23, 24), wool factories (25) and in military staff before and after deployment to countries with high endemicity (26). Regular periodical screening could provide the serological data needed to study the time-course (27) and the individual variation in the longitudinal antibody responses after *C. burnetii* infection in these professional groups, including the need for new cut-off levels and the likelihood of a booster effect in the context of continuous exposure (25, 28).

Outbreak investigations during the Dutch Q fever epidemic

Previous knowledge on this subject

Several large community Q fever outbreaks had occurred in Europe and elsewhere, but never on such an unprecedented scale as in the Netherlands from 2007-2009. Unusual outbreak features that distinguished the Dutch epidemic from historical Q fever outbreaks were its magnitude, and the protracted nature and supra-regional spread with multiple human clusters that could not be explained by one source (29-31). A recurrent seasonal pattern, with cases peaking during spring and early summer, was observed during three subsequent epidemic years.

From the early stages of the investigations there were strong indications that dairy goat herds were the main source of the Dutch Q fever epidemic. There had been active reports of abortion waves due to *C. burnetii* infection since 2005, with incidences up to 80% of goats spontaneously aborting (32, 33).
Moreover, the dairy goat population rapidly increased the last decade in this region, as had the average herd size.As reported in other Q fever outbreaks, there had been a diagnostic delay of several weeks in pathogen confirmation (34) of the unknown respiratory disease outbreak that was detected in the village of Herpen in May 2007, and later turned out to be acute Q fever.

Regional microbiology laboratories in Noord-Brabant province had already signaled a possible rise in Q fever cases during the previous months, March to June 2007, and reported this to the public health authorities. Nevertheless these six hospitalized patients with an atypical pneumonia and symptoms of high fever, dyspnea and headache could not be epidemiologically linked (29).

The large majority of acute Q fever outbreaks described in the literature involve single and locally restricted outbreaks with human cases arising during several months (35). These localized outbreaks could be explained by exposure to an infected ruminant source, mainly sheep or goats, while only few Q fever outbreaks have been attributed to cattle. Recurrent outbreaks in the same community during two subsequent years have occurred at relative small scale, most likely due to environmental resilience of *C. burnetii* (36). Information on the environmental investigation, including sampling of animal herds, records of reproductive events in the presumed animal source and supportive information on the weather conditions and prevailing wind direction and speed during the infectious period, is often included. The maximum distance of windborne *C. burnetii* spread is reported up to 18 kilometres in a community sheep-related outbreak in the United Kingdom (37) and at comparable distances during the mistral season that coincides with the main lambing season in the south of France (38). In some outbreak reports, distance-based attack rates were calculated to study the importance of proximity of meadows with grazing sheep flocks to the residential address of Q fever cases (39, 40).

What did these investigations add?

During the Dutch Q fever epidemic, several in-depth epidemiological investigations of separate acute Q fever clusters that occurred in different semi-urban and rural community settings were carried out. Four of them are described in this thesis in *Chapter 3* (41-44), and at least three other community outbreaks are published elsewhere (45-47).
When confronted with a national epidemic on this scale, it is imperative to keep collecting case-based information through trawling questionnaires and implement early notification of suspected animal sources and environmental reservoirs that might play a role in the epidemic. There were multiple *C. burnetii* emitting sources during this protracted epidemic, with cases arising simultaneously in different regions.

The investigated community outbreaks appeared to be related to the presence of nearby commercial dairy goat farms (41, 43, 46, 47), of which some had recorded a Q fever induced abortion storm in goats prior to outbreak detection (32). Commercial dairy-goat farms were soon considered the putative source in the Dutch Q fever epidemic as at least 23 farms experienced *C. burnetii* related abortion storms from 2005 through 2008. These abortion storms were reported voluntarily during the initial stages of the epidemic, but became notifiable in June 2008 (defined as ≥5% abortions in a herd).

As humans served in a way as sentinel for Q fever presence in nearby small ruminant populations, there was an important trigger to further improve abortion surveillance on small ruminant farms with mandatory notification of unexpectedly high abortion rates (>5%) in 2008 (30). PCR positivity of bulk tank milk (BTM) on dairy small ruminant farms was added to the notification criteria in October 2009, as on large farms the abortion rate was difficult to measure (32), or could be subjectively interpreted.

There were indications that direct contact with non-dairy sheep had also caused a limited number of acute Q fever cases. Two smaller sheep-related outbreaks were investigated: one among staff and employees of a healthcare institution (42) and the other one affected recreational visitors of a non-dairy sheep farm with a public function (44). These clusters showed that during the whole duration of the epidemic, it was important to disentangle the different veterinary sources and the responsible ruminant species. The non-dairy sheep farm that was later implicated as the source was first identified from free text fields in the trawling questionnaires that were used by the local public health service. Where exposure to infected animals could be avoided (i.e. visiting infected farms) the advice was to do so, and the farm involved in this latter investigation was closed to the public pending vaccination of the herd and reopened in the spring of 2011.

These outbreak investigations were carried out by or in close collaboration with the local public health services and regional laboratories. This is important as for a thorough outbreak investigation one needs to go to the field to obtain insight in the local context and outbreak dynamics in order to develop hypotheses on the possible outbreak source. Environmental sampling of different matrices was an integrated part of all investigations and appeared an important tool to provide supportive evidence by detection of *C. burnetii* in samples derived of epidemiologically incriminated sources.

First outbreak investigation suggested a causal link with a nearby dairy goat farm

The population-based case-control study in Herpen took place several months after the initial occurrence of acute Q fever cases in this village. This study showed a high attack rate as almost one quarter of the sampled inhabitants had a recent *C. burnetii* infection based on IFA. The identified risk factors suggested a strong plausible link with a nearby dairy goat farm that experienced an abortion storm in April 2007, one month before human Q fever cases arose. This was supported by *C. burnetii* DNA-positive environmental samples taken on the farm.

Due to presence of several other ruminant farms in this area, it was difficult to pinpoint this first community outbreak of acute Q fever in the Netherlands to one farm, as proximity to the specific dairy goat farm with abortion history or one of four postal codes to the east of the village ('area A') were both significant risk factors, hampering support for the immediate implementation of control measures. Between 2007 and 2010 this village was in the proximity of four *C. burnetii* bulk tank milk (BTM)-positive farms, of which two had *C. burnetii* induced abortions in 2007 and 2008. The closest farm was in the village itself and the furthest was six kilometers from the center of the village. Population screening 7 years after the initial outbreak to identify unrecognized chronic Q fever infections in this village required a considerable effort and investment, but had a small yield with just one newly diagnosed patient with chronic Q fever out of 513 inhabitants (0.2%) with *C. burnetii* antibodies (48). This is currently used as input for cost-benefit analyses of future post-epidemic screening options for chronic Q fever.

The urgency to establish the epidemiological link

Without uniform sufficiently discriminatory molecular typing methods for comparison of human, environmental and veterinary strains, more innovative spatial-temporal methods needed to be applied during the Dutch Q fever outbreak to establish an epidemiological link, as otherwise the outbreak source would remain unproven. It became imperative to study distance-response relations in detail, as sound epidemiological evidence was needed to support the implementation of veterinary control measures to stop the zoonotic transmission (49).

Spatial-temporal aspects of the Dutch Q fever epidemic

The proximity of the residential address of Q fever cases to multiple potential veterinary sources had not been studied extensively in previous outbreaks abroad. In search of comparable situations, we adapted a GIS-based approach that was successfully applied during an outbreak of legionellosis occurring in two adjacent cities in Norway (50).

As *C. burnetii* is an airborne pathogen, like *Legionella spp.,* that similarly spreads over considerable distances and is emitted from outdoor sources, it was assumed that this method could be applied in the ongoing Dutch Q fever epidemic. This method needed data with a high spatial resolution from both notified patients and ruminant farm locations. This kind of detailed information was not routinely available at the start of the Dutch Q fever epidemic, and if accessible, not easily shared between the public and veterinary health sectors. This data sharing gradually improved during the epidemic and as soon as exact small ruminant farm locations became available and six-digit postal codes of the case residence addresses were provided by the municipal public health services for this study purpose, the spatial-temporal relation between human clusters and potential farm sources lead to improved source finding.

Discrimination of potential sources through calculation of distance-based attack rates

The GIS-based method using concentric ring analyses was first introduced in the urban Q fever outbreak in Helmond in 2008, and later again applied in a recurrent outbreak in the same area in 2009, and in community clusters in Utrecht and Zuid-Limburg provinces in 2009 (46, 47, 51). In all these settings, distance-based attack rates were calculated in a retrospective cohort design, resembling the risk to acquire acute Q fever associated with exposure to each of several potential farm sources, using proximity of case residential address to the source as a proxy for exposure.

This rapid discerning method facilitated in discriminating sources that could already be ruled out after this GIS-based exercise (43, 47) and identifying epidemiologically suspect sources. This subset of suspected sources warranted further investigation including extensive environmental and veterinary sampling and collection of farm-based characteristics, such as herd size, recent animal movements, occurrence of reproductive events and status of bulk tank milk (BTM) investigations that were obtained during farm visits. This process could finally implicate a specific farm as the definite source (47). The evidence provided by clear distance-response relations in these outbreak settings was strengthened by a temporal relation between the occurrence of an abortion wave among small ruminants or the peak number of births during the kidding or lambing season and the peak week of illness onset in human Q fever cases, matching the 3 week mean incubation period of acute Q fever.

Investigation of an urban Q fever outbreak led to 5-kilometer (km) risk zones around BTM-positive farms
In retrospect, the urban outbreak in Helmond in 2008 could be epidemiologically linked to a single dairy goat farm as the primary outbreak source. This farm experienced a Q fever-related abortion wave a few weeks before the first human cases were notified. Residents living within 2 km of that farm had a 31 times higher risk to acquire clinical Q fever compared to the reference population living more than 5 km away. It was concluded that living close to an infected dairy goat farm with abortion problems caused by *C. burnetii* posed a risk for human Q fever.

This was considered strong epidemiological evidence that further supported the hypothesis of dairy goat farms as the main source of infection.Similar distance-response relationships were repeatedly found in other community outbreaks using the same initial method as in Helmond in 2008, and were later combined with more sophisticated spatial analyses and dispersion models (52). The findings from the Helmond study directly led to a national public information campaign targeted at residents that lived within a 5 km-radius around BTM-positive dairy goat and sheep farms [8]. It also convinced veterinary authorities of the indisputable relationship between dairy goat farms with abortion storms and Q fever cases among nearby residents.

A list of BTM-positive small ruminant farms was made available to the public on the website of the Food and Consumer Product Safety Authority and updated daily. Residents in the 5-km zone of these farms received a letter informing them on the presence of a Q fever-positive farm in their proximity that allowed persons with known risk factors to avoid these farm areas and raised awareness among residents of these areas to seek medical care if experiencing symptoms possibly related to Q fever.
In reality, it was however impossible for residents to avoid these risk zones as it was their direct residence or working area where they could experience long-lasting exposure to *C. burnetii*. Next to informing the public, physicians in this 5 km-zones were notified by the municipal public health services to raise awareness of possible consultation by Q fever cases and to successfully decrease the diagnostic delay, as was demonstrated in the notification data (53).

Proportion of Q fever cases explained through residence in 5 km-risk zones
In-depth outbreak investigations showed that the large majority of acute Q fever patients got infected following community-based exposure caused by *C. burnetii* transmission from nearby infected small ruminant farms. The proportion of cases that could be explained by community exposure through residence within these 5 km-risk zones, was 59% in 2009, meaning that 6 out of 10 notified cases in 2009 lived within a 5 km-radius of a BMT-positive dairy goat or dairy sheep farm.
The incidence of Q fever in 2009 was 69 per 100,000 population within, and 6 per 100,000 outside the 5 km-areas (31). Nevertheless, a considerable number of dairy goat farms were BTM-positive whereas the number of notified acute Q fever cases in these regions remained limited.
Therefore, several hypotheses arose including the high heterogeneity in shedding rates among farms, regional diversity in Q fever awareness and diagnostics (12, 29), presence of multiple *C. burnetii* strains with different virulence, and effect of the local environmental conditions on *C. burnetii* transmission (54, 55).

Residing near goat farms associated with Q fever-related outcomes and increased risk of pneumonia

The 5 km-radius was later also adopted in several policy advices which was in the light of the ongoing epidemic quite understandable. From an epidemiologists' perspective, extrapolation and the generalizability of study findings from one outbreak setting to other settings needs to be done cautiously as every outbreak situation is unique and has its own features. Atmospheric modelling studies showed that the affected area and timing of disease in each outbreak setting is strongly dependent on meteorological and local environmental conditions (47, 55, 56).

The distance of 5 kilometres from the putative farm source should not be rigidly interpreted, as shown by another study included in this thesis (*Chapter 4*). The presence of a BTM-positive dairy goat farm within 8 km-distance was a risk factor for *C. burnetii* seropositivity in dairy goats on herd and animal level, and a BTM-positive dairy goat farm within 16 km-radius was a risk factor for *C. burnetii* seropositivity in dairy goat farm residents *(Table 1).*

Another GIS-based study carried out in a livestock-dense area in the eastern part of North Brabant province and in the north of Limburg province (12) demonstrated a clear exposure-response relationship between the number of goats within a 5 km-radius of the residential address and Q fever related outcomes such as pneumonia and 'other infectious disease' incidence using records of general practitioners (57). This study also indicated a considerable residual risk for infection beyond this 5 kilometer radius as the increased pneumonia risk returned to background levels only beyond 10 kilometers from the nearest goat farm (57). This same association between goat density and pneumonia incidence was re-investigated in the same area, when the incidence of acute Q fever had dropped to pre-epidemic levels.

During the years 2009-2013, there is still a higher incidence of pneumonia in residents in the near vicinity of goat farms, even though the pneumonia incidence in this area has decreased since the Dutch Q fever epidemic. The number of additional cases of pneumonia in the research area attributable to the presence of goat farms is approximately 5.4% over the years 2009-2013, based on advanced kernel-analyses (58). The cause of this increase is still unclear and is most likely not caused by Q fever as individuals with pneumonia were not more often seropositive for *C. burnetii*.

Further studies are needed to confirm and identify other causes of pneumonia in this population and the potential role of non-infectious agents such as particulate matter or endotoxins (59).

Seroprevalence and risk factors of *C. burnetii* infection in residents and ruminants on commercial farms in a 'One Health' context (*Chapter 4*)

Previous knowledge on this subject

Maintenance of *C. burnetii* infection within a small ruminant farm might be influenced by persistently infected domestic ruminants, other animal reservoirs of infection, ticks, and husbandry practices that favor within herd transmission or environmental contamination (60). However there is considerable uncertainty about the relative importance of risk factors for maintenance of *C. burnetii* infection in domestic ruminant populations within a farm and risk factors for zoonotic spill-over to other ruminant farms and to humans. Also conclusive evidence in support of a direct link between increased animal density or farm density and risks for spillover from *C. burnetii* infected farms to nearby human and ruminant communities was lacking. In theory, such a link would be consistent with the consequences of increased pathogen pressure as infected goats kept at a higher density would result in a greater amount of bacterial contamination per square meter. This would put naïve goats introduced to the herd at a greater risk of exposure to *C. burnetii* and lead to an increased emission of *C. burnetii* for airborne transmission.

What did these studies add?

Q VIVE studies were at the forefront of the One Health approach in studying Q fever

Since 2007, a large multidisciplinary research portfolio using a 'One Health' approach was implemented that aimed at generating better knowledge about the Dutch Q fever situation with a clear expressed need and benefit for cross-sectoral collaboration. These integrated studies studying the seroprevalence and risk factors for *C. burnetii* infection both in farmers' households as in the ruminants present on the same farms were at the forefront of this 'One Health' research collaboration. Many institutional partners from both the veterinary and public health domains, including occupational health experts and environmental specialists were involved.

The 'One Health' set-up of these integrated studies has later been adapted and extended in other large population-based studies such as the so-called 'Livestock farming and the health of local residents' (VGO)-study focusing on the health risks associated with livestock farming in local residents in a livestock-dense area (12).

Identified risk factors in (small) ruminants on herd and animal level and among farm residents

The identified risk factors in all four ruminant sectors (dairy goat, dairy sheep, non-dairy sheep and dairy cattle) correspond with the dominant role of dairy goats during the Dutch Q fever epidemic.

For all sectors, a higher risk of *C. burnetii* infection was found for large-sized small ruminant farms ***(Table 1, blue)***, farms located in the south of the Netherlands ***(Table 1, purple)*** or at a short distance of a BTM-positive small ruminant farm ***(Table 1, orange)***. Several risk factors indicated plausible routes of *C. burnetii* introduction on farms ***(Table 1, red)***. These ruminant sectors were advised to improve biosecurity measures, such as a closed farming system, avoiding other introduction pathways and optimize good hygiene practices, including wearing of protective clothing, especially when participating in birthing. Also more insight in the role of airflow in indoor farms is needed, as covering air spaces in stables, whether for pest control or as a windbreak, has been identified as a risk factor for *C. burnetii* infection in goats: restricted air flow in an indoor environment could have a greater risk of exposure for ruminant livestock, as indicated by positive air samples in indoor flock premises several months after lambing, compared to facilities with continuous ventilation (61). Risk factors were often similar for farm residents and (small) ruminants within the same sector, but frequently differed between sectors.

