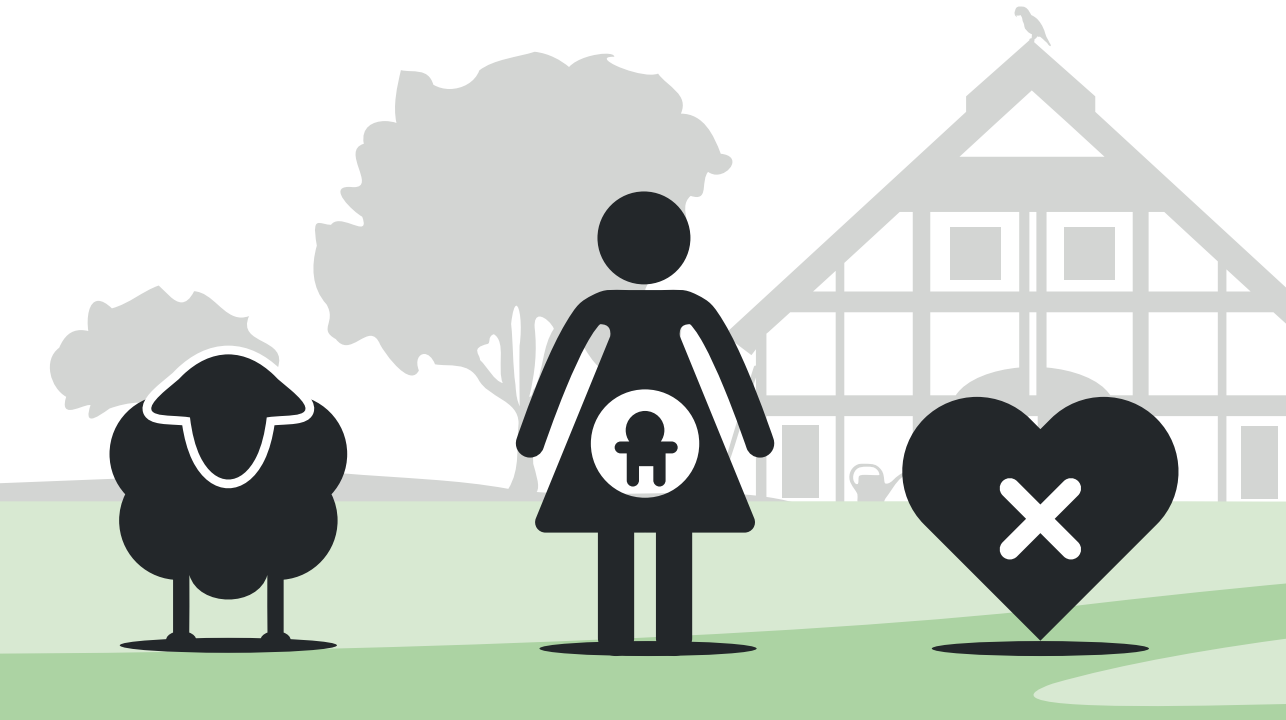
Q fever
in the Netherlands

Occupational exposure, pregnancy outcomes,
and chronic Q fever screening



- Marit de Lange -

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Q fever in the Netherlands

Occupational exposure, pregnancy outcomes, and chronic Q fever screening

Q-koorts in Nederland

Beroepsmatige blootstelling, zwangerschapsuitkomsten en chronische Q-koorts screening

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor
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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Q FEVER

Q fever is caused by the bacterium *Coxiella burnetii*. It is a worldwide zoonosis with goats, sheep, and cattle as primary source for human infections.[1] Infected animals mostly do not have symptoms. However, abortion may occur in pregnant goats and sheep, whereby many bacteria can spread into the environment.[2, 3] The bacterium is very stable to environmental conditions and can remain infectious for many months.[4] Humans are usually infected by inhalation of contaminated aerosols.[1] Inhaling of just a few bacteria can cause infection in humans. [5]

RECENT INFECTIONS

When humans are infected with the *C. burnetii* bacterium, around 60% remain asymptomatic.[1] The other 40% develop symptoms of acute Q fever, which mainly presents as febrile illness, atypical pneumonia, or hepatitis.[1, 6] Acute Q fever patients mostly recover without treatment. However, if treatment is needed, the first-choice antibiotic is doxycycline for 14 days.[7]

In the Netherlands, acute Q fever is a notifiable disease since 1975. Before 2007, there were on average 20 notifications of acute Q fever per year. From 2007 onwards, the number of acute Q fever notifications sharply increased, with a cumulative total of more than 4,000 notified patients by 2010, see Figure 1.1. It has been estimated that one notification represents 12.6 incident infections, suggesting that there were more than 50,000 incident *C. burnetii* infections in the Netherlands during this outbreak.[8] It was the largest outbreak ever reported.[9, 10] Abortion waves at dairy goat farms were identified as sources of this outbreak.[10-12] Additionally, sheep were also a source for some human infections during the Dutch outbreak.[13, 14] The Q fever outbreak was controlled at the end of 2009, when several veterinary measures were implemented, including compulsory vaccination of all dairy goat and sheep, and pre-emptive culling of all pregnant goats and sheep on infected farms.[10, 15] Since the end of the large outbreak in the Netherlands in 2010, still around 20 acute Q fever patients have been diagnosed each year. [15]

People with occupational exposure to farm animals such as farmers, veterinarians, and culling workers, are at high risk of a *C. burnetii* infection, as evident from high seroprevalence figures.[16-19] Previous studies have identified pregnant women as a specific risk group for complications from acute Q fever. Based on case histories, it has been described that if women are infected during pregnancy, they are at increased risk for spontaneous abortion, intrauterine fetal death, low birth weight, and premature delivery. [20, 21] However, the number of pregnant women for whom the pregnancy outcome is

described in the literature is low. Asymptomatic infections may carry the same risk for adverse pregnancy outcome as symptomatic cases.[22] However, in a Dutch study, the presence of antibodies against *C. burnetii* in 1,174 pregnant women was not significantly associated with preterm delivery, low birth weight or other outcome measures.[23]

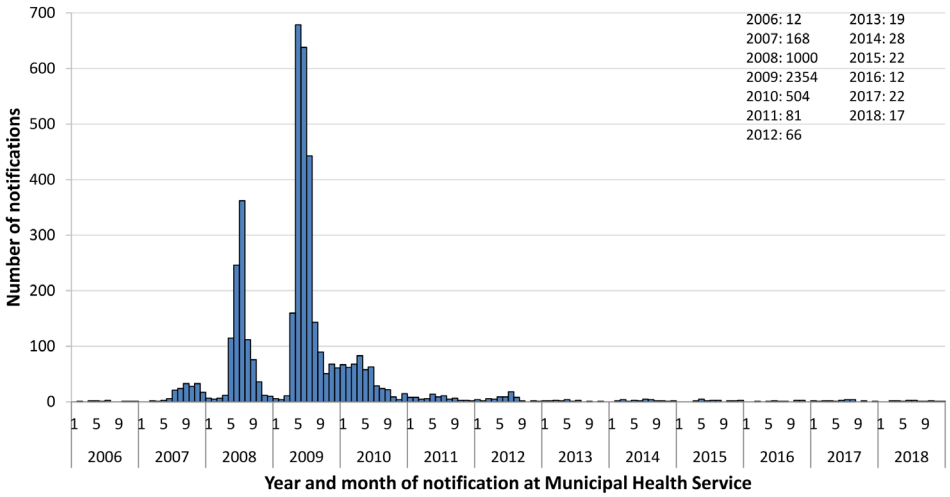

Figure 1.1. Q fever notifications in the Netherlands by month of notification, 2006–2018, original compiled by Frederika Dijkstra (RIVM)

CHRONIC Q FEVER

Acute Q fever is usually self-limiting. However, chronic Q fever can develop in 2-5% of all symptomatic acute Q fever patients.[24, 25] Additionally, people with an asymptomatic infection are also at risk for a chronic infection.[26] Laboratory signs of chronic Q fever are mostly evident within one year after acute Q fever diagnosis but diagnosis can be delayed for months, or even years. [25] Risk factors for chronic Q fever include older age, preexisting cardiac valvulopathy, vascular prosthesis and aneurysms, renal insufficiency, and immunosuppression.[27-29] The recommended treatment of chronic Q fever is a combination of doxycycline and hydroxychloroquine for 18 months or even longer.[7, 30] Studies from the south of France, where a lot of Q fever research is performed, show that endocarditis is the most common clinical manifestation, followed by infections of aortic aneuryms and vascular prostheses.[31] During and in the aftermath of the large outbreak in the Netherlands, however, more vascular infections were diagnosed. The Dutch consensus guideline subdivides chronic Q fever in proven, probable, and possible chronic Q fever.[32] Of the proven chronic Q fever patients, the case fatality rate was 25%,

and patients with complications have a higher risk for chronic Q fever-related mortality. Through January 2019, there were 519 possible, probable, and proven chronic Q fever together patients registered in the Dutch national chronic Q fever database (personal communication Jan-Jelrik Oosterheert, 10-7-2019).[26]

A possible strategy to detect chronic Q fever patients is serological follow-up of all acute Q fever patients at 12-months after diagnosis.[25] Additionally, French researchers advised to refer all known acute Q fever patients for echocardiography, as progression to endocarditis has been reported in patients with undiagnosed and clinically silent valvulopathies.[27, 33] In France, it is advised to give antibiotic prophylaxis for 12 months to patients with acute Q fever and cardiac valve lesions to prevent endocarditis.[34] However, the majority of the Dutch chronic Q fever patients were not notified as acute Q fever, and active screening of acute Q fever patients will therefore not identify or prevent all chronic infections.[26] Another possible strategy for chronic Q fever identification is serological screening of high-risk groups, like patients with a valvulopathy or an aneurysm.

AIMS AND OUTLINE OF THE THESIS

Many studies have already been performed based on the Dutch Q fever outbreak. However, research questions remain, often concerning topics on which the international literature is inconclusive, but that have a potentially large public health impact. This includes the best strategy to prevent or detect chronic infections, the risks for pregnant women, and the importance of occupational exposure to other animals than dairy goats.

In the first part of this thesis, we will focus on the presence of *C. burnetii* antibodies in two occupationally exposed groups. The *C. burnetii* seroprevalence has already been investigated for dairy goat and cattle farm residents in the Netherlands.[17, 18] However, the seroprevalence was still unknown for residents at dairy and non-dairy sheep farms. Therefore, we describe in **Chapter 2** the *C. burnetii* seroprevalence and associated risk factors in sheep farmers and farm residents. A crossectional seroprevalence study has also previously been performed among veterinary students [35], but of interest is the *C. burnetii* seroconversion and associated risk factors during veterinary training. **Chapter 3** describes the seroconversion rate and associated risk factors in this risk group. Study-related and non-study related risk factors are both investigated.

Furthermore, except for some case reports, it remained unknown what the public health relevance was for all pregnant women in areas with a high Q fever incidence. In part two of this thesis, pregnancy outcomes registered in The Netherlands Perinatal Registry in

areas with a high acute Q fever incidence are compared with pregnancy outcomes in areas with no registered Q fever in **Chapter 4**. In this study, we will look at the outcomes preterm delivery, small for gestational age, and perinatal mortality, but not abortion.

The last part of this thesis focuses on strategies for the identification of unrecognized chronic Q fever patients. Echocardiographic screening of acute Q fever patients and antibiotic prophylaxis for patients with cardiac valvulopathy is considered an important approach to prevent chronic Q fever-related endocarditis. In **Chapter 5**, we followed a cohort of acute Q fever patients to estimate the risk for developing chronic Q fever, and we evaluated the impact of screening in patients who were not yet known to have a valvulopathy. Next, patients with cardiac valvulopathy are at high risk to develop chronic Q fever after an acute infection. This patient group was not routinely screened in the Netherlands, so it is unknown whether all their chronic infections were diagnosed. **Chapter 6** describes how many chronic Q fever patients can be identified by routinely screening of patients with valvulopathy in a hospital in the epicenter of the outbreak, to establish whether the policy of not screening should be changed. Next, **Chapter 7** focusses on the cost-effectiveness of one-of screening program of certain risk groups to identify not yet diagnosed chronic Q fever patients, and which scenario is the most cost-effective. Last, a general discussion of the findings of the present thesis in relation to the international literature is given in **Chapter 8**.

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CHAPTER 2

COXIELLA BURNETII SEROPREVALENCE AND RISK FACTORS IN SHEEP FARMERS AND FARM RESIDENTS IN THE NETHERLANDS

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ABSTRACT

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.

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 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 nondairy 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 ≥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 ≥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 nonparticipating 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 [$P<0.20$ 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 ($P<0.20$), 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/km2). 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 ($P=0.03$).

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 ≥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 nonparticipating farms, 34.5 (range 1.8–143.6) and 47.5 (range 1.0–162.9) sheep/km2 in the 5-km radius (excluding own sheep), respectively (P=0.01). In addition, the number of sheep was borderline significantly higher at the participating farms (median 191 sheep, range 102–1310), compared to the nonparticipating farms (median 167 sheep, range 100–2857). For the other variables, no significant differences were found between participating and nonparticipating farms (Table 2.1).

Table 2.1. Non-response analyses of non-dairy sheep farms, comparison of participating and non-participating farms

	Participating farms (*N*=119)	**Non-participating farms (*N*=1193)**	
Numerical variables	**Median**	**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)*	13960	13806	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.

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 2.2 and 2.3.

Table 2.2. 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)	Seroprevalence (%)	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 at 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 health care 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 horse at own or other farm*§	Yes	145	59.3	2.07 (1.27-3.38)
	No	121	41.3	Reference
Contact with pig 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 goat 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 2.2 continued.

Variable	Category	Frequency (*N*) (*N*=266)	Seropre-valence (%)	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 manure cattle	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 agriculture 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 <5 m or touch animals.

¶ See animals <5 m.

Table 2.3. 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)*	Seropre-valence (%)	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 a 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 Q fever bulk-milk-positive dairy goat or dairy sheep farms in a10-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 farm (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 stable	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
Dairy cattle in stable†	Yes	66	71.2	3.37 (1.76-6.45)
	No	184	42.9	Reference

Table 2.3 continued.

Variable	Category	Frequency (*N*) (*N*=261)*	Seropre-valence (%)	OR (95% CI)
Type 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 normally lambed animal†	Leave in stable or pasture	50	58.0	Reference
	Direct or once a day render bucket	84	47.6	0.64 (0.30-1.36)
	Direct or once a day manure yard	100	51.0	0.72 (0.34-1.53)
	Other	20	30.0	0.31 (0.10-0.97)
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 farm-based questionnaire.

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 2.4). 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 2.5).

Table 2.4. 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 <5 m or touch animals.

Table 2.5. 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* seropositivity

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 stable 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).

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 2.6). 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 2.6. 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* seropositivity

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 trough door	Yes	0.47 (0.21-1.01)
	No	Reference
	No stable	8.34 (1.71-40.60)
Dairy cattle present during stable 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 <5 m or touch animals.

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 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 (≥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 Texelaar (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.

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 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 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 ≥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.

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CHAPTER 3

HIGH *COXIELLA BURNETII* SEROCONVERSION RATE IN VETERINARY STUDENTS, THE NETHERLANDS, 2006-2010

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ABSTRACT

We examined seroconversion rates by measuring IgG antibodies against *Coxiella burnetii* among two cohorts of veterinary students. During follow-up of 118 seronegative veterinary students, 23 students seroconverted. Although the clinical significance of the presence of antibodies is unknown, students should be informed about the potential risks of Q fever.

INTRODUCTION

Q fever is caused by the bacterium *Coxiella burnetii* and can present as acute or chronic illness. However, around 60% of infected people remain asymptomatic.[1] In particular, livestock veterinarians are at increased risk of a *C. burnetii* infection.[2] Previously, a high seroprevalence, range 11-19%, among veterinary medicine students was reported.[3-5] However, the incidence of Q fever and associated risk factors during veterinary training are still unknown. Therefore, we performed a longitudinal study in veterinary students in the Netherlands following incoming, seronegative veterinary students during a maximum of 4-year study period and registered potential study and non-study related associated factors for seroconversion.

THE STUDY

Veterinary medicine students who started in 2006 or 2008 (around 225 each year) at the Faculty of Veterinary Medicine of Utrecht University (FVMUU) were eligible for inclusion. This faculty provides the only veterinary medicine program in the Netherlands. Recruitment methods included informational class presentations and a mailed brochure. The Medical Ethical Commission of the University Medical Centre Utrecht approved the study protocol (no. 06/169). After obtaining written informed consent, a blood sample was collected at study start and participants completed a baseline questionnaire. From participants who started at the FVMUU in 2006 (cohort 2006), up to two additional blood samples were collected and follow-up questionnaires completed in 2008 and 2010. Students who started in 2008 (cohort 2008) provided only one follow-up blood sample and follow-up questionnaire, in 2010. Both the baseline and follow-up questionnaires included questions about animal contact, living situation, personal health situation, and smoking habits before and during the study period. The follow-up questionnaires also included questions about the study choices and study related courses.

Serum samples were tested for IgG antibodies against phase I and II of *C. burnetii*, using an indirect immunofluorescence assay (IFA) as previously described.[3] Those with IgG phase I or II antibodies ≥ 1:32 were classified as *C. burnetii* seropositive. Seroconversion was defined as a participant who was IgG seronegative at baseline and seropositive in one of the follow-up samples. Participants with an IgG phase I titer of ≥ 1:1024 had a serological indication for a chronic Q fever infection.[6]

All data were analyzed with SAS, version 9.4 (SAS Institute Inc., USA). First, differences in demographics and past animal exposure characteristics between seropositive

and seronegative participants at baseline were determined with a Fisher exact test (numerical characteristics) or Kruskal Wallis test (categorical characteristics). To estimate seroconversion rate and possible associated factors for seroconversion during follow-up, data from seronegative participants with at least one follow-up sample were used. The univariable logistic regression analyses were performed with generalized estimating equations (GEE) models with an exchangeable correlation matrix. These models were used to take into account correlations between the repeated measurements of serostatus within the same subject.[7] Participants' data were censured for the times after they were tested *C. burnetii* seropositive. The data from the two cohorts were analyzed together, because the data sets were too small to analyze them separately. The FVMUU starting year (cohort) and the number of years after the study start were always included as covariates in the model. Investigated characteristics were animal-related exposure outside the study, living situation, smoking habits, study duration, cohort, and chosen study direction; in total, we investigated 20 characteristics. Associations were considered significant at confidence level of $\alpha < 0.05$. All univariable associated characteristics were highly interrelated ($P<0.05$ in Fisher exact test). Therefore, multivariable logistic GEE analysis was not possible.

At the beginning of their veterinary training 447 students were invited to participate in the study of which 131 participated, of whom 13 (10%) were *C. burnetii* IgG seropositive at baseline. Students who were seropositive at baseline were more likely to have ever lived on a farm (Table 3.1). Similarly, they had more contact with farm animals than students who were seronegative at baseline, but the difference was only statistically significant for contact with cows and poultry. No other significant differences were found between *C. burnetii* seronegative and seropositive students at baseline (Table 3.1).

Of the 118 seronegative participants at baseline, 78 started their study in 2006 and 40 in 2008 (Figure 3.1). Of those students, 23 seroconverted during the follow-up period of 362 person-years, which results in an incidence of 0.06 per person-year. None of the seroconverted participants reported that their general practitioner or medical specialist diagnosed them with acute Q fever. Additionally, none of the participants had a serological indication for a chronic infection.

Of the 20 investigated characteristics, "living on a sheep or goat farm", "having contact with sheep outside the study", and "working with hay, straw, silage grass or animal feed" during their study period outside the FVMUU increased the odds of seroconversion ($P<0.05$), in analyses adjusted for time since start study and cohort. Performing animal nursing on the farm where they lived tended to increase seroconversion ($P=0.07$) (Table 3.2).

Table 3.1. Baseline questionnaire characteristics of two cohorts of veterinary students (cohort 1 started in 2006, cohort 2 started in 2008) at the Faculty of Veterinary Medicine of Utrecht University, the Netherlands

Characteristic	Seronegative participants at baseline in 2006 or 2008 N=118* Median or no/N (%)	Seropositive participants at baseline in 2006 or 2008 N=13 Median or no/N (%)	*P*-value
Age (years)	19	18	0.24
BMI	21	21	0.87
Gender			
Male	18/116 (16)	3/13 (23)	0.44
Female	98/116 (84)	10/13 (77)	
Smoking			
No smoking	109/115 (94)	13/13 (100)	1.00
Past smoker	3/115 (3)	0/13 (0)	
Current smoker	3/115 (3)	0/13 (0)	
Grew up in a			
Village (<15,000 inhabitants)	44/116 (38)	7/13 (54)	0.45
Town (15,000-80,000 inhabitants)	47/116 (40)	5/13 (38)	
City (>80,000 inhabitants)	25/116 (22)	1/13 (8)	
Ever lived on a farm			
Yes	11/116 (9)	7/13 (54)	<0.01
No	105/116 (91)	6/13 (46)	
Regular contact with cows before start of FVMUU			
Yes	19/116 (16)	9/13 (69)	<0.01
No	97/116 (84)	4/13 (31)	
Regular contact with goats before start of FVMUU			
Yes	18/116 (16)	5/13 (38)	0.06
No	98/116 (84)	8/13 (62)	
Regular contact with sheep before start of FVMUU			
Yes	20/116 (17)	5/13 (38)	0.13
No	96/116 (83)	8/13 (62)	
Regular contact with poultry before start of FVMUU			
Yes	31/116 (27)	8/13 (62)	0.02
No	85/116 (73)	5/13 (38)	
Regular contact with horses before start of FVMUU			
Yes	65/116 (56)	7/13 (54)	1.00
No	51/116 (44)	6/13 (46)	
Regular contact with pigs before start of FVMUU			
Yes	13/116 (11)	4/13 (31)	0.07
No	103/116 (89)	9/13 (69)	

Abbreviations: n=Number, N=Total number, BMI=Body Mass Index, FVMUU = Faculty of Veterinary Medicine of Utrecht University, NA=Not available.

* Two seronegative participants at baseline did not fill out a questionnaire.

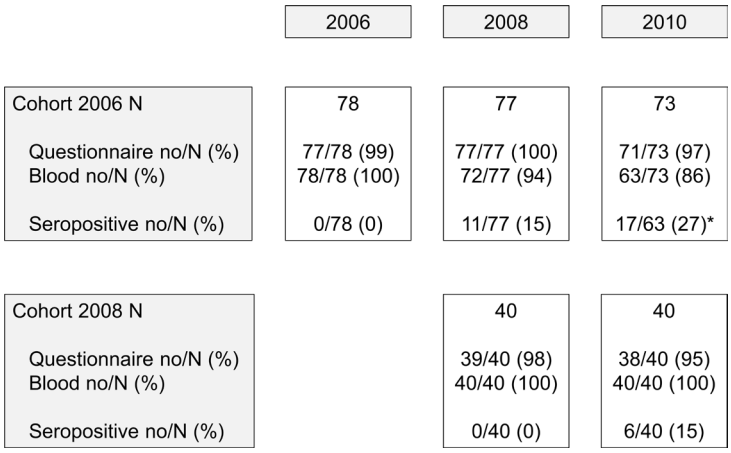

Figure 3.1. Follow-up timeline illustrating the number and percentages of seronegative participants at baseline, per follow-up moment

Abbreviations: no=the number in the corresponding group, N=total number of patients

*The 17 seropositive students in 2010 include the 11 seropositive students who already seroconverted between 2006 and 2008, who were censured from risk factor analysis in 2010.

Table 3.2. Characteristics from follow-up questionnaire in association with Q fever seroconversion among 118 veterinary students who were seronegative at start of their study in 2006 or 2008, the Netherlands

Characteristic	OR (95% CI)	*P*-value
Age (years)		
≤ 20	Reference	
21	0.9 (0.2-3.5)	0.85
≥ 22	1.3 (0.4-4.2)	0.69
Gender		
Male	Reference	
Female	0.7 (0.2-2.3)	0.53
Regularly exposed to cigarette smoke		
Yes	1.1 (0.4-2.8)	0.81
No	Reference	
Living on a farm with cows		
Yes	ND*	
No		
Living on a farm with sheep or goats		
Yes	6.2 (1.4-28.1)	0.02
No	Reference	
Living on a farm with pigs		
Yes	ND*	
No		
Living on a farm with chicken		
Yes	3.0 (0.3-35.0)	0.39
No	Reference	
Having regular contact with cows outside study		
Yes	0.3 (0.1-2.7)	0.31
No	Reference	

Table 3.2 continued.

Characteristic	OR (95% CI)	*P*-value
Having regular contact with goats outside study		
Yes	0.6 (0.1-3.8)	0.56
No	Reference	
Having regular contact with horses outside study		
Yes	0.7 (0.3-1.7)	0.40
No	Reference	
Having regular contact with pigs outside study		
Yes	ND*	
No		
Having regular contact with chicken outside study		
Yes	0.5 (0.1-3.8)	0.50
No	Reference	
Having regular contact with sheep outside study		
Yes	4.4 (1.2-16.7)	0.03
No	Reference	
Performing animal nursing on the farm where you lived		
Yes	3.6 (0.9-14.3)	0.07
No	Reference	
Working with straw/hay on the farm where you lived		
Yes	6.4 (1.6-26.1)	<0.01
No	Reference	
Working with fertilizers on the farm where you lived		
Yes	3.2 (0.5-19.6)	0.21
No	Reference	
Performing plant nursing on the farm where you lived		
Yes	3.1 (0.3-33.5)	0.35
No	Reference	
Number of years after start study†		
Two	Reference	
Four	1.0 (0.3-2.9)	0.96
Cohort‡		
2006	Reference	
2008	0.7 (0.3-2.0)	0.56
Chosen specialization during study		
Individually kept animals	Reference	
Veterinary public health / farm animals	1.6 (0.5-5.0)	0.38

Abbreviations: CI=confidence interval, ND=Not determined, OR=Odds Ratio.

* Not to be determined due to low numbers.

† Only adjusted for cohort.

‡ Only adjusted for number of years after the study.

CONCLUSION

In this longitudinal serological study among veterinary students, we found a *C. burnetii* incidence of 0.06 per person-year. None of the seroconverted participants self-reported that they were diagnosed with acute Q fever, suggesting all cases were mild or asymptomatic. Additionally, none of the participants had a serological indication for a chronic infection. In veterinarians, which is the studied group in an advanced stage of their career, a high IgG phase I seroprevalence was found. It remains debatable whether presence of antibodies in occupationally exposed people with frequent boosting is of clinical significance.[8]

Three non-study factors were associated with increased seroconversion. Proximity to (aborting) small ruminants, such as goats and sheep, has previously been reported as important risk factor in an outbreak in the Netherlands.[9] It is known that veterinary students have a high prevalence of animal contacts outside the study.[10] Next, contact with hay, straw, silage grass or animal feed, is a known risk factor for human Q fever.[11] A major acute Q fever outbreak occurred in the Netherlands from 2007 to 2010 [12], as a result of which some students may also have contracted the infection. Although increased seroprevalence of Q fever in veterinary students prior to the outbreak has been reported.[3] We were not able to investigate study-related potential risk factors, such as followed courses due to two reasons. First, the curriculum changed during the investigation, so participants from the 2006 and 2008 cohort followed different courses, causing a low power in the analysis. Second, per cohort of students, there was little variation in followed courses.