Ruminant farmers face daily exposure to livestock-associated pathogens in every aspect of their work. In general, it remains difficult to precisely determine the intensity of this livestock-human contact as an exposure-response relation (62). The occupational status of the farmer, a full time workweek and the number of daily (small) ruminant-related tasks (e.g. milking, feeding, supply and removal, general animal health care, birth assistance) served as a proxy for this intensity in the studies included in this thesis.

Dairy (small) ruminants seem more likely to contract *C. burnetii* infection than non-dairy animals. This is probably related to husbandry factors that make transmission more efficient or animals more vulnerable on dairy farms, resulting in relatively high within-herd prevalences. This has also been observed in international literature showing a higher seroprevalence in dairy cattle herds compared to beef cattle herds (63).

Compulsory Q fever vaccination in dairy small ruminants

Since 2010, compulsory vaccination is applicable to all farms with more than 50 dairy goats or sheep, farms with a public function such as social care farms, petting farms and for small ruminants that are participating in shows. The French phase one vaccine Coxevac® (CEVA Sante Animale) is an animal vaccine that should be given before pregnancy in order to be effective. Vaccination has been shown to strongly decrease the abortion risk due to *C. burnetii* and the amount of bacterial shedding into the environment. Thereby it prevents airborne C*. burnetii* spread to nearby animal populations and human residents (64).

Continuation of the annual vaccination at dairy small ruminant farms is of vital importance in this post-epidemic period. In the absence of vaccination, a birth cohort study that systematically investigated *C. burnetii* seroconversions among intensively-managed goats on infected farms showed that the majority of goats are infected early in life well before the goats were mated. The first *C. burnetii* IgM surge was observed in week 9 underlining the need for early vaccination in order to control *C. burnetii* in a heavily contaminated environment (65). This need is reinforced by the current steep increase in the mean herd size at commercial dairy goat farms since 2009 (66). Without the compulsory vaccination, there would be an increased risk of *C. burnetii* infection among ruminants as larger herd size was an identified risk factor on herd level (*Table 1*). Moreover, this rapidly increasing mean herd size conceivably makes the Dutch dairy goat sector more vulnerable for introduction of zoonotic pathogens other than *C. burnetii*, emphasizing the importance of implementing more general biosecurity measures beyond compulsory vaccination. End of 2016, a multidisciplinary panel of Dutch Q fever experts advised both the Ministry of Health and Ministry of Agriculture to continue the annual compulsory vaccination for dairy goat and sheep for an undetermined period.

Table 1. Summary of main identified risk factors for C. burnetii seropositivity in four ruminant sectors on animal and herd level and in residents, Q-VIVE study, 2009-2011

	Individual ruminant level	Herd level	Farm residents
Dairy goats	≥100 cattle per km^2 in farm municipality **<8 km distance to nearest BTM-positive SR* farm** presence of cats in the stable combat nuisance animals by covering air spaces unknown if signs of vermin present at farm use of windbreak curtains in the stable **use of artificial insemination**	≥100 cattle per km^2 in farm municipality **<8 km distance to nearest BTM-positive SR* farm** presence of cats and dogs in the stable combat nuisance animals by covering air spaces unknown if signs of vermin present at farm **origin of straw from abroad or unknown** **herd size ≥800 goats**	**<16 km distance to nearest BTM-positive SR* farm** presence of cats in the stable combat nuisance animals by covering air spaces **farm location in south of country** dairy goats on extended lactation other goat breeds present besides Dutch white goat residence <10 meters from nearest stable high number of daily farm tasks (≥3) no farm boots for staff lived as a child on ruminant farm**
Dairy sheep	n/a	n/a	n/a
Non-dairy sheep	**farm location in south of country** goat density per km^2 in 10-km radius ≥5.3 **flock size ≥225 sheep** windbreak curtains and/or windshields in the stable unknown if signs or no signs of vermin in roughage ≥ 6 stillborn lambs *(in 2009)* breeding lambs as main farm purpose	**farm location in south of country** **flock size ≥225 sheep** **≥1 supply address for ewes** using boots by professional farm visitors sheep located at marginal grounds	goat density per km^2 in 5 km radius ≥11.4 farm started since 1990 presence of Blessumer sheep breed on farm refresh bedding in stables every other day or more frequent air entry other than through door sheep supply from Groningen and Noord-Holland provinces lambing indoors age of farm resident 40-49 years** worked in cattle sector in past cattle at same pasture as sheep, but not simultaneously dairy cattle present during stable period sheep contact with cattle at own or other farm
Dairy cattle	n/a (no individual cows sampled)	**ELISA+:** **increasing herd size (per 10 cows)** **no use of automatic milking system** **≥2 purchase locations for cattle** clean bedding in stable every other day or less **PCR+:** **no use of automatic milking system** no goats or sheep present on farm ticks at dairy cattle present/unknown	**farm location in south of country** **increasing herd size** **no use of automatic milking system** age of farm resident ≥55 years part-time and full-time work on farm non-compliant use of gloves during cattle birth care pig contact at own or other farm indirect contact with rats and/or mice at farm presence of birds in stable beef cattle present on farm dairy cattle contact at other farms

**SR=small ruminant; ** borderline risk factor; n/a, not available*

The frequency of the BTM-monitoring program implemented in October 2009 has been reduced to monthly measurements as lambing is now more evenly spread over the year, while the rest of the preventive hygienic measures in the dairy small ruminant sector are kept in place (67). Potential health risks related to intensive animal husbandry has become a broadly debated topic in the Netherlands. These discussions have been intensified by the Q fever epidemic and the transmission of antibiotic resistant pathogens of animal origin to humans, debating the herd size, the farm density of an area and the close proximity of intense livestock farming to nearby residential areas.

Seroprevalence and occupational risk factors of *C. burnetii* infection in veterinary-associated populations (*Chapter 5*)

What is already known on this topic

Q fever has long been considered primarily an occupational zoonosis for abattoir workers, sheep shearers, livestock farmers, and especially veterinarians and veterinary students, because of their contact with potentially *C. burnetii* infected animals. In the 1980s there was already evidence that the large majority of Dutch livestock veterinarians, residents of dairy farms and wool spinners were serologically positive for *C. burnetii* antibodies (68), indicating that Q fever has been endemic for a long time among certain occupational groups in the Netherlands. A problem with comparing the results from studies of the 1980s is that the specificity of the indirect IFA used at that time is not known. For example also a high seroprevalence was found in the general population and antibody percentages in all age classes were much alike (68). Direct comparison with the current screening assays is not possible (69). The population-based seroprevalence survey in 2006-2007 confirmed that the presence of antibodies against *C. burnetii* was significantly associated with exposure to domesticated livestock by occupation or hobby (*Chapter 2*). The association with exposure to domesticated livestock and the agricultural industry was also shown in a large population-based study in the United States with agricultural workers six times more likely to have *C. burnetii* antibodies than those employed in other occupations (70).

Although Q fever is often cited as an occupational disease, most acute Q fever patients actually become infected during community outbreaks (71), while occupational Q fever mainly occurs during a lifespan as sporadic infections. Based on the Dutch Q fever notification data, direct occupational exposure did not play a major role in the Q fever epidemic as only a small percentage of notified Q cases worked in the agriculture sector (3.2%) and meat processing industry (0.5%). However, the percentage of cases working in these sectors was higher than in the general population and other control groups (29) and confirms the increased occupational risk to acquire clinical Q fever. Since 2009, there is more insight in occupational exposures of cases with a notifiable infectious disease as specific questions on occupational categories and type of work were added to the national infectious disease surveillance database (72).
Generally, high seroprevalence figures were found in occupational studies that screened farm residents (20, 73, 74), shepherds (75) and veterinarians for past *C. burnetii* infection in different countries (76-83).
Studies carried out in farming populations have suggested that the disease incidence is low and that farmers dealing with cattle are considerably more at risk of infection by *C. bumetii* than are other farming populations (84). In seroprevalence studies that focused on the risk of *C. burnetii* infection in veterinarians, intensive direct contact with infected ruminants and exposures during parturition were identified as occupational risk factors (77, 82). Among veterinary students, risk factors for Q fever such as practising with living animals, especially ruminants, following specific courses focusing on animal production and food inspection, and an increasing seroprevalence prior to graduation have been reported (21, 81, 85). As the possible health impact of the Dutch Q fever epidemic was unclear for livestock veterinarians and ruminant farmers including their household members in the Netherlands, it was relevant to study *C. burnetii* seroprevalence and assess individual and occupational risk factors for *C. burnetii* seropositivity.

An effective occupational Q fever vaccine is available in Australia

Q fever is in theory a vaccine preventable disease. An effective whole-cell formalin-inactivated vaccine for humans is produced and only licensed in Australia since 1989 for use in persons from 15 years of age (Q-Vax; CSL Biotherapies, Parkville, Victoria, Australia) (86). An Australian government-funded national vaccination program (NQFMP) for abattoir

workers, sheep shearers and farmers, their families and employees in the livestock-rearing industry ran from 2001 to 2006. Following introduction of this program, the number of notified Q fever cases halved with the greatest reductions among young men aged 15–39 years, consistent with high documented vaccine uptake among abattoir workers (87). Since 2007, after the NQFMP finished, the vaccine remained available on the private market targeted at different occupational risk groups according to its product information. There is also a high vaccine uptake among Australian veterinarians (88), however identified potential barriers to vaccination are lack of perceived risk, financial expense, time constraints and difficulty to find a vaccine provider.

The Q-Vax vaccine has been demonstrated to be highly effective and has a strong safety record in these occupational groups (89, 90). There are no indications for reduced immune responses with increasing time (91). Vaccine protection has not been evaluated in a randomized clinical trial; however, retrospective cohort studies estimate efficacy at >90% for those vaccinated at least 15 days prior to exposure (92). Following an abattoir Q fever outbreak where a Q fever vaccination program was underway, it has been proven that the Q-vax vaccine is effective if given in advance of likely *C. burnetii* exposure (93). Persons that already have a humoral or cellular immune response to *C. burnetii* can get severe adverse reactions at the injection site and are therefore contraindicated for vaccination.
Because of this, potential vaccine recipients should be evaluated both for *C. burnetii* antibodies and by a skin test (Q-Vax skin test) prior to vaccination. Both these tests need to be negative before vaccination is advised. Since these procedures are time-consuming and costly, they preclude the use of this whole-cell vaccine in a mass vaccination program. Ongoing research efforts are targeted to develop a safer, more effective new-generation vaccine, that will not cause adverse reactions when given to someone with pre-existing immunity (94). Recent studies have concentrated on identifying protective antigens, from where development of DNA vaccines will be the next step (95, 96).

What did these studies add?

High life-time risk to acquire *C. burnetii* infection in professionals with intensive livestock contact
For all occupational groups studied in this thesis, seroprevalence figures were comparable in international context, although more in the upper range. This high seroprevalence can be influenced by the selection of study populations: small ruminant farmers that have intensive exposure to possibly infected livestock on a daily basis during the Dutch Q fever epidemic, and the focus on the specific group of livestock veterinarians who in general have a higher seroprevalence due to exposure to ruminants compared to veterinarians that solely have contact with companion animals (79), or mixed groups of veterinarians (77, 82).
It is interesting to notice that the overall seroprevalence of 65% among Dutch livestock veterinarians during the Dutch Q fever epidemic was about 7% higher than in the south of Belgium (79) and 2–14% higher than in the south of Germany (77). This relative high seroprevalence can be the result of the inclusion of more recent infections, that mainly occurred among veterinary students in their final year of clinical rotations (28), or can be explained by a booster effect through continuous exposure as was suggested in studies among wool workers and livestock veterinarians (25, 28).

Seroprevalence studies among farm residents in all four ruminant sectors (dairy goat, dairy sheep, non-dairy sheep and dairy cattle) indicated a high lifetime risk for *C. burnetii* infection (*Chapter 4*). Seroprevalence figures were almost identical among farm residents of dairy goat, dairy sheep and dairy cattle, around 70%, and a bit lower in residents of meat- and breeding sheep farms (51%). One out of 9 farm residents living on a commercial dairy goat farm had evidence of a recent *C. burnetii* infection based on presence of IgM-phase 2 antibodies, regardless of symptoms, indicating recent exposure originating from BTM-positive small ruminant farms. There were less recent infections (1.2%) among residents of dairy cattle farms, indicating that the infection was generally in the more distant past, even though *C. burnetii* is widespread and enzootic in Dutch dairy cattle *(Chapter 4)*.

Veterinarians and veterinary students, ruminant farmers and other professionals intensely exposed to livestock and their physicians should be aware of this lifetime risk of Q fever to ensure a timely diagnosis and treatment, especially in case of predisposing conditions for developing chronic Q fever (20, 21, 77, 97).

Among the seropositive participants in the Q-VIVE project, 11 residents of dairy goat farms, 2 residents of a dairy cattle farm, 1 of a dairy sheep farm and 7 livestock veterinarians showed a serological profile compatible with possible chronic Q fever (*Chapter 4 and 5*), defined by an IgG phase I titer ≥1:1024 (98). None of them could be confirmed that these truly were chronic Q fever cases because clinical information (e.g., presence of vascular infection, endocardial involvement, or other clinical risk factors) was lacking. Although apparently healthy, proper serological follow-up as well as a medical examination should be recommended in study participants with this serological profile (79). Few periodical screening studies have been conducted to examine *C. burnetii* seroconversion rates and associated risk factors among exposed workers (82, 99).
By following incoming, seronegative veterinary students through their veterinary program during a four-year study period, almost one out of five veterinary students seroconverted (*personal communication Wim van der Hoek, RIVM*), resembling the seroconversion rate in the culling workers, however during a longer time-span and not in an outbreak context. A short follow-up period of several months was sufficient in high exposure settings as was done in the culling worker study. In low-intensity settings a longer follow-up period is useful to assess the seroconversion rate and clinical impact, as was done in a cohort of first-year veterinary students participating in the Veterinarians' Health study (VHS II) (22, 100). More efforts could be made in existing data to identify predictor variables for specific serological profiles including potential chronic *C. burnetii* infection as was done in Belgium livestock veterinarians using classification and regression tree analysis. Contact with manure during the prior month was found to be significantly associated with a higher risk of *C. burnetii* seropositivity while frequent contacts with bovine birth products in veterinarians aged 34 years or older could explain all potentially chronic *C. burnetii* infections (79).

Occupational risk factors for *C. burnetii* infection in professionals with intensive exposure to livestock
Identified risk factors for occupational past *C. burnetii* infection in Dutch livestock veterinarians were the number of hours with animal contact per week, number of years graduated as veterinarian, rural or suburban residence area, being a practicing veterinarian and occupational contact with swine. In Dutch veterinary students, advanced year of study, experiencing a zoonotic disease since starting the veterinary medicine curriculum and having ever lived on a ruminant farm were significant risk factors for being *C. burnetii* IgG-phase 2 seropositive (*Chapter 5*).
Workers involved in the culling of more than 50,000 sheep and goats in December 2009 up to June 2010 were recruited into a prospective cohort study to examine *C. burnetii* seroconversion and related morbidity while culling. Despite the fact that these culling workers were given instructions in advance through a common presentation, advised to read the occupational health and hygiene regulations, and were provided with personal protective equipment (PPE) including FFP3 masks ('Face Filtering Pieces' thought to filter at least 99% of airborne particles), 17.5% seroconverted for antibodies to *C. burnetii* and one third of them reported a clinical episode with fever, headache and cough after culling. Working in close proximity to the animals inside the stable and prolonged contact (>100 hours per week) were risk factors for seroconversion. Anecdotally, culling workers reported removing PPE during coffee breaks and lunch breaks, which may have contributed to the high proportion of seroconversions recorded. In the context of inevitable exposure during animal culling, workers should be carefully instructed on PPE compliance in designated risk areas. Vaccination should be provided in professionals working short-term in high-exposure settings with inevitable exposure, as was the case in the group of culling workers (*Chapter 5*), but unfortunately this option was barely considered and not introduced in the Netherlands (101, 102).

Vaccination against Q fever as an occupational control measure
Q fever is a serious disease with a possible severe outcome and a high disability adjusted life years (DALY) burden (103). Vaccination of occupational risk groups is a feasible preventive option as specific risk groups have been clearly defined, with a specific focus on employees with intensive livestock contact. The availability of a Q fever vaccine is of particular interest to veterinary professionals, ruminant farmers and abattoir employees in the context of high lifetime risk for *C. burnetii* infection. The European Centre for Disease Prevention and Control (ECDC) has also called for the availability and use of Q-Vax among at-risk groups in Europe, including professionals coming into contact with livestock, besides persons with cardiovascular disease and immunosuppressed patients, while a new-generation vaccine is being developed (96, 104, 105).