Seroconversion incidence data is scarce. Of 246 seronegative culling workers 36 became IgG positive during the half year follow-up period, corresponding to an incidence of 0.29 per person-year [13], which is higher than the incidence in veterinary students (0.06 per person-year).

A nationally funded Q fever vaccination program for occupationally exposed people was introduced in Australia in 2002.[14] At this moment, the Health Council of the Netherlands does not advice to vaccinate occupationally exposed people.[15] The results of this study should be taken into account when reconsidering this advice.

In conclusion, we found a considerable *C. burnetii* seroconversion rate among veterinary students. Although the clinical significance of the presence of antibodies is unknown, it is advisable to inform students at the beginning of the study about the potential risks of acute and chronic Q fever.

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CHAPTER 4

NATIONWIDE REGISTRY-BASED ECOLOGICAL ANALYSIS OF Q FEVER INCIDENCE AND PREGNANCY OUTCOME DURING AN OUTBREAK IN THE NETHERLANDS

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ABSTRACT

OBJECTIVE

Whether areas affected by Q fever during a large outbreak (2008–2010) had higher rates of adverse pregnancy outcomes than areas not affected by Q fever.

DESIGN

Nationwide registry-based ecological study.

SETTING

Pregnant women in areas affected and not affected by Q fever in the Netherlands, 2003–2004 and 2008–2010.

PARTICIPANTS

Index group (N=58,737): pregnant women in 307 areas with more than two Q fever notifications. Reference group (N=310,635): pregnant women in 921 areas without Q fever notifications. As a baseline, pregnant women in index and reference areas in the years 2003–2004 were also included in the reference group to estimate the effect of Q fever in 2008–2010, and not the already existing differences before the outbreak.

MAIN OUTCOME MEASURES

Preterm delivery, small for gestational age, perinatal mortality.

RESULTS

In 2008–2010, there was no association between residing in a Q fever-affected area and both preterm delivery (adjusted OR 1.01 (95% CI 0.94 to 1.08)), and perinatal mortality (adjusted OR 0.87 (95% CI 0.72 to 1.05)). In contrast, we found a weak significant association between residing in a Q fever-affected area in 2008–2010 and small for gestational age (adjusted OR 1.06 (95% CI 1.01 to 1.12)), with a population-attributable fraction of 0.70% (95% CI 0.07% to 1.34%). We observed no dose–response relation for this outcome with increasing Q fever notifications, and we did not find a stronger association for women who were in their first trimester of pregnancy during the months of high human Q fever incidence.

CONCLUSIONS

This study found a weak association between residing in a Q fever-affected area and the pregnancy outcome small for gestational age. Early detection of infection would require mass screening of pregnant women; this does not seem to be justified considering these results, and the uncertainties about its efficacy and the adverse effects of antibiotic treatment.

INTRODUCTION

Q fever, caused by *Coxiella burnetii*, is a worldwide occurring zoonosis, with goats and sheep as primary sources of human infections.[1]. Infected goat and sheep herds can have high abortion rates, with massive contamination of the environment from infectious birth products.[2] The Netherlands faced the world's largest reported outbreak of Q fever, starting in 2007 and reaching a peak in 2009.[3] There are indications that the increase in acute Q fever had already started before 2007.[4]

A number of case descriptions and case series reports of pregnant women have documented that untreated acute or chronic *C. burnetii* infections may result in adverse pregnancy outcome in up to 81% of the cases.[5] The risk of adverse events on the fetus is highest when infection occurs during first trimester.[5] In addition, reactivation of a latent *C. burnetii* infection might also cause an adverse pregnancy outcome.[6] These adverse outcomes include spontaneous abortion, perinatal death, preterm delivery, and low birth weight.[5, 7-11] These reports were supported by community-based studies among pregnant women in Canada and Spain in whom serological titres consistent with an acute or recent Q fever infection were found to have a twofold higher risk for poor obstetrical outcomes.[6, 12] However, studies in France, the Netherlands and Denmark found no evidence of adverse pregnancy outcome among women with a serological indication of a *C. burnetii* infection.[13-15] Moreover, a prospective controlled clinical trial conducted during the outbreak in the Netherlands showed no benefits of screening for antibodies against *C. burnetii* during pregnancy.[16]

The inconsistent findings from studies analysing the risks of potentially serious obstetric complications and the lack of an accurate algorithm to identify pregnancies at risk preclude the implementation of evidence-based preventive public health measures in case of increased exposure. Therefore, during the Dutch outbreak, large-scale preventive screening was not implemented. The aim of this study was to assess whether Q fever-affected areas had higher rates of adverse pregnancy outcome than areas not affected by Q fever and thus, to evaluate the policy of not implementing large-scale screening among pregnant women during the outbreak.

METHODS

STUDY DESIGN AND SETTING

This was a nationwide registry-based ecological study. Data on Q fever incidence and pregnancy outcome were obtained for the 3 years with the highest Q fever incidence in the Netherlands (2008–2010). In addition, data were obtained for the years 2003–2004, which preceded this large outbreak.

DEFINING AREAS AFFECTED AND NOT AFFECTED BY Q FEVER IN 2008–2010

As in most other European countries, acute Q fever is a notifiable disease in the Netherlands. For notification, requirements include a positive laboratory result indicating a recent *C. burnetii* infection and a matching clinical presentation of fever, pneumonia or hepatitis. The laboratory criteria were a fourfold IgG titre rise or more measured by immunofluorescence assay, ELISA or complement fixation test, a positive IgM phase II antibody test or detection by PCR of *C. burnetii* DNA in blood or respiratory material. These data as well as patient age, gender, and four-digit postal-code area are recorded in the national infectious diseases database.[3]

For this study, postal-code areas were divided into those without notifications of Q fever and those with two or more notifications (Q fever-affected areas) in one of the outbreak years 2008–2010. Population numbers for the year 2009 were used to calculate Q fever incidence for Q fever-affected areas. The Q fever-affected areas were subdivided into four quartiles: <4.59, 4.59–10.61, 10.62–21.50 and ≥21.51 notifications per 10,000 inhabitants. Areas with only one notification in a year were excluded to minimise the risk of falsely identifying an area as Q fever affected, as isolated infections are more likely to have occurred outside the area of residence.

Areas not affected by Q fever were selected in two stages. First, four-digit postal-code areas with zero Q fever notifications in 2008 through 2010 were identified. Then, for each Q fever-affected area, we selected three postal-code areas not affected by Q fever (Figure 4.1), which resembled the affected area in their proportion of adverse pregnancy outcomes in 2003 through 2004 (before the Q fever outbreak). With this method, we created two area types (areas affected and not affected by Q fever) which were comparable with respect to the risk of obstetric complications before the start of the Q fever outbreak.

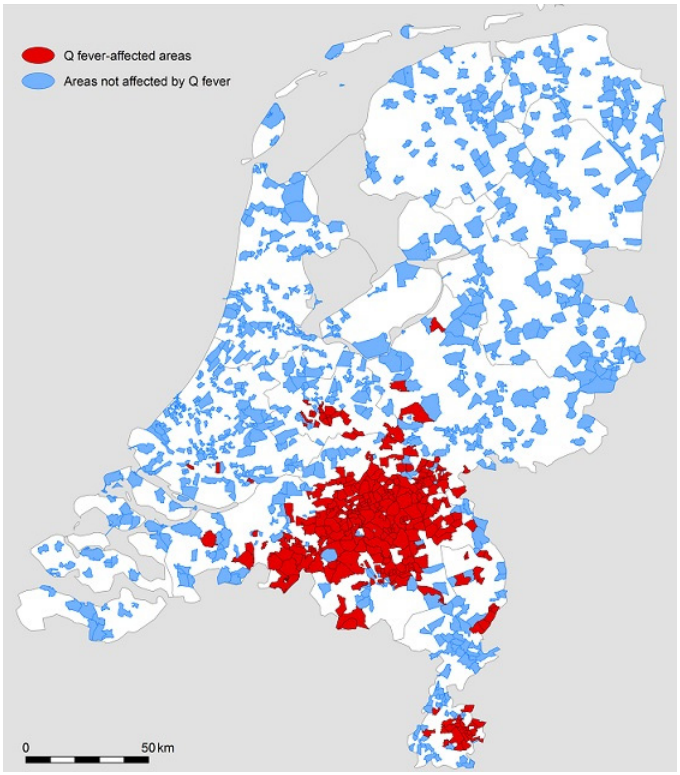

Figure 4.1. Postal-code areas affected by Q fever (2 or more notifications in 1 year during the years 2008, 2009 and 2010) and postal-code areas not affected by Q fever

PREGNANCY OUTCOME

Information on pregnancy outcome at the individual level was obtained from The Netherlands Perinatal Registry (PRN). This registry is a joint effort of the professional organisations of midwives, gynaecologists, obstetrically trained general practitioners and paediatricians in the Netherlands. The PRN covers 96% of all births in the Netherlands.[17]

Our analyses included singleton births only, from 20 weeks of gestation onwards, for which the mother's postal-code was known. Births of children with congenital malformations were excluded, because these malformations could lead to termination of the pregnancy. Therefore, this could introduce bias for preterm delivery and perinatal mortality. Additionally, a congenital malformation is often accompanied with the outcome child small for gestational age; this could also introduce bias.

We investigated three outcome variables, namely preterm delivery, a child small for gestational age and perinatal mortality. Preterm delivery was defined as a delivery

before a gestational age of 37 weeks. Small for gestational age was defined as a birth weight below the 10th centile, as derived from sex-specific, parity-specific and ethnic background-specific reference curves.[18] Finally, perinatal mortality was defined as fetal (from 20 weeks of gestation onwards) or neonatal (up to 7 days after birth).

CONFOUNDING VARIABLES

Information was collected on a number of a priori determined confounding variables known to be associate with both *C. burnetii* infection and adverse pregnancy outcome. At the individual level, these included maternal age, ethnic background and smoking behaviour. Additionally, socioeconomic status (SES), degree of urbanisation and animal densities (goat, sheep, and cattle) were included at the level of the four-digit postal code area. Maternal age was categorised as younger than 20, 20–34 and 35 years or older.[19] Ethnic background of the mother was classified by the healthcare provider as Western or non-Western, the latter consisting largely of ethnic groups from Surinam, Morocco and Turkey.[19] Smoking behaviour was classified by the healthcare provider as heavy (>20 cigarettes per day) and non-heavy.[20]

At four-digit postal-code area level, SES, degree of urbanisation and animal densities (goat, sheep and cattle) were included in the analysis as confounding variables. SES was estimated from the woman's postal-code (four-digits) using mean income level, employment and education level.[21, 22] The SES was categorised as low (≤25th centile), average (26–74th centile), and high (≥75th centile). Degree of urbanisation was based on information at municipality level supplied by Statistics Netherlands and was translated to four-digit postal-code area.[23] For the year 2003, no degree of urbanisation was available from Statistics Netherlands. We assumed that it differed little from the following year and therefore, applied figures for 2004 to the year 2003. Degree of urbanisation was categorised into five categories ranging from highly urbanised (≥2500 addresses per km^2) to not urbanised (<500 addresses per km^2). As Q fever-affected areas generally have high livestock densities, we assumed that zoonotic infections other than Q fever might occur in these areas. Some of the infections that can cause adverse pregnancy outcome in humans are brucellosis, toxoplasmosis, and infections with the bacteria *Chlamydia psittaci* and *Chlamydia abortus*.[24-28] Therefore, as a proxy for those zoonotic infections, we considered the animal densities as confounders. The number of goats, sheep and cattle was based on information at municipality level supplied by Statistics Netherlands and was translated to four-digit postal-code area. In the number of cattle, we excluded veal calves, because they skewed the expected distribution of cattle. The animal densities per square kilometre were calculated per four-digit postal-code area and divided into three categories of equal size for all postal-code areas in the Netherlands.

DATA ANALYSIS

To investigate whether there were statistically significant differences in characteristics between the areas affected and not affected by Q fever, for the periods 2003–2004 and 2008–2010, and to investigate differences for the three pregnancy outcomes between the areas affected and not affected by Q fever in different years, we used the χ^2 test. To determine the association between residing in a Q fever-affected area and the three adverse pregnancy outcomes, we performed multivariable multilevel analyses, using the four-digit postal-code number as cluster variable, adjusting for maternal age, ethnic background, smoking behaviour, SES, urbanisation degree, and animal densities (goat, sheep and cattle). An interaction term consisting of period (before and during the Q fever outbreak) with residing in a Q fever-affected area (yes or no) was included in the model. The index group was defined as the pregnancy outcomes in 2008–2010 in the areas affected by Q fever. The reference group was defined as the pregnancy outcomes in the areas not affected by Q fever in 2008–2010 combined with outcomes in areas affected and unaffected in the preoutbreak years of 2003–2004. In our study, the interaction term was the most important result, as we were able to estimate only the effect of residing in a Q fever-affected area during 2008–2010, and not the already existing differences before the outbreak. Next, this analysis was repeated with a Q fever incidence variable in which incidence was divided into four categories to determine whether there was a dose–response relationship. In addition, we estimated for the statistically significant associations the population-attributable fraction (PAF) with accompanying 95% CIs [29], which represents the estimated proportion of the adverse pregnancy outcomes that is attributable to residing in a Q fever-affected area, if in fact there is a causal relation. With the PAF, we estimated the number of women who had a negative pregnancy outcome due to residing in a Q fever-affected area in the worst-case scenario. The worst-case scenario was based on the published estimate that one acute Q fever notification represents 12.6 incident infections.[30] Finally, we performed a stratified analysis for women who were in their first trimester or in their second to third trimester of pregnancy in April and May, the months with highest Q fever transmission to humans.[3] A *P*-value <0.05 indicated statistical significance. Missing values occurred for only 1% of the outcome variable and for all confounders. These were imputed once with single imputation, using R software.[31, 32] The other analyses were performed using SAS software V.9.3 (SAS institute, Cary, North Carolina, USA).

RESULTS

We identified 307 postal-code areas with two or more Q fever notifications in one of the outbreak years, and 921 areas not affected by any Q fever notification (Figure 4.1). There

was a statistically significant difference in all recorded characteristics between the areas affected and not affected by Q fever, for the periods 2003–2004 and 2008–2010. We found the largest differences for ethnic background, SES, urbanisation, goat density and cattle density (Table 4.1).

Table 4.1. Descriptive statistics of all births in Q fever-affected areas and areas not affected by Q fever, in the years 2003 through 2004 and 2008 through 2010

Category	2003-2004		2008-2010	
	Q fever-affected area N, (%)	Area not affected by Q fever N, (%)	Q fever-affected area N, (%)	Area not affected by Q fever N, (%)
Maternal age				
< 20 years	604 (1.4)	2,561 (2.3)	767 (1.3)	2,821 (1.7)
20-34 years	34,311 (79.8)	86,984 (78.5)	46,146 (78.1)	124,123 (77.4)
≥ 35 years	8,094 (18.8)	21,231 (19.2)	12,136 (20.6)	33,474 (20.9)
Data missing	0 (0.0)	7 (<0.1)	0 (0.0)	3 (<0.1)
Ethnic background				
Western	38,482 (89.5)	87,754 (79.2)	52,121 (88.3)	125,101 (78.0)
Non-Western	4,247 (9.9)	22,194 (20.0)	6,745 (11.4)	34,350 (21.4)
Missing	280 (0.6)	835 (0.8)	183 (0.3)	970 (0.6)
Smoking				
Heavy smokers	244 (0.6)	344 (0.3)	210 (0.4)	373 (0.2)
Non-heavy smokers	42,765 (99.4)	110,439 (99.7)	58,839 (99.6)	160,048 (99.8)
Data missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Socio-economic status				
Low	9,248 (21.5)	46,882 (42.3)	15,214 (25.8)	68,886 (42.9)
Average	23,225 (54.0)	45,733 (41.3)	30,085 (50.9)	62,389 (38.9)
High	10,536 (24.5)	18,168 (16.4)	13,750 (23.3)	29,146 (18.2)
Data missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Urbanisation degree				
Very high urban area	3,126 (7.3)	30,643 (27.7)	4,922 (8.3)	48,714 (30.4)
High urban area	7,429 (17.3)	26,779 (24.2)	11,679 (19.8)	39,855 (24.8)
Moderate urban area	14,330 (33.3)	16,632 (15.0)	18,803 (31.8)	24,013 (15.0)
Minor urban area	9,285 (21.6)	18,339 (16.5)	12,689 (21.5)	23,622 (14.7)
Rural area	8,839 (20.5)	18,390 (16.6)	10,956 (18.6)	24,197 (15.1)
Data missing	0 (0.0)	0 (0.0)	0 (0.0)	20 (<0.1)
Goat density				
Low	11,649 (27.1)	46,963 (42.4)	18,829 (31.9)	73,678 (45.9)
Medium	9,577 (22.3)	39,763 (35.9)	8,812 (14.9)	51,179 (31.9)
High	21,783 (50.6)	24,057 (21.7)	31,408 (53.2)	35,564 (22.2)
Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sheep density				
Low	17,893 (41.6)	51,111 (46.1)	28,589 (48.4)	75,135 (46.8)
Medium	17,810 (41.4)	36,943 (33.4)	20,016 (33.9)	51,945 (32.4)
High	7,306 (17.0)	22,729 (20.5)	10,444 (17.7)	33,341 (20.8)
Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cattle density				
Low	10,057 (23.4)	61,176 (55.2)	17,202 (29.1)	92,118 (57.4)
Medium	19,006 (44.2)	29,694 (26.8)	23,571 (39.9)	40,774 (25.4)
High	13,946 (32.4)	19,913 (18.0)	18,276 (31.0)	27,529 (17.2)
Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

In 2003 and 2004, the proportions preterm delivery, child small for gestational age, and perinatal mortality were not statistically significant different in Q fever-affected areas compared with unaffected areas (Table 4.2). In 2008 and 2009, the proportion of child small for gestational age was statistically significantly higher in Q fever-affected areas compared to unaffected areas. There was no statistically significant difference in preterm delivery between the areas affected and not affected by Q fever in all years. The proportion of perinatal mortality was higher in areas not affected by Q fever except for 2010.

Table 4.2. Pregnancy outcome in Q fever-affected areas and areas not affected by Q fever

Year	Area	Number of 4-digit postal-code areas	Number of births	% Preterm delivery†	% Child small for gestational age‡	% Perinatal mortality
2003/ 2004	Q fever	307	42686	6.1	10.4	0.9
	No Q fever	921	109012	6.3	10.2	1.0
2008	Q fever	307	19735	5.9	9.2*	0.7*
	No Q fever	921	52503	6.1	8.5*	0.9*
2009	Q fever	307	19936	5.9	9.3*	0.6*
	No Q fever	921	53221	6.1	8.6*	0.7*
2010	Q fever	307	19066	6.3	8.9	0.7
	No Q fever	921	53213	6.0	8.5	0.7

* *P*-value <0.05

† Preterm delivery outcome was missing for: 2003/2004 (n=2095), 2008 (n=523), 2009 (n=656), 2010 (n=537).

‡ Child small for gestational age outcome was missing for: 2003/2004 (n=1918), 2008 (n=55), 2009 (n=549), 2010 (n=545).

The multivariable analysis confirmed these results (Table 4.3), in which we adjusted for maternal age, ethnic background, smoking behaviour, SES, urbanisation degree, cattle density, goat density and sheep density. For the pregnancy outcome child small for gestational age, the variable residing in a Q fever-affected area had an adjusted OR of 1.10 (95% CI 1.05 to 1.14) and the interaction term residing in a Q fever-affected area × period had an adjusted OR of 1.06 (95% CI 1.01 to 1.12). The first OR of 1.10 reflects that the affected areas and not affected areas already differed before the Q fever outbreak (2003-2004). The second OR of 1.06 implies that the differences between areas were further increased in the period 2008–2010. This means that the selection of the three not affected areas per affected area was not perfect in this study. Therefore, the interaction term is our term of interest.

Table 4.3. Multivariable adjusted association between residing in a Q fever-affected area in 2008-2010 and three adverse pregnancy outcomes

Variable	Category	Preterm delivery OR [95% CI]	Child small for gestational age OR [95% CI]	Perinatal mortality OR [95% CI]
Residing in a Q fever-affected area	Yes	1.00 [0.95-1.05]	1.10 [1.05-1.14]	0.96 [0.85-1.09]
	No	Reference	Reference	Reference
Period	2003-2004	Reference	Reference	Reference
	2008-2010	0.92 [0.89-0.95]	0.82 [0.80-0.84]	0.71 [0.65-0.77]
Interaction term:	Yes, in 2008-2010	1.01 [0.94-1.08]	1.06 [1.01-1.12]	0.87 [0.72-1.05]
Residing in a Q fever-affected area x period†	Reference group*	Reference	Reference	Reference
Age of the mother	< 20 years	1.47 [1.34-1.61]	1.26 [1.17-1.36]	1.17 [0.91-1.51]
	20-35 years	Reference	Reference	Reference
	≥ 35 years	1.02 [0.98-1.06]	1.06 [1.03-1.09]	1.33 [1.21-1.45]
Ethnic background	Western	Reference	Reference	Reference
	Non-Western	1.02 [0.98-1.06]	1.22 [1.18-1.26]	1.68 [1.53-1.85]
Smoking	Heavy smoking	1.71 [1.39-2.09]	3.36 [2.92-3.87]	1.67 [0.98-2.84]
	Non-heavy smoking	Reference	Reference	Reference
Socio-economic status	Low	1.17 [1.11-1.23]	1.33 [1.27-1.39]	1.19 [1.05-1.35]
	Average	1.08 [1.03-1.14]	1.13 [1.09-1.18]	1.09 [0.96-1.22]
	High	Reference	Reference	Reference
Urbanisation degree	Very high urban area	0.94 [0.88-1.00]	1.05 [0.99-1.10]	0.89 [0.77-1.04]
	High urban area	0.97 [0.92-1.03]	1.13 [1.08-1.19]	0.94 [0.81-1.08]
	Moderate urban area	0.98 [0.93-1.04]	1.08 [1.02-1.13]	0.97 [0.85-1.12]
	Minor urban area	0.92 [0.87-0.98]	1.01 [0.97-1.06]	0.86 [0.74-0.99]
	Rural area	Reference	Reference	Reference
Cattle density	Low	Reference	Reference	Reference
	Medium	1.00 [0.95-1.05]	1.02 [0.98-1.06]	1.05 [0.93-1.19]
	High	1.03 [0.97-1.10]	1.00 [0.95-1.05]	1.03 [0.89-1.20]
Goat density	Low	Reference	Reference	Reference
	Medium	1.01 [0.97-1.05]	0.98 [0.95-1.02]	0.98 [0.88-1.09]
	High	0.94 [0.89-0.99]	0.97 [0.93-1.02]	0.99 [0.88-1.13]
Sheep density	Low	Reference	Reference	Reference
	Medium	0.97 [0.92-1.01]	0.95 [0.92-0.98]	1.00 [0.92-1.14]
	High	0.97 [0.93-1.02]	0.92 [0.88-0.96]	1.01 [0.89-1.10]

Number of observations used: 312,420.

* Reference group included pregnancy outcomes in areas not affected by Q fever in 2008-2010 combined with outcomes in areas affected and unaffected by Q fever in the pre-outbreak years of 2003-2004.

† Interaction term of interest, adjusted for confounders age of the mother, ethnic background, smoking, socio-economic status, urbanisation degree, cattle density, goat density, and sheep density.

In contrast, we found no statistically significant association for preterm delivery and perinatal mortality in the Q fever-affected areas in 2008–2010, compared to the reference group.

As expected, there were stronger associated factors for the three adverse pregnancy outcomes in the multivariable analyses compared to residing in a Q fever-affected area, notably heavy smoking, young maternal age, non-Western ethnic background of the mother, and residence in an area with low SES. The PAF for the significant relationship with the outcome child small for gestational age was 0.70% (95% CI 0.07% to 1.34%). This implies that—if there is a causal relation between residing in a Q fever-affected area and adverse pregnancy outcome and if Q fever had not occurred—0.7% of the children

small for gestational age in the Q fever-affected areas could have been prevented. Accordingly, of 5,381 children small for gestational age for women residing in a Q fever-affected area in 2008–2010, 38 could have been attributable to residing in the area and in the worst-case scenario, whereby each notified case represents 12.6 infected people, 475 could have been attributable to residing in that area.

However, we found no clear dose–response relation between a higher incidence of Q fever notifications and all three adverse pregnancy outcomes (Table 4.4 for the main outcomes and Appendix Table 4.A1 for the detailed information).

Table 4.4. Multivariable adjusted association between Q fever incidence in 2008-2010 and three adverse pregnancy outcomes

Variable	Category	Preterm delivery OR [95% CI]	Child small for gestational age OR [95% CI]	Perinatal mortality OR [95% CI]
Interaction term: Incidence Q fever postal-code area x period†	<4.59 notifications/ 10,000 inhabitants, in 2008-2010	1.01 [0.91-1.11]	1.04 [0.96-1.13]	0.86 [0.65-1.14]
	4.59-10.61 notifications/10,000 inhabitants, in 2008-2010	1.04 [0.92-1.16]	1.13 [1.03-1.24]	0.84 [0.62-1.14]
	10.62-21.50 notifications/10,000 inhabitants, in 2008-2010	0.87 [0.76-1.00]	1.03 [0.92-1.15]	0.80 [0.54-1.19]
	≥ 21.51 notifications/ 10,000 inhabitants, in 2008-2010	1.13 [0.98-1.31]	1.04 [0.92-1.16]	1.04 [0.70-1.57]
	Reference group*	Reference	Reference	Reference

Number of observations used: 312,420.

* Reference group included pregnancy outcomes in areas not affected by Q fever in 2008-2010 combined with outcomes in areas affected and unaffected by Q fever in the pre-outbreak years of 2003-2004.

† Adjusted for confounders age of the mother, ethnic background, smoking, socio-economic status, urbanisation degree, cattle density, goat density, and sheep density.

Lastly, we found no evidence for a stronger association between residing in a Q fever-affected area and adverse pregnancy outcomes for women who were in their first trimester of pregnancy during months of high human Q fever incidence, compared to women who were in their second or third trimester (see Appendix Tables 4.A2–4.A4).

DISCUSSION

During the years 2008–2010 of the Q fever outbreak, pregnant women residing in a Q fever-affected area had slightly higher rates of having children small for gestational age compared to the reference group. There were no differences between the two groups in rates of preterm delivery and perinatal mortality. A higher incidence of Q fever notifications was not associated with higher rates of the three adverse pregnancy outcomes. Of the 5,381 children small for gestational age of women residing in a Q fever area in 2008–2010,

38 could have been attributable to residing in that area. In a worst-case scenario, this could have been 475 children. However, this is conditional given the causal relationship between residing in a Q fever-affected area and adverse pregnancy outcome, an assumption for which the present ecological study design can provide no evidence.

STRENGTHS AND LIMITATIONS

Our study has several strengths. First, the registry-based approach with nationwide coverage of Q fever notifications and pregnancies allowed for accurate estimation of regional differences in Q fever incidence and adverse pregnancy outcome. Second, by using a multivariable model with an interaction term, we were able to compare pregnancy outcomes in Q fever areas in 2008–2010 with both the outcomes of the areas without Q fever in 2008–2010 and the outcomes in the period before the Q fever outbreak (in areas with and without Q fever). Therefore, we were able to estimate the effect of residing in a Q fever-affected area in 2008–2010, and not the already existing differences before the outbreak. Finally, adjustment for potential confounding variables at the individual level was possible for those variables that are routinely recorded in PRN.

However, our study has some limitations. First, maternal smoking behaviour is an established risk factor for adverse pregnancy outcome. However, as the PRN records only heavy smoking, the role of smoking may have been underestimated. Second, information on some other potential confounding variables were available only at postal-code area level. For instance, we used livestock animal densities at a postal-code area level, as an indicator for individual exposure to livestock animals. Third, other well-known risk factors for an adverse pregnancy outcome, like body mass index, were not included in the PRN database; therefore, we were not able to adjust for these.[33] Fourth, as the PRN registry does not contain information on early pregnancies, we could not study spontaneous abortion as a possible adverse outcome from acute Q fever infection. Results from previous studies on spontaneous abortion, as an adverse outcome from a *C. burnetii* infection, are inconclusive.[12, 34] Fifth, there were statistically significant differences between areas affected and not affected by Q fever for all characteristics in the periods 2003–2004 and 2008–2010. However, we added these factors to the multivariable model and therefore, adjusted for these differences between the cohorts. Sixth, the classification as 'Q fever-affected area' or 'area not affected by Q fever' was based on notifications of acute Q fever. Such notification requires a positive laboratory result indicating a recent *C. burnetii* infection with a matching clinical presentation (fever, pneumonia or hepatitis). Cases could be over-reported because laboratory criteria cannot always discriminate between acute or past resolved infection because of long-lasting persistence of IgM antibodies and aspecific clinical symptoms.[35] The opposite, that is, under-reporting, applies to Q fever as well as to many other infectious diseases,

because people with illness might not seek medical care or the attending physician might not request microbiological tests. To compensate for under-reporting, we performed a worst case scenario analysis. Next, some misclassification might have occurred, as people might acquire the infection in a different postal code area as in which they live. We assumed this is the case for only a small proportion of infected people as previous studies have shown that residential address is a good proxy for environmental exposure.[36, 37] Lastly, any ecological study is subject to bias when it is used to make inferences about individual effects and is a weaker design compared with an observational study with individual data.

INTERPRETATION OF FINDINGS

Several case reports indicate a high risk for adverse pregnancy outcome after Q fever infection during pregnancy[5, 7-11], but several large community-based studies could not find such a relationship.[13-15] The percentage of people with *C. burnetii* antibodies in the Dutch population increased from 2.4% before the Q fever outbreak to about 12.2% after the outbreak.[38, 39] Despite this very high-attack rate, the present study found that the large Q fever outbreak in the Netherlands posed no major public health threat to pregnant women. However, individual cases with an adverse pregnancy outcome, especially children small for gestational age, might have occurred.

Early detection of infected pregnant women would require nationwide screening, and a previous trial showed that screening to detect acute Q fever infection was not clinically effective.[16] Given the difficulty of making a consistent diagnosis of an acute infection because of the lack of discriminating factors or even absence of clinical factors, repeated serological screening of all pregnant women would be needed to identify a case at risk. In addition, there are uncertainties about the efficacy and adverse effects of antibiotic treatment, as only observational studies have been performed on this subject. In retrospect, these findings justify the Dutch approach of not implementing nationwide screening during the 2007–2010 outbreak.

CONCLUSIONS AND IMPLICATIONS

We report a weak association between residing in a Q fever-affected area and the pregnancy outcome of having a child small for gestational age. Early detection of infection would require mass screening of pregnant women and this seems not to be justified based on the results of the present study, the difficulty of making a consistent diagnosis of an acute infection, the lack of discriminating factors or even the absence of clinical factors, and uncertainties about efficacy and adverse effects of antibiotic treatment. However, a case-by-case approach, that is, early diagnosis and treatment of pregnant women with acute Q fever, is recommended.

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CONTRIBUTORS

CWPMH, JMM, PMS and WvdH designed the study. CWPMH, JMM and MMAdL conducted the data analyses. MMAdL drafted the final manuscript. All authors contributed to the analysis of results and writing of the manuscript.

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COMPETING INTERESTS

None declared.

ETHICS APPROVAL

The Board of the PRN approved the study; this included an assessment by a privacy commission.

PROVENANCE AND PEER REVIEW

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DATA SHARING STATEMENT

No additional data are available.

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APPENDIX CHAPTER 4

Table 4.A1. Multivariable adjusted association between Q fever incidence in 2008-2010 and three adverse pregnancy outcomes

Variable	Category	Preterm delivery OR [95% CI]	Child small for gestational age OR [95% CI]	Perinatal mortality OR [95% CI]
Incidence of Q fever in postal-code area	0 notifications/ 10,000 inhabitants	Reference	Reference	Reference
	<4.6 notifications/ 10,000 inhabitants	0.97 [0.90-1.04]	1.10 [1.04-1.18]	0.94 [0.65-1.13]
	4.6-<10.6 notifications/ 10,000 inhabitants	0.97 [0.89-1.06]	1.02 [0.95-1.10]	1.07 [0.72-1.18]
	10.6-<21.5 notifications/ 10,000 inhabitants	1.08 [0.98-1.19]	1.13 [1.04-1.23]	0.92 [0.88-1.31]
	≥ 21.5 notifications/ 10,000 inhabitants	1.03 [0.92-1.15]	1.16 [1.06-1.27]	0.86 [0.78-1.12]
Period	2003-2004	Reference	Reference	Reference
	2008-2010	0.92 [0.89-0.95]	0.82 [0.80-0.84]	0.71 [0.65-0.77]
Interaction term: Incidence Q fever postal-code area x period†	<4.59 notifications/ 10,000 inhabitants, in 2008-2010	1.01 [0.91-1.11]	1.04 [0.96-1.13]	0.86 [0.65-1.14]
	4.59-10.61 notifications/10,000 inhabitants, in 2008-2010	1.04 [0.92-1.16]	1.13 [1.03-1.24]	0.84 [0.62-1.14]
	10.62-21.50 notifications/10,000 inhabitants, in 2008-2010	0.87 [0.76-1.00]	1.03 [0.92-1.15]	0.80 [0.54-1.19]
	≥ 21.51 notifications/ 10,000 inhabitants, in 2008-2010	1.13 [0.98-1.31]	1.04 [0.92-1.16]	1.04 [0.70-1.57]
	Reference group*	Reference	Reference	Reference
Age of the mother	< 20 years	1.47 [1.34-1.61]	1.26 [1.17-1.36]	1.18 [0.91-1.52]
	20-35 years	Reference	Reference	Reference
	≥ 35 years	1.02 [0.98-1.06]	1.06 [1.03-1.09]	1.33 [1.21-1.45]
Ethnic background	Western	Reference	Reference	Reference
	Non-Western	1.02 [0.98-1.06]	1.22 [1.18-1.26]	1.68 [1.53-1.85]
Smoking	Heavy smoking	1.72 [1.40-2.10]	3.38 [2.94-3.89]	1.67 [0.98-2.85]
	Non-heavy smoking	Reference	Reference	Reference
Socio-economic status	Low	1.17 [1.11-1.22]	1.33 [1.27-1.39]	1.19 [1.05-1.34]
	Average	1.08 [1.03-1.13]	1.14 [1.09-1.18]	1.09 [0.96-1.22]
	High	Reference	Reference	Reference
Urbanisation degree	Very high urban area	0.94 [0.88-1.00]	1.05 [0.99-1.11]	0.90 [0.77-1.04]
	High urban area	0.98 [0.92-1.03]	1.13 [1.08-1.19]	0.94 [0.82-1.08]
	Moderate urban area	0.98 [0.93-1.04]	1.08 [1.03-1.13]	0.98 [0.85-1.12]
	Minor urban area	0.93 [0.88-0.98]	1.02 [0.97-1.07]	0.86 [0.74-0.99]
	Rural area	Reference	Reference	Reference
Cattle density	Low	Reference	Reference	Reference
	Medium	1.00 [0.95-1.05]	1.02 [0.98-1.07]	1.05 [0.93-1.18]
	High	1.03 [0.97-1.09]	1.00 [0.94-1.05]	1.03 [0.89-1.20]
Goat density	Low	Reference	Reference	Reference
	Medium	1.01 [0.97-1.05]	0.98 [0.95-1.02]	0.98 [0.88-1.08]
	High	0.93 [0.88-0.98]	0.97 [0.93-1.01]	1.00 [0.88-1.13]
Sheep density	Low	Reference	Reference	Reference
	Medium	0.96 [0.93-1.00]	0.95 [0.92-0.98]	1.00 [0.91-1.10]
	High	0.97 [0.92-1.02]	0.92 [0.88-0.96]	1.01 [0.90-1.14]

Number of observations used: 312,420.

* Reference group included pregnancy outcomes in areas not affected by Q fever in 2008-2010 combined with outcomes in areas affected and unaffected by Q fever in the pre-outbreak years of 2003-2004.

† Interaction term of interest, adjusted for confounders age of the mother, ethnic background, smoking, socio-economic status, urbanisation degree, cattle density, goat density, and sheep density.

Table 4.A2. Multivariable adjusted association between residing in a Q fever-affected area in 2008-2010 and preterm delivery, comparison who was and was not in their 1st trimester during the months with the highest human Q fever transmission

Variable	Category	In 1st trimester OR [95% CI]	In 2nd or 3rd trimester OR [95% CI]
Residing in a Q fever-affected area	Yes	0.99 [0.91-1.07]	1.00 [0.94-1.07]
	No	Reference	Reference
Period	2003-2004	Reference	Reference
	2008-2010	0.92 [0.87-0.97]	0.92 [0.88-0.96]
Interaction term:	Yes, in 2008-2010	1.00 [0.90-1.11]	1.01 [0.93-1.10]
Residing in a Q fever-affected area x period†	Reference group*	Reference	Reference
Age of the mother	< 20 years	1.53 [1.32-1.77]	1.43 [1.26-1.61]
	20-35 years	Reference	Reference
	≥ 35 years	1.03 [0.97-1.09]	1.01 [0.97-1.06]
Ethnic background	Western	Reference	Reference
	Non-Western	1.00 [0.94-1.07]	1.03 [0.98-1.09]
Smoking	Heavy smoking	1.68 [1.21-2.32]	1.73 [1.33-2.24]
	Non-heavy smoking	Reference	Reference
Socio-economic status	Low	1.16 [1.08-1.25]	1.17 [1.10-1.25]
	Average	1.06 [0.98-1.14]	1.10 [1.03-1.16]
	High	Reference	Reference
Urbanisation degree	Very high urban area	0.96 [0.87-1.05]	0.93 [0.86-1.00]
	High urban area	1.00 [0.92-1.09]	0.96 [0.90-1.03]
	Moderate urban area	1.02 [0.93-1.11]	0.96 [0.89-1.02]
	Minor urban area	0.99 [0.91-1.07]	0.88 [0.82-0.95]
	Rural area	Reference	Reference
Cattle density	Low	Reference	Reference
	Medium	1.00 [0.93-1.07]	1.00 [0.94-1.06]
	High	1.04 [0.95-1.14]	1.03 [0.95-1.11]
Goat density	Low	Reference	Reference
	Medium	1.05 [0.98-1.12]	0.99 [0.94-1.04]
	High	0.97 [0.89-1.04]	0.92 [0.87-0.98]
Sheep density	Low	Reference	Reference
	Medium	0.94 [0.89-1.00]	0.99 [0.94-1.03]
	High	0.96 [0.89-1.03]	0.98 [0.92-1.04]

Number of observations used: pregnancy outcomes of women in their 1st trimester: 186,236, pregnancy outcomes of women in their 2nd or 3rd trimester: 126,184.

* Reference group included pregnancy outcomes in areas not affected by Q fever in 2008-2010 combined with outcomes in areas affected and unaffected by Q fever in the pre-outbreak years of 2003-2004.

† Interaction term of interest, adjusted for confounders age of the mother, ethnic background, smoking, socio-economic status, urbanisation degree, cattle density, goat density, and sheep density.

Table 4.A3. Multivariable adjusted association between residing in a Q fever-affected area in 2008-2010 and child small for gestational age, comparison who was and was not in their 1st trimester during the months with the highest human Q fever transmission

Variable	Category	In 1st trimester OR [95% CI]	In 2nd or 3rd trimester OR [95% CI]
Residing in a Q fever-affected area	Yes	1.14 [1.07-1.22]	1.07 [1.02-1.13]
	No	Reference	Reference
Period	2003-2004	Reference	Reference
	2008-2010	0.80 [0.76-0.83]	0.83 [0.80-0.86]
Interaction term:	Yes, in 2008-2010	1.03 [0.95-1.12]	1.08 [1.01-1.16]
Residing in a Q fever-affected area x period†	Reference group*	Reference	Reference
Age of the mother	< 20 years	1.18 [1.04-1.34]	1.33 [1.21-1.47]
	20-35 years	Reference	Reference
	≥ 35 years	1.03 [0.98-1.08]	1.07 [1.03-1.12]
Ethnic background	Western	Reference	Reference
	Non-Western	1.21 [1.15-1.27]	1.25 [1.20-1.30]
Smoking	Heavy smoking	2.99 [2.38-3.75]	3.58 [3.00-4.28]
	Non-heavy smoking	Reference	Reference
Socio-economic status	Low	1.33 [1.25-1.42]	1.35 [1.28-1.42]
	Average	1.13 [1.06-1.20]	1.14 [1.08-1.20]
	High	Reference	Reference
Urbanisation degree	Very high urban area	1.09 [1.01-1.18]	1.00 [0.94-1.06]
	High urban area	1.15 [1.06-1.23]	1.11 [1.05-1.18]
	Moderate urban area	1.10 [1.03-1.19]	1.04 [0.98-1.11]
	Minor urban area	1.03 [0.96-1.11]	1.00 [0.94-1.06]
	Rural area	Reference	Reference
Cattle density	Low	Reference	Reference
	Medium	1.00 [0.94-1.07]	1.03 [0.95-1.08]
	High	0.99 [0.91-1.07]	1.01 [0.95-1.07]
Goat density	Low	Reference	Reference
	Medium	0.97 [0.92-1.03]	0.98 [0.94-1.03]
	High	0.95 [0.89-1.02]	0.98 [0.93-1.04]
Sheep density	Low	Reference	Reference
	Medium	0.96 [0.91-1.01]	0.94 [0.90-0.98]
	High	0.95 [0.89-1.01]	0.90 [0.85-0.94]

Number of observations used: pregnancy outcomes of women in their 1st trimester: 186,236, pregnancy outcomes of women in their 2nd or 3rd trimester: 126,184.

* Reference group included pregnancy outcomes in areas not affected by Q fever in 2008-2010 combined with outcomes in areas affected and unaffected by Q fever in the pre-outbreak years of 2003-2004.

† Interaction term of interest, adjusted for confounders age of the mother, ethnic background, smoking, socio-economic status, urbanisation degree, cattle density, goat density, and sheep density.

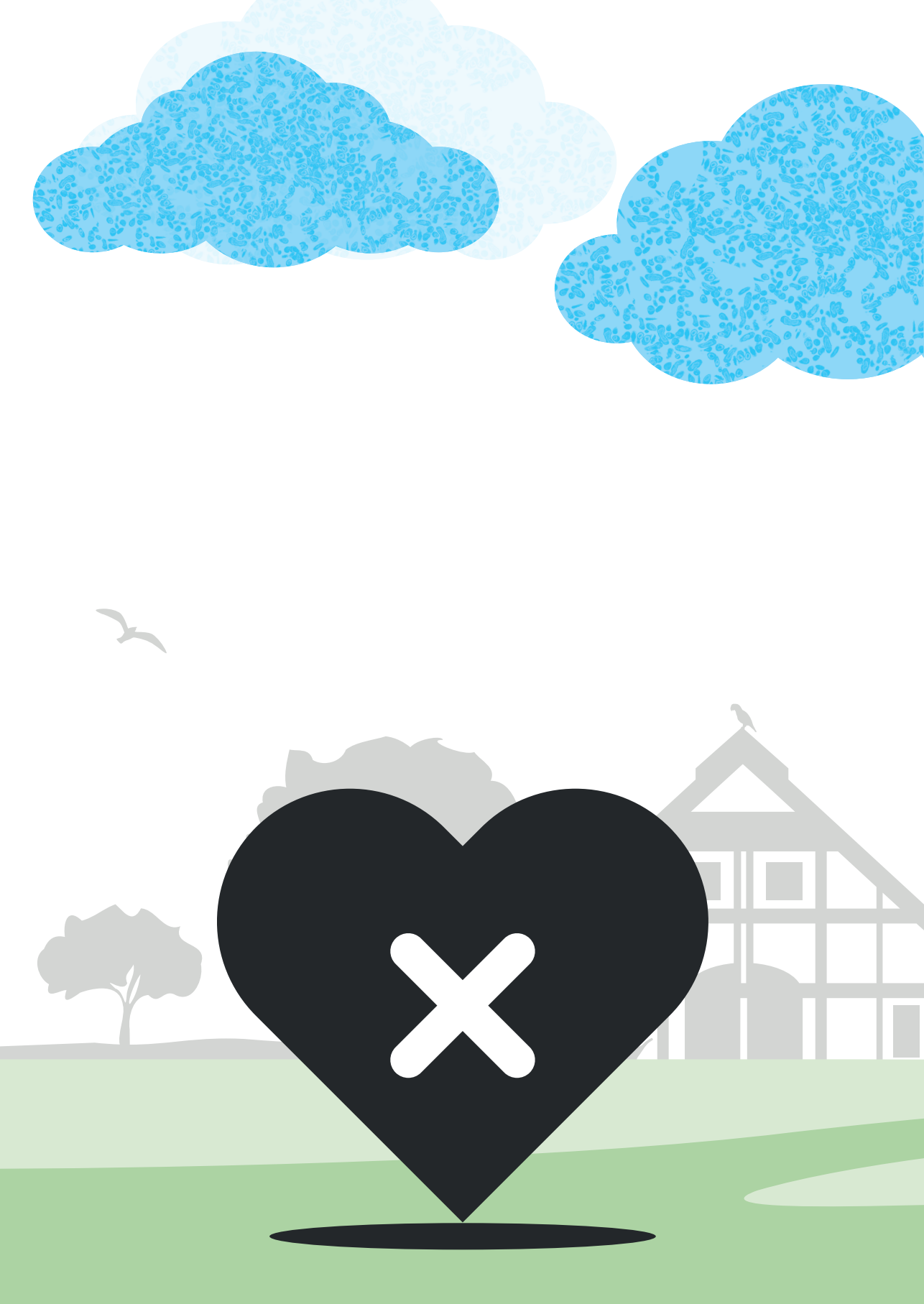
Table 4.A4. Multivariable adjusted association between residing in a Q fever-affected area in 2008-2010 and perinatal death, comparison who was and was not in their 1st trimester during the months with the highest human Q fever transmission

Variable	Category	In 1st trimester OR [95% CI]	In 2nd or 3rd trimester OR [95% CI]
Residing in a Q fever-affected area	Yes	0.94 [0.77-1.14]	0.97 [0.93-1.14]
	No	Reference	Reference
Period	2003-2004	Reference	Reference
	2008-2010	0.64 [0.55-0.74]	0.76 [0.68-0.85]
Interaction term:	Yes, in 2008-2010	0.80 [0.59-1.08]	0.91 [0.72-1.14]
Residing in a Q fever-affected area x period†	Reference group*	Reference	Reference
Age of the mother	< 20 years	1.29 [0.88-1.89]	1.10 [0.78-1.55]
	20-35 years	Reference	Reference
	≥ 35 years	1.25 [1.08-1.45]	1.38 [1.23-1.55]
Ethnic background	Western	Reference	Reference
	Non-Western	1.61 [1.38-1.87]	1.75 [1.55-1.98]
Smoking	Heavy smoking	2.14 [1.01-4.56]	1.36 [0.64-2.89]
	Non-heavy smoking	Reference	Reference
Socio-economic status	Low	1.28 [1.05-1.56]	1.14 [0.98-1.33]
	Average	1.17 [0.97-1.42]	1.03 [0.89-1.20]
	High	Reference	Reference
Urbanisation degree	Very high urban area	1.07 [0.84-1.35]	0.80 [0.66-0.97]
	High urban area	1.00 [0.79-1.25]	0.91 [0.76-1.08]
	Moderate urban area	1.12 [0.89-1.40]	0.90 [0.76-1.06]
	Minor urban area	0.96 [0.77-1.22]	0.80 [0.67-0.95]
	Rural area	Reference	Reference
Cattle density	Low	Reference	Reference
	Medium	1.10 [0.91-1.32]	1.03 [0.88-1.20]
	High	1.00 [0.78-1.27]	1.06 [0.88-1.28]
Goat density	Low	Reference	Reference
	Medium	1.12 [0.95-1.31]	0.89 [0.78-1.02]
	High	0.96 [0.78-1.17]	1.01 [0.86-1.19]
Sheep density	Low	Reference	Reference
	Medium	0.95 [0.82-1.10]	1.04 [0.93-1.17]
	High	0.96 [0.79-1.16]	1.05 [0.90-1.22]

Number of observations used: pregnancy outcomes of women in their 1st trimester: 186,236, pregnancy outcomes of women in their 2nd or 3rd trimester: 126,184.

* Reference group included pregnancy outcomes in areas not affected by Q fever in 2008-2010 combined with outcomes in areas affected and unaffected by Q fever in the pre-outbreak years of 2003-2004.

† Interaction term of interest, adjusted for confounders age of the (1)mother, ethnic background, smoking, socio-economic status, urbanisation degree, cattle density, goat density, and sheep density.



CHAPTER 5

SHOULD ACUTE Q FEVER PATIENTS BE SCREENED FOR VALVULOPATHY TO PREVENT ENDOCARDITIS?

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ABSTRACT

BACKGROUND

Echocardiographic screening of acute Q fever patients and antibiotic prophylaxis for patients with cardiac valvulopathy is considered an important approach to prevent chronic Q fever-related endocarditis. During a large Q fever epidemic in the Netherlands, routine screening echocardiography was discontinued, raising controversy in the international literature. We followed a cohort of acute Q fever patients to estimate the risk for developing chronic Q fever, and we evaluated the impact of screening in patients who were not yet known to have a valvulopathy.

METHODS

The study population consisted of patients diagnosed with acute Q fever in 2007 and 2008. We retrospectively reviewed all screening echocardiographs and checked for development of chronic Q fever 8 years after the acute episode. Risks of developing chronic Q fever in relation to the presence or absence of valvulopathy were analyzed with logistic regression.

RESULTS

The cohort included 509 patients, of whom 306 received echocardiographic screening. There was no significant difference (*P*-value = 0.22) in occurrence of chronic Q fever between patients with a newly detected valvulopathy (2/84, 2.4%) and those with no valvulopathy (12/202, 5.9%). Two patients with a newly detected valvulopathy, who did not receive antibiotic prophylaxis, developed chronic Q fever at a later stage.

CONCLUSIONS

We found no difference in outcome between patients with and without a valvulopathy newly detected by echocardiographic screening. In retrospect, the 2 above-mentioned patients could have benefitted from antibiotic prophylaxis, but its omission must be weighed against the unnecessary large-scale and long-term use of antibiotics that would have resulted from universal echocardiographic screening.

INTRODUCTION

Q fever is a zoonosis caused by the bacterium *Coxiella burnetii*.[1] Acute Q fever mainly presents as febrile illness, atypical pneumonia, or hepatitis. However, almost 60% of acute *C. burnetii* infections remain asymptomatic, and 1–5% of acute Q fever infections will develop into chronic Q fever.[1, 2]

In the Netherlands, vascular infection is the most common clinical presentation of chronic Q fever, in contrast to other countries, such as France, where endocarditis predominates.[3, 4] Clinical manifestations leading to the diagnosis of endocarditis are not specific, posing a challenge to the clinician.[1] Early detection and treatment of endocarditis and other presentations of chronic infection may prevent prolonged morbidity, complications, and fatal outcomes.[2, 5, 6] Main risk factors for developing endocarditis are older age and underlying cardiac valvulopathy.[7, 8] In symptomatic acute Q fever patients with valvulopathy, the estimated risk of developing endocarditis is 39%.[8] Progression to endocarditis has been reported in patients with undiagnosed and clinically silent valvulopathies.[9] Physicians are therefore encouraged to detect at-risk patients by echocardiographic screening of all acute Q fever patients for presence of valvulopathy.[8, 9] In France, indeed, echocardiography is part of the standard work-up for all acute Q fever patients.

Initially, when the Netherlands faced its first outbreak of Q fever in 2007, referral of all acute Q fever patients for echocardiography was recommended to all treating physicians. However, when the number of acute Q fever patients sharply increased in 2008, cardiologists became increasingly reluctant to continue routine screening, as it drained a lot of resources and yielded many valvulopathies classified as "minor," with no clinical significance. Additionally, none of the patients with mostly minor valvulopathies were diagnosed with chronic Q fever.[10, 11] Thus, screening was discontinued, and French studies estimate that 100 Dutch cases of endocarditis may have been missed and that half of them could have been prevented with antibiotic prophylaxis.[12]

In the present study, we followed a large cohort of acute Q fever patients over 8 years to estimate the risk for developing chronic Q fever.[13] Our aim was to evaluate the impact of echocardiographic screening in patients who were not yet known to have a valvulopathy. Our results may help to improve screening policies in future outbreaks.

METHODS

PATIENT ENROLLMENT

We invited patients who were diagnosed with Q fever in 2007 and 2008 and who had participated in the Q-HORT study. This was a 4-year follow-up study of patients diagnosed with acute Q fever in 2007–2009 that aimed to detect chronic Q fever cases.[13] All patients enrolled in the Q-HORT study were diagnosed with Q fever at the Laboratory of Medical Microbiology of the Jeroen Bosch Hospital, the regional diagnostic facility serving Bernhoven Hospital in Uden, Jeroen Bosch Hospital in 's-Hertogenbosch, and most general practitioners in the catchment areas of these 2 hospitals. The laboratory is located in the epicenter of the 2007–2010 outbreak, where approximately 80% of all reported Q fever cases in 2007 and 2008 were diagnosed.[14] The present study included Q-HORT patients diagnosed in 2007 and 2008, because only during this period were clinicians instructed to refer acute Q fever patients to a cardiologist for a single screening by echocardiography.