The Health Council of the Netherlands released two advisory reports on Q fever vaccination in 2010. The Q fever vaccine can be of direct benefit to those with inevitable occupational exposure to *C. burnetii* or be beneficial to those with background medical conditions that might increase complication risks after having been infected *with C. burnetii.* In the first report, the Health Council Committee advised a Q fever vaccine campaign for patients at high risk of developing cardiovascular complications. It was designed as a one-of campaign in July 2011 and resulted in a limited coverage due to this late implementation and ending of the epidemic (106, 107).

In the second report, the Committee decided not to provide this vaccine to current occupational groups with intense livestock contact such as dairy ruminant farmers, sheep shearers, laborers and veterinarians, or future professionals at risk for *C. burnetii* infection. While the vaccine effectiveness and occurrence of possible adverse reactions was in favor for the use of Q-Vax in these professional groups, the Committee anticipated on a low disease burden in the current professionals with intense livestock contact, as the large majority have already acquired *C. burnetii* antibodies, but also concluded the vaccine would be of limited efficiency in future professionals, using the same argument of a low anticipated disease burden in terms of severity and magnitude in the future (101, 102).

The Health Council Committee acknowledged that the advice was based on limited research data and recommended further research regarding *C. burnetii* infection, underreporting and disease burden among future professionals coming into contact with livestock, such as veterinary students (102). The Committee also stated that another approach, using a more precautionary principle guaranteeing a healthy working environment for employees at all times, could lead to a different recommendation. Nevertheless, an important consideration not to advice Q-Vax for occupational groups and future professionals with livestock contact is that Q-Vax is currently not registered for use in the Netherlands. The Health Council Committee deemed it unlikely that such registration could still take place, given the available data and the relevant requirements (102). Without a license, Q-Vax can only be administered after the patients' physician has signed a doctor's statement and the recipient of the vaccine has signed an informed consent form, which are both significant hurdles. In 2009, the same issue was debated in the United Kingdom by the Joint Committee on Vaccination and Immunization (JCVI) after a Scottish Q fever outbreak in a slaughterhouse and cutting plant affecting a large group of short-term migrant workers who were lost for serological follow-up (108, 109). This Committee also concluded that more detailed studies were needed to assess the burden of disease in occupational risk groups, including exposed or potentially exposed workers before a decision could be made if the vaccine could be routinely implemented in an occupational context.

Given the lack of data on occupational exposure and risk of infection, and the adverse reaction profile of Q-vax, this Committee stated that it could not recommend Q-fever vaccination (110). However, with the available research data as also included in this thesis, the true clinical burden in livestock-associated occupational groups has not yet been established as low, but rather is unknown. Although there is enough evidence that veterinarians and especially those working with livestock are highly exposed *to C. burnetii* during their lifespan, clinical disease is mostly not directly recognized because of the asymptomatic and often benign and mild, nonspecific presentation of the infection.

The assumed and perceived low clinical burden of disease in occupational risk groups, might not reflect the true clinical burden, because of several reasons:

1. Based on seroprevalence data reaching a plateau at young adult age in rural areas (111) and seroconversion rates among veterinary students (personal communication Wim van der Hoek), it is likely that professionals with a high a priori risk acquire a *C. burnetii* infection at a relatively early stage of their professional career, which can be decades before study inclusion to assess seroprevalence and risk factors for *C. burnetii* infection such as during the Dutch Q fever epidemic. That explains why the livestock veterinarians and dairy cattle farmers were probably less likely to develop clinical Q fever during the epidemic years 2007-2009, due to previous *C. burnetii* exposure.

2. Several clinical Q fever cases within one occupational group during the Dutch Q fever epidemic, e.g. livestock veterinarians, appear to be a low absolute number, especially when compared to the high case numbers notified during the Dutch Q fever epidemic. However if one realizes that sporadic occupational cases keep occurring during the whole lifespan, a few notified veterinarians or livestock farmers with acute Q fever during one year can still represent a high attack rate within this occupational group, as the denominator data of these occupational groups are much smaller compared to the larger population at risk from where community Q fever cases arose.

3. There is a reported lower healthcare utilization by persons with increased livestock exposure, as well as a lower latent demand indicated by self-reported symptoms (112). It is hypothesized that this population has a different attitude towards reporting of symptoms and visiting healthcare providers that could lead to a selection bias when using GP-recorded data, and thereby underestimating the true clinical Q fever burden in these groups.

Further studies are therefore warranted that are more centered around the time of infection in order to assess the true clinical burden in professional groups with a high a priori occupational risk for *C. burnetii* infection. Such a study could be combined with the assessment of other zoonotic risks or non-infectious diseases as part of a targeted occupational surveillance, including periodical serological screening, monitoring relevant occupational exposures and self-reported symptoms, collection of sick leave registrations and health care visits. In order to establish the currently unknown occupational burden of chronic Q fever, information on occupation and specific occupational exposures should also be collected as part of the Dutch chronic Q fever database that until May 2016 includes over 400 proven, probable and possible chronic Q fever patients (113).

Vaccination of future livestock farmers, veterinarians and short-term professionals working in high-exposure settings with inevitable exposure

Seroprevalence findings in Dutch livestock veterinarians, veterinary students, culling workers and small ruminant and dairy cattle farmers, even though the complete clinical picture is absent, are in favor of seriously discussing the eventual Q fever vaccine benefits as they all encounter a high lifetime risk of *C. burnetii* infection (*Chapter 4 and 5*). The timing of vaccination is important to determine in the different professional groups that could benefit of this vaccine, e.g. first-year veterinary students at the beginning of their degree or new farm employees that start to work on a ruminant farm or pet farm.

With the current available studies in occupational settings, including those included in this thesis in Chapter 4 and 5, not vaccinating future professionals coming into extensive and close contact with livestock is a missed opportunity. It remains unclear why the same occupational groups that are entitled to receive an occupational Q fever vaccine in Australia, would not benefit from this vaccine when administered in an occupational setting in the Netherlands. However, caution must be taken when interpreting the current effect of *C. burnetii* vaccination as significant heterogeneity amongst publications was observed in recent meta-analyses (114, 115). Extensive knowledge on the size of the target occupational groups, the actual risk of occupational exposures and the number needed to screen are needed to assess the cost effectiveness for this intervention. However an effective occupational preventive measure does not necessarily need to be cost-effective as long as the occupational risk is effectively reduced. A recent call for a large, double-blind, multi-center randomized controlled trial was made to better help to establish the clinical effect of vaccination amongst occupationally exposed groups (115). So far, the evidence included may not be sufficiently robust to extrapolate the effect of vaccination beyond the population of relatively healthy and young abattoir workers with a high risk to acquire Q fever in Australia.

It is imperative to involve occupational health experts, employers and representatives of occupational groups in this debate of using Q fever vaccination as effective occupational preventive measure. The Netherlands Centre for Occupational Disease released a background document as part of the registration guideline for Q fever as an occupational disease in July 2016 (116), but unfortunately failed to discuss the advantages of Q fever vaccination for professionals at risk for Q fever in an occupational setting as it just briefly copied the recommendations made by the Health Council of the Netherlands (101, 102).

References (Chapter 6)

1. Anderson AD, Kruszon-Moran D, Loftis AD, McQuillan G, Nicholson WL, Priestley RA, et al. Seroprevalence of Q fever in the United States, 2003-2004. Am J Trop Med Hyg. 2009;81(4):691-4.
2. Wegdam-Blans MC, Wielders CC, Meekelenkamp J, Korbeeck JM, Herremans T, Tjhie HT, et al. Evaluation of commonly used serological tests for detection of *Coxiella burnetii* antibodies in well-defined acute and follow-up sera. Clin Vaccine Immunol. 2012;19(7):1110-5.
3. Blaauw GJ, Notermans DW, Schimmer B, Meekelenkamp J, Reimerink JH, Teunis P, et al. The application of an enzyme-linked immunosorbent assay or an immunofluorescent assay test leads to different estimates of seroprevalence of *Coxiella burnetii* in the population. Epidemiology and infection. 2012;140(1):36-41.
4. Anderson A, Bijlmer H, Fournier PE, Graves S, Hartzell J, Kersh GJ, et al. Diagnosis and management of Q fever--United States, 2013: recommendations from CDC and the Q Fever Working Group. MMWR Recomm Rep. 2013;62(RR-03):1-30.
5. Maurin M, Raoult D. Q fever. Clinical microbiology reviews. 1999;12(4):518-53.
6. Dupuis G, Peter O, Peacock M, Burgdorfer W, Haller E. Immunoglobulin responses in acute Q fever. Journal of clinical microbiology. 1985;22(4):484-7.
7. Guigno D, Coupland B, Smith EG, Farrell ID, Desselberger U, Caul EO. Primary humoral antibody response to *Coxiella burnetii*, the causative agent of Q fever. Journal of clinical microbiology. 1992;30(8):1958-67.
8. Worswick D, Marmion BP. Antibody responses in acute and chronic Q fever and in subjects vaccinated against Q fever. J Med Microbiol. 1985;19(3):281-96.
9. Dupont HT, Thirion X, Raoult D. Q fever serology: cutoff determination for microimmunofluorescence. Clin Diagn Lab Immunol. 1994;1(2):189-96.
10. Limonard GJ, Nabuurs-Franssen MH, Weers-Pothoff G, Wijkmans C, Besselink R, Horrevorts AM, et al. One-year follow-up of patients of the ongoing Dutch Q fever outbreak: clinical, serological and echocardiographic findings. Infection. 2010;38(6):471-7.
11. Hogema BM, Slot E, Molier M, Schneeberger PM, Hermans MH, van Hannen EJ, et al. *Coxiella burnetii* infection among blood donors during the 2009 Q-fever outbreak in The Netherlands. Transfusion. 2012;52(1):144-50.
12. Maassen KS, L. Wouters, I. van Duijkeren, E. Janse, I. Hagenaars, T. IJzermans, J. van der Hoek, W. Heederik, D. Livestock farming and the health of local residents (in Dutch). Bilthoven, the Netherlands: National Institute of Public Health and the Environment; 2016 05-07-2016. Contract No.: RIVM Report 2016-0058.
13. van der Hoek W, Meekelenkamp JC, Dijkstra F, Notermans DW, Bom B, Vellema P, *et al*. Proximity to goat farms and *Coxiella burnetii* seroprevalence among pregnant women. Emerging infectious diseases. 2011;17(12):2360-3.
14. Kampschreur LM, Hagenaars JC, Wielders CC, Elsman P, Lestrade PJ, Koning OH, et al. Screening for *Coxiella burnetii* seroprevalence in chronic Q fever high-risk groups reveals the magnitude of the Dutch Q fever outbreak. Epidemiology and infection. 2013;141(4):847-51.
15. Schimmer B, Lenferink A, Schneeberger P, Aangenend H, Vellema P, Hautvast J, et al. Seroprevalence and risk factors for *Coxiella burnetii* (Q fever) seropositivity in dairy goat farmers' households in The Netherlands, 2009-2010. PloS one. 2012;7(7):e42364.
16. Van den Brom R, Schimmer B, Schneeberger PM, Swart WA, van der Hoek W, Vellema P. Seroepidemiological survey for *Coxiella burnetii* antibodies and associated risk factors in Dutch livestock veterinarians. PloS one. 2013;8(1):e54021.
17. Whelan J, Schimmer B, Schneeberger P, Meekelenkamp J, Ijff A, van der Hoek W, et al. Q fever among culling workers, the Netherlands, 2009-2010. Emerging infectious diseases. 2011;17(9):1719-23.
18. van der Hoek W, Schneeberger PM, Oomen T, Wegdam-Blans MC, Dijkstra F, Notermans DW, et al. Shifting priorities in the aftermath of a Q fever epidemic in 2007 to 2009 in The Netherlands: from acute to chronic infection. Euro surveillance. 2012;17(3):2009.
19. Wielders CC, Teunis PF, Hermans MH, van der Hoek W, Schneeberger PM. Kinetics of antibody response to *Coxiella burnetii* infection (Q fever): Estimation of the seroresponse onset from antibody levels. Epidemics. 2015;13:37-43.
20. Thomas DR, Treweek L, Salmon RL, Kench SM, Coleman TJ, Meadows D, et al. The risk of acquiring Q fever on farms: a seroepidemiological study. Occupational and environmental medicine. 1995;52(10):644-7.
21. Sanchez A, Prats-van der Ham M, Tatay-Dualde J, Paterna A, de la Fe C, Gomez-Martin A, et al. Zoonoses in Veterinary Students: A Systematic Review of the Literature. PloS one. 2017;12(1):e0169534.
22. IRAS UU. VHS study II: cohort study among allergic and non-allergic health risks starting with first year veterinary students at the University of Utrecht, the Netherlands: study brochure. Utrechtt; 2009. Available: http://www.iras.uu.nl/vhs/
23. Chu H, Yoo SJ, Hwang KJ, Lim HS, Lee K, Park MY. Seroreactivity to Q Fever Among Slaughterhouse Workers in South Korea. J Prev Med Public Health. 2017;50(3):195-200.
24. Greig JE, Patel MS, Clements MS, Taylor NK. Control strategies for Q fever based on results of pre-vaccination screening in Victoria, 1988 to 2001. Australian and New Zealand journal of public health. 2005;29(1):53-7.
25. Wattiau P, Boldisova E, Toman R, Van Esbroeck M, Quoilin S, Hammadi S, et al. Q fever in Woolsorters, Belgium. Emerging infectious diseases. 2011;17(12):2368-9.
26. Anderson AD, Smoak B, Shuping E, Ockenhouse C, Petruccelli B. Q fever and the US military. Emerging infectious diseases. 2005;11(8):1320-2.
27. Villumsen S, Jorgensen CS, Smith B, Uldum S, Schiellerup P, Krogfelt KA. Determination of new cutoff values for indirect immunofluorescence antibody test for Q fever diagnosis in Denmark. Diagnostic microbiology and infectious disease. 2009;65(2):93-8.