During the outbreak, the standard work-up after diagnosis of acute Q fever consisted of serological testing at 3, 6, and 12 months. After 4 years, an additional test was performed in the context of Q-HORT. Before Q-HORT enrolment, 4 years after their acute Q fever diagnosis, potential participants received an informed consent form. Next, the persons who participated in the Q-HORT study filled in a questionnaire. The questionnaire gathered data on general demographics and risk factors for chronic Q fever. Patients who were diagnosed with chronic Q fever based on the first blood sample submitted to the laboratory were excluded, as it was too late to follow their course of Q fever development. Patients with an immunoglobulin G (IgG) phase II titer ≤1:32 in all follow-up samples were likewise excluded, as they did not meet the case definition of a laboratory-confirmed acute Q fever case.

ACUTE Q FEVER DIAGNOSIS

One of 3 laboratory criteria had to be met for the diagnosis of acute Q fever [13]:

(1) Both immunoglobulin M (IgM) and IgG phase II antibody titers ≥1:32 at diagnosis as determined by immunofluorescence assay (IFA; Focus Diagnostics, Inc., Cypress, CA, USA), with IgG phase II ≥1:64 during follow-up;
(2) IgM phase II positive and IFA IgG phase II ≥1:32 at diagnosis by enzyme-linked immunosorbent assay (Virion\Serion, Würzburg, Germany), with IgG phase II ≥1:64 during follow-up;
(3) a positive polymerase chain reaction (PCR; in-house assay [15]) result preceding seroconversion in IFA, with IgG phase II ≥1:64 during follow-up.

ECHOCARDIOGRAPHY

During the Q fever outbreak in 2007, public health authorities informed treating physicians as to the need for echocardiographic screening after every notification of acute Q fever.[8, 9]

Patients were screened with transthoracic echocardiography (TTE), and transoesophageal echocardiography was performed when the TTE results were inconclusive. The cardiologists of both involved hospitals interpreted all echocardiographs according to the European Society of Cardiology guidelines. The aortic, mitral, pulmonic, and tricuspid valves were examined for regurgitation and stenosis. Per diagnosis (e.g. aortic regurgitation, aortic stenosis, mitral regurgitation), patients were subdivided into groups with no, mild, moderate, or severe valvulopathy.[16] For the present study, we collected echocardiographic results by reviewing medical records, excluding patients with an echocardiograph taken more than 1 year after acute Q fever diagnosis.

DEFINITIONS OF BASELINE CHARACTERISTICS AND CHRONIC Q FEVER

For baseline characteristics, we used the Q-HORT questionnaire data, which were collected 4 years after the acute Q fever diagnosis. For the patients who died between this diagnosis and the Q-HORT invitation, and for whom therefore no questionnaire was available, we obtained baseline characteristics from medical files at both hospitals. Additionally, serological results at time of diagnosis, at 3-, 6-, and 12-month follow-up, and at 4 years after diagnosis were obtained from the Q-HORT database. In the Netherlands, chronic Q fever is not a notifiable disease. However, since 2007, all chronic Q fever cases are included in a national chronic Q fever database, which is maintained by the University Medical Centre in Utrecht. In 2016, approximately 8 years after the diagnosis, we checked the national chronic Q fever database to see whether more patients had been diagnosed with chronic Q fever. For the patients who underwent echocardiography at time of diagnosis, we checked whether they were known to have valvulopathy before the Q fever diagnosis. Next, we searched the medical records for information about the chronic Q fever cases, such as additional risk factors and antibiotic treatment. Based on all available information, cases were subdivided into no, possible, probable, or proven chronic Q fever, as suggested by the Dutch Q fever Consensus Group. See Appendix Table 5.A1.[17] We categorized patients into the highest classification they received during follow-up.

STATISTICAL ANALYSIS

In order to prevent survivor bias, we included patients who died between the diagnosis of acute Q fever and the Q-HORT invitation 4 years later. We performed the χ^2 test, Fisher exact test, and the unpaired T-test to compare the characteristics of patients with and without echocardiographic screening. We performed univariable and multivariable

logistic regression analysis, corrected for sex and age at time of diagnosis, to investigate whether the development of chronic Q fever differed significantly between patients with and without valvulopathy. In this analysis, we excluded patients with known valvulopathy before Q fever diagnosis, as echocardiographic screening was not intended for that patient group. Lastly, we performed univariable and multivariable analyses restricted to probable and proven chronic Q fever cases. A *P*-value of <0.05 was considered statistically significant. SAS version 9.4 was used for the analyses (SAS Institute Inc., USA).

ETHICAL PERMISSION

The Medical Ethical Committee Brabant (METC Brabant) approved the Q-HORT study. The Internal Review Board of Jeroen Bosch Hospital approved the present study. The Q-HORT informed consent form included permission to access medical data. We enrolled only patients who gave permission to review all available echocardiographic, clinical, and laboratory data. We obtained information about deceased patients in accordance with the Medical Treatment Contracts Act (article 458).

RESULTS

In 2011 and 2012, 519 acute Q fever patients, who were diagnosed in 2007 or 2008, participated in the Q-HORT study. We excluded 10 persons from analyses, as 1 already had chronic Q fever at time of the diagnosis; 3 had an IgG phase I and/or IgG phase II titer ≤1:32 in all follow-up samples; 3 underwent echocardiography >1 year after diagnosis, and for 3 the echocardiography report could not be traced.

Table 5.1 shows baseline characteristics for the remaining 509 patients, 4 years after their acute Q fever diagnosis. Of the total, 306 (60.1%) patients underwent echocardiographic screening at time of diagnosis. Of these 306, 20 were already diagnosed with 1 or more valvulopathies before the acute Q fever episode (Figure 5.1). In those not screened, 8 of the 203 (3.9%) were diagnosed with chronic Q fever, 1 of whom had proven chronic Q fever and 7 had possible chronic Q fever. In screened patients with unknown valvulopathy status at time of acute Q fever diagnosis, 14 of 286 (4.9%) patients were diagnosed with chronic Q fever during 4-year follow-up. Of these, 10 had no valvulopathy or other risk factor at time of screening and were classified as possible chronic Q fever based on serological findings. Of the other 4 patients, 2 were classified as probable chronic Q fever, and 2 as proven chronic Q fever. Additionally, among 20 screened patients with known valvulopathy at time of acute Q fever diagnoses, 4 (20.0%) were diagnosed with chronic Q fever, of whom 1 had proven and 3 had probable chronic Q fever. Of the 5 probable chronic Q fever cases in this cohort of 509 acute Q fever patients, 1 was

immunocompromised and the other 4 had a valvulopathy. Approximately 8 years after the diagnosis, or 4 years after Q-HORT participation, no additional patients were diagnosed with chronic Q fever. One person in the entire cohort received antibiotic prophylaxis. This person was known to have a valvulopathy (mild stenosis of aortic valve after valve replacement with a mechanical prosthesis) at the time of acute Q fever diagnosis and did not develop chronic Q fever. None of the patients with a newly detected valvulopathy was treated prophylactically with antibiotics.

Table 5.1. Baseline characteristics of acute Q fever patients, four years after their diagnosis in 2007 or 2008

Characteristic	Patients without echocardiography (N=203) number/N (%)	Patients with echocardiography (N=306)* number/N (%)	*P*-value†
Sex, male	117/203 (57.6)	178/306 (58.2)	0.905
Age at time acute Q fever diagnosis, years (± SD)	49.9 ± 13.1‡	53.0 ± 12.3‡	0.008§
Aortic aneurysm	3/203 (1.5)	2/305 (0.7)	0.393¶
Vascular prosthesis	4/203 (2.0)	5/305 (1.6)	1.000¶
Heart valve prosthesis	1/203 (0.5)	5/305 (1.6)	0.410¶
Myocardial infarction	13/203 (6.4)	14/305 (4.6)	0.372
Coronary artery procedure#	15/203 (7.4)	22/305 (7.2)	0.940
Peripheral arterial procedure**	8/203 (3.9)	1/305 (0.3)	0.004¶
Pacemaker	1/203 (0.5)	9/305 (3.0)	0.057¶
Rheumatoid arthritis	15/203 (7.4)	16/305 (5.3)	0.323
Crohn's disease/ ulcerative colitis	1/203 (0.5)	3/305 (1.0)	1.000¶
Malignancy in last five years	19/203 (9.4)	23/305 (7.5)	0.466
Chronic renal disease	1/203 (0.5)	4/305 (1.3)	0.653¶
Asthma	7/203 (3.5)	11/305 (3.6)	0.925
COPD	9/203 (4.4)	12/305 (3.9)	0.782
Diabetes	18/203 (8.9)	24/305 (7.9)	0.689
Organ transplantation	0/203 (0.0)	1/304 (0.3)	1.000¶
Pregnancy in last 5 years††	4/82 (4.9)	1/124 (0.8)	0.083¶

Abbreviations: N, total number of that group; SD, standard deviation; COPD, chronic obstructive pulmonary disease.
* Data is missing for one patient, as no questionnaire was completed.
† *P*-values are calculated by chi-square test unless otherwise indicated.
‡ Age is shown as mean ± SD, as age was normally distributed.
§ *P*-values are calculated by unpaired T-test.
¶ *P*-values are calculated by Fisher's exact test.
# Coronary artery procedure includes bypass surgery, percutaneous coronary intervention, or a stent.
** Peripheral arterial procedure includes bypass surgery, angioplasty, or a stent.
†† Calculated for women only.

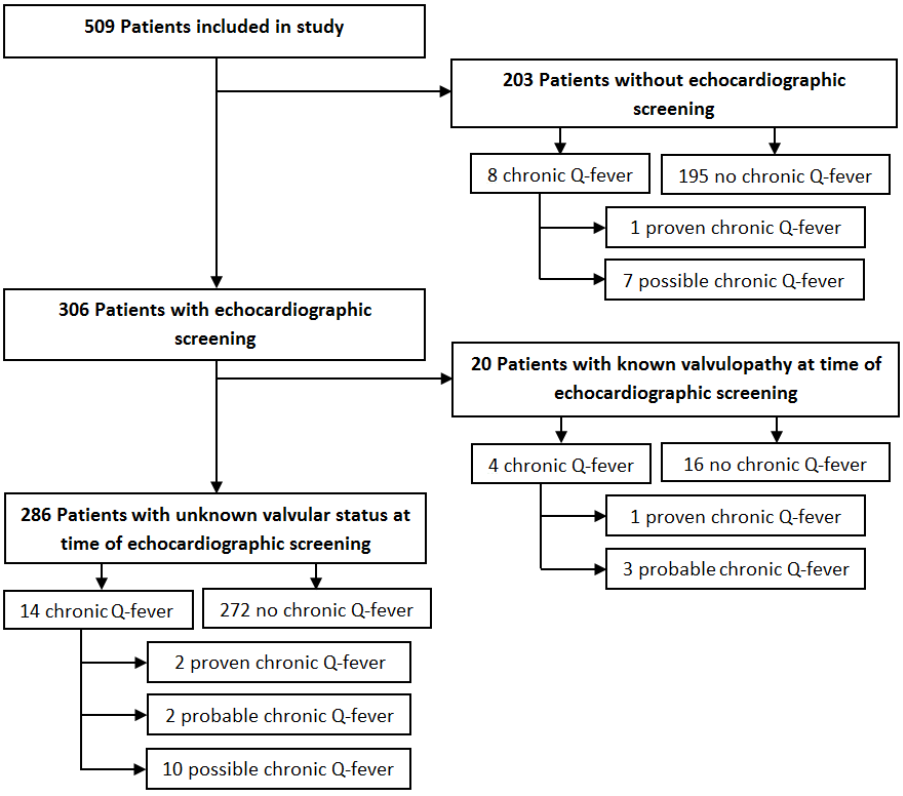

Figure 5.1. Flowchart of included patients

Of the 4 proven chronic Q fever cases in the cohort of 509 acute Q fever patients, 1 had an endocarditis, and 3 had an infected aneurysm (Table 5.2).

In Table 5.3, we present the results of echocardiographic screening after acute Q fever diagnosis, combined for patients with known and unknown valvulopathy at the time of the diagnosis. The prevalence of valvulopathy in this group was 33.7% (103/306). Of the 103 patients with valvulopathy, 85 (82.5%) were classified with 1 or more mild valvulopathies, 16 (15.5%) with 1 or more moderate valvulopathies, and 2 (2.0%) with a severe valvulopathy. Chronic Q fever developed at a later stage in 3 of the 85 patients (3.5%) with mild valvulopathy, 2 of the 16 (12.5%) with moderate valvulopathy, and 1 of the 2 patients (50%) with severe valvulopathy.

Table 5.2. Details of proven chronic Q fever patients

Sex	Age	Known valvulo-pathy*	Valvulo-Pathy†	Diagnosis chronic Q fever (time after acute Q fever)‡	IgG phase I§	Clinical presentation chronic Q fever	PCR result	Antibiotic treatment¶	Antibiotic prophylaxis
M#	70-79	No	No	19 months	1:2048	Infected aneurysm	+	Yes	No
F	70-79	No	Yes; mild AoS	2 months	1:2048	Endocarditis	-	Yes**	No
M	60-69	Yes	Yes; moderate MR, mild TR	3 months	1:4096	Infected aneurysm	+	Yes	No
M#††	50-59	Unknown (no screening echo made)	Unknown (no screening echo made)	3 months	1:4096	Infected aneurysm	+	Yes	No

Abbreviations: echo: echocardiography; IgG, immunoglobulin G; PCR, polymerase chain reaction; M, male; F, female; MR, mitral regurgitation; TR, tricuspid regurgitation; AoS, aortic stenosis.
* Already diagnosed with valvulopathy before diagnosis of acute Q fever.
† Valvulopathy detected at time of screening.
‡ Time between acute Q fever diagnosis and when IgG phase I titre was for the first time ≥ 1:1024, or other clinical sign of chronic infection.
§ Highest IgG phase I during follow-up after diagnosis of acute Q fever.
¶ Consisted of doxycyclin and hydroxychloroquine.
# Died within four years after acute Q fever diagnosis.
** Treated with claritromycin because of an intolerance to doxycyclin, hydroxychloroquine, moxifloxacin and ciproxin.
†† Patient also known to have Klinefelter syndrome.

Table 5.3. Valvulopathy per category of chronic Q fever infection, including patients with known valvulopathy at time of echocardiographic screening, in acute Q fever patients diagnosed in 2007 or 2008*

	Chronic Q fever, number				
	No	Possible	Probable	Proven	Total number (%)
Aortic regurgitation					
No	272	10	5	3	290 (94.8)
Mild	15	0	0	0	15 (4.9)
Moderate	1	0	0	0	1 (0.3)
Severe	0	0	0	0	0 (0.0)
Total	288	10	5	3	306
Aortic stenosis					
No	281	10	4	2	297 (97.1)
Mild	6	0	0	1	7 (2.3)
Moderate	1	0	0	0	1 (0.3)
Severe	0	0	1	0	1 (0.3)
Total	288	10	5	3	306
Mitral regurgitation					
No	232	10	1	2	245 (80.1)
Mild	46	0	3	0	49 (16.0)
Moderate	9	0	1	1	11 (3.6)
Severe	1	0	0	0	1 (0.3)
Total	288	10	5	3	306
Mitral stenosis					
No	286	10	5	3	304 (99.4)
Mild	1	0	0	0	1 (0.3)
Moderate	1	0	0	0	1 (0.3)
Severe	0	0	0	0	0 (0.0)
Total	288	10	5	3	306

Table 5.3 continued.

	Chronic Q fever, number				
	No	Possible	Probable	Proven	Total number (%)
Tricuspid regurgitation					
No	234	10	4	2	250 (81.7)
Mild	50	0	1	1	52 (17.0)
Moderate	4	0	0	0	4 (1.3)
Severe	0	0	0	0	0 (0.0)
Total	288	10	5	3	306
Tricuspid stenosis					
No	287	10	5	3	305 (99.7)
Mild	1	0	0	0	1 (0.3)
Moderate	0	0	0	0	0 (0.0)
Severe	0	0	0	0	0 (0.0)
Total	288	10	5	3	306
Pulmonic regurgitation†					
No	286	10	5	3	304 (99.4)
Mild	2	0	0	0	2 (0.6)
Moderate	0	0	0	0	0 (0.0)
Severe	0	0	0	0	0 (0.0)
Total	288	10	5	3	306
Pulmonic stenosis†					
No	288	10	5	3	306 (100.0)
Mild	0	0	0	0	0 (0.0)
Moderate	0	0	0	0	0 (0.0)
Severe	0	0	0	0	0 (0.0)
Total	288	10	5	3	306

* Multiple valvulopathies are possible in one patient.

† The pulmonic valve is difficult to visualise and not described in every report.

5

Of the 84 screened patients with newly detected valvulopathy at time of acute Q fever diagnosis, two patients developed chronic Q fever (2.4%) during follow-up (Table 5.4). In the group of 202 patients who had no newly detected valvulopathy at time of screening, 12 were diagnosed with chronic Q fever (5.9%). In univariable analysis, valvulopathy was not significantly associated with chronic Q fever (taking all levels together) (odds ratio [OR] = 0.39, 95% confidence interval [CI]: 0.09–1.76, *P*-value = 0.22). In multivariable logistic regression analysis corrected for differences in age and sex, valvulopathy was again no risk factor for developing chronic Q fever (OR = 0.26, 95% CI: 0.06–1.24, *P*-value = 0.09). Additionally, we performed univariable analysis for only the probable and the proven cases, as the possible cases are less likely to be true chronic infections. Again, no statistically significant difference was found between the patients with and without newly detected valvulopathy (OR = 2.32, 95% CI: 0.32–16.73, *P*-value = 0.40). We were not able to perform multivariable analysis for this subgroup, due to the low number of chronic cases.

Table 5.4. Number of chronic Q fever cases, by presence of valvulopathy during echocardiographic screening at the time of acute Q fever diagnosis in 2007 or 2008

	Number chronic Q fever		
	No	**Yes**	**Total**
Valvulopathy			
No	190	12	202
Yes	82	2	84
Total	272	14	286

DISCUSSION

We found no statistically significant difference in development of chronic Q fever between acute Q fever patients with and without valvulopathy detected with screening echocardiography. However, 2 patients with a newly detected valvulopathy did not receive antibiotic prophylaxis and were diagnosed with chronic Q fever later on.

Early in the large Q fever epidemic in the Netherlands, public health authorities instructed clinicians to perform echocardiographic screening in all reported acute Q fever patients. However, the responsibility of referring patients for echocardiography lay with the treating physician, and cardiologists were sometimes reluctant to perform echocardiography without clear clinical indication. Therefore, even at that stage, only slightly more than half of all patients received echocardiographic screening. Furthermore, as no professional guideline existed on how to prevent chronic infection in patients with newly detected valvulopathy, no such patients were prophylactically treated with antibiotics. Later in the epidemic, a small cohort study found no chronic Q fever in patients with newly detected valvulopathy, and echocardiographic screening was not further promoted.[10, 11]

The present study seems to confirm that vascular infection is more common than endocarditis as clinical manifestation of chronic infection.[3] Therefore, screening for aortic aneurysms in patients who present with a primary infection, could be considered as Eldin *et al*. has suggested.[18] However, in France, where many chronic Q fever research is performed, endocarditis seems to predominate [4], due possibly to differences in the virulence of circulating *C. burnetii* strains [1, 19] and different case definitions for chronic Q fever.[20] Additionally, reports from a national reference centre are prone to selection bias, whereas we studied a non-selected group of acute Q fever patients, eliminating sampling and selection bias. Moreover, in our study the time of acute illness was established, and how the patients were diagnosed was well described.

The difference in the incidence of endocarditis may further be explained by a lack of specificity in describing valvular defect severity in retrospective reports.[2, 8, 21] In one

study, the risk for endocarditis in patients with valvulopathy was estimated to be 39%, but many of these patients had a prosthetic valve.[8] In our study, a much lower percentage had prosthetic valves, and mainly minor valvulopathies were diagnosed. Minor, clinically insignificant valvulopathy has a high prevalence in any unselected population.[22] Finally, the vascular infections diagnosed in the Netherlands may be an embolic consequence of clinically silent endocarditis, as Million *et al*. suggested.[4]

Our results have reduced the likelihood of 2 other possible explanations for the difference between the Dutch and French literature. First, given the 8-year follow-up of this study, a possibly prolonged incubation period for endocarditis to become manifest is unlikely to explain the difference. Second, we performed a systematic screening and still found more vascular infections than endocarditis. It seems therefore unlikely that a lack of systematic screening led to underdiagnoses of endocarditis in the Netherlands. However, the numbers are low because the number of chronic Q fever cases in this study was smaller than anticipated.

The primary strength of this study is the large sample size. The Q fever epidemic in the Netherlands provides the largest group of acute Q fever patients ever reported, and the largest one with echocardiographic results. Second, we included a prolonged follow-up with serological results at 3, 6, 12 months, and 4 years. After approximately 8 years, we checked to see if additional acute Q fever patients had been diagnosed with chronic Q fever. Finally, we included all deceased patients to avoid survival bias.

Among study limitations are the potential that the risk of proven chronic Q fever was underestimated, as long-term antibiotic treatment was initiated in 4 patients (1 possible and 3 probable cases). If left untreated, proven chronic Q fever might have developed. However, the risk of chronic Q fever may have been overestimated, as our patients were categorized into the highest classification they received during the 4-year follow-up. For example, 12 patients were classified as possible chronic Q fever based on an IgG phase I titer ≥1:1024, without a risk factor for chronic Q fever. During the follow-up, the titers showed a spontaneous decline in phase I IgG titers (data not shown). Arguably, these patients should not be classified as having a chronic infection. Another limitation is that although the screened and not-screened acute Q fever patients were largely comparable with respect to baseline characteristics, we had no information on the decision process of doctors and patients with respect to screening. Therefore, we cannot rule out some degree of selection bias. Finally, almost 60% of acute Q fever patients are known to remain asymptomatic. These as-yet-unidentified acute Q fever patients could of course not be included in our study despite being at risk for progression to chronic infection.[23]

To decide whether a new screening approach should be implemented, often the Wilson and Jungner criteria are used.[24] Some criteria support screening. First, chronic Q fever is a serious health problem that may lead to death. Next, Dutch hospitals have the health infrastructure to implement this possible screening. Last, an echocardiography is not invasive and is therefore acceptable as screening test. However, some criteria are not supportive. First, if screening is implemented, many acute Q fever patients will receive antibiotic prophylaxis even if chronic Q fever is unlikely to develop without this prophylaxis. Second, as in an earlier study performed in the Netherlands, mostly minor valvulopathies were diagnosed in our study.[10] In Dutch guidelines drawn after the large Q fever outbreaks, definitions are unclear as to which valvular defect and which grade of defect are important risk factors for developing chronic Q fever.[17] Because of our low number of diagnosed chronic Q fever patients, this study can provide no new insight on this issue. Third, the treatment (antibiotic prophylaxis) is not generally accepted, having been investigated only in France in a selected group of patients.[12] Next, long-term treatment with doxycycline and hydroxychloroquine is not without potential complications. Drug-induced photosensitivity is a notorious adverse effect of doxycycline, and long-term use of hydroxychloroquine can lead to retinopathy.[25, 26] Last, the cost-effectiveness of the screening has not been investigated.

In conclusion, we found no difference in Q fever outcome between patients with or without a newly detected valvulopathy at the time of their acute Q fever episode. Additionally, echocardiographic screening would lead to an unnecessary long-term antibiotic use, which is not desirable and which must be included in cost-benefit analysis of screening in future outbreaks. We recommend that Dutch guidelines regarding chronic Q fever should further specify the types and grades of valvulopathy that are most important to prognosis.

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POTENTIAL CONFLICTS OF INTEREST

All authors: No reported conflicts of Interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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APPENDIX CHAPTER 5

Table 5.A1. Dutch consensus guideline on chronic Q fever diagnosis*

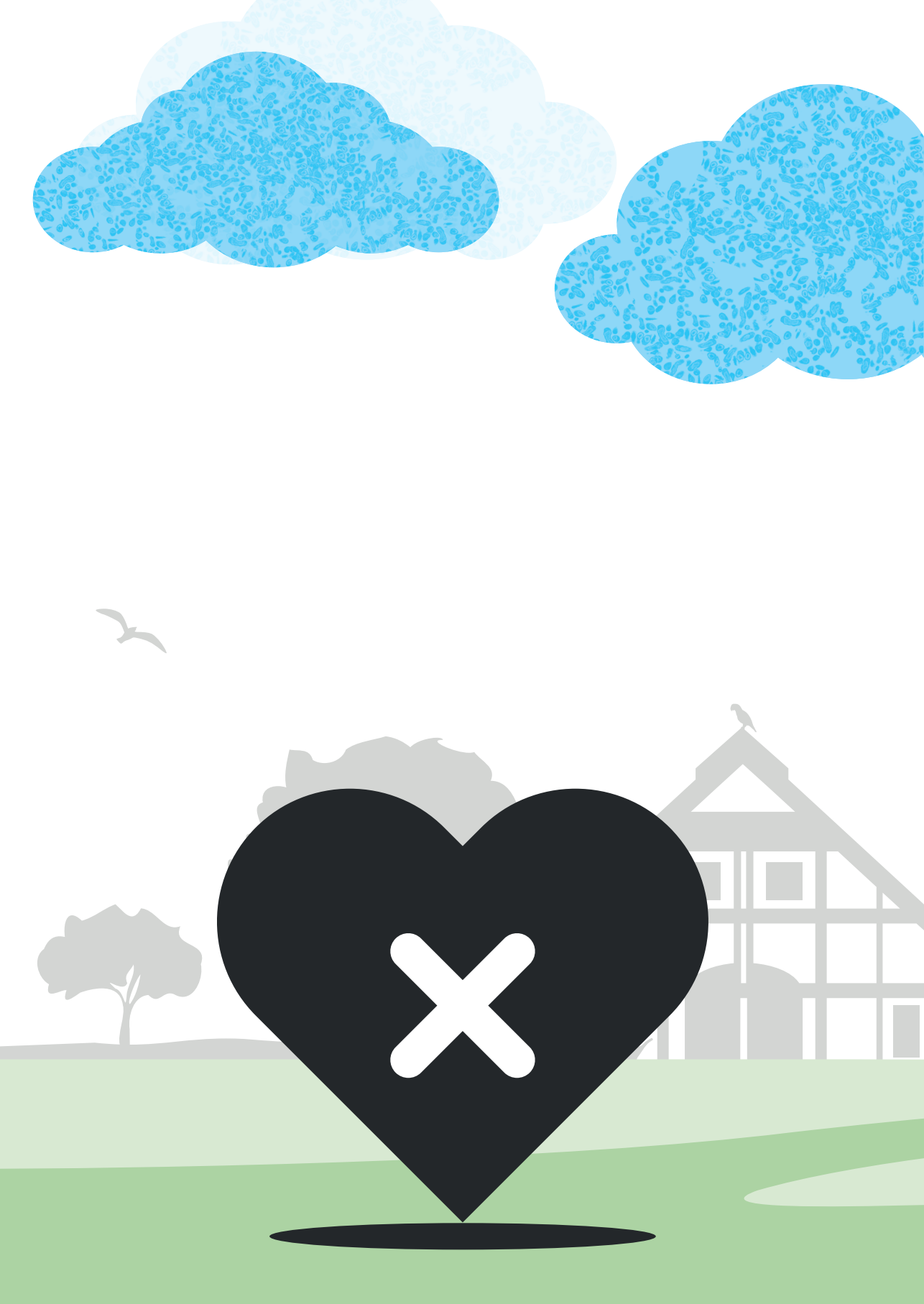
Proven chronic Q fever	Probable chronic Q fever	Possible chronic Q fever
1. Positive *C. burnetii* PCR in blood or tissue† OR 2. IFA ≥ 1:1024 for *Coxiella burnetii* phase I IgG AND - Definite endocarditis according to the modified Duke criteria‡ OR - Proven large vessel or prosthetic infection by imaging studies (^{18}FDG-PET, CT, MRI or AUS)	IFA ≥ 1:1024 for *Coxiella burnetii* phase I IgG AND one or more of following criteria: - Valvulopathy not meeting the major criteria of the modified Duke criteria‡ - Known aneurysm and/or vascular or cardiac valve prosthesis without signs of infection by means of TEE/TTE, ^{18}FDG-PET, CT, MRI or abdominal doppler ultrasound - Suspected osteomyelitis or hepatitis as manifestation of chronic Q fever - Pregnancy - Symptoms and signs of chronic infection, such as fever, weight loss and night sweats, hepato-splenomegaly, persistent raised ESR and CRP - Granulomatous tissue inflammation, proven by histological examination - Immunocompromised state	IFA ≥ 1:1024 for *Coxiella burnetii* phase I IgG without manifestations meeting the criteria for proven or probable chronic Q fever

Abbreviations: PCR, polymerase chain reaction; IFA, immunofluorescence assay; FDG-PET, fluorodeoxyglucosepositron emission tomography; CT, computer tomography; MRI, magnetic resonance imaging; AUS, abdominal ultrasound; TEE, transesophageal echocardiography; TTE, Transthoracic echocardiography, ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

* 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.