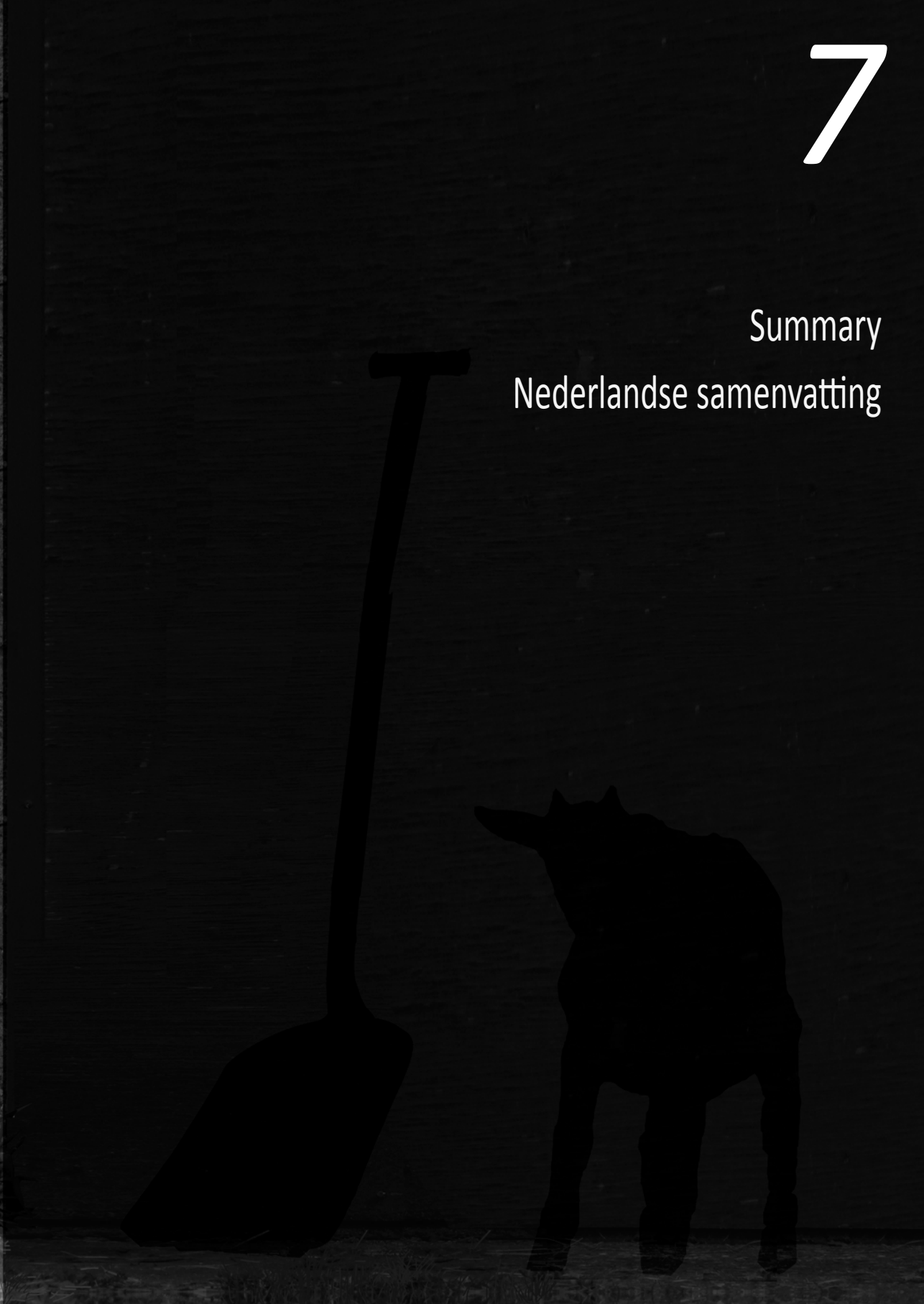
28. Wielders CC, Boerman AW, Schimmer B, van den Brom R, Notermans DW, van der Hoek W, et al. Persistent high IgG phase I antibody levels against *Coxiella burnetii* among veterinarians compared to patients previously diagnosed with acute Q fever after three years of follow-up. PloS one. 2015;10(1):e0116937.
29. Dijkstra F, van der Hoek W, Wijers N, Schimmer B, Rietveld A, Wijkmans CJ, et al. The 2007-2010 Q fever epidemic in The Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. FEMS immunology and medical microbiology. 2012;64(1):3-12.
30. Schimmer B, Morroy G, Dijkstra F, Schneeberger PM, Weers-Pothoff G, Timen A, et al. Large ongoing Q fever outbreak in the south of The Netherlands, 2008. Euro surveillance. 2008;13(31).
31. van der Hoek W, Dijkstra F, Schimmer B, Schneeberger PM, Vellema P, Wijkmans C, et al. Q fever in the Netherlands: an update on the epidemiology and control measures. Euro surveillance.2010;15(12).
32. Roest HI, Tilburg JJ, van der Hoek W, Vellema P, van Zijderveld FG, Klaassen CH, et al. The Q fever epidemic in The Netherlands: history, onset, response and reflection. Epidemiology and infection. 2011;139(1):1-12.
33. Wouda W, Dercksen DP. [Abortion and stillbirth among dairy goats as a consequence of *Coxiella burnetii*]. Tijdschrift voor diergeneeskunde. 2007;132(23):908-11.
34. Karagiannis I, Morroy G, Rietveld A, Horrevorts AM, Hamans M, Francken P, et al. Q fever outbreak in the Netherlands: a preliminary report. Euro surveillance. 2007;12(8):E070809 2.
35. Georgiev M, Afonso A, Neubauer H, Needham H, Thiery R, Rodolakis A, et al. Q fever in humans and farm animals in four European countries, 1982 to 2010. Euro surveillance. 2013;18(8).
36. Hilbert A, Reith P, Brockmann SO, Tyczka J, Fischer SF, Piechotowski I, et al. [Epidemiological enquiries in two Q fever outbreaks in a community of Baden-Wurttemberg during 2008 and 2009]. Berliner und Munchener tierarztliche Wochenschrift. 2011;124(7-8):295-302.
37. Hawker JI, Ayres JG, Blair I, Evans MR, Smith DL, Smith EG, et al. A large outbreak of Q fever in the West Midlands: windborne spread into a metropolitan area? Communicable disease and public health / PHLS. 1998;1(3):180-7.
38. Tissot-Dupont H, Amadei MA, Nezri M, Raoult D. Wind in November, Q fever in December. Emerging infectious diseases. 2004;10(7):1264-9.
39. 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. Epidemiology and infection. 2008;136(8):1084-7.
40. Porten K, Rissland J, Tigges A, Broll S, Hopp W, Lunemann M, et al. A super-spreading ewe infects hundreds with Q fever at a farmers' market in Germany. BMC infectious diseases. 2006;6:147.
41. Karagiannis I, Schimmer B, Van Lier A, Timen A, Schneeberger P, Van Rotterdam B, et al. Investigation of a Q fever outbreak in a rural area of The Netherlands. Epidemiology and infection. 2009;137(9):1283-94.
42. Koene RP, Schimmer B, Rensen H, Biesheuvel M, de Bruin A, Lohuis A, et al. A Q fever outbreak in a psychiatric care institution in The Netherlands. Epidemiology and infection. 2011;139(1):13-8.
43. Schimmer B, Ter Schegget R, Wegdam M, Zuchner L, de Bruin A, Schneeberger PM, et al. The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. BMC infectious diseases. 2010;10:69.
44. Whelan J, Schimmer B, de Bruin A, van Beest Holle MR, van der Hoek W, ter Schegget R. Visits on 'lamb-viewing days' at a sheep farm open to the public was a risk factor for Q fever in 2009. Epidemiology and infection. 2012;140(5):858-64.
45. Brandsen-Schreijer AJ. RB, Schimmer B, Cox CP, van den Bergh JP, Hulshof F, Hulshof(K, van der Hoek W, Woonink F. Q-koortsuitbraak in de provincie Utrecht in 2009 Infectieziekten Bulletin. 2010;21(9):334-41.
46. Hackert VH, van der Hoek W, Dukers-Muijrers N, de Bruin A, Al Dahouk S, Neubauer H, et al. Q fever: single-point source outbreak with high attack rates and massive numbers of undetected infections across an entire region. Clinical infectious diseases. 2012;55(12):1591-9.
47. Ladbury GA, Van Leuken JP, Swart A, Vellema P, Schimmer B, Ter Schegget R, et al. Integrating interdisciplinary methodologies for One Health: goat farm re-implicated as the probable source of an urban Q fever outbreak, the Netherlands, 2009. BMC infectious diseases. 2015;15:372.
48. Morroy G, van der Hoek W, Albers J, Coutinho RA, Bleeker-Rovers CP, Schneeberger PM. Population Screening for Chronic Q-Fever Seven Years after a Major Outbreak. PloS one. 2015;10(7):e0131777.
49. de Valk H. Q fever: new insights, still many queries. Euro surveillance. 2012;17(3):20062.
50. Nygard K, Werner-Johansen O, Ronsen S, Caugant DA, Simonsen O, Kanestrom A, et al. An outbreak of legionnaires disease caused by long-distance spread from an industrial air scrubber in Sarpsborg, Norway. Clinical infectious diseases. 2008; 46(1):61-9.
51. Bacterial and viral zoonoses: report of a WHO Expert Committee with participation of FAO. Geneva: WHO; 1982.
52. van Leuken JP, Havelaar AH, van der Hoek W, Ladbury GA, Hackert VH, Swart AN. A model for the early identification of sources of airborne pathogens in an outdoor environment. PloS one. 2013;8(12):e80412.
53. van der Hoek W, Dijkstra F, Wijers N, Rietveld A, Wijkmans CJ, van Steenbergen JE, et al. [Three years of Q fever in the Netherlands: faster diagnosis]. Nederlands Tijdschrift voor Geneeskunde. 2010;154:A1845.
54. Van Leuken JPG, Swart AN, Brandsma J, Terink W, Van de Kassteele J, Droogers P, et al. Human Q fever incidence is associated to spatiotemporal environmental conditions. One Health. 2016;2:77-87.
55. van der Hoek W, Hunink J, Vellema P, Droogers P. Q fever in The Netherlands: the role of local environmental conditions. Int J Environ Health Res. 2011;21(6):441-51.

56. van Leuken JP, van de Kassteele J, Sauter FJ, van der Hoek W, Heederik D, Havelaar AH, et al. Improved correlation of human Q fever incidence to modelled *C. burnetii* concentrations by means of an atmospheric dispersion model. Int J Health Geogr. 2015;14:14.
57. Smit LA, van der Sman-de Beer F, Opstal-van Winden AW, Hooiveld M, Beekhuizen J, Wouters IM, et al. Q fever and pneumonia in an area with a high livestock density: a large population-based study. PloS one. 2012;7(6):e38843.
58. Hagenaars T, Hoeksma P, de Roda Husman AM, Swart A, Wouters I. Livestock farming and the health of local residents (additional studies). Analysis of health effects, risk factors and emission of bio-aerosols (in Dutch). Bilthoven, The Netherlands: RIVM; 2017. Report No.: RIVM-2017-0062.
59. Freidl GS, Spruijt IT, Borlee F, Smit LA, van Gageldonk-Lafeber AB, Heederik DJ, et al. Livestock-associated risk factors for pneumonia in an area of intensive animal farming in the Netherlands. PloS one. 2017;12(3):e0174796.
60. EFSA Panel on Animal Health and Welfare (AHAW); Scientific Opinion on Q Fever. EFSA Journal 2010; 8(5):1595. [114 pp.].
61. Astobiza I, Barandika JF, Ruiz-Fons F, Hurtado A, Povedano I, Juste RA, et al. *Coxiella burnetii* shedding and environmental contamination at lambing in two highly naturally-infected dairy sheep flocks after vaccination. Research in veterinary science. 2011;91(3):e58-63.
62. Klous G, Huss A, Heederik DJJ, Coutinho RA. Human-livestock contacts and their relationship to transmission of zoonotic pathogens, a systematic review of literature. One Health. 2016;2:65-76.
63. Paul S, Agger JF, Agerholm JS, Markussen B. Prevalence and risk factors of *Coxiella burnetii* seropositivity in Danish beef and dairy cattle at slaughter adjusted for test uncertainty. Preventive veterinary medicine. 2014;113(4):504-11.
64. Hogerwerf L, van den Brom R, Roest HI, Bouma A, Vellema P, Pieterse M, et al. Reduction of *Coxiella burnetii* prevalence by vaccination of goats and sheep, The Netherlands. Emerging infectious diseases. 2011;17(3):379-86.
65. Muleme M, Campbell A, Stenos J, Devlin JM, Vincent G, Cameron A, et al. A longitudinal study of serological responses to *Coxiella burnetii* and shedding at kidding among intensively-managed goats supports early use of vaccines. Veterinary research. 2017;48(1):50.
66. ZuivelNL. Dutch dairy in figures 2016. The Hague; 2017.
67. Van Dissel JT. Advisory letter on the Dutch expert panel meeting on zoonoses regarding Q fever. 2 December 2016.
68. Richardus JH, Donkers A, Dumas AM, Schaap GJ, Akkermans JP, Huisman J, et al. Q fever in the Netherlands: a sero-epidemiological survey among human population groups from 1968 to 1983. Epidemiology and infection. 1987;98(2):211-9.
69. Richardus JH, Akkermans JP. [Q fever already present for a longer time in the Netherlands]. Tijdschrift voor diergeneeskunde. 2010;135(9):373-4.
70. Walsh MG. Assessing Q fever in a representative sample from the United States population: identification of a potential occupational hazard. Epidemiology and infection. 2012;140(1):42-6.
71. Dorko E, Rimarova K, Pilipcinec E. Influence of the environment and occupational exposure on the occurrence of Q fever. Central European journal of public health. 2012;20(3):208-14.
72. Heimeriks K, Beaujean D, Maas J. Surveillance work-related infectious diseases- Analysis work-related infectious diseases 2009. Bilthhoven: RIVM; 2010. Report No.: RIVM Report 205014007.
73. Cisak E, Chmielewska-Badora J, Mackiewicz B, Dutkiewicz J. Prevalence of antibodies to *Coxiella burnetii* among farming population in eastern Poland. Annals of agricultural and environmental medicine : AAEM. 2003;10(2):265-7.
74. Monno R, Fumarola L, Trerotoli P, Cavone D, Giannelli G, Rizzo C, et al. Seroprevalence of Q fever, brucellosis and leptospirosis in farmers and agricultural workers in Bari, Southern Italy. Annals of agricultural and environmental medicine : AAEM. 2009;16(2):205-9.
75. Dolce P, Belanger MJ, Tumanowicz K, Gauthier CP, Jutras P, Masse R, et al. *Coxiella burnetii* seroprevalence of shepherds and their flocks in the lower Saint-Lawrence River region of Quebec, Canada. The Canadian journal of infectious disease.. 2003;14(2):97-102.
76. Abe T, Yamaki K, Hayakawa T, Fukuda H, Ito Y, Kume H, et al. A seroepidemiological study of the risks of Q fever infection in Japanese veterinarians. European journal of epidemiology. 2001;17(11):1029-32.
77. Bernard H, Brockmann SO, Kleinkauf N, Klinc C, Wagner-Wiening C, Stark K, et al. High seroprevalence of *Coxiella burnetii* antibodies in veterinarians associated with cattle obstetrics, Bavaria, 2009. Vector borne and zoonotic diseases. 2012;12(7):552-7.
78. Chang CC, Lin PS, Hou MY, Lin CC, Hung MN, Wu TM, et al. Identification of risk factors of *Coxiella burnetii* (Q fever) infection in veterinary-associated populations in southern Taiwan. Zoonoses and public health. 2010;57(7-8):e95-101.
79. Dal Pozzo F, Martinelle L, Leonard P, Renaville B, Renaville R, Thys C, et al. Q Fever Serological Survey and Associated Risk Factors in Veterinarians, Southern Belgium, 2013. Transboundary and emerging diseases. 2017;64(3):959-66.
80. Dorko E, Rimarova K, Kecerova A, Pilipcinec E, Dudrikova E, Lovayova V, et al. Potential association between *Coxiella burnetii* seroprevalence and selected risk factors among veterinary students in Slovakia. Annals of agricultural and environmental medicine : AAEM. 2011;18(1):47-53.
81. Valencia MC, Rodriguez CO, Punet OG, de Blas Giral I. Q fever seroprevalence and associated risk factors among students from the Veterinary School of Zaragoza, Spain. European journal of epidemiology. 2000;16(5):469-76.
82. Whitney EA, Massung RF, Candee AJ, Ailes EC, Myers LM, Patterson NE, et al. Seroepidemiologic and occupational risk survey for *Coxiella burnetii* antibodies among US veterinarians. Clinical infectious diseases. 2009;48(5):550-7.
83. Meadows SL, Jones-Bitton A, McEwen SA, Jansen J, Patel SN, Filejski C, et al. Prevalence and risk factors for *Coxiella burnetii* seropositivity in small ruminant veterinarians and veterinary students in Ontario, Canada. The Canadian veterinary journal. 2017;58(4):397-9.
84. Davies TR, Edwards Y, Morgan A, Caul EO. Prevalence of Q fever in a rural practice. J Public Health Med. 1997;19(3):324-7.

85. Schnurrenberger PR, Helwig JH, Bashe WJ, Jr. The Incidence of Zoonotic Infections in Veterinary Students. J Am Vet Med Assoc. 1964;144:384-6.
86. Palmer C, McCall B, Jarvinen K, Krause M, Heel K. "The dust hasn't settled yet": the National Q fever Management Program, missed opportunities for vaccination and community exposures. Australian and New Zealand journal of public health. 2007;31(4):330-2.
87. Gidding HF, Wallace C, Lawrence GL, McIntyre PB. Australia's national Q fever vaccination program. Vaccine. 2009;27(14):2037-41.
88. Sellens E, Norris JM, Dhand NK, Heller J, Hayes L, Gidding HF, et al. Q Fever Knowledge, Attitudes and Vaccination Status of Australia's Veterinary Workforce in 2014. PloS one. 2016;11(1):e0146819.
89. Ackland JR, Worswick DA, Marmion BP. Vaccine prophylaxis of Q fever. A follow-up study of the efficacy of Q-Vax (CSL) 1985-1990. Med J Aust. 1994;160(11):704-8.
90. Marmion BP, Ormsbee RA, Kyrkou M, Wright J, Worswick DA, Izzo AA, et al. Vaccine prophylaxis of abattoir-associated Q fever: eight years' experience in Australian abattoirs. Epidemiology and infection. 1990;104(2):275-87.
91. Kersh GJ, Fitzpatrick KA, Self JS, Biggerstaff BJ, Massung RF. Long-Term immune responses to C*oxiella burnetii* after vaccination. Clin Vaccine Immunol. 2013;20(2):129-33.
92. Chiu CK, Durrheim DN. A review of the efficacy of human Q fever vaccine registered in Australia. N S.W Public Health Bull. 2007;18(7-8):133-6.
93. Gilroy N, Formica N, Beers M, Egan A, Conaty S, Marmion B. Abattoir-associated Q fever: a Q fever outbreak during a Q fever vaccination program. Australian and New Zealand journal of public health. 2001;25(4):362-7.
94. Ruiz S, Wolfe DN. Vaccination against Q fever for biodefense and public health indications. Front Microbiol. 2014;5:726.
95. Zhang G, Samuel JE. Vaccines against *Coxiella* infection. Expert Rev Vaccines. 2004;3(5):577-84.
96. Reeves PM, Paul SR, Sluder AE, Brauns TA, Poznansky MC. Q-vaxcelerate: A distributed development approach for a new *Coxiella burnetii* vaccine. Hum Vaccin Immunother. 2017:0.
97. Bosnjak E, Hvass AM, Villumsen S, Nielsen H. Emerging evidence for Q fever in humans in Denmark: role of contact with dairy cattle. Clin Microbiol Infect. 2010;16(8):1285-8.
98. van der Hoek W, Versteeg B, Meekelenkamp JC, Renders NH, Leenders AC, Weers-Pothoff I, et al. Follow-up of 686 patients with acute Q fever and detection of chronic infection. Clinical infectious diseases. 2011;52(12):1431-6.
99. Fenga C, Gangemi S, De Luca A, Calimeri S, Lo Giudice D, Pugliese M, et al. Seroprevalence and occupational risk survey for *Coxiella burnetii* among exposed workers in Sicily, Southern Italy. Int J Occup Med Environ Health. 2015;28(5):901-7.
100. Samadi S, Spithoven J, Jamshidifard AR, Berends BR, Lipman L, Heederik DJ, et al. Allergy among veterinary medicine students in The Netherlands. Occupational and environmental medicine. 2012;69(1):48-55.
101. Health Council of the Netherlands. Human vaccination against Q fever. The Hague: 2010; publication no. 2010/08E.
102. Health Council of the Netherlands. Human vaccination against Q fever: second advisory report. The Hague: 2010; publication no. 2010/18E
103. Brooke RJ, Van Lier A, Donker GA, Van Der Hoek W, Kretzschmar ME. Comparing the impact of two concurrent infectious disease outbreaks on The Netherlands population, 2009, using disability-adjusted life years. Epidemiology and infection. 2014;142(11):2412-21.
104. ECDC Technical Report. Risk assessment on Q fever. Stockholm: ECDC; May 2010. Report No.: ISBN 978-92-9193-210-8.
105. Forland F, De Carvalho Gomes H, Nokleby H, Escriva A, Coulombier D, Giesecke J, et al. Applicability of evidence-based practice in public health: risk assessment on Q fever under an ongoing outbreak. Euro surveillance. 2012;17(3):20060.
106. Isken LD, Kraaij-Dirkzwager M, Vermeer-de Bondt PE, Rumke HC, Wijkmans C, Opstelten W, et al. Implementation of a Q fever vaccination program for high-risk patients in the Netherlands. Vaccine. 2013;31(23):2617-22.
107. Vermeer-de Bondt PE, Schoffelen T, Vanrolleghem AM, Isken LD, van Deuren M, Sturkenboom MC, et al. Coverage of the 2011 Q fever vaccination campaign in the Netherlands, using retrospective population-based prevalence estimation of cardiovascular risk-conditions for chronic Q fever. PloS one. 2015;10(4):e0123570.
108. Wilson LE, Couper S, Prempeh H, Young D, Pollock KG, Stewart WC, et al. Investigation of a Q fever outbreak in a Scottish co-located slaughterhouse and cutting plant. Zoonoses and public health. 2010;57(7-8):493-8.
109. Pollock KG, Mellor DJ, Browning LM, Wilson L, Donaghy M. Q fever in migrant workers, Scotland. Emerging infectious diseases. 2007;13(12):1963-4.
110. Joint Committee on Vaccination and Immunisation (JCVI). Minutes of JCVI meeting held on 14 October 2009. London; 2009.
111. McCaughey C, McKenna J, McKenna C, Coyle PV, O'Neill HJ, Wyatt DE, et al. Human seroprevalence to *Coxiella burnetii* (Q fever) in Northern Ireland. Zoonoses and public health. 2008;55(4):189-94.
112. van Dijk CE, Smit LA, Hooiveld M, Zock JP, Wouters IM, Heederik DJ, et al. Associations between proximity to livestock farms, primary health care visits and self-reported symptoms. BMC Fam Pract. 2016;17:22.
113. Kampschreur LM, Delsing CE, Groenwold RH, Wegdam-Blans MC, Bleeker-Rovers CP, de Jager-Leclercq MG, et al. Chronic Q fever in the Netherlands 5 years after the start of the Q fever epidemic: results from the Dutch chronic Q fever database. Journal of clinical microbiology. 2014;52(5):1637-43.
114. Gefenaite G, Munster JM, van Houdt R, Hak E. Effectiveness of the Q fever vaccine: a meta-analysis. Vaccine. 2011; 29(3):395-8.
115. O'Neill TJ, Sargeant JM, Poljak Z. The effectiveness of *Coxiella burnetii* vaccines in occupationally exposed populations: a systematic review and meta-analysis. Zoonoses and public health. 2014;61(2):81-96.
116. Maas JG, F.; Frings-Dresen, M.; van der Molen H. Background document for registration guideline C004 Q fever as an occupational disease CAS-code A433. Amsterdam: Netherlands Centre for Occupational Disease; 2016 July 2016.

7

Summary
Nederlandse samenvatting



Summary

The 2007 – 2009 Q fever epidemic in the Netherlands caused widespread morbidity in the human population. This thesis presents several outbreak investigations focusing on the detection of farm sources and identification of distance-response relations, and a follow-up study focusing on long-term antibody responses of patients diagnosed with acute Q fever. Moreover, this large epidemic also provided opportunities to gain knowledge about the occupational nature of this zoonotic infection in several occupational groups with intensive livestock contact. Seroprevalence and associated risk factors with *C. burnetii* seropositivity were studied in an integrated research project, including both farm residents and on-farm herds in four different ruminant sectors, in order to formulate better control strategies to reduce the animal and human Q fever burden.

In **Chapter 2,** sera and questionnaires from 5654 individuals containing epidemiological data were obtained in a representative nationwide seroprevalence survey carried out in 2006–2007 and used to evaluate the National Immunisation Programme in the Netherlands each decade since 1997 (the PIENTER Study). Sera were tested by ELISA for presence of *C. burnetii* IgG phase-2 antibodies in order to provide a nationwide estimate of seroprevalence and main determinants for *C. burnetii* seropositivity. The overall seroprevalence was estimated to be 2.4%. Seropositivity increased with age, from 0.48% in young children aged 0–4 years to 2.30% in persons aged 60 years and older. *C. burnetii* seropositivity was not associated with municipal ruminant densities of goat, sheep and cattle. Independent risk factors for seropositivity were keeping ruminants during the past 5 years and being born in Turkey, emphasizing the risk of direct ruminant contact and a country of birth where *C. burnetii* is highly prevalent in the human population, respectively. This population-based study supports the hypothesis that the nationwide seroprevalence was low just prior to the start of the epidemic, and suggests that low population immunity levels may have contributed to the magnitude of the Dutch Q fever epidemic. This study served as a baseline for seroprevalence studies carried out during and after the Q fever epidemic. The 2007-2009 Dutch Q fever epidemic also provided an opportunity for long-term follow-up of acute Q fever patients.
As presented in **Chapter 2,** a cohort of 344 adult patients diagnosed with acute Q fever between 2007 and 2009 by the regional Laboratory of Medical Microbiology at the Jeroen Bosch Hospital were offered routine serological follow-up at three, six and twelve months after diagnosis. *C. burnetii* IgM and IgG phase 1 and 2 antibodies were determined in all sera by IFA. These data were used to study longitudinal antibody responses and to model the seroresponse profiles. A mathematical model of the dynamic interaction of serum antibodies and pathogens was used in a mixed model framework to quantitatively analyze responses to *C. burnetii* infection. Responses show strong heterogeneity, with individual serum antibody responses widely different in magnitude and shape. Features of the response, peak titer and decay rate, are used to characterize the diversity of the observed responses. Binary mixture analysis of IgG peak levels (phases 1 and 2) revealed a class of patients with high IgG peak titers that decay slowly and may represent potential chronic cases. When combining the results of mixture analysis into an odds score, it is concluded that not only high IgG phase 1 may be predictive for chronic Q fever, but also that high IgG phase 2 may aid in detecting such putative chronic cases.

The Netherlands experienced several large outbreaks of acute Q fever during 2007 until 2009. In **Chapter 3**, four of these outbreaks are reported. The first recognized community Q fever outbreak occurred during late spring and early summer 2007 in and around Herpen, a rural village in Noord-Brabant province. A total of 696 inhabitants residing in the cluster area were invited to complete a questionnaire and provide a blood sample to be screened for presence of IgM and IgG antibodies against phase 1 and 2 of *C. burnetii* using an immunofluorescence assay. Risk factors for the acquisition of a recent *C. burnetii* infection were studied in a frequency-matched case-control study involving 332 seronegative controls and 73 cases with a recent *C. burnetii* infection, including previously identified Q fever cases. Current smoking, contact with agricultural products such as manure, hay and straw and residence in the east of the cluster area were risk factors associated with a recent *C. burnetii* infection. In this area, several livestock farms were situated including a commercial dairy goat farm where an abortion wave had occurred in April 2007. Limited sampling of environmental and animal matrices for *C. burnetii* DNA was done on this commercial goat farm and a hobby goat farm. All *C. burnetii* DNA-positive samples originated from the commercial goat farm.

Record-warm and extreme dry weather with prominent winds from the east during April 2007 may have contributed to the spread of *C. burnetii* contaminated aerosols. Information leaflets on Q fever were distributed to ruminant farms in this area early 2008 and included hygiene measures to reduce the risk of animal to human spread.

In May 2008, a community outbreak in the urban area of Helmond in Noord-Brabant province involved 96 patients with acute Q fever. The distribution and timing of acute Q fever cases suggested a common veterinary source. A generic geographic information system (GIS) was used to develop a method for source detection. All notified Q fever cases in the area were interviewed. Postal codes of cases and of small ruminant farms (size >40 animals) located within 5 kilometers of the cluster area were geo-referenced as point locations in a GIS-model. For each farm, attack rates and relative risks were calculated for 5 concentric zones adding 1 kilometer at a time, using the 5-10 kilometer zone as reference. These data were linked to the results of veterinary investigations. Persons living within 2 kilometers of a commercial dairy goat farm that experienced an abortion wave due to Q fever in April 2008 had a strongly increased risk (Relative risk 31.1 [95% CI 16.4-59.1]) to acquire acute Q fever compared to residents living more than 5 kilometers away. This study supported the hypothesis that this single dairy goat farm was the most likely source of this urban outbreak, proved that GIS-based attack rate analysis is a promising tool to facilitate source detection in an outdoor environment and played an important role in convincing the veterinary authorities that abortion storms among goats were the source of the human Q fever epidemic.

An institutional sheep-related Q fever outbreak occurred in May 2008 in a long-term psychiatric care institution situated near the city of Nijmegen. Active case finding among the residents, employees and visitors of the institution resulted in 28 laboratory-confirmed cases among staff and in-clients. A small sheep flock was present on the institution premises and had produced 5 newborn lambs during April and the first week of May 2008. One of those lambs had been abandoned. Attack rates of acute Q fever were highest in the building where the abandoned lamb was cuddled by several in-clients, followed by buildings adjacent to the small meadows where the flock was grazing or the rabbit cage where the abandoned lamb was placed 3 days after its birth. This merely descriptive investigation pointed to the small sheep flock with newborn lambs as the most probable source of exposure, supported by *C. burnetii* DNA-positive samples from 3 ewes and the abandoned lamb. A large sheep flock that had been grazing outside the premises was considered less likely as an alternative source, as 10 random sheep all tested *C. burnetii* DNA-negative. Care institutions maintaining flocks of sheep should take adequate preventive measures to avoid contact between often vulnerable patients and lambing sheep, and take adequate hygienic measures during lambing and handling of birth products.

Another sheep-related outbreak occurred between February and May 2009. The regional MHS identified this outbreak based on trawling questionnaires during preliminary investigations into an urban Q fever outbreak in the same region. The MHS suspected a non-dairy sheep farm as an alternative source for 42 acute Q fever cases that had all visited this farm during 'lamb-viewing days'. A matched case-control study was initiated and included 146 acute Q fever cases and 431 address-matched controls. Multivariable logistic regression analysis confirmed the association between visiting a non-dairy sheep farm and development of acute Q fever in this MHS region. Other risk factors identified were being a smoker, having a past medical history and being over 40 years of age. This non-dairy sheep farm was open to the public and had received 12,000 visitors during the lambing season in 2009 with over 1200 lambs born and only 3 abortions. The large majority of vaginal swabs was PCR positive for multicopy target IS*1111.* Vaccination of sheep and goats on farms open to the public should help to reduce the future occurrence of acute Q fever cases related to farm visits. Dairy goat farms were the main implicated source during the Dutch Q fever epidemic. However, many questions remained concerning the burden of *C. burnetii* infection in presumably highly exposed farm residents and their ruminant populations.

Chapter 4 presents studies that were part of an integrated research project carried out in 4 different ruminant sectors during 2009-2011, the socalled Q-VIVE project. The overall aim of this research project was to assess the *C. burnetii* seroprevalence in farm residents and assess herd and animal prevalence based on a representative sample per farm and to identify farm and person-based risk factors for *C. burnetii* seropositivity in order to formulate targeted advice and better control strategies to reduce the animal and human Q fever burden.

In total, 141 out of 334 eligible commercial dairy goat farms (≥ 100 goats), 14 out of 33 dairy sheep farms (≥ 100 sheep), 119 out of 1344 non-dairy sheep farms (≥100 sheep) and 311 out of a representative sample of 3000 dairy cattle farms (≥50 adult cows) were included in this research project. On each participating farm, up to 3 farm residents were requested to provide a blood sample and to complete an individual questionnaire, and the farm manager was asked to complete a farm questionnaire. Sera of 268 dairy goat farm residents, 27 dairy sheep farm residents, 271 non-dairy sheep farm residents and 755 cattle farm residents were screened for *C. burnetii* IgG and IgM phase 1 and 2 antibodies using IFA Seroprevalence was high for residents of dairy goat farms (69% of residents seropositive), dairy sheep farms (67%) and dairy cattle farms (72%), indicating a high lifetime risk to acquire a *C. burnetii* infection. A slightly lower seroprevalence was found in residents of non-dairy sheep farms (51%). Although the overall *C. burnetii* seroprevalence was high among residents on dairy cattle farms, relatively little evidence was found for recent infections among C. *burnetii* IgG phase 2 seropositive dairy cattle farm residents (1.2% had presence of *C. burnetii* IgM phase 2 antibodies versus 11.2% in dairy goat farm residents, 11.1% in residents on dairy sheep farms and 2.6% at non-dairy sheep farms). Among *C. burnetii* IgG phase 2 seropositive farm residents, 0.3% of dairy cattle farm residents, 4.1% of dairy goat farm residents, 4% of the dairy sheep farmers and none of the non-dairy sheep farm residents had an IgG phase 1 end titer of ≥1:1024 serologically indicative for a possible chronic *C. burnetii* infection.
On dairy goat farms and sheep farms that participated in these studies, presence of *C. burnetii* antibodies was determined in sera taken from a random sample of 21 goats and 62 sheep by ELISA. A farm was considered positive when at least 1 goat or sheep tested ELISA-positive. A bulk tank milk (BTM)-sample was collected on each participating dairy cattle farm for ELISA and PCR testing to assess the farm status, while dairy cows were not individually sampled. In 2009-2010, the *C. burnetii* seroprevalence was assessed in dairy goats and sheep before the mandatory Q fever vaccination started at the participating farm. At individual animal level, the highest seroprevalence figures were found in dairy goats (21%) and dairy sheep (19%). In 2010-2011, the seroprevalence was significantly lower in non-dairy sheep (2%). At herd level, 43% of the dairy goat farms, 79% of the dairy sheep farms and 31% of the non-dairy sheep farms had at least one infected animal present in the random sample screened. The mean within-herd prevalence was 46.6% on *C. burnetii* positive goat farms, 23.5% on *C. burnetii* positive dairy sheep farms and 6.4% on non-dairy sheep farms, respectively. In 2010-2011, 82% of the dairy cattle farms were BTM-positive by ELISA and 19% by PCR.

Risk factors for *C. burnetii* seropositivity were usually similar for residents and animals within a sector, but frequently differed between the sectors. For all sectors, a higher risk of infection was found for farms with a large herd size. Also farm location was a risk factor; being located in the provinces of Noord-Brabant and Limburg or in an area near (within 8-16 kilometers) BTM-positive small ruminant farms. This corresponds with the dominant role of BTM-positive dairy goat farms during the Dutch Q fever epidemic with goat-related risk factors being important for *C. burnetii* infections on non-dairy sheep farms. In addition, several risk factors indicated pathways for introduction of *C. burnetii* on dairy goat farms, such as use of imported straw, artificial insemination and presence of companion animals in the stables.

For dairy cattle farms, the risk increased with wild bird presence in the stables, when rats and mice were present on the farm, if the dairy cattle had ticks and if multiple addresses were used for supply of calves. Supply of ewes from multiple addresses was a risk factor for the non-dairy sheep sector. Finally, some factors were identified that seemed to reduce the *C. burnetii* infection risk, such as the consistent use of gloves during birth assistance, the use of automatic milking in the dairy cattle sector (less direct contact), the use of boots by the farmer or employees at dairy goat farms (less introduction and spread) and lambing outside in the paddock at the meat- and breeding sheep farms (less accumulation and spread of bacteria). These ruminant sectors were advised to use a closed farming system, to avoid other pathways by vermin control and to optimize hygiene especially around birth, including the use of protective clothing. Moreover, Q fever vaccination as a preventive strategy could be considered in dairy ruminant farmers and their adult household members residing on the farm after pre-screening. During the Dutch Q fever epidemic, little was known about the degree of exposure to *C. burnetii* and related health consequences for veterinary professionals directly working with small ruminants and dairy cattle.