† In absence of acute infection.

‡ Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr, Ryan T, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis, 2000;30:633-8.



CHAPTER 6

RISK OF CHRONIC Q FEVER IN PATIENTS WITH CARDIAC VALVULOPATHY, SEVEN YEARS AFTER A LARGE EPIDEMIC IN THE NETHERLANDS

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ABSTRACT

BACKGROUND

From 2007 through 2010, a large epidemic of acute Q fever occurred in the Netherlands. Patients with cardiac valvulopathy are at high risk to develop chronic Q fever after an acute infection. This patient group was not routinely screened, so it is unknown whether all their chronic infections were diagnosed. This study aims to investigate how many chronic Q fever patients can be identified by routinely screening patients with valvulopathy and to establish whether the policy of not screening should be changed.

METHODS

In a cross-sectional study (2016–2017) in a hospital at the epicentre of the Q fever epidemic, a blood sample was taken from patients 18 years and older who presented with cardiac valvulopathy. The sample was tested for IgG antibodies against phase I and II of *Coxiella burnetii* using an immunofluorescence assay. An IgG phase II titre of ≥1:64 was considered serological evidence of a previous Q fever infection. An IgG phase I titre of ≥1:512 was considered suspicious for a chronic infection, and these patients were referred for medical examination.

RESULTS

Of the 904 included patients, 133 (15%) had evidence of a previous *C. burnetii* infection, of whom 6 (5%) had a chronic infection on medical examination.

CONCLUSIONS

In a group of high-risk patients with a heart valve defect, we diagnosed new chronic Q fever infections seven years after the epidemic, emphasizing the need for screening of this group to prevent complications in those not yet diagnosed in epidemic areas.

INTRODUCTION

In the Netherlands, a large epidemic of Q fever occurred from 2007 through 2010, with more than 4,000 reported acute Q fever patients [1], whose most common clinical presentation was pneumonia.[2] These 4,000 reported cases are estimated to reflect more than 50,000 acute infections with *Coxiella burnetii*, including asymptomatic patients and symptomatic patients not seeking medical care or not being diagnosed with *C. burnetii* infection.[3]

Chronic Q fever can develop in 5% of all symptomatic acute Q fever patients.[4] A serious disease with high morbidity and mortality, it most often presents in patients with risk factors such as cardiac valve and vascular disease or immunodeficiency.[5-7] Long-term treatment with antibiotics of at least 18 months, consisting of the combination of doxycycline and hydroxychloroquine, and cardiovascular surgical procedures can improve the prognosis.[7-9] Predominant clinical presentations of chronic Q fever are endocarditis and endovascular infection.[5-7] In the aftermath of the Dutch epidemic, more vascular chronic infections were diagnosed, compared to endocarditis.[10] However, in the south of France, where much research on chronic Q fever has been performed, the opposite is seen: more endocarditis is diagnosed than vascular chronic infection. In the Netherlands to date, only patients with a history of valvular replacement were screened for chronic Q fever, in only one hospital.[11] The entire group of patients with valvulopathy, irrespective of surgical treatment, has not been screened and therefore, chronic *C. burnetii* infections may have been missed or diagnosed late.

The objective of this study is to investigate how many chronic Q fever patients can still be identified, several years after the epidemic, by routinely screening of patients with valvulopathy in the high incidence area. This finding will be important to inform policy on screening during future Q fever outbreaks.

METHODS

PATIENT ENROLMENT

The study was performed in the Bernhoven hospital, which is located in the small town of Uden, in the centre of the North Brabant province, where Q fever was epidemic (Figure 6.1). This hospital has a catchment area of around 300.000 people. Over a one-year period (15 February 2016 through 17 February 2017), patients aged 18 years and older were eligible for inclusion if newly diagnosed with or already known to have a valvulopathy at the cardiology outpatient clinic, or who were admitted to the cardiology ward. We

invited patients with a mild, moderate, or severe insufficiency or stenosis of aortic or mitral valves that were natural or artificial. The eligible patients received the following study documents: information letter, a laboratory form for the blood collection, and an informed consent letter. We asked the participants for permission to examine their electronic patient records for possible risk factors for chronic infection (age, gender, postal code area, cardiac and non-cardiac medical conditions). All participants provided their written consent to participate in this study. We excluded patients already known to have chronic Q fever infection.

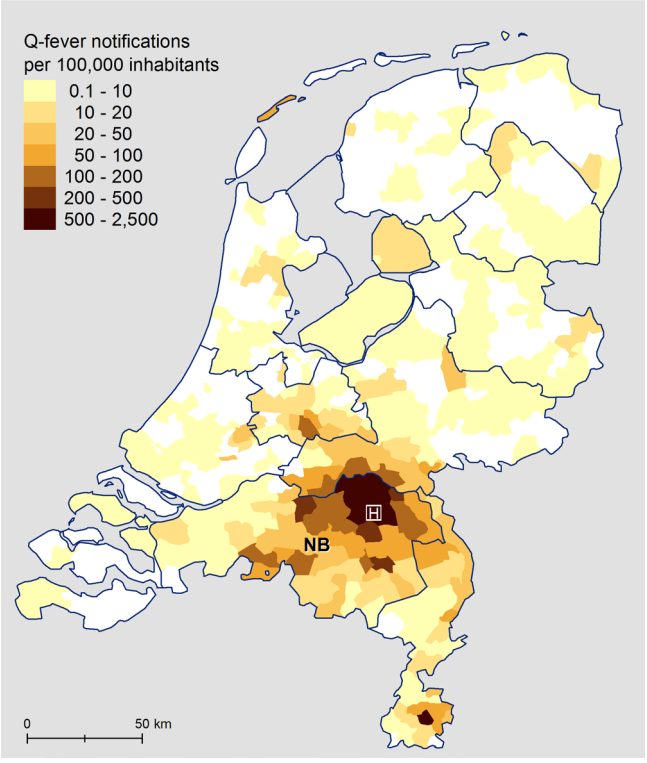

Figure 6.1. Q fever notifications per 100,000 inhabitants with location of Bernhoven hospital
NB = province Noord-Brabant. Source of the data: Notification system (OSIRIS), and Statistics Netherlands (CBS).

The Medical Ethics Review Committee of Brabant examined the study protocol and concluded that the Medical Research Involving Human Subjects Act (WMO) was not applicable and that the study complies with the Data Protection Act (WBP), the Medical Treatment Contracts Act (WGBO), and the Code of Conduct for Health Research, and the Code of Conduct for Responsible Use. Additionally, the management board of the Bernhoven hospital approved the local feasibility.

ECHOCARDIOGRAPHY

The eleven cardiologists of the Bernhoven hospital interpreted all echocardiographs according to the European Society of Cardiology (ESC) guidelines.[12] The aortic and mitral valves were examined for regurgitation and stenosis. Echocardiographic results were retrieved from electronic patient records by one of the authors (MMAdL) and support staff at the cardiology department. If the description was not clear, the researcher consulted one of the cardiologists (AS). Per diagnosis, patients were subdivided into groups with no, mild, moderate, or severe valvulopathy.

DIAGNOSIS

We performed screening on serum samples obtained by venepuncture. First, sera were screened for IgG antibodies against phase I and II of *C. burnetii* using an immunofluorescence assay (IFA; Focus Diagnostics, INC., Cypress, CA, USA) with a detection cut-off titre of ≥1:64. We considered patients with a phase II IgG titre of ≥1:64 to have serological evidence of a previous *C. burnetii* infection. An IgG phase I titre of ≥1:512 was suspicious for a chronic Q fever infection. If phase I IgG antibodies were present at or above this cut-off, we determined the exact antibody titre. We performed real-time PCR for *C. burnetii* DNA if the phase I IgG titre was ≥1:512.[13] This is below the chronic Q fever definition cut-off titre of ≥1:1024 (see next paragraph) and was chosen to increase the probability of capturing all cases. Participants with antibodies present at or above 1:512 were advised to be referred to the Internal Medicine Department in the Bernhoven Hospital for further examination. Eight months after the last patient was included, we checked the outcome of those who were further evaluated because of an IgG phase I titre ≥1:512. As suggested by the Dutch Q fever Consensus group, we categorised the chronic Q fever infections into probable or proven chronic Q fever. All participants with an IgG phase I titre of ≥1:1024 against *C. burnetii* were classified as probable cases of chronic Q fever, as they all possess a risk factor for a chronic infection, namely a valvulopathy. If additionally the patient had a PCR positive for *C. burnetii* in blood or tissue, had a definite endocarditis according to the modified Duke criteria, or had a proven large vessel or prosthetic infection, then they were classified as having a proven chronic Q fever infection (Appendix Table 6.A1).[14]

DATA ANALYSIS

We calculated frequencies and percentages for the baseline characteristics. Next, we performed univariable logistic regression to estimate possible risk factors for chronic infection, compared to patients who had serological evidence of a previous *C. burnetii* infection but had no chronic Q fever infection. We considered age, gender, various health complaints/diseases, and heart valve diseases as possible risk factors. Additionally, we performed univariable logistic regression to estimate possible risk factors for chronic

infection, compared to those who had no chronic infection (patients who had serological evidence of a previous *C. burnetii* infection but had no chronic Q fever infection and seronegative patients taken together). The small number of identified chronic infections precluded multivariable analysis. We performed all analyses using SAS software version 9.4 (SAS institute, Cary, North Carolina, USA).

RESULTS

We invited 1023 people for the study, of whom 968 were willing to participate (Figure 6.2). Of those 968 persons, 64 were excluded because inclusion or blood sampling was not performed according to the study protocol; because valvulopathy was not found on reviewing the echocardiographic results; or because the echocardiographic images could not be retrieved. Therefore, 904 patients were included in the analysis. None was already known to have a chronic Q fever infection. Of the 904 participants, 133 (15%) had serological evidence of a previous *C. burnetii* infection, as they had an IgG phase II titre of 1:64 or higher. Of these 133 participants, 11 had a phase I titre of 1:512 or higher. Of these, further clinical examination at the Internal Medicine Department showed that five had no chronic infection, as they had an IgG phase I titre of 1:512 and no other signs of chronic infection. The other six (5%) were diagnosed with a chronic Q fever infection.

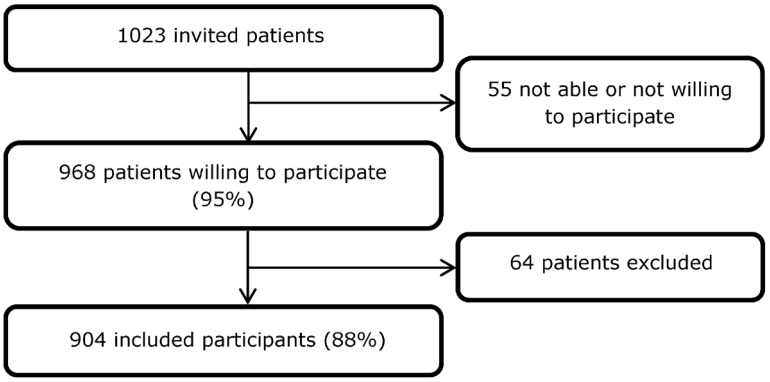

Figure 6.2. Flow diagram participant inclusion

Table 6.1 shows the baseline characteristics of the complete cohort. Baseline characteristics are presented in Appendix Table 6.A2 separately for patients with no previous *C. burnetii* infection, patients with serological evidence of a previous *C. burnetii* infection but no chronic Q fever infection, and patients with a chronic infection. Patients with chronic Q fever had a higher prevalence of COPD, vascular prosthesis of the large body vessels, and vascular abnormalities of the large body vessels than persons with or without serological evidence of a previous *C. burnetii* infection.

Table 6.1. Characteristics of the study participants*

Characteristic		
Gender	n/N (% male)	487/904 (54)
Age	Median (min – max) (years)	73 (26-95)
Diabetes	n/N (%)	157/886 (18)
COPD	n/N (%)	80/886 (9)
Asthma	n/N (%)	40/886 (5)
Impaired kidney function or chronic kidney disease†	n/N (%)	238/886 (27)
Stroke	n/N (%)	60/886(7)
Hematologic cancer‡	n/N (%)	4/886 (<1)
Cancer, other than hematologic cancer‡	n/N (%)	90/886 (10)
Autoimmune disease	n/N (%)	107/886 (12)
HIV	n/N (%)	0/886 (0)
Vascular prosthesis of the large body vessels§	n/N (%)	30/886 (3)
Vascular abnormality of the large body vessels¶	n/N (%)	86/886 (10)

Abbreviations: n=Number, N=total number

* 18 Participants gave no permission to collect data from the electronic patient record.

† Estimated kidney function (modification of diet in renal disease) in majority of tests in recent years smaller than 60.

‡ Cancer present in last five years before inclusion.

§ Vascular prosthesis of the aorta, femoral artery, or common iliac artery.

¶ Aneurysm or vascular dilatation of the aortic arch (>29mm) or ascending aorta (>40 mm) described in echocardiographic report or dilatation of abdominal aorta, femoral artery, or common iliac artery mentioned in the electronic patient record.

Table 6.2 provides details of the six chronic Q fever patients. Three had a proven chronic infection, of which two had an infection of the aortic tube, and one an endocarditis. Two patients had a probable chronic infection, as they had an IgG phase I titre ≥1:1024 with valvulopathy as risk factor but had no evidence of actual chronic infection. One patient with high IgG phase I titre did not want to be referred to the Internal Medicine Department, as he experienced no disease symptoms, so the classification of his infection remained unknown. Only one of the six patients was aware of a previous acute *C. burnetii* infection, having participated in an earlier Q fever serosurvey.

Table 6.2. Characteristics of the six participants with a chronic infection

Patient	IgG phase I	IgG phase II	Gender	Age	Underlying disease	Clinical presentation
1	1:8192	1:8192	Male	76	Moderate mitral regurgitation	Probable chronic Q fever, no focus discovered
2	1:4096	1:4096	Male	81	Mild mitral regurgitation, aortic tube prosthesis	Proven chronic Q fever, aortic tube infection localised
3	1:4096	1:4096	Male	83	Moderate mitral regurgitation, aortic prosthesis valve, impaired kidney function	Proven chronic Q fever, abdominal aortic infection localised
4	1:2048	1:2048	Male	61	Mild aortic regurgitation and moderate aortic stenosis of bicuspid valve, aneurysm of aorta ascendens	Proven chronic Q fever, endocarditis
5	1:2048	1:4096	Male	83	Mild mitral regurgitation	Unknown chronic Q fever status
6	1:512*	1:1024	Female	68	Moderate mitral regurgitation, COPD GOLD 1, aneurysm of abdominal aorta, cholangiocarcinoma	Probable chronic Q fever, no focus discovered

Abbreviations: IgG = immunoglobulin.

* In follow-up sample, the IgG phase I titre was 1:1024.

Table 6.3 compares the presence of valvulopathy per group of patients. The most common valvular defect was mild mitral regurgitation, mild aortic regurgitation, or mild aortic stenosis.

Lastly, we performed univariable logistic regression, in order to determine risk factors for developing chronic Q fever (Appendix Tables 6.A3 and 6.A4). There was only one significant risk factor, namely stenosis of a bicuspid aortic valve. Due to low numbers, we were not able to investigate all possible risk factors that are mentioned in Tables 6.1 and 6.3, and were therefore not displayed in Appendix Tables 6.A3 and 6.A4.

Table 6.3. Valvulopathy of patients with no serological evidence of a previous *C. burnetii* infection, patients with serological evidence of a previous *C. burnetii* infection but no chronic Q fever infection, and patients with a chronic Q fever infection

Heart valve disease		Patients with no serological evidence of a previous *C. burnetii* infection	Patients with serological evidence of a previous *C. burnetii* infection, no chronic infection	Patients with chronic Q fever
		N=771	N=127	N=6
Mitral valve total	**n (%)**	**608 (79)**	**98 (77)**	**5 (83)**
Mild mitral regurgitation	n (%)	403 (52)	68 (54)	2 (33)
Moderate mitral regurgitation	n (%)	174 (23)	26 (20)	3 (50)
Severe mitral regurgitation	n (%)	20 (3)	3 (2)	0 (0)
Mitral regurgitation with prolapse	n (%)	41 (5)	8 (6)	0 (0)
Mild mitral stenosis	n (%)	14 (2)	1 (<1)	0 (0)
Moderate mitral stenosis	n (%)	2 (<1)	1 (<1)	0 (0)
Severe mitral stenosis	n (%)	1 (<1)	0 (0)	0 (0)
Mitral stenosis with prolapse	n (%)	0 (0)	0 (0)	0 (0)
Mitral paravalvular leakage	n (%)	0 (0)	0 (0)	0 (0)
Mitral prosthetic valve	n (%)	6 (<1)	0 (0)	0 (0)
Mitral valve repair	n (%)	13 (2)	1 (<1)	0 (0)

Table 6.3 continued.

Heart valve disease		Patients with no serological evidence of a previous *C. burnetii* infection	Patients with serological evidence of a previous *C. burnetii* infection, no chronic infection	Patients with chronic Q fever
		N=771	N=127	N=6
Aortic valve total	**n (%)**	**431 (56)**	**75 (59)**	**2 (33)**
Mild aortic regurgitation	n (%)	245 (32)	39 (31)	1 (6)
Moderate aortic regurgitation	n (%)	42 (5)	6 (5)	0 (0)
Severe aortic regurgitation	n (%)	0 (0)	1 (<1)	0 (0)
Aortic regurgitation bicuspid valve	n (%)	2 (<1)	0 (0)	1 (17)
Mild aortic stenosis	n (%)	133 (17)	29 (23)	0 (0)
Moderate aortic stenosis	n (%)	63 (8)	8 (6)	1 (17)
Severe aortic stenosis	n (%)	29 (4)	4 (3)	0 (0)
Aortic stenosis bicuspid valve	n (%)	1 (<1)	1 (<1)	1 (17)
Aortic paravalvular leakage	n (%)	1 (<1)	1 (<1)	0 (0)
Aortic prosthetic valve	n (%)	24 (3)	5 (4)	1 (17)

Abbreviations: N = number.

DISCUSSION

We found in this study that 5% of the patients with a valvulopathy who had serological evidence of a previous *C. burnetii* infection, had a chronic Q fever infection seven years after the end of the large Q fever epidemic. This percentage is higher than reported after a screening of the general population of a village in the epidemic area, where 34% of the participants had antibodies against *C. burnetii*, but only 1% of them had a chronic infection.[15] In contrast, screening studies of patients in areas affected by the Q fever epidemic showed a risk of 8% for those with a history of cardiac valve surgery and 31% for those with an abdominal aortic/iliac aneurysm or aorto-iliac reconstruction.[11, 16] However, these studies were performed closer to the end of the epidemic than our study.

It has been estimated that 703 patients with a known heart valve defect or prosthesis were chronically infected throughout the Netherlands when the epidemic ended in 2010.[17] In the same study, the authors estimated that between 2010 and 2017, 369 of these patients were diagnosed with chronic Q fever or died due to Q fever or another cause. Accordingly, in 2017, an estimated 334 chronic infections were not yet diagnosed in this patient group. Screening for chronic Q fever in patients with valvulopathy, in areas that have experienced moderate to high Q fever incidence, was found to be cost-effective.[17]

In this study, we found that patients with stenosis of a bicuspid aortic valve had a higher risk for chronic infection than patients with other valvulopathies. Having a bicuspid aortic valve was earlier described as a risk factor for chronic Q fever.[18] Interestingly, we did not find that patients with a higher-grade valvulopathy had a higher risk for developing a chronic infection. This corresponds with a study in patients with clinically silent valvulopathies, which showed that patients with a only minor vavulopathy had an

increased risk for developing chronic Q fever.[18]

The Dutch National Chronic Q fever database contains clinical data on chronic Q fever patients that are treated in various hospitals. In this database, the infection in most patients has a vascular focus. Of the 323 probable and proven chronic Q fever patients, 153 have a vascular focus, 84 have a cardiac focus, 43 have a combination of cardiac and vascular infection, and 11 have another focus (personal communication Sonja van Roeden, 19-12-2016, [10]). It is noteworthy that in our screening programme of patients with cardiac valvulopathy, two of the six patients detected with chronic Q fever had a vascular focus of infection, rather than endocarditis. However, in France, endocarditis seems to predominate.[7] As earlier described, several possible explanations could account for this difference [19]: differing case definitions for chronic infection, differing virulence of circulating *C. burnetii* strains, possible selection bias in a national reference centre, and a lack of specificity in describing valvular defect severity in retrospective reports.[6, 7, 20-23] Lastly, the vascular infections diagnosed in the Netherlands may be an embolic consequence of clinically silent endocarditis, as Million et al suggested.[24] On the other hand, our results reduce the likelihood of one explanation for the discrepancy between the Dutch and French literature. As our systematic screening found more vascular infections than endocarditis, it is unlikely that a lack of systematic screening led to underdiagnoses of endocarditis in the Netherlands.

A major strength of this study was the high participation rate of 95%. We therefore assume that the study population is representative of the general Dutch population with a heart valve defect living in areas that were affected by the Q fever epidemic. The high participation rate may have been influenced by reports on websites and regional media plus a short video on the study that ran continuously in the waiting room area of the Bernhoven hospital.

However, our study also has some limitations. We first screened patients for IgG phase II antibodies against *C. burnetii*. Because IgG phase II antibodies wane over the years, some patients with previous acute infection may have been missed, and overall seroprevalence may have been underestimated.[4, 25] Therefore, we might have overestimated the percentage of chronic Q fever patients among those who had serological evidence of a previous *C. burnetii* infection. With a lower IgG phase II cut-off value, we would have missed fewer previous infections. However, we chose the cut-off value of IgG phase II of 1:64, to enable comparison of our results with other screening studies, and to minimise false-positive results.[15, 16] Conversely, we assumed that all patients with chronic Q fever have an IgG phase II titre $\geq$1:64.[4] Not all valvulopathy patients who attended a cardiologist in the Bernhoven hospital during the study year were invited to participate in this study.

The main reason was the high workload of the cardiologists during a reorganisation of the department. A further limitation is that we cannot exclude some form of survival bias in this study, as not all valvulopathy patients who died of an unknown cause during the study year were tested for chronic Q fever. Unfortunately, it is unknown how large this group has been. Therefore, it is possible that we underestimated the chronic Q fever prevalence. Next, patients might have acquired the *C. burnetii* infection in the years after the large outbreak. However, the risk was low, as the number of acute Q fever sharply declined after the outbreak. Lastly, we are uncertain whether all six chronic Q fever patients who were diagnosed in the present study were actually infected during the 2007–2010 Q fever epidemic. Acute Q fever remains endemic at very low levels, and chronic Q fever could have been the result of a more recent acute infection.

In conclusion, among 904 patients with a heart valve defect, we diagnosed six cases of chronic Q fever seven years after the end of a large epidemic. Screening of this patient group in other areas affected by the Q fever epidemic may yield additional cases of chronic infection. Diagnosis of chronic Q fever in patients with valvulopathy can be beneficial in preventing future complications of this chronic disease. Results of this study are used as input in a cost-effectiveness study.[17] At this time, a screening program is started in high-risk groups. GPs are now selecting and inviting high risk patients for the screening who live in an area that was affected by the Q fever epidemic.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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APPENDIX CHAPTER 6

Table 6.A1. Dutch consensus guideline on chronic Q fever diagnosis*

Proven chronic Q fever	Probable chronic Q fever	Possible chronic Q fever
1. Positive *C. burnetii* PCR in blood or tissue† OR 2. IFA ≥ 1:1024 for *Coxiella burnetii* phase I IgG AND - Definite endocarditis according to the modified Duke criteria‡ OR - Proven large vessel or prosthetic infection by imaging studies (^{18}FDG-PET, CT, MRI or AUS)	IFA ≥ 1:1024 for *Coxiella burnetii* phase I IgG AND one or more of following criteria: - Valvulopathy not meeting the major criteria of the modified Duke criteria‡ - Known aneurysm and/or vascular or cardiac valve prosthesis without signs of infection by means of TEE/TTE, ^{18}FDG-PET, CT, MRI or abdominal doppler ultrasound - Suspected osteomyelitis or hepatitis as manifestation of chronic Q fever - Pregnancy - Symptoms and signs of chronic infection, such as fever, weight loss and night sweats, hepato-splenomegaly, persistent raised ESR and CRP - Granulomatous tissue inflammation, proven by histological examination - Immunocompromised state	IFA ≥ 1:1024 for *Coxiella burnetii* phase I IgG without manifestations meeting the criteria for proven or probable chronic Q fever

Abbreviations: PCR, polymerase chain reaction; IFA, immunofluorescence assay; PET, positron emission tomography; CT, computer tomography; MRI, magnetic resonance imaging; AUS, abdominal ultrasound; TEE, transesophageal echocardiography; TTE, Transthoracic echocardiography, ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

*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.

† In absence of acute infection.

‡ Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr, Ryan T, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis, 2000;30:633-8.