In **Chapter 5** the seroprevalence of *C. burnetii* IgG antibodies among Dutch livestock veterinarians was determined, and occupational risk factors for seropositivity identified. Sera and questionnaires from 189 veterinarians, including veterinary students in their final study year were collected at a national veterinary conference held in November 2008. C*. burnetii* antibodies were detected in 65.1% of livestock veterinarians. Independent risk factors associated with seropositivity were number of animal contact hours per week, number of working years since graduation as veterinarian, being a practicing veterinarian, occupational contact with swine, and residence in a rural or suburban area. Livestock veterinarians, as well as their physicians, should be aware of these occupational risks.
Risk factors associated with *C. burnetii* infection in veterinary medicine students have been scarcely studied. *C. burnetii* seroprevalence and associated risk factors were assessed among Dutch veterinary medicine students for all 6 study years in a cross-sectional study based on 674 sera and questionnaires obtained in 2006. Among Dutch veterinary medicine students *C. burnetii* seroprevalence was considerable (18.7%). Several study-related risk factors were identified such as study direction 'farm animals', advanced year of study and having had a zoonosis since study enrollment. Having ever resided on a ruminant farm was a separate risk factor and a dose-response relation with *C. burnetii* seropositivity was identified for increasing number of farm residence years. Bearing in mind that the burden of disease among veterinarians remains uncertain, vaccination of veterinary students at the start of their study should be seriously considered.
These cross-sectional studies in livestock veterinarians and veterinary students mainly focused on risk factors for past *C. burnetii* infection. The risk for seroconversion was studied in a group of seronegative culling workers that were involved in the culling of 51,820 dairy goats on BTM-positive small ruminant farms end 2009 and first months of 2010. These workers were therefore highly exposed to *C. burnetii* during the culling activities.
Among 517 culling workers, despite use of personal protective equipment (PPE) and instructions how to avoid infection during their work, 17.5% seroconverted for antibodies to *C. burnetii*. One third of seroconverted culling workers reported symptoms compatible with acute Q fever such as fever and/or rigors, malaise, headache, cough or myalgia after December 1, 2009. Mean duration of illness was 7.6 (range 1–14) days. Total hours worked, herd size, and working inside the stable were significant risk factors in the univariate model. In the multivariable model working more than 100 hours on the farms and working inside the stable remained independent risk factors for seroconversion. The compliance with PPE was reportedly high; however, a key problem was not wearing PPE during lunch breaks while staying on the farm. Vaccination of culling workers should be provided if animal culling in the small ruminant sector is advised in the future.

Finally, a general discussion of the findings of the present thesis is given in **Chapter 6.** This describes the information already known before the studies in this thesis were performed, what these studies added and several recommendations arising from these studies.

Nederlandse samenvatting

Dit proefschrift bevat epidemiologische studies die zijn uitgevoerd ten tijde van de Q-koorts epidemie in Nederland van 2007 tot en met 2010. Deze epidemie bestond uit meerdere seizoensgebonden uitbraken en resulteerde uiteindelijk in meer dan 4000 gemelde gevallen van acute Q-koorts, vooral in de provincies Noord-Brabant, Gelderland en Limburg. Q-koorts werd een grote dreiging voor de volksgezondheid. Ten tijde van de eerste Q-koorts uitbraak was nog veel onbekend. Dit proefschrift bevatenkele gedetailleerde descriptieve en analytische uitbraakstudies waarbij tijd, plaats en persoon worden gebruikt voor het aantonen van de meest waarschijnlijk veterinaire bronnen.
Tevens bevat het studies naar de seroprevalentie en risicofactoren van een doorgemaakte infectie met *Coxiella burnetii (C. burnetii)* in de algemene Nederlandse bevolking en voor degenen die beroepsmatig worden blootgesteld aan *C. burnetii*.
De studies in dit proefschrift werden uitgevoerd binnen het Rijksinstituut voor Volksgezondheid en Milieu (RIVM), vaak samen met onderzoekers werkzaam bij andere organisaties zoals de GGD Hart voor Brabant en GGD Brabant-Zuidoost, Academische Werkplaats AMPHI, Jeroen Bosch Ziekenhuis, de Gezondheidsdienst voor Dieren en het Institute for Risk Assessment Sciences (IRAS) van de Universiteit Utrecht.

In **Hoofdstuk 1** wordt een algemene inleiding gegeven over Q-koorts en worden de inhoud en de doelen van dit proefschrift beschreven.

In het ***eerste artikel van Hoofdstuk 2*** zijn sera en vragenlijsten van 5654 personen gebruikt die in 2006-2007 hebben deelgenomen aan het PIENTER-project (Peiling Immunisatie Effect Nederland ter Evaluatie van het Rijksvaccinatieprogramma). Het PIENTER-project is een groot landelijk onderzoek dat eens in de 10 jaar georganiseerd wordt, primair ter evaluatie van het Rijksvaccinatieprogramma. In het kader van dit project is sinds 1997 een serumbank opgericht, die sera bevatten van een representatieve steekproef van de Nederlandse bevolking van 0-79 jaar uit 40 gemeentes**.**
Blootstelling aan *C. burnetii* leidt tot de vorming van antistoffen. Analyse van de aanwezigheid van die antistoffen bij groepen mensen geeft, in de vorm van de seroprevalentie, een indicatie van de doorgemaakte blootstelling. De tweede PIENTER-studie bood een mooie gelegenheid om retrospectief de seroprevalentie van *C. burnetii* in Nederland te bestuderen, vlak voordat de eerste Q-koortsuitbraak eind mei 2007 werd opgemerkt. IgG antistoffen tegen fase 2-*C. burnetii* werden gemeten met ELISA (Serion ELISA classic, Virion/Serion, Würzburg, Duitsland) om een nationale schatting van de seroprevalentie te verkrijgen. Om de sensitiviteit te verhogen werden zwakpositieve uitslagen als positief beschouwd. Verder werden 504 ELISA-negatieve monsters voor validatie onderzocht met de sensitievere immunofluorescentie (IFA; Focus Diagnostics, Cypress, Californië, VS).
Gecorrigeerd voor de IFA-resultaten, werd er een relatief lage seroprevalentie gevonden van 2,4%. De ELISA-seroprevalentie nam toe met de leeftijd van 0,5% in jonge kinderen tot 2,3% voor 60-79 jarigen. Significante risicofactoren waren onder andere het geboren zijn in Turkije, beroepsmatig contact hebben met dieren en het houden van landbouwhuisdieren in de laatste 5 jaren. Deze risicofactoren benadrukken het bekende risico van direct contact met herkauwers en afkomst uit een voor Q-koorts hoog-endemisch land. Er werd geen verhoogde seroprevalentie van *C. burnetii* gevonden in Noord-Brabant. Er was geen relatie met de geiten-, schapen-, of runderdichtheid per gemeente. Deze studie ondersteunt de hypothese dat de seroprevalentie van *C. burnetii* antistoffen in de Nederlandse bevolking vlak voor de eerste Q-koorts uitbraak laag was, ook in vergelijking met andere Europese landen. De grote omvang van vatbaren in de Nederlandse bevolking heeft mogelijk bijgedragen aan de omvang van de Q-koortsepidemie in Nederland in de periode 2007-2009.
De Q-koortsepidemie in Nederland bood de mogelijkheid om de antistofresponsen van een grote groep patiënten met acute Q-koorts te bestuderen gedurende een langere periode. Het ***tweede artikel in Hoofdstuk 2*** beschrijft de resultaten van een cohort van volwassen acute Q-koortspatiënten die tussen 2007 en 2009 in het Laboratorium Medische Microbiologie in het Jeroen Bosch Ziekenhuis in **'**s-Hertogenbosch zijn gediagnosticeerd. Deze 344 patiënten kregen drie, zes en twaalf maanden na de diagnose standaard serologische controles aangeboden. *C. burnetii* IgM en IgG fase 1 en 2 antistoffen werden bepaald met behulp van IFA in serum. Deze data zijn gebruikt om de longitudinale

respons van deze vier verschillende antistoffen tegen *C. burnetii* antigenen te bestuderen. Een wiskundig model gericht op de dynamiek tussen serumantistoffen en pathogenen is gebruikt om kwantitatief de profielen van de *C. burnetii* serorespons te modelleren. Er werd een aanzienlijke mate van heterogeniteit in de individuele antistofresponsen gezien. Kenmerken van deze respons, zoals de piektiter en de vervalsnelheid werden gebruikt om deze respons te beschrijven. De binaire mixtureanalyse van de *C. burnetii* IgG piektiters (fase 1 en 2) liet een groep patiënten zien met hoge IgG piektiters die langzaam afnamen en mogelijk patiënten met chronische Q koorts zouden kunnen representeren. Nadat de uitkomsten van de binaire mixtureanalyse in een odds score werden verwerkt, kon worden geconcludeerd dat naast een hoge IgG fase 1-titer, ook hoge IgG fase 2-titers, voorspellend kunnen zijn voor de detectie van patiënten met mogelijke chronische Q koorts.

In **Hoofdstuk 3** worden vier uitbraakonderzoeken beschreven.

In het ***eerste artikel van Hoofdstuk 3*** wordt het patiënt-controle onderzoek beschreven dat in en rondom Herpen, een plattelandsdorp met 2.850 inwoners gelegen in de provincie Noord Brabant tussen Oss en Nijmegen, is uitgevoerd. Huisartsen in dit dorp meldden eind mei 2007 een opvallende toename van het aantal patiënten - tussen de 30 en de 60 jaar - met een pneumonie of andere respiratoire klachten aan de regionale GGD. In september 2007 werden in totaal 696 inwoners en 35 reeds gediagnosticeerde patiënten met acute Q-koorts uit dit clustergebied uitgenodigd om deel te nemen aan een vragenlijstonderzoek en een eenmalige bloedafname voor screening op *C. burnetii* IgG and IgM fase 1 en 2 antistoffen met IFA met een 1:64 serum verdunning.

Uit dit onderzoek bleek dat 73 (16.5%) van de deelnemende inwoners een recente *C. burnetii* infectie had doorgemaakt. Het patiënt-controle onderzoek omvatte 332 seronegatieve inwoners als controlepersonen en 73 inwoners met een recente *C. burnetii* infectie, waaronder reeds gediagnosticeerde patiënten met acute Q-koorts. Er bleek een verhoogd risico op recente Q-koorts voor mensen die roken en contact hadden met landbouwproducten zoals mest, hooi en stro. Tevens gaf het onderzoek aanwijzingen dat de veterinaire bron van deze Q-koorts uitbraak zich bevond in een gebied aan de oostelijke kant van Herpen. Binnen dit gebied bevonden zich 3 melkveehouderijen met runderen, 1 melkgeitenbedrijf, 1 schapenfokkerij en 3 hobbyboerderijen. In april 2007 had het grote melkgeitenbedrijf een abortusstorm onder de melkgeiten gehad. Onderzocht abortusmateriaal door de Gezondheidsdienst voor Dieren (GD) werd positief bevonden voor *C. burnetii*. Omgeving- en diermonsters afgenomen op 2 verschillende bedrijven, waaronder dit melkgeitenbedrijf en een hobbyboerderij met geiten, waren eveneens positief voor *C. burnetii* volgens een nieuw ontwikkelde kwantitatieve Q-PCR. Analyse van de weersomstandigheden net voor de uitbraakperiode lieten een record warme en droge aprilmaand zien, met significant meer oostelijke wind in vergelijking tot voorgaande 30 jaren. Deze weersomstandigheden hebben mogelijk bijgedragen aan een effectieve aerosolverspreiding vanuit het brongebied. Eind februari 2008 zijn er informatiefolders gestuurd aan geitenhouders in Noord-Brabant, waarin hygiënemaatregelen werden aanbevolen.

In het ***tweede artikel van Hoofdstuk 3*** is onderzocht in hoeverre het risico op acute Q-koorts afneemt met toenemende afstand tussen woonhuis en een melkgeitenbedrijf met een door *C. burnetii* veroorzaakt abortusprobleem. Deze mogelijkheid werd geboden door een in tijd en plaats goed afgebakend cluster van 96 acute Q-koortspatiënten in en rondom de stad Helmond tussen week 16 en week 32, 2008. Er was sprake van een duidelijke clustering in 2 woonwijken. Toen lag dit cluster nog buiten het hoogincidentie gebied van Q-koorts in Noord Brabant. Q-koorts als oorzaak van de abortusstorm werd bevestigd na onderzoek van placentamateriaal door de GD. Een generiek **g**eografisch informatiesysteem werd gebruikt als hulpmiddel bij bronopsporing. Voor 7 bedrijven met meer dan 40 schapen of geiten werden incidenties en relatieve risico's berekend voor cirkels met toenemende afstand rond het bedrijf, waarbij de 5-10 kilometer (km)-zone als referentie werd gebruikt. Mensen die binnen 2 km van het besmette melkgeitenbedrijf woonden hadden een veel hoger risico (relatief risico 31,1; 95% betrouwbaarheidsinterval 16,4-59,1) dan mensen die op meer dan 5 km afstand van dit bedrijf woonden. De chronologie van de abortusstorm, dagen met oost- tot noordoostenwind en incubatietijd van Q-koorts maakten het verband tussen het melkgeitenbedrijf en het humane cluster van acute Q-koorts zeer waarschijnlijk.

De gebruikte GIS-methode is snel inzetbaar en biedt de mogelijkheid om op basis van epidemiologische gegevens onderscheid te maken in de waarschijnlijkheid dat bedrijven een rol spelen in de transmissie. De methode kan echter niet differentiëren tussen bedrijven die dicht bij elkaar liggen, en houdt geen rekening met windrichting en windsnelheid.

Het ***derde artikel van Hoofdstuk 3*** omvat een beschrijvende epidemiologische studie van een Q-koorts uitbraak in een psychiatrische inrichting in Nijmegen in mei 2008. Actieve case finding onder de inwonende cliënten, werknemers en bezoekers van de instelling leverde 45 personen op met klinische verschijnselen passend bij Q-koorts, waarvan 28 cliënten en werknemers na laboratoriumtesten werden bevestigd met acute Q-koorts. Een kleine kudde van zes schapen met vijf pasgeboren lammeren was de meest waarschijnlijke bron van deze institutionele uitbraak. Deze kudde werd op het terrein van deze instelling gehouden om te grazen en had in april en begin mei afgelammerd.
Een lammetje dat verstoten werd door de moeder werd geplaatst in een konijnenhok 3 dagen na geboorte. De incidentie (attack rate) van acute Q-koorts werden berekend per gebouw op deze instelling en bleek het hoogst in het gebouw waar het verstoten lammertje was geaaid en verzorgd door meerdere cliënten, gevolgd door de gebouwen die grensden aan de schapenweide en het konijnenhok. Monsters afgenomen van 3 ooien uit deze kleine kudde en het verstoten lammetje waren positief voor *C. burnetii* DNA. Een grote schapenkudde die net buiten de instelling had gegraasd tijdens de uitbraakperiode was minder waarschijnlijk als alternatieve bron aangezien 10 random geselecteerde schapen alle negatief testen op *C. burnetii* DNA. Besmette kleine herkauwers vormen het grootste risico op het verspreiden van *C. burnetii* als ze aflammeren of verwerpen. Het is belangrijk dat zorginstellingen het contact tussen vaak kwetsbare patiëntengroepen en aflammerende schapen op hun terrein vermijden, en adequate hygiënemaatregelen treffen ten tijde van het aflammeren en het hanteren van de (na)geboorteproducten.

In het ***vierde artikel van Hoofdstuk 3*** wordt een andere schaapgerelateerde uitbraak beschreven die plaatsvond in de periode februari tot en met mei 2009. De GGD Brabant- Zuidoost identificeerde een schapenboerderij als mogelijk additionele bron tijdens het lopende uitbraakonderzoek naar de Q-koorts uitbraak in Helmond (*artikel 2 in Hoofdstuk 3*) aan de hand van een door patiënten ingevulde bronopsporingsvragenlijst. Van de in deze uitbraak gemelde gevallen ('cases') nam 62% (162/248) deel aan het onderzoek en werden vergeleken met 433 gematchte controlepersonen uit dezelfde omgeving. Er werd onderzocht of een bezoek aan deze specifieke schapenboerderij een risicofactor was voor acute Q-koorts in de lokale bevolking. Er werd een gecorrigeerde odds ratio (OR) van 43,3 voor een bezoek aan deze schapenboerderij gevonden. Andere risicofactoren voor het oplopen van acute Q-koorts waren roken, overige medische problemen en een leeftijd ouder dan 40 jaar. Deze boerderij had een publieksfunctie en ontving 12.000 bezoekers tijdens het lammerseizoen in 2009 waarin 1200 lammeren werden geboren en er slechts 3 verwerpingen plaatsvonden. Het merendeel van de vaginale swabs afgenomen van de schapen was PCR positief. Deze boerderij werd gesloten voor publiek gedurende de lente van 2010. Nadat alle schapen tegen Q-koorts gevaccineerd waren werd de schapenboerderij voor publiek heropend in de lente van 2011. Deze uitbraak illustreert het belang van het instellen van een vaccinatieplicht voor geiten- en schapenbedrijven met een publieksfunctie zoals bedrijven met lammetjesaaidagen, kinder- en zorgboerderijen, en rondtrekkende schapenkuddes in natuurgebieden.