Table 6.A2. Characteristics of the study participants, per group of infection status*

Characteristic		Patients with no serological evidence of a previous *C. burnetii* infection	Patients with serological evidence of a previous *C. burnetii* infection, no chronic infection	Patients with chronic Q fever
Gender	n/N (% male)	397/771 (51)	85/127 (67)	5/6 (83)
Age	Median (min – max) (years)	73 (26-95)	72 (39-89)	78 (61-83)
Comorbidity				
Diabetes	n/N (%)	138/755 (18)	19/125 (15)	0/6 (0)
COPD	n/N (%)	66/755 (9)	13/125 (10)	1/6 (17)
Asthma	n/N (%)	45/755 (6)	4/125 (3)	0/6 (0)
Impaired kidney function or chronic kidney disease†	n/N (%)	207/755 (27)	30/125 (24)	1/6 (17)
Stroke	n/N (%)	52/755 (7)	8/125 (6)	0/6 (0)
Hematologic cancer‡	n/N (%)	3/755 (<1))	1/125 (1)	0/6 (0)
Cancer, other than hematologic cancer‡	n/N (%)	79/755 (10)	11/125 (9)	0/6 (0)
Autoimmune disease	n/N (%)	97/755 (13)	10/125 (8)	0/6 (0)
HIV	n/N (%)	0/755 (0)	0/125 (0)	0/6 (0)
Vascular prosthesis of the large body vessels§	n/N (%)	25/755 (3)	4/125 (3)	1/6 (17)
Vascular abnormality of the large body vessels¶	n/N (%)	73/755 (10)	11/125 (9)	2/6 (33)

Abbreviations: n=Number, N=total number

* 18 Participants gave no permission to collect data from the electronic patient record.

† eGFR (MDRD) in majority of tests in recent years smaller than 60.

‡ Cancer present in last five years before inclusion.

§ Vascular prosthesis of the aorta, femoral artery, or common iliac artery.

¶ Aneurysm or vascular dilatation of the aortic arch (>29mm) or, ascending aorta (>40 mm) described in echocardiographic report or dilatation of abdominal aorta, femoral artery, or common iliac artery mentioned in the electronic patient record.

Table 6.A3. Univariable risk analysis for chronic Q fever patients versus patients with serological evidence of a previous *C. burnetii* infection but no chronic Q fever infection (reference category).

Characteristic	Odds ratio (95% CI)	*P*-value
Mild mitral regurgitation (yes vs. no)	0.43 (0.08 – 2.45)	0.34
Moderate mitral regurgitation (yes vs. no)	3.89 (0.74 – 20.38)	0.11
Mild aortic regurgitation (yes vs. no)	0.45 (0.05 – 3.99)	0.47
Moderate aortic stenosis (yes vs. no)	2.98 (0.31 – 28.60)	0.35
Stenosis of bicuspid aortic valve (yes vs. no)	25.20 (1.37 – 463.64)	0.03
Aortic prosthetic valve (yes vs. no)	4.88 (0.48 – 49.95)	0.18
Age (≥75 years vs. <75 years)	2.54 (0.45 – 14.35)	0.29
Gender (male vs. female)	2.47 (0.28 – 21.82)	0.42
COPD (yes vs. no)	1.72 (0.19 – 15.91)	0.63
Impaired kidney function (yes vs. no)	0.63 (0.07 – 5.64)	0.68
Vascular prosthesis of the large body vessels (yes vs. no)	6.05 (0.57 – 64.51)	0.14
Aneurysm large body vessels (yes vs. no)	5.18 (0.85 – 31.56)	0.07

Abbreviations: CI = confidence interval.

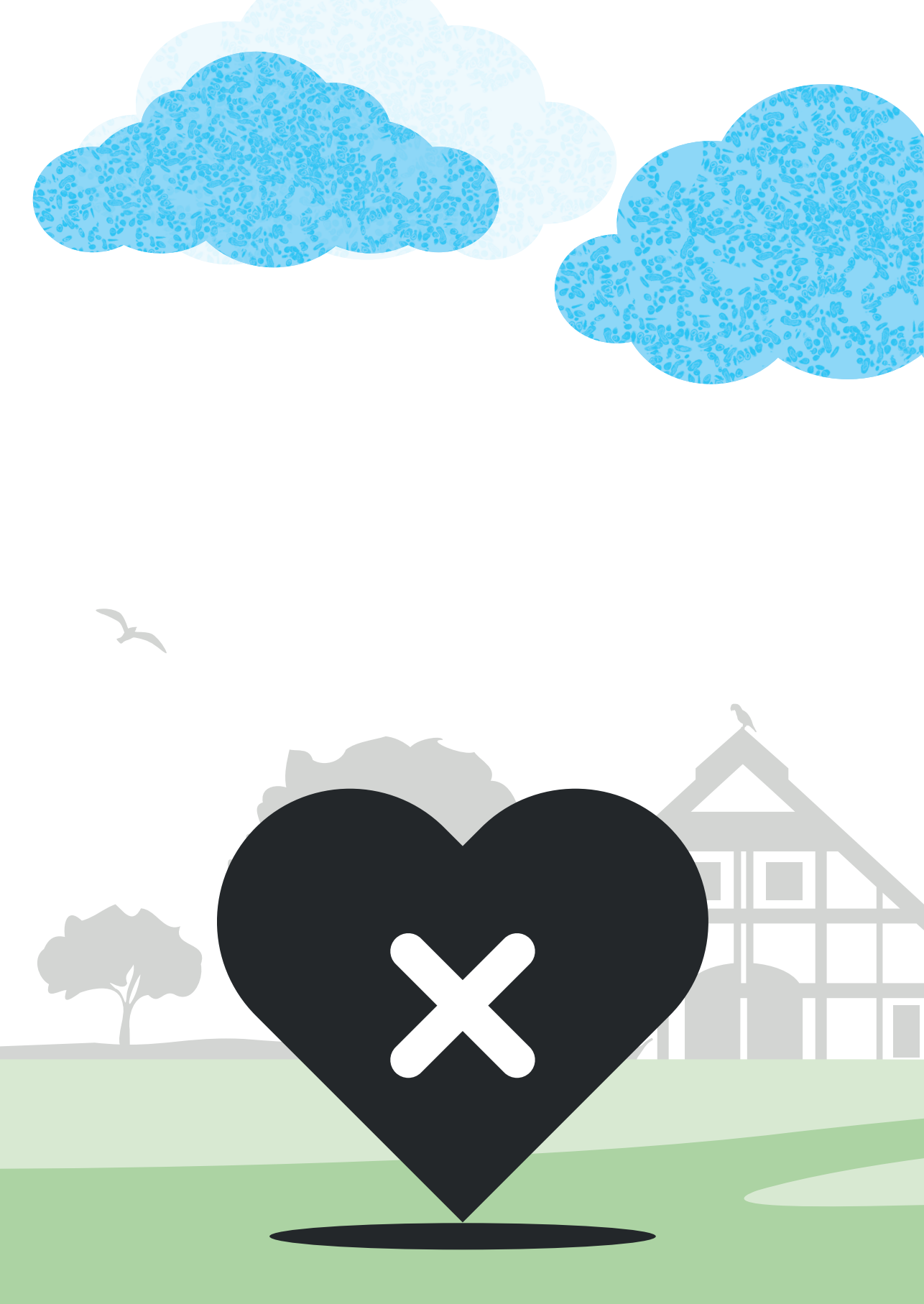
Due to low numbers, we were not able to investigate all possible risk factors that are mentioned in Table 6.1 and 6.3. Here we show the results of the characteristics for which we could perform a risk factor analysis.

Table 6.A4. Univariable risk analysis for chronic Q fever patients versus patients with no chronic Q fever (patients with serological evidence of a previous *C. burnetii* infection and patients with serological evidence of a previous *C. burnetii* infection but no chronic Q fever infection taken together (reference category)).

Characteristic	Odds ratio (95% CI)	*P*-value
Mild mitral regurgitation (yes vs. no)	0.45 (0.08 – 2.49)	0.36
Moderate mitral regurgitation (yes vs. no)	3.49 (0.70 – 17.43)	0.13
Mild aortic regurgitation (yes vs. no)	0.43 (0.05 – 3.72)	0.45
Moderate aortic stenosis (yes vs. no)	2.33 (0.27 – 20.21)	0.44
Aortic prosthetic valve (yes vs. no)	5.99 (0.68 – 52.95)	0.11
Age (≥75 years vs. <75 years)	2.56 (0.47 – 14.03)	0.28
Gender (male vs. female)	4.32 (0.50 – 37.09)	0.18
COPD (yes vs. no)	2.03 (0.23 – 17.6)	0.52
Impaired kidney function (yes vs. no)	0.54 (0.06 – 4.67)	0.58
Vascular prosthesis of the large body vessels (yes vs. no)	5.87 (0.66 – 51.85)	0.11
Aneurysm large body vessels (yes vs. no)	4.74 (0.86 – 26.26)	0.08

Abbreviations: CI = confidence interval.

Due to low numbers, we were not able to investigate all possible risk factors that are mentioned in Table 6.1 and 6.3. Here we show the results of the characteristics for which we could perform a risk factor analysis.



CHAPTER 7

COST-EFFECTIVENESS OF SCREENING PROGRAM FOR CHRONIC Q FEVER IN THE NETHERLANDS

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ABSTRACT

In the aftermath of a large Q fever (QF) epidemic in the Netherlands during 2007–2010, new chronic QF (CQF) patients continue to be detected. We developed a health-economic decision model to evaluate the cost-effectiveness of a 1-time screening program for CQF 7 years after the epidemic. The model was parameterized with spatial data on QF notifications for the Netherlands, prevalence data from targeted screening studies, and clinical data from the national QF database. The cost-effectiveness of screening varied substantially among subpopulations and geographic areas. Screening that focused on cardiovascular risk patients in areas with high QF incidence during the epidemic ranged from cost-saving to €31,373 per quality-adjusted life year gained, depending on the method to estimate the prevalence of CQF. The cost per quality-adjusted life year of mass screening of all older adults was €70,000 in the most optimistic scenario.

INTRODUCTION

Chronic Q fever (CQF) is a potentially lethal condition that develops in 2% of Q fever (QF) patients.[1] QF is caused by infection with *Coxiella burnetii*, a gram-negative bacterium that has its main reservoir in livestock and can infect humans by airborne transmission. CQF can become apparent months to years after infection and usually manifests as endocarditis or vascular infection.[2] Risk factors for CQF include heart valve disorders, aortic aneurysms, vascular prostheses, older age, and a compromised immune system.[3–5] Prognosis is poor despite long-term antimicrobial drug treatment; 28% of patients need surgery, and 15% die from CQF-related complications.[6]

During 2007–2010, the Netherlands faced the world's largest QF epidemic ever documented. More than 4,000 patients with acute QF were notified. However, QF often occurs asymptomatically [1], and the total number of infections has been estimated at 50,000.[7] Through May 2016, a substantial number of CQF infections occurred, and at least 74 patients died.[8] Because early detection of CQF might result in a better prognosis, local hospitals initiated multiple targeted screening studies for clinical risk groups living in areas affected by the epidemic. These studies revealed that 7%–20% of screened patients had serologic evidence of *C. burnetii* infection, of whom 5%– 31% had CQF.[9–11]

In 2017, new diagnoses of CQF continued to appear in the Netherlands, often with severe complications, and led to a call from multiple concerned parties, including politicians, the QF patient association, and medical doctors for a national CQF screening program. One aspect considered for such a screening program is whether its costs are economically balanced with the expenditure.[12,13] To answer this question, we assessed the cost-effectiveness of a screening program for CQF in the Netherlands.

METHODS

OVERVIEW

We developed a health-economic decision model to compare estimated costs and effects of a 1-time screening program for CQF with no such screening program (Figure 7.1). The screening was assumed to occur in 2017, seven years after the epidemic. We estimated comparative outcomes of the model in terms of clinical events, quality-adjusted life years (QALYs), and costs from a societal perspective. We used a lifetime time horizon. Costs were annually discounted at 4% and QALYs at 1.5%.[14]

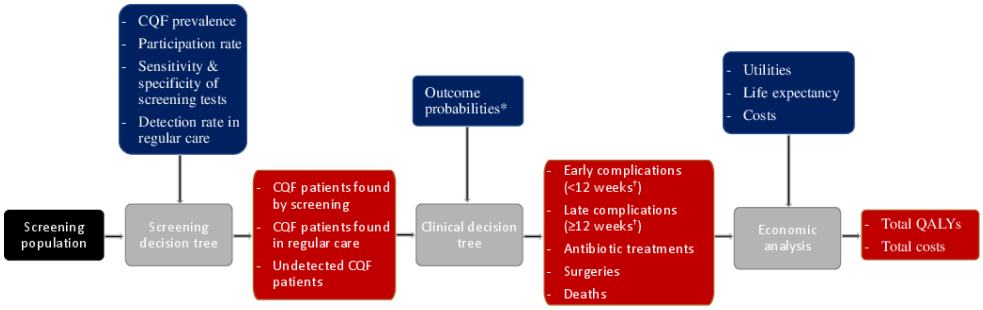


Figure 7.1. Schematic overview of the health-economic model in a study of the cost-effectiveness of screening for CQF, the Netherlands, 2017. Black square represents model input; grey squares are model processes; blue squares are model parameters; and red squares are model outputs. Individual decision trees for screening and clinical outcomes are shown in Appendix Figure 7.A1

CQF: Chronic Q fever, QALY: Quality-adjusted life year.

* Outcome probabilities differe among patients found by screening, patients found in regular care, and patients who remained undetected.

† Weeks after diagnoses.

SCREENING POPULATION

The analysis focused on adults ≥18 years of age. Because the prevalence of CQF is not uniformly distributed in the population (most QF patients resided in the south of the Netherlands; patients can have risk factors for CQF), we considered different subgroups for screening. We used the Netherlands population data from 2017.[15] First, we stratified the population on the basis of residence area between high, middle, and low QF incidence areas. For this stratification, we used spatial data on QF notifications and farms with QF outbreaks during the epidemic period (2007–2010). Next, we further divided these subgroups on the basis of a risk factor for CQF between persons with a cardiovascular risk factor, an immunocompromised status, or an unknown risk status. The last group was labeled as unknown because the prevalences of heart valve disorders and aortic aneurysms are underreported. Because these cardiovascular prevalences increase with age, the unknown subgroup was split between persons <60 years and ≥60 years of age. Thus, we considered 12 (3 × 4) subgroups (Table 7.1). We obtained prevalences of diagnosed and undiagnosed risk factors from the literature [16-21] (Appendix Table 7.A1).

Table 7.1. Subgroup criteria in a study of the cost-effectiveness of screening for CQF, the Netherlands, 2017*

Category	Condition
Area of residence	
High incidence	≥50 acute QF notifications/100,000 inhabitants *and* ≥2 acute QF notifications OR presence of a farm with QF abortion wavest within a 5-km range during the epidemic period.
Middle incidence	10–49 acute QF notifications/100,000 inhabitants *and* ≥2 acute QF notifications OR presence of a farm that tested positive in the mandatory bulk tank milk monitoring initiated during the QF epidemic.
Low incidence	<10 acute QF notifications/100,000 inhabitants OR <2 notifications during the epidemic period.
Preexisting risk factor	
Diagnosed cardiovascular risk factor	Heart valve disorder (all types of defects), heart valve prosthesis, aortic aneurysm, prosthesis/stent, history of endocarditis and congenital heart anomalies.
Immunocompromised patients	HIV infection, asplenia, spleen disorder, malignancy or bone marrow transplantations and patients using immunosuppressant drugs. As proxy for patients using immunosuppressant drugs, prevalence data were used of rheumatoid arthritis patients and patients with inflammatory bowel disease, assuming these patients frequently use immunosuppressant medication.
Unknown, ≥60 y	Age ≥60 y AND no or undiagnosed cardiovascular risk factor, e.g., heart valve disorder, aortic aneurysm.
Unknown 18–59 y	Age 18–59 y AND no or undiagnosed cardiovascular risk factor, e.g., heart valve disorder, aortic aneurysm.

QF, Q fever.
*The epidemic period was 2007–2010.
†Abortion of >5% of pregnant goats in a farm over a 4-week period.

MODEL

We used a decision-tree model that consisted of 2 parts: a screening part and a clinical part (Appendix Figure 7.A1). CQF is usually characterized by persistent high IgG against *C. burnetii* phase I, often in the presence of high IgG against phase II.[2,3] In the current clinical setting in the Netherlands, patients suspected of having CQF are tested with immunofluorescence assay (IFA) for IgG against phase I. However, IFA is a nonautomated and subjective test, and its use might not be feasible for a large-scale screening program.[22] Therefore, we proposed an initial screening round with the ELISA for IgG against phase II, and positive samples were tested with IFA for IgG against phase I. In the sensitivity analysis, we explored a scenario with direct testing with IFA for IgG against phase I.

In the clinical part, patients were first classified among proven, probable, or possible CQF, according to the guideline of the Dutch Q Fever Consensus Group.[23] This classification ranks the probability of having CQF based on PCR, serology, clinical parameters, imaging techniques, and pathologic findings (Appendix Table 7.A2). Next, patients were divided by focus of infection and whether CQF led to an early complication (before diagnosis or within 12 weeks after diagnosis). Complications considered were heart failure, symptomatic aneurysm, arterial embolic complication, and other complications. After diagnosis, antimicrobial treatment can be initiated, possibly combined with a surgical

procedure. Then, patients may have a late complication (≥12 weeks after diagnosis) and can die of CQF.

CQF PREVALENCE

The prevalence of CQF 7 years after the QF epidemic is uncertain because the average duration between infection and development of CQF is unknown. Therefore, we considered 2 scenarios, a low CQF prevalence scenario and a high CQF prevalence scenario. For both scenarios, we estimated the prevalence of CQF in 3 consecutive steps: 1) define the risk for *C. burnetii* infection per QF incidence area, 2) multiply by the risk for CQF given infection per risk group, and 3) adjust the CQF prevalence from directly after the epidemic to the year of screening 7 years later. This final step accounts for a decrease of CQF prevalence over time, for instance, because of death or earlier diagnosis.

We selected parameter values for the low and high CQF prevalence scenarios (Table 7.2). In the low CQF prevalence scenario, we assumed that only patients with a *C. burnetii* infection during the epidemic period were at risk for CQF. We divided them among high, middle, and low QF incidence areas using small geographic areas (4-digit postal code) and used incidence rates of QF notifications during the epidemic period for each incidence area. To adjust for underreporting, we multiplied the incidence rates by 12.6.[7] In the high CQF prevalence scenario, we assumed that all patients who seroconverted after the epidemic can develop CQF. For this scenario, we used larger geographic areas (3-digit postal code areas) and *C. burnetii* seroprevalences for each incidence area from the literature.[24,25] In the second step, we estimated the risk for CQF using targeted screening studies for CQF conducted during or immediately after the epidemic (Appendix Table 7.A4).[9–11,26,27] In the third step, we based the adjustment of the CQF prevalence from directly after the epidemic to the year of screening for the low CQF prevalence scenario on the reduction of CQF patients in the national CQF database over time.[28] For the high prevalence scenario, we estimated this adjustment factor on the risk for CQF among patients with a heart valve disorder in studies conducted immediately after the outbreak [9,10] and a study conducted in 2016–2017 [29] (Appendix).

Table 7.2. Prevalence scenarios explored in a study of the cost-effectiveness of screening for CQF, the Netherlands, 2017*

Parameter	Low CQF prevalence scenario	High CQF prevalence scenario
Risk for *Coxiella burnetii* infection	Based on incidence rates of new infections during the epidemic period, adjusted for underreporting	Based on overall seroprevalences from the literature
High incidence area, %	2.15	10.70
Middle incidence area, %	0.15	2.30
Low incidence area, %	0.027	1.00
Risk for CQF after *C. burnetii* infection	Equal for low and high CQF prevalence scenarios. Risk for CQF after infection is 7% for patients with heart valve disorders/prostheses, 29.3% for patients with vascular disorders/prostheses, and 6.9% for immunocompromised patients (probable or proven CQF). Risk for possible CQF in patients without risk factor is 0.2%.	
Adjustment factor to account for reduction of CQF prevalence from directly after epidemic (2010–2012) to year of screening (2017)	0.25	0.52

CQF, chronic Q fever.
*The epidemic period was 2007–2010.

DETECTION RATE OF SCREENING AND REGULAR CARE

We assumed a participation rate in the screening program of 50%, which is the lower bound of previous targeted screening programs for CQF in the Netherlands.[10,27,30] The prevalence of CQF was assumed to be equal between participating and nonparticipating persons; hence, the participation rate affects only the number of CQF patients detected but not the cost-effectiveness of screening. We obtained sensitivity and specificity of ELISA from the literature; these values accounted for decreasing sensitivity over time after infection [31] (Appendix Table 7.A5). CQF patients with high IgG against phase I were assumed to also have high IgG against phase II (C.C.H. Wielders, unpub. data [32]), which implies that all CQF patients test positive with ELISA. In the second screening round using IFA, patients with an IgG ≥1:512 against phase I were clinically evaluated. The detection rate of CQF in regular care is unknown; we used a detection rate of 80% for proven CQF, 50% for probable CQF, and 10% for possible CQF.

OUTCOME PROBABILITIES

We estimated outcome probabilities using data from the national CQF database (Appendix Table 7.A6). This database contains information about 439 CQF patients in the Netherlands, of whom 249 had proven, 74 had probable, and 116 had possible CQF. [6] To estimate the effectiveness of screening, we stratified outcome data between CQF patients detected by regular healthcare (358 patients) and CQF patients detected by screening (78 patients). Proven CQF patients detected through screening had a 4.0 (95% CI 3.3–4.7) times lower risk for an early complication, 2.8 (95% CI 2.2–3.3) times lower risk for surgery, and 1.8 (95% CI 1.1–2.5) times lower risk for CQF-related death compared with proven CQF patients detected through regular care. The risk for a late complication

did not differ significantly (risk ratio 0.7 [95% CI 0.1–1.4]) and was assumed to be equal between screening and regular care. For probable CQF patients, outcome probabilities were not significantly lower for screened patients than for patients identified through regular care. To avoid overestimation of the effect of screening, we conservatively assumed no effectiveness of screening for probable CQF patients and explored a scenario in which probable CQF patients benefit from screening in the sensitivity analysis. No clinical events were assumed in possible CQF patients.[6] For undetected CQF patients, we used a higher risk for a late complication and death than for patients found through regular care.

QALYs AND COSTS

We estimated QALYs by multiplying the utility value associated with a certain health status by the years lived in that status. We obtained utility data for CQF-related complications from the literature [33–36] (Appendix Table 7.A7). We applied a disutility for antimicrobial treatment.[37,38] Average life expectancies of patients with premature CQF-related death were obtained from the national CQF database [6] (Appendix Table 7.A8). For patients without premature CQF-related death, we assumed life expectancy to be half the life expectancy of a person at that age from the general population.[39] We also obtained utility values for the general population from the literature [40] (Appendix).

We calculated costs in 2016 Euros (Appendix Table 7.A9). Direct healthcare costs include costs of screening, diagnostic procedures, surgical procedures, antimicrobial drugs, specialist consultations, and lifelong costs of chronic complications. According to the national cost-effectiveness guideline [41], indirect healthcare costs (healthcare costs unrelated to CQF in life-years gained) should be taken into account, which we estimated using a prespecified tool.[42] Because guidelines from other countries do not consider indirect healthcare costs, we show results without including indirect healthcare costs in the sensitivity analysis. Direct nonhealthcare costs include travel costs, and indirect nonhealthcare costs include productivity losses resulting from work absence (Appendix).

COST-EFFECTIVENESS AND SENSITIVITY ANALYSIS

We calculated the incremental cost-effectiveness ratio (ICER) of screening versus no screening by dividing the difference in costs by the difference in QALYs. We conducted a multivariate probabilistic sensitivity analysis using 10,000 simulations in which we varied a set of parameters at the same time within their uncertainty distributions. We conducted univariate sensitivity analyses, in which we varied several parameters one by one.

RESULTS

CQF PREVALENCE

Depending on the size of the areas, 16% of the population (3-digit postal codes) or 12% of the population (4-digit postal codes) live in high QF incidence areas (Figure 7.2; Appendix Table 7.A10). For the low CQF prevalence scenario, we estimated the number of *C. burnetii* infections at 42,143, resulting in 414 CQF patients directly after the epidemic and 102 CQF patients in the year of screening. For the high CQF prevalence scenario, the number of *C. burnetii*–infected persons was estimated to be 391,188, resulting in 3,842 CQF patients directly after the epidemic and 1,844 CQF patients in 2017. We also stratified the population by risk factor (Appendix Table 7.A11). The prevalence of CQF varied substantially among risk groups and by residence area (Table 7.2); the highest prevalence occurred in cardiovascular risk patients living in high incidence areas (Appendix Table 7.A12).

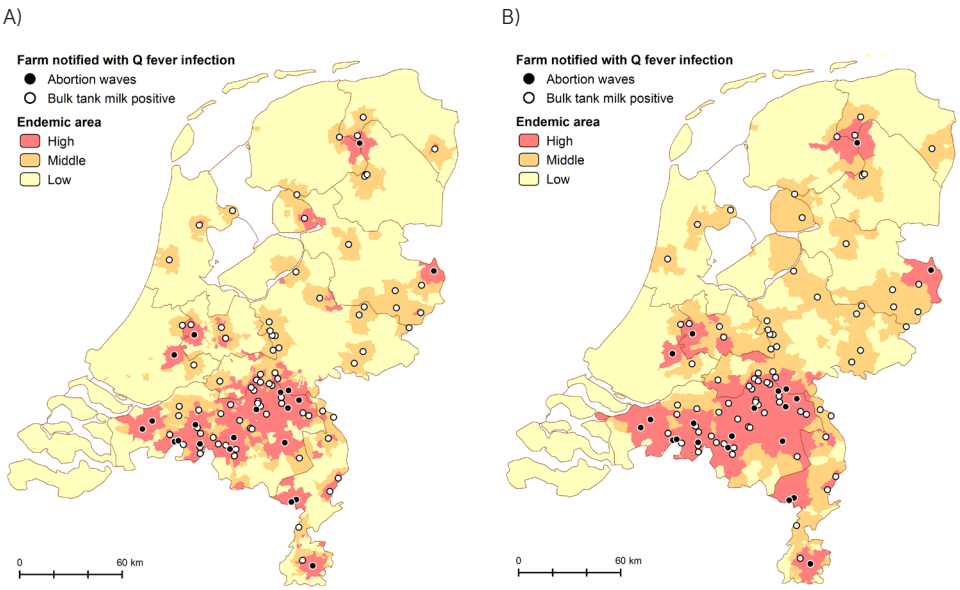


Figure 7.2. Geographic categorization of high, middle, and low Q fever incidence in the Netherlands using (A) 4-digit postal code areas and (B) 3-digit postal code areas. Incidence level was based on acute Q fever notifications and the proximity of farms with Q fever during the epidemic period (2007-2010).

Table 7.3. Outcomes of screening for chronic Q fever when a participation rate of 50% was assumed, the Netherlands, 2017*

Target population	CQF prevalence scenario	CQF prevalence†	Persons screened	CQF patients detected	Proven CQF patients detected	Complications prevented	Surgeries prevented	Deaths prevented	QALYs gained	Total cost difference (€, millions)	ICER (€/QALY gained)
High IA											
CVRF patients	Low	644	27,911	18.0	12.4	8.4	4.3	2.1	17.1	0.54	31,737
	High	6,245	36,098	225.4	155.4	104.7	53.9	25.8	214.9	-0.07	Cost-saving
IC patients	Low	364	26,898	9.8	6.7	4.5	2.3	1.1	9.3	0.62	66,145
	High	3,525	34,789	122.6	84.5	56.9	29.3	14.0	116.9	0.27	2,312
≥60y, unknown RF	Low	41.6	219,247	9.1	4.8	3.2	1.6	0.8	6.6	4.46	679,136
	High	305	283,564	86.4	59.6	40.1	20.7	9.9	82.4	5.70	69,208
18-59y, unknown RF	Low	11.0	551,381	6.1	0.2	0.1	0.1	0.0	0.2	16.23	76,308,665
	High	3.9	713,133	2.8	1.9	1.3	0.7	0.3	2.7	21.41	8,029,064
Middle IA											
CVRF patients	Low	45.5	44,586	2.0	1.4	0.9	0.5	0.2	1.9	0.96	495,918
	High	1,342	61,503	82.6	56.9	38.3	19.7	9.4	78.7	1.02	12,929
IC patients	Low	25.7	42,969	1.1	0.8	0.5	0.3	0.1	1.1	1.04	990,755
	High	758	59,273	44.9	30.9	20.9	10.7	5.1	42.8	1.23	28,755
≥60y, unknown RF	Low	2.9	350,237	1.0	0.5	0.4	0.2	0.1	0.7	7.12	9,610,222
	High	65.5	483,129	31.7	21.8	14.7	7.6	3.6	30.2	9.80	324,632
18-59y, unknown RF	Low	0.78	880,807	0.7	0.0	0.0	0.0	0.0	0.0	25.83	1,077,459,984
	High	0.84	1,215,017	1.0	0.7	0.5	0.2	0.1	1.0	35.80	36,661,479
Low IA											
CVRF patients	Low	8.2	158,759	1.3	0.9	0.6	0.3	0.1	1.2	3.43	2,757,608
	High	584	133,654	78.0	53.8	36.2	18.7	8.9	74.4	2.60	34,912
IC patients	Low	4.64	153,001	0.7	0.5	0.3	0.2	0.1	0.7	3.72	5,495,846
	High	329	128,807	42.4	29.2	19.7	10.1	4.9	40.5	2.93	72,544
≥60y, unknown RF	Low	0.53	1,247,109	0.7	0.3	0.2	0.1	0.1	0.5	25.35	53,126,291
	High	28.5	1,049,899	29.9	20.6	13.9	7.2	3.4	28.5	21.32	747,603
18-59y, unknown RF	Low	0.14	3,136,344	0.4	0.0	0.0	0.0	0.0	0.0	91.94	5,955,497,518
	High	0.37	2,640,382	1.0	0.7	0.4	0.2	0.1	0.9	77.57	84,075,394

CQF, chronic Q fever; CVRF, cardiovascular risk factor; IA, incidence area; IC, immunocompromised; ICER, incremental cost-effectiveness ratio; QALY, quality-adjusted life year; RF, risk factor; y, years of age.

* Results are stratified by target population and prevalence.

† Per million population.

CLINICAL IMPACT

We determined the number of CQF patients and prevented clinical events for each subgroup (Table 7.3; Appendix Tables 7.A13, 7.A14). Most CQF-related events are prevented by screening of cardiovascular risk groups living in high incidence areas. At an assumed participation rate of 50%, 8 complications, 4 surgeries, and 2 premature deaths are prevented for the low CQF prevalence scenario and 105 complications, 54 surgeries, and 26 premature deaths for the high CQF prevalence scenario. Screening of immunocompromised patients or all adults ≥60 years of age living in high-risk incidence areas, or screening of cardiovascular risk groups in middle-incidence areas, also could prevent a substantial number of clinical events.

COST-EFFECTIVENESS

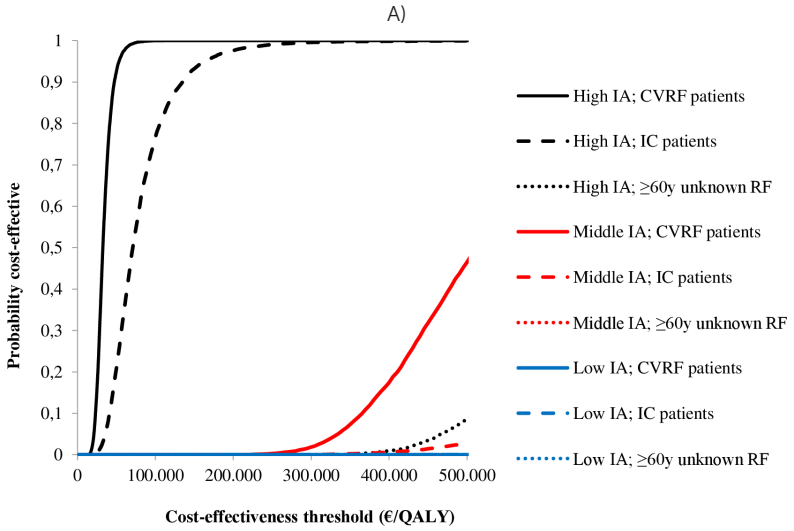
We determined the incremental costs, incremental QALYs, and ICERs for each subgroup (Table 7.3; Appendix Tables 7.A15–7.A17). The ICER of screening of cardiovascular risk groups living in high QF incidence areas was €31,737 per QALY for the low CQF prevalence scenario and cost-saving for the high CQF prevalence scenario. The next most cost-effective strategy would be screening of immunocompromised patients living in high incidence areas; ICERs were €66,145 per QALY for the low CQF prevalence scenario and €2,312 per QALY for the high CQF prevalence scenario. The ICER of screening for cardiovascular risk groups would increase substantially outside the high QF incidence area. For the high CQF prevalence scenario, the ICER increased from cost-saving to €12,929 per QALY in middle QF incidence areas and to €34,912 per QALY in low QF incidence areas. The ICER of screening for adults ≥60 years of age with an unknown risk factor living in high QF incidence areas was €679,136 per QALY in the low CQF prevalence scenario and €69,208 per QALY in the high CQF prevalence scenario. Screening of adults 18–59 years of age with an unknown risk factor was at least €8 million per QALY.

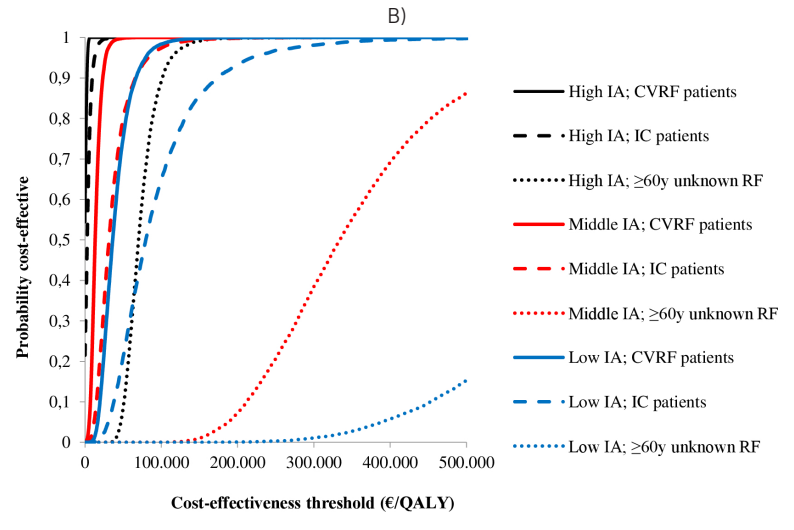
SENSITIVITY ANALYSIS

We conducted a multivariate probabilistic sensitivity analysis (Figure 7.3; Appendix Figure 7.A2). In the low CQF prevalence scenario, screening of cardiovascular risk patients living in high incidence areas had a 3.1% chance of an ICER <€20,000 per QALY and 92.5% chance of an ICER <€50,000 per QALY (Figure 7.3, panel A). In the high CQF prevalence scenario, screening had a 54.4% chance of being cost-saving and 100% chance of an ICER <€20,000 per QALY (Figure 7.3, panel B) for this subgroup.

The ICER was most sensitive to the lifetime costs of complications, the life expectancy of CQF patients, and the effectiveness of the screening program. For the low CQF prevalence scenario, the ICER varied from €17,561 to €63,449 per QALY (Figure 7.3, panel

C). Adding the effectiveness of screening for probable CQF patients changed the ICER from €31,737 to €29,585 per QALY. Exclusion of indirect healthcare costs reduced the ICER to €25,681 per QALY (ICERs without the inclusion of indirect healthcare costs of other subgroups are shown in Appendix Table 7.A18). Adding additional program costs of €11.36 per participant increased the ICER to €53,639 per QALY. For the high CQF prevalence scenario, the ICER remained cost-saving in most scenarios explored, and the highest ICER found was €1,903 per QALY (Figure 7.3, panel D).





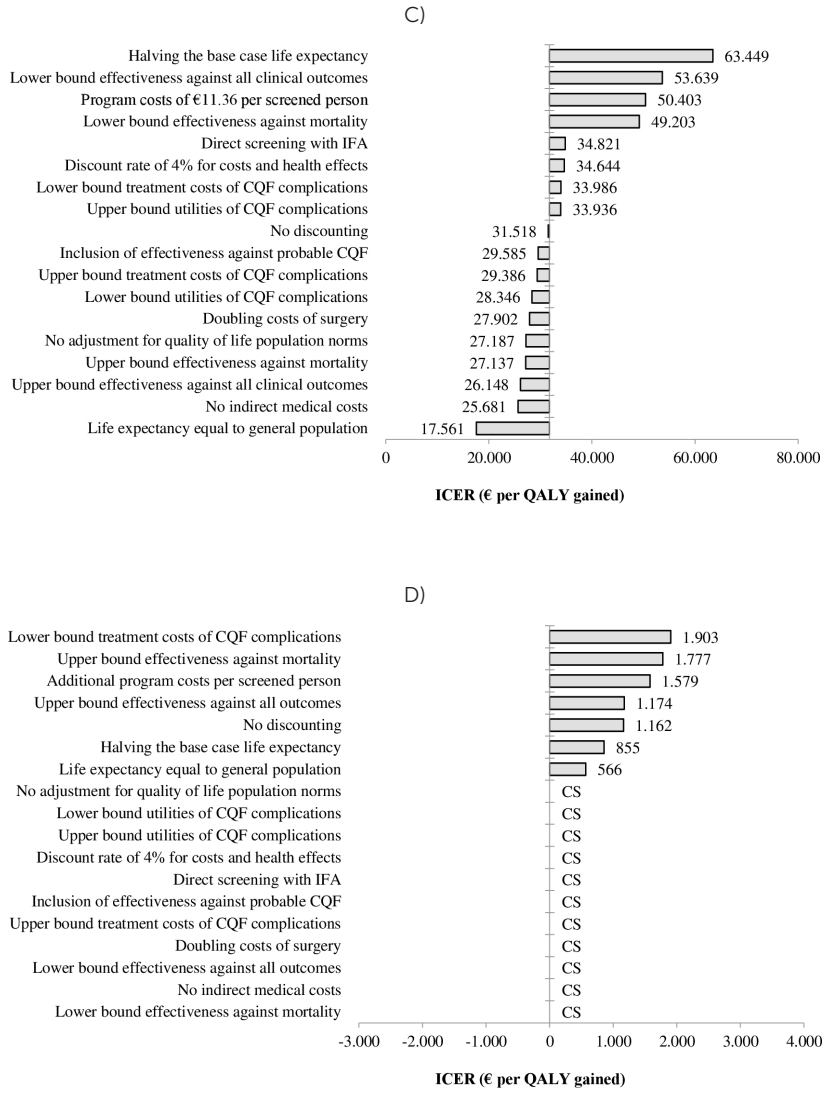


Figure 7.3. Sensitivity analysis of a screening program for CQF 7 years after the 2007–2010 epidemic, the Netherlands. A, B) Results of the multivariate probabilistic sensitivity analysis of screening in various target groups for a low CQF prevalence scenario (A) and a high CQF prevalence scenario (B). C, D) Results of a univariate sensitivity analysis of screening for chronic Q fever in patients with CVRFs living in high incidence areas for a low CQF prevalence scenario (C) and a high CQF prevalence scenario (D).

CS, cost-saving; CQF, chronic Q fever; CVRF, cardiovascular risk factor; IA, incidence area; IC, immunocompromised; ICER, incremental cost-effectiveness ratio; IFA, immunofluorescence assay; QALY, quality-adjusted life year; RF, risk factor.

DISCUSSION

We assessed the cost-effectiveness of a 1-time screening program for CQF in the Netherlands 7 years after a large QF epidemic. Cost-effectiveness varied substantially among areas and risk groups, and the results are highly sensitive to the prevalence of CQF. In a high CQF prevalence scenario, screening of cardiovascular risk patients living in high QF incidence areas during the epidemic was estimated cost-saving, whereas in a low CQF prevalence scenario the ICER was €31,737 per QALY for this subgroup. We found substantially higher ICERs for screening in areas with lower QF incidence during the epidemic or for screening of adults with an unknown risk factor for CQF.

A limitation is that the true prevalence of CQF 7 years after the epidemic is unknown. This prevalence can be affected by many factors, such as death from CQF or other causes, earlier diagnosis in regular care, and the background QF incidence after the epidemic. To account for uncertainty in CQF prevalence, we conducted a low and high CQF prevalence analysis. The estimated 42,000 new *C. burnetii* infections and 411 CQF patients during or after the epidemic low CQF prevalence scenario estimated correspond with previous estimates from the literature [7] or CQF patients included in the national database until May 2016.[6] However, these numbers are thought to be the absolute minimum. Only 23% of the proven CQF patients had a diagnosed acute QF episode [6], and a postmortem study among patients with a history of heart valve surgery in the epidemic area indicates that CQF possibly contributed to the death in 15% of the patients.[9] The high CQF prevalence scenario could be the upper range because it does not account for preexisting immunity from before the epidemic. It is therefore likely that the true prevalence falls within the reported ranges.

Recent seroprevalence studies performed outside high QF incidence areas are lacking. Underreporting of QF could be higher in these areas because medical doctors are less familiar with QF symptoms.[7] Furthermore, the geographic division between high, middle, and low QF incidence areas is arbitrary. Persons could be infected while traveling, and the extent to which farms with positive bulk milk samples contribute to disease spread is uncertain because 1 infected goat could yield a positive result.

The effectiveness of screening on the prevention of CQF-related complications and premature death is not well documented. We estimated the effectiveness by comparing outcome data between patients detected by screening and by regular care. We did this comparison separately for different CQF categories (proven, probable, or possible), but the effectiveness of screening can still be biased by uncontrolled confounders, such as age and presence of underlying conditions. The effectiveness of antimicrobial treatment

for CQF has never been assessed in a randomized clinical trial. Surgery is known to have a positive effect on survival of CQF patients with vascular infection.[3]

Our cost-effectiveness analysis is based on data from several sources in the Netherlands, such as spatial data on notifications of acute QF, seroprevalence data of *C. burnetii* infections, risk factor–specific probabilities of CQF given infection, and clinical data from a large number of CQF patients. However, combining data from different sources could also introduce biases when study populations do not exactly overlap or screening studies are conducted at different time-points.

Results of our study could also be relevant for other countries, where CQF also might be underreported. For instance, the seroprevalence of *C. burnetii* infection in the United States was estimated at 3.1% [43], representing millions of infections and potentially thousands of CQF cases, but no high numbers of CQF have been reported. An explanation may be that *C. burnetii* infections in the United States originate from cattle. The *C. burnetii* strains circulating in cattle differ from and are considered less pathogenic than the strains in small ruminants.[3] In France, however, *C. burnetii* causes 5% of all endocarditis [44], and in Israel, *C. burnetii* infection was found in 9% of patients undergoing valve surgical procedure caused by endocarditis.[45]

Cost-effectiveness is not the only criterion in deciding whether a screening program is justified.[12] Screening for CQF is based on an antibody profile suggesting a chronic infection but cannot always be linked to a focus of infection (probable or possible CQF patients). Therefore, physicians must make difficult decisions about whether long-term antimicrobial treatment should be initiated when the outcome is uncertain and adverse events frequently occur. Raoult [46] has recently proposed alternative definition criteria for CQF from the consensus guideline in the Netherlands; these criteria could exclude most probable and possible CQF patients from follow-up but also may be less sensitive in the diagnosis of proven CQF.[47]

When screening for CQF would be limited to subgroups for which screening is most cost-effective, a substantial proportion of CQF patients will remain undetected. Serologic follow-up for patients with acute QF is therefore recommended, even in absence of a risk factor for CQF.[32] However, compliance with this recommendation was suboptimal during the epidemic [48], and many patients experience an acute infection asymptomatically or do not have the infection diagnosed. Alongside a standalone screening program, case finding could be implemented in regular care, in which the physician decides whether a patient should be screened according to a risk profile. Also, a combination of case-finding and screening programs among high-risk groups could

be initiated; this approach has also been suggested for hepatitis B and hepatitis C.[49]

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APPENDIX CHAPTER 7

PREVALENCE OF RISK FACTORS FOR CHRONIC Q FEVER

Prevalence rates of risk factors are shown in Table 7.A1. Prevalence rates of cardiovascular risk factors by age group were based on data from a general practice research database in the Netherlands.[1] We used prevalence data of patients with heart valve defect, aortic aneurysm/prosthesis, congenital heart anomaly and endocarditis. As patients can have more than one risk factor, we used prevalence rates of any of these diagnosed cardiovascular risk factors and assigned these patients to the individual cardiovascular risk factors in proportion with the prevalence rates of the risk factor-specific prevalence rates. As the prevalence of aortic aneurysms and heart valve disorders are underreported, we also considered people with undiagnosed cardiovascular risk factors to be at increased risk of chronic Q fever (CQF). Prevalence rates of these undiagnosed cardiovascular risk factors were based on screening studies in the general population and prevalence rates of diagnosed risk factors were then subtracted from these. For heart valve disorders we used prevalence rates of clinically relevant heart valve disorders in ≥65 year-olds from the UK [2], and for aortic aneurysms we used prevalence rates of abdominal aortic aneurysms in ≥55 year-olds from the Netherlands.[3]

The prevalence of patients being immunocompromised due to an underlying disease by age was obtained from a study in the UK and includes patients with HIV infection, asplenia, spleen dysfunction, malignancy (e.g. leukemia) or bone marrow transplant. [4] As proxy for the prevalence of immunosuppressive drug users, we used prevalence rates by age of rheumatoid arthritis and inflammatory bowel disease.[5,6] These are the largest patient groups that use immunosuppressive drugs and we assumed that all these patients use these drugs continuously or have used these drugs at least temporarily. To avoid counting patients twice, we adjusted the prevalence rates of immunocompromised patients for the probability of having a cardiovascular risk factor. As the risk of developing CQF in patients with cardiovascular risk factors is thought to be higher than in immunocompromised patients [7], we considered patient with both a cardiovascular risk factor and an immunocompromised status in our model as a patient with cardiovascular risk factor.

Table 7.A1. Prevalence of risk factors for chronic Q fever (per 10,000 persons)

Population	18-19y	20-29y	30-39y	40-49y	50-59y	60-69y	70-79y	80-89y	≥90y
Diagnosed cardiovascular risk factor	74	66	60	132	171	476	948	1,666	1,845
Heart valve disorders or -prosthesis	14	19	28	87	122	373	793	1,375	1,760
Aortic aneurysm or -prosthesis	5	4	0	11	34	85	222	339	172
Congenital heart anomaly	70	51	34	39	25	31	7	35	0
Endocarditis	0	2	6	6	13	27	21	28	0
Undiagnosed cardiovascular risk factor									
Heart valve disorder*	0	0	0	0	0	57	251	941	1,220
Aortic aneurysm†	0	0	0	0	10	101	120	194	255
Immunocompromised									
Underlying disease‡	90	90	90	90	90	158	230	230	230
Medication use									
Rheumatoid arthritis	21	39	68	115	177	273	353	465	507
Inflammatory bowel disease	14	39	32	35	46	81	119	95	95

y: years of age.

* Only clinical relevant heart valve disorder.

† Abdominal aortic aneurysms only.

‡ Includes HIV infection, asplenia, spleen dysfunction, malignancy (e.g. leukemia) or bone marrow transplant.

MODEL DESIGN

Figure 7.A1 shows the decision tree of the screening part (Figure 7.A1, panel A) and the clinical part (Figure 7.A1, panel B).

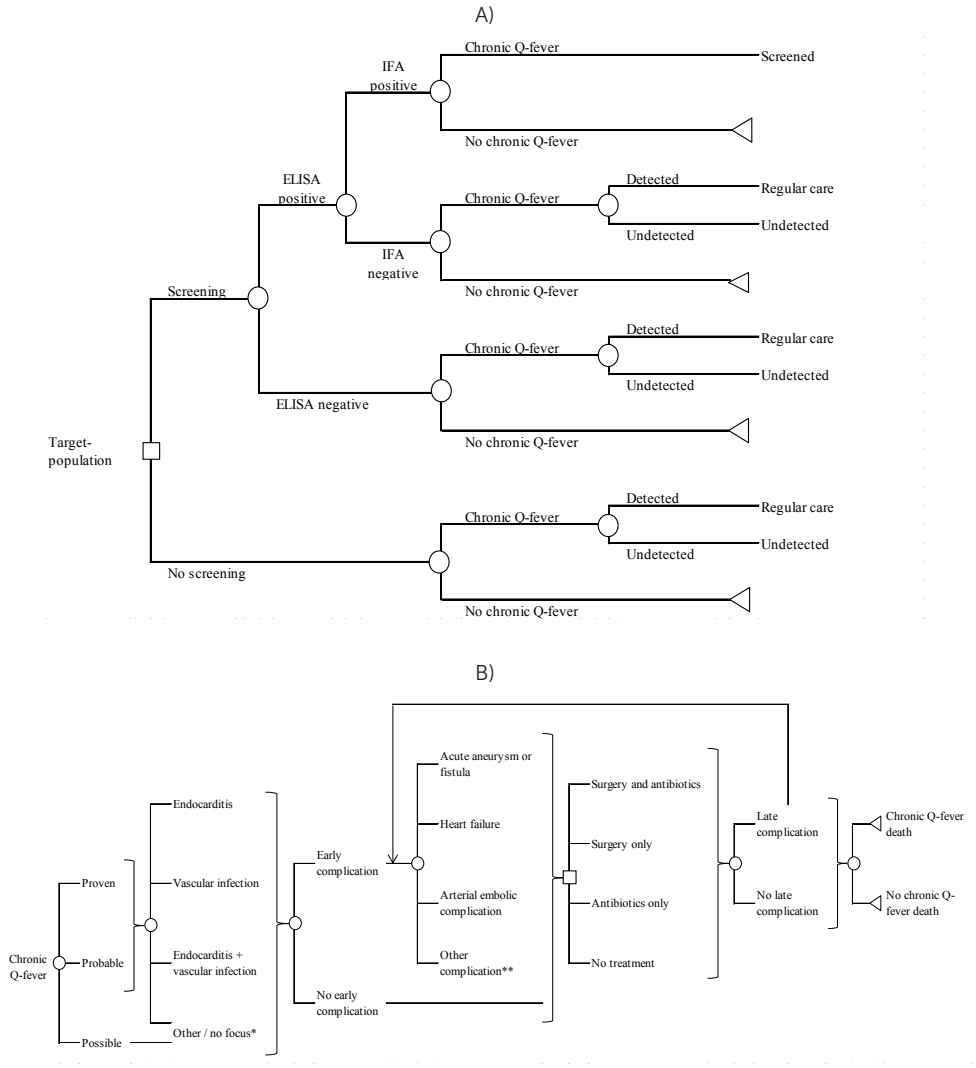

Figure 7.A1. Decision tree model.

ELISA: Enzyme-linked immune sorbent assay, IFA: Immunofluorescence assay.

(A) Decision tree for detection of chronic Q fever in presence or absence of a screening program. A square represents a decision node, a circle represents a chance node and a triangle represents a terminal node.

(B) Decision tree for the clinical outcomes of chronic Q fever after screening, regular care or undetected (outcome of the decision tree of screening).

* Contains less prevalent presentations, i.e. osteomyelitis, pericarditis and spondylodiscitis.

** Includes non-cardiac abscess, spondylodiscitis and osteomyelitis.

DEFINITION OF CHRONIC Q FEVER

Table 7.A2 shows the definition of chronic Q fever according to the Dutch Q fever consensus group [8].

Table 7.A2. Diagnostic criteria for chronic Q fever as defined by the Dutch Q fever consensus group

Category	Criteria
Proven chronic Q fever	1) Positive *C. burnetii* PCR in blood or tissue in absence of an acute Q fever infection OR 2) IFA ≥ 1:1024 for *C. burnetii* phase I IgG, AND one or more of the following criteria: - Definite endocarditis according to the modified Duke criteria [9] OR - Proven large vessel or prosthetic infection, confirmed by imaging studies (e.g. PET-CT)
Probable chronic Q fever	IFA ≥ 1:1024 for *C. burnetii* phase I IgG AND one or more of the following criteria: - Valvulopathy not meeting the major criteria of the modified Duke criteria [9] - Known aneurysm or vascular or cardiac valve prosthesis without signs of infection (by means of TEE/ TTE, PET-CT, other imaging studies) - Suspected osteomyelitis, pericarditis or hepatitis as manifestation of chronic Q fever - Pregnancy - Symptoms and signs of chronic infection, such as fever, weight loss and night sweats, hepato-splenomegaly, persistent raised ESR and CRP - Granulomatous tissue inflammation proven by histological examination - Immunocompromised state
Possible chronic Q fever	IFA ≥ 1:1024 for *C. burnetii* phase I IgG without meeting the criteria for proven or probable chronic Q fever

CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, IFA: immunofluorescence assay, PET-CT: positron emission tomography-computed tomography, PCR: polymerase chain reaction, IgG: Immunoglobulin G, TEE: transesophageal echocardiography, TTE: transthoracal echocardiography

Prevalence of chronic Q fever

We estimated the prevalence of CQF in three steps:

1) Estimating the number of patients with a *C. burnetii* infection. This was done separately for high, middle and low QF incidence areas during the epidemic.
2) Estimating the number of patients that develop CQF after *C. burnetii* infection. This was separately done for risk groups (heart valve disorder, aortic aneurysm, compromised immune system, or none of the aforementioned risk factors).
3) Estimating the number of CQF patients that are still alive and undetected in the year screening seven years after the epidemic.

Given the uncertainty around the prevalence of CQF seven years after the epidemic, we analyzed two scenarios: i) a low prevalence scenario and, ii) a high prevalence scenario.

Estimating the number of patients with a C. burnetii infection

The low prevalence scenario assumes that only patients infected with *C. burnetii* during the epidemic (period 2007-2010) are able to develop CQF; hence, individuals that were seroconverted before the epidemic only had an immune boost but no risk of developing CQF. These boosted individuals are treated as seronegative in the model. The risk of a *C. burnetii* infection during the epidemic is based on Dutch incidence rates

of QF notifications for areas that were qualified as high, middle and low incidence area. The distribution of the population between high, middle and low incidence areas was estimated using the incidence of QF notifications and the proximity of a farm with QF abortion waves or the proximity of a farm that tested positive in the mandatory bulk tank milk monitoring within a range of 5 kilometers during the epidemic (See Table 7.1 of the main article for more details). To account for underreporting because of asymptomatic infections or symptomatic infections that were not medically-attended or diagnosed, we multiplied these notification rates by 12.6. This multiplication factor was based on a study from the Netherlands that compared QF notification rates with seroconversion rates in blood donors from whom serial samples were available.[10] The adjusted risk of *C. burnetii* infection during the epidemic was then estimated at 2.15% in high incidence areas, 0.15% in middle incidence areas and 0.027% in low incidence areas.