In ***Hoofdstuk 4*** zijn een zestal studies opgenomen die deel uitmaken van het Q-VIVE project, een geïntegreerd humaan-veterinair onderzoeksproject. Het doel van dit 'One-Health' project was om betere adviezen en controlemaatregelen te formuleren om de impact van Q-koorts en het infectierisico met *C. burnetii* bij zowel melkgeiten, melkschapen, vlees-en fokschapen en melkrundvee in Nederland als binnen het veehouderijgezinnen te reduceren.
Het ging om bedrijven met minstens 100 melkgeiten (141 deelnemende bedrijven; 334 uitgenodigd), 100 melkschapen (14 deelnemende bedrijven; 33 uitgenodigd), 100 vlees- en fokschapen (119 deelnemende bedrijven; 1344 uitgenodigd) en bedrijven met minstens 50 volwassen melkrunderen (311 deelnemende bedrijven uit een representatieve steekproef van 3000 melkveebedrijven). De veehouder of bedrijfsmanager werd verzocht om een bedrijfsvragenlijst in te vullen met karakteristieken over het bedrijf en de veestapel. Bij elk deelnemend geiten- en schapenbedrijf werd van een representatieve steekproef (21 geiten, 62 schapen) bloed afgenomen dat werd getest op aanwezigheid van *C. burnetii* antistoffen met een enzyme-linked immunosorbent assay (ELISA).

Het hoogste percentage ELISA-positieve dieren werd gevonden op de melkgeiten- en melkschapenbedrijven, respectievelijk 21% en 19%. Voor vlees- en fokschapen lag dit met 2% beduidend lager. Op bedrijfsniveau bleek 43% van de melkgeitenbedrijven, 79% van de 14 melkschapenbedrijven en 31% van de vlees/fokschaapbedrijven minimaal 1 besmet dier te houden. Voor de runderbedrijven werd een tankmelkmonster onderzocht, waarvan 82% positief bleek te zijn voor de antistoffen op basis van ELISA en 19% positief was voor aanwezigheid van bacterieel DNA.

De seroprevalentie van *C. burnetii* infecties onder de onderzochte kleine herkauwers komt goed overeen met de dominante rol die melkgeiten en melkschapen gespeeld hebben in de Nederlandse Q-koorts epidemie. Vergelijking met eerdere seroprevalentieschattingen uit onderzoek in 2008 toen 5,7% van de onderzochte melkschapen en 14,7% van de onderzochte melkgeiten positief was, laat zien dat in deze sectoren de bacterie zich tijdens de epidemie duidelijk verder heeft kunnen verspreiden. Voor de vlees- en fokschapen werd die toename niet gezien (ook in 2008 2% positief). Voor deze schapen was er tijdens de epidemie ook enkel een relatie met Q-koorts patiënten als er direct contact met de schapen had plaatsgevonden op bedrijven waar de bacterie voorkwam. Een rol voor verwaaiing naar omwonenden werd nooit gevonden, wat past bij het duidelijk lagere risico voor verspreiding van de bacterie vanuit deze schapenbedrijven in verhouding tot de melkgeiten en melkschapenbedrijven zoals gevonden in ons onderzoek in de stal en stalomgeving. Daar werden op de vlees-en fokschapenbedrijven minder onderzochte omgevingsmonsters positief bevonden voor *C. burnetii*, en deze bevatten doorgaans ook een lagere hoeveelheid van de bacterie.

Per bedrijf werd aan 3 personen gevraagd een individuele vragenlijst in te vullen (bij voorkeur de veehouder zelf, de partner en een inwonend kind van 12 jaar of ouder of eventueel een bedrijfswerknemer) en werd bij hen bloed afgenomen. In totaal zijn 268 sera van bewoners op melkgeitenbedrijven, 27 sera van bewoners van melkschapenbedrijven, 271 sera van bewoners op vlees- en fokschaapbedrijven en 755 sera van bewoners van melkrundveebedrijven gescreend op de aanwezigheid van *C. burnetii* IgG en IgM fase 1 en 2 antistoffen met IFA (Focus Diagnostics, Cypress, Californië, VS). Het percentage veehouderijbewoners met *C. burnetii* IgG fase 2 antistoffen was vergelijkbaar hoog voor de melkgeiten-(69%), melkschapen- (67%) en melkrundveehouders (72%). Een wat lager percentage werd gevonden voor de veehouderijgezinnen op de vlees- en fokschaapbedrijven (51%). Dit laat zien dat veehouders en hun gezinsleden in Nederland een grote kans hebben om gedurende hun leven een infectie met *C. burnetii* door te maken, al dan niet met gezondheidsklachten.
Hoewel de seroprevalentie op basis van aanwezigheid van *C. burnetii* IgG fase 2 antistoffen bij de melkrundveehouders hoog was, werden er relatief weinig aanwijzingen gevonden voor recente infecties op basis van aanwezigheid van *C. burnetii* IgM fase 2 antistoffen (1,2% versus 11% bij de bewoners van melkgeiten- en melkschapenhouderijen). Dit suggereert dat de klinische impact van deze infecties in deze bedrijfstak mogelijk beperkt is. Dit gold tevens voor mogelijke chronische Q-koorts. Geen van de bewoners op de vlees- en fokschaapbedrijven en 0,3% van de bewoners van melkrundhouderijen had een IgG fase 1 eindtiter van 1:1024 of hoger, terwijl 4% van de bewoners op melkgeitenhouderijen en melkschapenhouderijen deze hoge IgG fase 1 titer had, hetgeen indicatief is voor mogelijke chronische Q-koorts.

Risicofactoren voor infectie met *C. burnetii* waren doorgaans redelijk vergelijkbaar voor de veehouders en dieren binnen een bedrijfstak, maar verschilden regelmatig tussen de bedrijfstakken. Voor alle bedrijfstakken werd een hoger risico gevonden voor bedrijven met een grote bedrijfsomvang en met een bedrijfslocatie in de provincies Noord-Brabant en Limburg of een kortere afstand tot het dichtstbijzijnde tankmelkpositieve melkgeitenbedrijf (<8-16 kilometer). Dit laatste laat zien dat de epidemie onder melkgeiten ook invloed heeft gehad op het voorkomen van *C. burnetii* op andere soorten veehouderijen. Daarnaast gaven diverse factoren verschillende risico's van insleep weer. Voor geitenbedrijven werd een hoger risico op infecties gevonden bij aanwezigheid van honden en katten in de geitenstallen, bij aankoop van buitenlands stro, en bij toepassing van kunstmatige inseminatie. Voor melkrunderbedrijven was het risico verhoogd bij aanwezigheid van wilde vogels in de stallen, als ratten en muizen op het bedrijf aanwezig waren, als de runderen teken hadden en als meerdere aanvoeradressen werden gebruikt voor nieuwe kalveren. Aanvoer van ooien van elders was een risicofactor voor de vlees- en fokschapensector.

Tenslotte werden ook enkele factoren gevonden die het risico juist leken te reduceren, zoals het consequent gebruiken van handschoenen bij geboortehulp en het gebruik maken van een melkrobot voor het melken in de rundersector, het gebruik van laarzen door veehouder/personeel op de geitenbedrijven (minder in-/versleep) en het buiten laten aflammeren op de vlees- en fokschaapbedrijven (minder ophoping en verwaaiing van bacteriën). Aan de verschillende bedrijfstakken is geadviseerd zoveel mogelijk een gesloten bedrijfsvoering te voeren, ook verder bronnen van insleep te beperken en goede hygiëne, vooral rondom geboorte, en biosecurity toe te passen op het bedrijf, waaronder het gebruik van beschermende kleding. Daarnaast zou op grond van de hoge seroprevalentie, de relatief hoge frequentie van recente infecties en het regelmatig voorkomen van seroprofielen indicatief voor een chronische infectie, Q-koorts vaccinatie als preventieve strategie moeten worden overwogen voor melkveehouderij gezinnen en werknemers op deze bedrijven.

In het ***eerste artikel van Hoofdstuk 5*** is de seroprevalentie van *C. burnetii* IgG fase 1 en 2 antistoffen bepaald onder Nederlandse landbouwhuisdierenartsen en zijn beroepsgerelateerde risico's voor seropositiviteit bestudeerd. Van 189 dierenartsen, inclusief studenten diergeneeskunde in het laatste jaar van hun studie, is bloed afgenomen en tevens hebben zij een vragenlijst ingevuld. *C. burnetii* antistoffen werden aangetoond bij 123 (65,1%) van de 189 dierenartsen. Praktiserende dierenartsen hadden een nog hogere seroprevalentie (88%), terwijl de seroprevalentie onder laatstejaarsstudenten diergeneeskunde 30% was. Zeven deelnemers, onder wie vier diergeneeskundestudenten bleken recent een infectie met *C. burnetii* te hebben doorgemaakt. Risicofactoren die gerelateerd bleken aan seropositiviteit waren het aantal uren diercontact per week, aantal jaren afgestudeerd als dierenarts, wonend op het platteland of aan de rand van de stad, werkzaam als praktiserend dierenarts en beroepsmatig contact met varkens. Slechts bij een klein deel van de deelnemende dierenartsen was Q-koorts ooit aangetoond als oorzaak van ziekteverschijnselen.
In het ***tweede artikel van Hoofdstuk 5*** wordt de seroprevalentie van *C. burnetii* IgG fase 1 bepaald bij 674 studenten diergeneeskunde in alle 6 studiejaren op basis van sera afgenomen in het kader van een dwarsdoorsnedeonderzoek (Veterinarians' Health Study I) in de periode november 2006 tot en met juni 2007. Risicofactoren die gerelateerd bleken aan seropositiviteit waren het volgen van de studierichting '-landbouwhuisdieren-en-veterinaire-volksgezondheid', gevorderd studiejaar en het hebben doorgemaakt van een zelf gerapporteerde zoönose sinds het begin van de studie diergeneeskunde. Ook het ooit hebben gewoond op een boerderij met herkauwers was een risicofactor voor seropositiviteit, waarbij een tijd-responsrelatie werd gezien bij een toenemend aantal woonjaren op de boerderij. In ogenschouw nemend dat de ziektelast van Q-koorts bij de groep diergeneeskundestudenten onbekend is, zou een Q-koorts vaccinatie moeten worden overwogen voor 'toekomstige professionals', zoals studenten diergeneeskunde, aan het begin van hun studie.
In het ***derde artikel van Hoofdstuk 5*** beschrijven we de studie onder 517 medewerkers die betrokken waren bij het ruimen van meer dan 50.000 drachtige melkgeiten en schapen tijdens de Q-koorts epidemie eind 2009 en begin 2010. Van de ruimers had 83 procent voorafgaand aan de werkzaamheden geen antistoffen tegen *C. burnetii.* Om te voorkomen dat de ruimers besmet raakten werden persoonlijke beschermende maatregelen (PPE) genomen in de vorm van handschoenen, overalls en maskers (FFP3 maskers, 'face filtering pieces', waarvan aangenomen wordt dat ze tenminste 99% van de deeltjes in lucht tegenhouden). Van hen ontwikkelden 17.5% gedurende de werkzaamheden antistoffen tegen *C. burnetii*, een zogeheten seroconversie, ondanks het gebruik van PPE. Een derde van hen rapporteerde ook symptomen die compatibel zijn met acute Q-koorts. Langdurig werken (>100 uur) en het werken in de stal bleken significante risicofactoren voor seroconversie in het multivariate model. Een enkele keer hadden de arbeiders tijdens de koffie- en lunchpauze hun PPE (deels) verwijderd. Dit kan bijgedragen hebben aan het hoge aantal seroconversies, hoewel we dit niet konden testen. Er is vervolgens in deze studie geadviseerd dat, indien er in de toekomst een vergelijkbare situatie van ruimen van dieren noodzakelijk zou zijn, Q-koorts vaccinatie van de ruimingswerkers serieus overwogen moet worden en dat een serologische follow-up op de langere termijn nodig is.

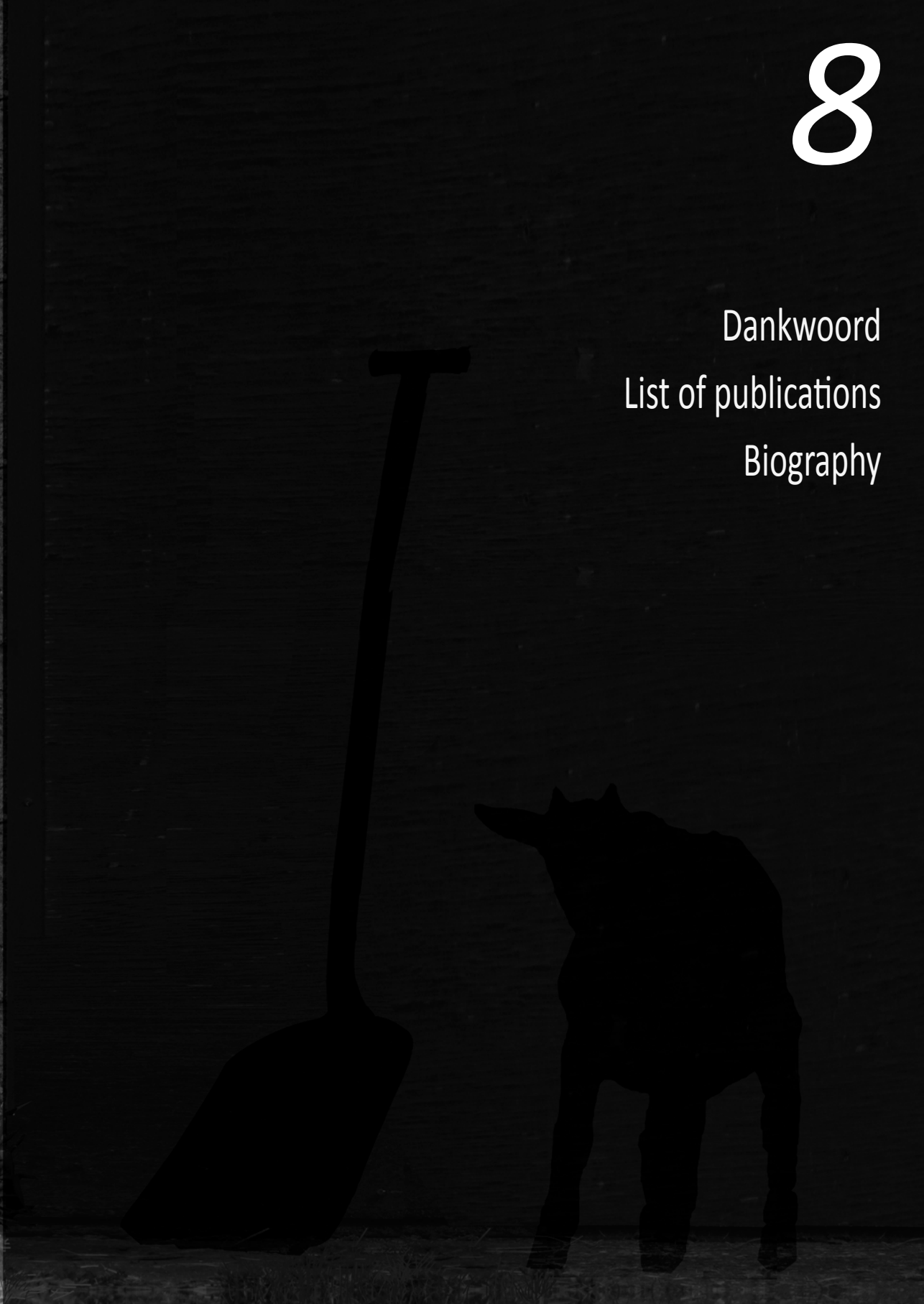
Tot slot wordt een algemene discussie van de bevindingen van de diverse studies in dit proefschrift gepresenteerd in **Hoofdstuk 6.** In dit hoofdstuk wordt beschreven welke gegevens al bekend waren, wat de bevindingen in dit proefschrift toevoegen en welke aanbevelingen uit deze studies naar voren komen.

8

Dankwoord

List of publications

Biography



Dankwoord

"There will be days you don't think you can run a marathon. There will be a lifetime of knowing you have."

Na een lange incubatietijd van bijna 10 jaar met een aantal onderbrekingen waarin ik serieus heb overwogen het bijltje er bij neer te gooien is dit proefschrift toch af! Tijdens zo'n lange weg kan het niet anders dat ik veel mensen wil bedanken die mij hebben begeleid, met wie ik heb samengewerkt of die op een andere wijze hebben bijgedragen aan de totstandkoming van dit boekje.

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Bij jou thuis aan de keukentafel schreven we verschillende ZonMw onderzoeksvoorstellen. Als het laat werd kon ik blijven slapen en jouw kinderen, die toen net zo oud waren als Rousanne en Gideon nu, wekten mij in de ochtend. Jouw onophoudelijke werklust, persoonlijke toewijding en effectiviteit bewonder ik zeer. Je pragmatische insteek raakte bij mij de juiste snaar. Zonder een woord te hoeven zeggen wist jij waar mijn knelpunten en onzekerheden zaten. Je deur stond altijd open en daar maakte ik dan ook graag gebruik van.

Wim, jij bent een rots in de branding. Toen jij in 2008 bij EPI kwam werken als hoofd respiratoire infecties ben je zelf diep in de Q-koorts materie gedoken en heb je in sneltreinvaart je eigen proefschrift afgerond. Door jou kwam er binnen EPI structuur en afstemming in het Q-koorts onderzoek. Ook werd er advies van buitenlandse experts ingewonnen. Je bent ogenschijnlijk de rust zelve. Ondertussen weet je precies met welke mensen je moet samenwerken in roerige tijden om voortgang te boeken. Dank dat je altijd open stond voor mijn ideeën en dat jij mij weer hebt betrokken bij andere onderzoeken. Het benoemen van de relevantie van dit traject in het grotere geheel was een enorme stimulans. Ik dank je voor het doorgeven van jouw ervaring en kennis.

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En nu is dit boekje af en zet ik de laatste **.**

List of publications

Publications on Q fever

Ladbury GA, Van Leuken JP, Swart A, Vellema P, **Schimmer B**, Ter Schegget R, Van der Hoek W. Integrating interdisciplinary methodologies for One Health: goat farm re-implicated as the probable source of an urban Q fever outbreak, the Netherlands, 2009. BMC Infect Dis. 2015 Sep 3;15:372.

Wielders CC, Hackert VH, **Schimmer B**, Hodemaekers HM, de Klerk A, Hoebe CJ, Schneeberger PM, van Duynhoven YT, Janssen R. Single nucleotide polymorphisms in immune response genes in acute Q fever cases with differences in self-reported symptoms. Eur J Clin Microbiol Infect Dis. 2015 May;34(5):943-50.

Wielders CC, Boerman AW, **Schimmer B**, van den Brom R, Notermans DW, van der Hoek W, Schneeberger PM. Persistent high IgG phase I antibody levels against *Coxiella burnetii* among veterinarians compared to patients previously diagnosed with acute Q fever after three years of follow-up. PLoS One. 2015 Jan 20;10(1):e0116937.

Van der Hoek W, **Schimmer B**, Hogerwerf L. Q fever in Africa: an emerging infectious disease. MT*b*, 2015 Mar 20, 53(1): 3-5.

Schimmer B, de Lange MM, Hautvast JL, Vellema P, van Duynhoven YT. *Coxiella burnetii* seroprevalence and risk factors on commercial sheep farms in The Netherlands. Vet Rec. 2014 Jul 5;175(1):17.

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

Schimmer B, Schotten N, van Engelen E, Hautvast JL, Schneeberger PM, van Duijnhoven YT. *Coxiella burnetii* seroprevalence and risk for humans on dairy cattle farms, the Netherlands, 2010-2011. Emerg Infect Dis. 2014 Mar;20(3):417-25.

De Lange MM, **Schimmer B**, Vellema P, Hautvast JL, Schneeberger PM, Van Duijnhoven YT. *Coxiella burnetii* seroprevalence and risk factors in sheep farmers and farm residents in The Netherlands. Epidemiol Infect. 2014 Jun;142(6):1231-44.

van der Hoek W, van de Kassteele J, Bom B, de Bruin A, Dijkstra F, **Schimmer B**, Vellema P, ter Schegget R, Schneeberger PM. Smooth incidence maps give valuable insight into Q fever outbreaks in The Netherlands. Geospat Health. 2012 Nov;7(1):127-34.

Schimmer B, Lenferink A, Schneeberger P, Aangenend H, Vellema P, Hautvast J, van Duynhoven Y. Seroprevalence and risk factors for *Coxiella burnetii* (Q fever) seropositivity in dairy goat farmers' households in The Netherlands, 2009-2010.PLoS One. 2012;7(7):e42364.

Van den Brom R, **Schimmer B**, Schneeberger PM, Swart WA, van der Hoek W,Vellema P. Seroepidemiological survey for *Coxiella burnetii* antibodies and associated risk factors in Dutch livestock veterinarians. PLoS One. 2013;8(1):e54021.

van der Hoek W, Wielders CC, **Schimmer B**, Wegdam-Blans MC, Meekelenkamp J, Zaaijer HL, Schneeberger PM. Detection of phase I IgG antibodies to *Coxiella burnetii* with EIA as a screening test for blood donations. Eur J Clin Microbiol Infect Dis. 2012 Nov;31(11):3207-9.

Teunis PF, **Schimmer B**, Notermans DW, Leenders AC, Wever PC, Kretzschmar ME, Schneeberger PM. Time-course of antibody responses against *Coxiella burnetii* following acute Q fever. Epidemiol Infect. 2013 Jan;141(1):62-73.

de Rooij MM, **Schimmer B**, Versteeg B, Schneeberger P, Berends BR, Heederik D, van der Hoek W, Wouters IM. Risk factors of *Coxiella burnetii* (Q fever) seropositivity in veterinary medicine students. PLoS One. 2012;7(2):e32108.

de Bruin A, van der Plaats RQ, de Heer L, Paauwe R, **Schimmer B**, Vellema P, van Rotterdam BJ, van Duynhoven YT. Detection of *Coxiella burnetii* DNA on small-ruminant farms during a Q fever outbreak in the Netherlands. Appl Environ Microbiol. 2012 Mar;78(6):1652-7.

Schimmer B, Luttikholt S, Hautvast JL, Graat EA, Vellema P, Duynhoven YT. Seroprevalence and risk factors of Q fever in goats on commercial dairy goat farms in the Netherlands, 2009-2010. BMC Vet Res. 2011 Dec 30;7:81.

Dijkstra F, van der Hoek W, Wijers N, **Schimmer B**, Rietveld A, Wijkmans CJ, Vellema P, Schneeberger PM. The 2007–2010 Q fever epidemic in The Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. FEMS Immunol Med Microbiol. 2012 Feb;64(1):3-12.

Whelan J, **Schimmer B**, Schneeberger P, Meekelenkamp J, Ijff A, van der Hoek W, Robert-Du Ry van Beest Holle M. Q fever among culling workers, the Netherlands, 2009-2010. Emerg Infect Dis. 2011 Sep;17(9):1719-23.

Whelan J, **Schimmer B**, de Bruin A, van Beest Holle MR, van der Hoek W, ter Schegget R. Visits on 'lamb-viewing days' at a sheep farm open to the public was a risk factor for Q fever in 2009. Epidemiol Infect. 2012 May;140(5):858-64.

Schimmer B, Notermans DW, Harms MG, Reimerink JH, Bakker J, Schneeberger P, Mollema L, Teunis P, van Pelt W, van Duynhoven Y. Low seroprevalence of Q fever in The Netherlands prior to a series of large outbreaks. Epidemiol Infect. 2012 Jan;140(1):27-35.

Blaauw GJ, Notermans DW, **Schimmer B**, Meekelenkamp J, Reimerink JH, Teunis P, Schneeberger PM. The application of an enzyme-linked immunosorbent assay or an immunofluorescent assay test leads to different estimates of seroprevalence of *Coxiella burnetii* in the population. Epidemiol Infect. 2012 Jan;140(1):36-41.

Dijkstra F, Riphagen-Dalhuisen J, Wijers N, Hak E, Van der Sande MA, Morroy G, Schneeberger PM, **Schimmer B**, Notermans DW, Van der Hoek W. Antibiotic therapy for acute Q fever in The Netherlands in 2007 and 2008 and its relation to hospitalization. Epidemiol Infect. 2011 Sep;139(9):1332-41.

van den Wijngaard CC, Dijkstra F, van Pelt W, van Asten L, Kretzschmar M, **Schimmer B**, Nagelkerke NJ, Vellema P, Donker GA, Koopmans MP. In search of hidden Q-fever outbreaks: linking syndromic hospital clusters to infected goat farms. Epidemiol Infect. 2011 Jan;139(1):19-26.

van der Hoek W, Dijkstra F, **Schimmer B**, Schneeberger PM, Vellema P, Wijkmans C, ter Schegget R, Hackert V, van Duynhoven Y. Q fever in the Netherlands: an update on the epidemiology and control measures. Euro Surveill. 2010 Mar 25;15(12).

Schimmer B, Ter Schegget R, Wegdam M, Züchner L, de Bruin A, Schneeberger PM, Veenstra T, Vellema P, van der Hoek W. The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. BMC Infect Dis. 2010 Mar 16;10:69.

Koene RP, **Schimmer B**, Rensen H, Biesheuvel M, de Bruin A, Lohuis A, Horrevorts A, Lunel FV, Delsing CE, Hautvast JL. A Q fever outbreak in a psychiatric care institution in The Netherlands. Epidemiol Infect. 2011 Jan;139(1):13-8.

Schimmer B, Dijkstra F, Vellema P, Schneeberger PM, Hackert V, ter Schegget R, Wijkmans C, van Duynhoven Y, van der Hoek W. Sustained intensive transmission of Q fever in the south of the Netherlands, 2009. Euro Surveill. 2009 May 14;14(19). pii: 19210.

Karagiannis I, **Schimmer B**, Van Lier A, Timen A, Schneeberger P, Van Rotterdam B, De Bruin A, Wijkmans C, Rietveld A, Van Duynhoven Y. Investigation of a Q fever outbreak in a rural area of The Netherlands. Epidemiol Infect. 2009 Sep;137(9):1283-94.

Schimmer B, Morroy G, Dijkstra F, Schneeberger PM, Weers-Pothoff G, Timen A, Wijkmans C, van der Hoek W. Large ongoing Q fever outbreak in the south of The Netherlands, 2008. Euro Surveill. 2008 Jul 31;13(31). pii: 18939.

Publications on other topics

Pijnacker R, Goris MG, Te Wierik MJ, Broens EM, van der Giessen JW, de Rosa M, Wagenaar JA, Hartskeerl RA, Notermans DW, Maassen K, **Schimmer B**. Marked increase in leptospirosis infections in humans and dogs in the Netherlands, 2014. Euro Surveill. 2016 Apr 28;21(17).

de Man H, Mughini Gras L, **Schimmer B**, Friesema IH, DE Roda Husman AM, van Pelt W. Gastrointestinal, influenza-like illness and dermatological complaints following exposure to floodwater: a cross-sectional survey in The Netherlands. Epidemiol Infect. 2016 May;144(7):1445-54.

Severi E, Verhoef L, Thornton L, Guzman-Herrador BR, Faber M, Sundqvist L, Rimhanen-Finne R, Roque-Afonso AM, Ngui SL, Allerberger F, Baumann-Popczyk A, Muller L, Parmakova K, Alfonsi V, Tavoschi L, Vennema H, Fitzgerald M, Myrmel M, Gertler M, Ederth J, Kontio M, Vanbockstael C, Mandal S, Sadkowska-Todys M, Tosti ME, **Schimmer B**, O Gorman J, Stene-Johansen K, Wenzel JJ, Jones G, Balogun K, Ciccaglione AR, O' Connor L, Vold L, Takkinen J, Rizzo C. Large and prolonged food-borne multistate hepatitis A outbreak in Europe associated with consumption of frozen berries, 2013 to 2014. Euro Surveill. 2015 Jul 23;20(29):21192.

Sane J, Reimerink J, Harms M, Bakker J, Mughini-Gras L, **Schimmer B**, van Pelt W. Human hantavirus infections in the Netherlands. Emerg Infect Dis. 2014 Dec;20(12):2107-10.

Rebolledo J, Garvey P, Ryan A, O'Donnell J, Cormican M, Jackson S, Cloak F, Cullen L, Swaan CM, **Schimmer B**, Appels RW, Nygard K, Finley R, Sreenivasan N, Lenglet A, Gossner C, McKeown P. International outbreak investigation of Salmonella Heidelberg associated with in-flight catering. Epidemiol Infect. 2014 Apr;142(4):833-42.

Friesema IH, Schimmer B, Ros JA, Ober HJ, Heck ME, Swaan CM, de Jager CM, Peran i Sala RM, van Pelt W. A regional *Salmonella enterica* serovar Typhimurium outbreak associated with raw beef products, The Netherlands, 2010. Foodborne Pathog Dis. 2012 Feb;9(2):102-7.

Jakopanec I, **Schimmer B**, Grjibovski AM, Klouman E, Aavitsland P. Self-reported sexually transmitted infections and their correlates among men who have sex with men in Norway: an Internet-based cross-sectional survey. BMC Infect Dis. 2010 Sep 6;10:261.

Bosman A, **Schimmer B**, Coulombier D. Contribution of EPIET to public health workforce in the EU, 1995-2008. Euro Surveill. 2009 Oct 29;14(43). pii: 19381.

Karagiannis I, **Schimmer B**, de Roda Husman AM. Compliance with boil water advice following a water contamination incident in the Netherlands in 2007. Euro Surveill. 2009 Mar 26;14(12). pii: 19156.

Friesema I, Sigmundsdottir G, van der Zwaluw K, Heuvelink A, **Schimmer B**, de Jager C, Rump B, Briem H, Hardardottir H, Atladottir A, Gudmundsdottir E, van Pelt W. An international outbreak of Shiga toxin-producing Escherichia coli O157 infection due to lettuce, September-October 2007. Euro Surveill. 2008 Dec 11;13(50). pii: 19065.

Schimmer B, Nygard K, Eriksen HM, Lassen J, Lindstedt BA, Brandal LT, Kapperud G, Aavitsland P. Outbreak of haemolytic uraemic syndrome in Norway caused by stx2-positive Escherichia coli O103:H25 traced to cured mutton sausages. BMC Infect Dis. 2008 Apr 3;8:41.

Nygård K, **Schimmer B**, Søbstad Ø, Walde A, Tveit I, Langeland N, Hausken T, Aavitsland P. A large community outbreak of waterborne giardiasis-delayed detection in a non-endemic urban area. BMC Public Health. 2006 May 25;6:141.

Schimmer B, Ihekweazu C. Polio eradication and measles immunisation in Nigeria. Lancet Infect Dis. 2006 Feb;6(2):63-5.

Guha-Sapir D, **Schimmer B**. Dengue fever: new paradigms for a changing epidemiology. Emerg Themes Epidemiol. 2005 Mar 2;2(1):1.

Telgt DS, van der Ven AJ, **Schimmer B**, Droogleever-Fortuyn HA, Sauerwein RW. Serious psychiatric symptoms after chloroquine treatment following experimental malaria infection. Ann Pharmacother. 2005 Mar;39(3):551-4.

Schimmer B, Sprenger HG, Wismans PJ, van Genderen PJ. Three patients with orf (ecthyma contagiosum). Ned Tijdschr Geneeskd. 2004 Apr 17;148(16):788-91.

Biography

Barbara Schimmer was born on 2 August, 1975 in Schiedam, the Netherlands. In 1993, she completed her secondary education at the Montessori Lyceum in Rotterdam and obtained a grant through the Netherlands America Commission for Educational Exchange (now known as Fulbright Center), for a freshman year of study in the United States, at the University of Wisconsin-Eau Claire. In 1995, she completed her undergraduate degree in International Business Studies from the University of Maastricht.

Barbara studied medicine at the University of Groningen, beginning in 1995. During this period, she was an active board member of the Faculty Council and the medical student society, Panacea. She functioned as UNESCO liaison officer for the International Federation of Medical Students' Associations. In 1999, she did her research elective on the screening of colorectal cancer at the prestigious Mayo Clinic in Rochester (Minnesota) through the International School of Hepatology and Tropical Medicine GISH-T. In 2002, Barbara completed a multidisciplinary Master's programme in humanitarian assistance (NOHA) at the University of Louvain-la-Neuve in Belgium, where she did an internship at the Centre for Research on the Epidemiology of Disasters **(**CRED)**.** After her final medical elective at the Harbour Hospital in Rotterdam, she decided to continue in the field of infectious diseases and international health.

After obtaining her medical degree in 2002, Barbara worked as a research physician at the Centre for Clinical Malaria Studies of the Radboud University Medical Center in Nijmegen, and as staff adviser at the Department for Medical and Legal Affairs at the VU University Medical Center in Amsterdam. From 2004-2006, she was a fellow in the European Programme for Intervention Epidemiology Training (EPIET), based at the Norwegian Institute for Public Health in Oslo, where she developed a keen interest in the epidemiology of zoonoses and gastroenteritis. As an EPIET-fellow, she participated in international field missions to Pakistan, Nigeria and Northwest Russia.
After completion of her fellowship, she was active in the board of the EPIET Alumni Network, as secretary and president, to help develop and maintain a network of European public health epidemiologists.

Since January 2007, Barbara has worked in the Epidemiology and Surveillance Unit, of the Centre for Infectious Disease Control (Cib) at the National Institute for Public Health and the Environment (RIVM) of the Netherlands. She was zoonoses coordinator in the department of Gastroenteritis and Zoonoses. In 2009, she started her PhD 'on the job' project, on the epidemiology of Q fever within a 'One Health' context, under the supervision of Prof. dr. Roel Coutinho, Dr. Yvonne van Duijnhoven and Dr. Wim van der Hoek, which resulted in this thesis.

Since 2016, she works within the department of Early Warning and Surveillance and is co-chair of the weekly Early Warning Meeting hosted by the Centre for Infectious Disease Control. She is also the content chair of the annual Transmission Day and editorial advisor to the *Eurosurveillance* editorial board.