In the high prevalence scenario, the risk of *C. burnetii* infection was based on Dutch seroprevalence studies. This scenario assumes that all patients tested seropositive after the epidemic are able to develop CQF, independent whether they were already infected before the epidemic and immune during the epidemic or not. The seroprevalence in high incidence areas was estimated at 10.7%. This estimate was based on a large seroprevalence study in areas with high QF incidence during the epidemic in 2014-2015 finding a seroprevalence of 6.0%. However, the used ELISA test for IgG phase II is known to decrease over time and the seroprevalence study was conducted 5 years after the epidemic in 2007-2010. Follow-up data over 4 years showed a decreasing trend of ELISA sensitivity after *C. burnetii* infection over time (C.C.H. Wielders, unpublished data from [21]) and, after extrapolation of this decreasing to 5 years after *C. burnetii* infection using a lognormal curve, we found that 55.9% of the patients test would still test positive after 5 years. We adjusted the seroprevalence to 10.7% using longitudinal data on sensitivity of. In absence of serological studies in middle and low incidence areas, we used data from a study that measured the seroprevalence of *C. burnetii* using IFA for IgG phase II in an area that covered high, middle and low incidence areas in 2008 (before the epidemic in this part of the country) and in 2010 (the final year of the QF epidemic). The seroprevalence of 3.2% after the epidemic was used for middle incidence areas and the seroprevalence of 1.0% before the epidemic was used for low incidence areas. More details of the studies are listed in Table 7.A3.

Not relevant for the cost-effectiveness within a specific incidence area, but relevant for the absolute number of cases, is the size of the areas that are divided between high, middle, and low incidence areas. For the low prevalence scenario, we based this division based on 4-digit postal code areas and for the high prevalence scenario we used 3-digit postal codes (larger areas). Use of 4-digit postal code areas result in a lower number of

infections, as the areas that are assigned to high or moderate incidence areas due to the proximity of an infected farm are smaller.

Table 7.A3. Prevalence of *C. burnetii* infection by chronic Q fever prevalence scenario and incidence area

Area	Deterministic	Sd*	Distribution*	Source
Low CQF prevalence scenario				
High IA	0.0215	95%CI: 0.0208-0.0223	Lognormal	Based on the incidence of QF notifications in areas with low QF incidence (see main article Table 7.1 for criteria) during the period 2007-2010, adjusted for underreporting by multiplying with 12.6.[10]
Middle IA	0.00152	95%CI: 0.00137-0.00168	Lognormal	Based on the incidence of QF notifications in areas with middle QF incidence (see main article Table 7.1 for criteria) during the period 2007-2010, adjusted for underreporting by multiplying with 12.6.[10]
Low IA	0.000275	95%CI: 0.000243-0.000311	Lognormal	Based on the incidence of QF notifications in areas with low QF incidence (see main article Table 7.1 for criteria) during the period 2007-2010, adjusted for underreporting by multiplying with 12.6.[10]
High CQF prevalence scenario				
High IA	0.107	95%CI: 0.088-0.131	Lognormal	Pijnacker, 2017.[17] The seroprevalence of QF was adjusted from 6.0% to 10.7% to account for a decreasing sensitivity of ELISA over time (unpublished data from [21]).
Middle IA	0.0230	95%CI: 0.0140-0.0380	Lognormal	Brandwacht, 2010.[18] Based on seroprevalence data of 2010 in areas of the Netherlands that covered high, middle and low incidence areas.
Low IA	0.0100	95%CI: 0.0050-0.0190	Lognormal	Brandwacht, 2010.[18] Based on seroprevalence data of 2008 from before the area was affected during the epidemic.

CI: Confidence interval, CQF: Chronic Q fever, IA: Incidence area, QF: Q fever, Sd: Standard deviation.
* Used for the multivariate probabilistic sensitivity analysis.

Estimating the number of patients that develop CQF after C. burnetii infection

The second step of estimating the risk of developing CQF after *C. burnetii* infection was assumed to be equal for the two prevalence scenarios. The risk of CQF given *C. burnetii* infection in risk groups was based on targeted screening studies for CQF from the Netherlands that were conducted during or directly after the epidemic (Table 7.A4). Most of these studies defined CQF as an IgG titer of 1:512 or 1,024 against *C. burnetii* phase I or a positive PCR not related to acute QF. The risk of CQF differs by pre-existing risk factor, estimated at 8.7% for patients with heart valve disorders/prostheses [11, 12], 29.3% for patients with vascular disorders/prostheses [12, 13] and 6.9% for immunocompromised patients.[14] In accordance with the Dutch consensus guideline, detected CQF patients in these studies are by definition proven or probable CQF patients because they have a risk factor.[15] We applied the same risk of CQF for diagnosed and undiagnosed cardiovascular risk factors. For people without a risk factor, we estimated that 0.2% had possible CQF based on a Dutch screening study in the general population.[16]

Table 7.A4. Dutch screening studies on the risk of chronic Q fever among individuals tested seropositive for *Coxiella burnetii*

Risk condition	Study	Population	Incidence area	Study period	Test and cut-off value	CQF given seropositive for *C. burnetii* infection	% CQF	Sd*	Distribution*	Additional information
Screening studies conducted directly after the Q fever epidemic of 2007-2010										
Aortic aneurysm / prosthesis	Hagenaars, 2014 [13]	Patients with abdominal aortic- or ileac aortic aneurysm or reconstruction	High	2009-2012	IFA IgG phase I ≥1:512	40/130	30.8			
	Wegdam-Blans, 2013 [12]	Patients with abdominal aortic aneurysm or vascular prosthesis	High	2010-2011	IFA IgG phase I ≥1:1.024 or positive PCR	7/30	23.1			
	Total					47/160	29.3	0.02	Beta	All proven or probable CQF†
Heart valve disorder / prosthesis	Wegdam-Blans, 2013 [12]	Patients with heart valve prosthesis	High	2010-2011	IFA IgG phase I ≥1:1.024 or positive PCR	3/22	13.8			
	Kampschreur, 2012 [11]	Patients with history of heart valve surgery	High	2010-2011	IFA IgG phase I ≥1:512	9/116	7.8			
	Total					12/138	8.7	0.04	Beta	All proven or probable CQF†
Immunocompromised patients	Schoffelen, 2014 [14]	Patients with rheumatoid arthritis	High	2011-2012	Not reported	7/102	6.9	0.03	Beta	All proven or probable CQF†
Non-risk patients	Morroy, 2015 [16]	All adults	High	2014	IFA IgG phase I ≥1:512	1/491‡	0.2	0.001	Beta	All possible CQF
Screening studies conducted close to the year of the screening in 2017										
Heart valve disorder / prosthesis	De Lange, 2019 [19]	Patients with heart valve disorder	High	2016-2017	IFA IgG phase I ≥1:512	6/133	4.5			All proven or probable CQF†

IFA: Immunofluorescence assay, ELISA: Enzyme-linked immunosorbent essay, IgG: immunoglobulin G, PCR: Polymerase chain reaction, Sd: Standard deviation.

* Used for the multivariate probabilistic sensitivity analysis.

† According to the Dutch consensus guideline patients with risk factors and titer IgG phase I ≥1:512 automatically qualify for probable or proven CQF [8].

‡ Patients with a cardiovascular risk factor or immunocompromised status were excluded.

Estimating the prevalence of CQF patients in the year screening

The targeted screening studies referred to in the second step were conducted during or directly after the epidemic (2010-2012), while the screening program was assumed to take place in 2017. As the prevalence of CQF is expected to decline over time due to CQF-related mortality or mortality from another cause and due to detection via regular care, we adjusted the prevalence downwards. This adjustment factor was different in the low and high CQF prevalence scenario. In the low prevalence scenario, we based this adjustment factor on the numbers of CQF patients in the Dutch national CQF database over time. This database includes all diagnosed CQF patients in the Netherlands and shows a high number of proven CQF patients reported in 2010-2011, which drops substantially in the year 2012 and remains relatively stable after 2012.[20] The adjustment factor was the division of the average annual number of proven CQF cases in the period 2012-2017 by the average annual number of CQF cases in the period 2010-2011, resulting in an adjustment factor of 0.25. For the high prevalence scenario, we compared the risk of proven or probable CQF given *C. burnetii* infection among people with heart valve disorders between screening studies conducted during or directly after the epidemic [17, 18], and a recent screening study conducted in 2016-2017.[19] This resulted in an adjustment factor of 0.52 (4.5%/8.7%; see Table 7.A4).

SENSITIVITY AND SPECIFICITY OF TESTING

Sensitivity of ELISA for IgG phase II and IFA for IgG phase II and phase I are shown in Table 7.A5. Sensitivity of ELISA seven years after the epidemic was estimated by extrapolating longitudinal data on sensitivity of ELISA over the first 4 years after infection (C.C.H. Wielders, unpublished data from [21]). The specificity was based on a study from Germany.[22] Cut-off for ELISA positivity was according to the manufacturer's instruction, considering borderline samples as positive. We assumed that all CQF patients had high IgG phase II titres (C.C.H. Wielders, unpub. data from [21]), hence testing positive for ELISA. In the second screening round using IFA, patients were tested for having an IgG titre of ≥1:512 against phase I are clinical examined. As patients with an IgG titre of ≥1:512 against phase I do not necessarily have CQF according to the Dutch consensus guideline (the guideline uses an IgG titre threshold of ≥1:1,024 against phase I). Targeted screening studies in patients with heart valve disorder showed that 8 of 234 patients had an IgG titre of 512 but no CQF [19, 23], resulting in a specificity of IFA of 0.966. Similarly, in individuals with no risk factor 2/512 patients had an IgG titre of 512, resulting in a specificity of 0.996.[16]

Table 7.A5. Sensitivity and specificity of ELISA IgG phase II and IFA IgG phase I

Diagnostic test	Deterministic	Sd*	Distribution*	Source
ELISA IgG phase II				
Historic QF only				
Sensitivity	0.50	95% range: 0.39-0.63	Lognormal	Extrapolation of sensitivity data of first 4 years after infection to 7 years after infection (C.C.H. Wielders, unpub. data from [21]
Specificity	0.980	0.014	Beta	Frosinski, 2016 [18]
CQF				
Sensitivity	1			
IFA IgG phase I titer 1:512				
Proven / probable CQF				
Sensitivity	1			
Specificity	0.966	0.012	Beta	Estimated from Kampschreur 2013 and De Lange 2019 [19, 23]
Possible CQF				
Sensitivity	1			
Specificity	0.996	0.003	Beta	Estimated from Morroy, 2016 [16]

CQF: Chronic Q fever, QF: Q fever, Sd: Standard deviation.
* Used for the multivariate probabilistic sensitivity analysis.

OUTCOME PROBABILITIES OF CQF

The outcome probabilities of CQF are listed in Table 7.A6. The outcome probabilities are stratified by CQF category (proven and probable) and by outcome of the screening decision tree (detected by screening, detected in regular care, not detected at all). Clinical outcome probabilities are obtained from the Dutch national CQF database. Proven and probable patients were stratified between patients detected via screening and patients detected in regular care. We found that proven CQF patients detected by screening had a significantly reduced risk of an early complication, surgery and CQF-related mortality as compared to patients detected in regular care, but not a significantly reduced risk of a late complication. For probable CQF patients, we found no significant reduction in any clinical outcome. Therefore, we conservatively assumed that screening had no effectiveness against probable CQF. In the sensitivity analysis we included a scenario in which screening had effectiveness against an early complication. No complications, surgeries or mortality was reported for possible CQF patients in the national CQF database.

Table 7.A6. Outcome probabilities of proven or probable chronic Q fever

Parameter	Deter-ministic	Sd*	Distribution*	Scenario	Reference and comments
Classification of proven/probable CQF					
Proven CQF	0.689	0.054	Beta		CQF database [20], distribution based on 74 proven and probable CQF patients that were found via screening.
Probable CQF	0.311				Calculated as 1-proven CQF
Type of infection					
Proven CQF					
Endocarditis	0.273	0.028	Dirichlet		CQF database [20], distribution based on 249 proven CQF patients.
Vascular infection	0.502	0.032	Dirichlet		
Endocarditis & vascular infection	0.161	0.023	Dirichlet		
Other /no infection focus	0.064	0.016	Dirichlet		
Probable CQF					
Endocarditis	0.216	0.048	Dirichlet		CQF database [20], distribution based on 74 probable CQF patients.
Vascular infection	0.378	0.056	Dirichlet		
Endocarditis & vascular infection	0.041	0.023	Dirichlet		
Other /no infection focus	0.365	0.056	Dirichlet		
Early complication					
Proven CQF					
Late detected by regular care or not detected	0.548	0.04	Beta		CQF database.[20] Early complication detected in 108/197 patients detected via regular care. Not detected was assumed equal to late detected, as late detected will usually be diagnosed after a complication occurred.
RR due to early detection by screening	3.99	95% CI: 3.30-4-69	Lognormal	Lower and upper bound of 95% CI	CQF database.[20] Early complication in 7/51 patients detected via screening (RR 4.0 [95%CI: 3.3-4.7] as compared to detected via regular care).
Early detected by screening	0.137				Probability late detected divided by RR
Probable CQF					
Late detected by regular care or not detected	0.095	0.034	Beta	0.118	CQF database.[20] Early complication detected in 8/73 patients. Not detected was assumed equal to late detected, as late detected will usually be diagnosed after a complication occurred.
RR due to early detection by screening	1			2.7	No significant difference between patients detected via screening or regular care. (RR 2.7 [95%CI: 0.6-4.8])
Early detected by screening	0.095			0.043	Probability late detected divided by RR
Type of complication					
Proven CQF					
Acute aneurysm / fistula	0.542	0.04	Beta		CQF database.[20] On the basis of 153 complications. Other complications include spondylodiscitis/osteomyelitis and non-cardiac abscess.
Heart failure	0.327	0.04	Beta		
Arterial embolic complication	0.124	0.03	Beta		
Other complication	0.248	0.04	Beta		
Probable CQF					
Acute aneurysm / fistula	0.364	0.15	Beta		CQF database.[20] On the basis of 11 complications. Other complications include spondylodiscitis/osteomyelitis and non-cardiac abscess.
Heart failure	0.455	0.15	Beta		
Arterial embolic complication	0.091	0.09	Beta		
Other complication	0.091	0.09	Beta		

Table 7.A6 continued.

Parameter	Deter-ministic	Sd*	Distribution*	Scenario	Reference and comments
Surgery					
Proven CQF					CQF database.[20] Surgery at 107/197 patients detected via regular care and at 10 of 51 detected via screening (RR 2.8 [95%CI: 2.2-3.3]).
Late detected by regular care or not detected	0.543				
RR due to early detection by screening	2.77	95% CI: 2.20-3.34	Lognormal	Lower and upper bound of 95% CI	
Early detected by screening	0.196				Probability late detected divided by RR
Probable CQF					
Late detected by regular care or not detected	0.081				CQF database.[20] Surgery at 6/74 patients
RR due to early detection by screening	1				No significant difference between patients detected via screening or regular care (RR 0.5 [95%CI: 0-2.0])
Early detected by screening	0.081				Probability late detected divided by RR
Antibiotic treatment initiated					
Proven CQF	0.912	0.02	Beta		CQF database [20], 227/249 patients.
Probable CQF	0.662	0.05	Beta		CQF database [20], 49/74 patients.
Possible CQF	0				Assumption based on current standard work-up of possible CQF patients (CP. Bleeker-Rovers, pers. comm)
Late complication					
Proven CQF					
Not detected	0.452				Assuming that all undetected patients will have a CQF complication; calculated as (1 – probability of early complication)
Late detected by regular care	0.153	0.02	Beta		CQF database.[20] Late complication in 38/249 patients
RR due to early detection by screening	1				CQF database.[20] No significant difference between patients detected via screening or regular care (RR 0.7 [95%CI: 0.1-1.4]).
Early detected by screening	0.153				Probability late detected divided by RR
Probable CQF					
Not detected	0.095				Assumed equal to early complication.
Late detected by regular care	0.054	0.03	Beta		CQF database.[20] Late complication in 38/249 probable CQF patients, with
RR due to early detection by screening	1				CQF database.[20] No significant difference between patients detected via screening or regular care (RR 1.4 [95%CI: 0-3.6].
Early detected by screening	0.054				Probability late detected divided by RR

Table 7.A6 continued.

Parameter	Deter-ministic	Sd*	Distribution*	Scenario	Reference and comments
CQF-related mortality					
Proven CQF					CQF database.[20] CQF-related mortality at 55/197 proven CQF patients detected via regular care.
Not detected	0.497				Assumed that the RR between non-detected and regular care was equal to between regular care and non-detected. This approximates a 60% death rate among CQF patients in the 1970s, when effective antibiotic treatment was not available and there was a large diagnostic delay.[24]
Late detected by regular care	0.279	0.032	Beta		CQF database.[20] CQF-related mortality at 55/197 proven CQF patients detected via regular care.
RR due to early detection by screening	1.78	95% range: 1.11-2.45	Lognormal	Lower and upper bound of 95% CI	CQF database.[20] CQF-related mortality in 8/51 patients detected via screening (RR 1.78 [95%CI: 1.11-2.45] as compared to late detected).
Early detected by screening	0.157				Probability late detected divided by RR
Probable CQF					
Late detected by regular care or not detected	0.041	0.023	Beta		CQF database.[20] CQF-related mortality in 3/74 probable CQF patients
RR due to early detection by screening	1				No significant difference between patients detected via screening or regular care (RR not given due to small numbers)
Early detected by screening	0.041				Probability late detected divided by RR

CQF: Chronic Q fever, CI: Confidence interval, RR: Risk ratio, Sd: Standard deviation.
* Used for the multivariate probabilistic sensitivity analysis.

QUALITY ADJUSTED LIFE YEARS

The number of quality-adjusted life years (QALYs) for CQF patients was calculated by multiplying the utilities (preference based measure of health-related quality of life) for each health state with the time spent in that health state.

Utilities

Utilities of the different health states used in this model are shown in Table 7.A7. As the average age of CQF patient in the national CQF database is 65 years [25], we used population norms of ≥50 year-olds for the general population.[26] In a sensitivity analysis we also explored a scenario in which the utility of the general population is 1. Utility data of CQF patients is lacking. Before a complication occurs, CQF is usually asymptomatic or it presents as flu-like symptoms. We assumed that for proven or probable CQF, the utility is equal to the utility of a patient with a heart valve prosthesis.[27] We based the utilities of the different health states on quality of life data of the complications. The utility of

an aneurysm or fistula was based on patients in need of a surgery for a symptomatic abdominal aortic aneurysm [28]. The utility of heart failure was based on patients with New York Heart Association class III or IV hear failure.[29] The utility of patients with an embolic complication was based on patients with a stroke with mild impairment.[30] We assumed that long-term antibiotic use leads to a reduction of the utility. According to data from France, long-term antibiotic use to treat CQF led to gastrointestinal adverse events in 7% [24] of the patients. The disutility of this adverse event was assumed to be 0.105.[31] Possible CQF patients were assumed to have no reduction of the utility.

Table 7.A7. Utilities of the different health states

Health state	Input	Sd*	Distribution*	Scenario	Source
Utilities					
General population	0.857	0.0086	Beta	1	Versteegh, 2016 [26]
Proven or probable CQF					
Uncomplicated CQF	0.855	0.0051	Beta		Franklin, 2016 [27]
Symptomatic aneurysm or fistula	0.690	0.048	Beta		Timmers, 2013 [28]
Heart failure	0.610	0.015	Beta		Calvert, 2005 [29]
Arterial embolic complication	0.640	0.063	Beta		Stouthard, 1997 [30]
Dead	0				
Utility adaption					
Gastroenteritis due to antibiotic use	-0.007	0.0028	Beta		Million, 2010 [24], WHO, 2004 [31]

CQF: Chronic Q fever, Sd: Standard deviation.
* Used for the multivariate probabilistic sensitivity analysis.

Time spent in each health state

Time spent in each health state is shown in Table 7.A8. It is assumed that patients with a complication remain in the indicated health state for the rest of their lives. The life expectancy of proven or probable CQF patients with premature CQF-related death was based on survival data of patients included in the Dutch national CQF database.[25] The life expectancy of patients not dying prematurely due to CQF was based on the life expectancy of a comparable person at that age from the general population. We obtained the average age at diagnosis of proven and probable CQF patients from the national CQF database, being 69 years and 64 years, respectively.[25] Using lifetables of the Netherlands, the life expectancies in the general Dutch population at these ages are 16.8 years and 20.8 years. [32] However, the life expectancy of proven and probable CQF patients is expected to be lower than the life expectancy of an average person at that age due to the presence of a cardiovascular risk condition. Based on the comparison of the life expectancy of patients with heart valve prosthesis at the age of 60 years [33] with the life expectancy of patients in the general population at that age from the literature, we halved the life expectancy of proven and probable CQF patients to 8.4 years and 10.4 years, respectively. In the sensitivity analysis we explored life expectancies of the general population or halving the base case life-expectancies to 4.2 years for proven CQF and 5.2 years for probable CQF.

For those receiving antibiotic treatment, the duration of treatment was obtained from the national Dutch CQF database for proven and probable CQF patients.[34]

Table 7.A8. Time spent in health state

Outcome	Input	Sd*	Distribution*	Scenario	Source
Life expectancy					
CQF-related mortality					
Proven CQF	0.6				Van Roeden, 2018 [25]
Probable CQF	2.6				Van Roeden, 2018 [25]
No CQF-related mortality					
Proven CQF	8.4			16.8 and 4.2	Average age of diagnosis Van Roeden, 2018 [25], life expectancy from Statistics Netherlands [32], adjustment factor for comorbidity from Van Geldorp, 2009 [33]
Probable CQF	10.4			20.8 and 5.2	Average age of diagnosis from Van Roeden, 2018 [25], life expectancy from Statistics Netherlands [32], adjustment factor for comorbidity from Van Geldorp, 2009 [33]
Duration of antibiotic treatment (weeks)					
Proven CQF	96	7.8	Gamma		Van Roeden, 2018 [34]
Probable CQF	83	9.1	Gamma		Van Roeden, 2018 [34]

CQF: Chronic Q fever, Sd: Standard deviation.
* Used for the multivariate probabilistic sensitivity analysis.

Costs

In accordance with the Dutch guideline on health economic evaluation in healthcare, we adopted a societal perspective. Costs considered in our analysis are:

- Direct healthcare costs: blood collection, diagnostic tests, surgeries, antibiotics, specialist visits.
- Indirect healthcare costs: costs unrelated to CQF in gained life years of averted premature CQF-related deaths.
- Direct non-healthcare costs: travel costs.
- Indirect non-healthcare costs: Productivity losses due to work absence.

Table 7.A9 shows the costs inputs presented in 2016 euros (€). Costs from other years were converted to the 2016 price year using the Dutch consumer price index.[35] A positive ELISA test will be followed by a IFA test for IgG titer of ≥1:512 against phase I (IFA screen) and a positive IFA screen test will be confirmed with a IFA titration to determine the exact titer. Patients with IgG titer of ≥1:512 against *C. burnetii* phase I will then be clinically evaluated by a medical specialist using different serological tests and imaging techniques (initial diagnostic procedure) whether the patient has proven, probable or possible CQF. In the base case analysis we ignored program costs because the screening of risk groups may also occur during routine visits. In the sensitivity analysis we explored a scenario in which we assumed that the program costs would be €11.36 per screened

person for selecting and inviting patients. We based these program costs on the tariff a GP currently receives for the selection, invitation and administration of influenza vaccination within the national influenza immunization program.

Cost of a surgery is the weighted average of vascular surgeries, heart valve surgeries and other kind of surgeries (according to surgery data from S.E. van Roeden, pers. comm., and cost data from the literature [36, 37]). Surgeries gathered under 'other surgeries' mostly consist of the drainage of a non-cardiac abscess and we used the cost of a pulmonary drainage for this parameter. The cost of antibiotics is based on a treatment with doxycycline and hydroxychloroquine and includes also costs of blood tests to determine the antibiotic levels. The duration of antibiotic treatment is shown in Table 7.A8. During treatment, patients visit the medical specialist every three months for serological follow up and CQF patients with a vascular infection have a PET scan every year. Follow-up of proven and probable CQF patients is life-long and consists of medical specialist visits and serological tests of which the frequency reduces over time. Possible CQF patients are followed until the IgG titer against *C. burnetii* phase I has been decreased to <1:1,024. We assumed that the average follow-up of possible CQF patients is one year. Concerning CQF-related complications, we assumed that the treatment of acute aneurysm, heart failure and arterial embolic complication would be lifetime. Treatment costs are obtained from the literature and include annual treatment costs as well as costs of future complications. For an arterial embolic complication we used costs of a stroke.

Indirect healthcare costs, also referred to as healthcare costs unrelated to CQF in gained life years, were estimated by using the remaining life-expectancy at the age of death (Table 7.A8) and age-specific healthcare costs from a specifically developed tool labelled Practical Application to Include Disease Costs (PAID).[38] This tool distinguishes healthcare costs incurred in the last year of life and costs incurred in other years by sex, age and healthcare provider. To avoid a possible double count of influenza-related costs, we excluded healthcare costs of the disease category heart failure and diseases of arteries. We included costs of all healthcare providers available in the tool and the weighted average of men and women was estimated using age-specific sex distributions of the Dutch population. The total indirect healthcare costs in the remaining life years was estimated using lifetables, attributing the cost incurred in a final life year to a person that died in the lifetable and cost incurred in other years to a person that survives in the lifetable. As the inclusion of indirect healthcare costs is specific for the Dutch guideline, we present results without the inclusion of indirect medical costs in the sensitivity analysis.

Direct non-medical costs include travel costs to the medical doctor, hospital and pharmacy. We assumed that blood collection for screening was conducted at the

medical doctor. Average distances to the different healthcare facilities and travel costs per kilometer were obtained from the Dutch guideline for economic evaluations in healthcare.

Indirect non-medical costs included productivity losses due to work absence were counted for screening, clinical evaluation and complications. The duration of absence was adjusted for age-specific labor participation rates and age-specific working hours per week from Statistics Netherlands of 2016.[39] The duration of absence was assumed to be half an hour for blood collection and 1.5 day for clinical evaluation. Given the seriousness of CQF-related complications, we assumed permanent work absence after developing a symptomatic aneurysm, heart failure or arterial embolic complication. In accordance with the Dutch guideline on economic evaluations in healthcare, we used the friction approach. This method assumes that work absence is limited to a certain friction period, as an unemployed person has replaced the deceased person after this period. We used a friction period of 85 days.[40] Productivity loss per absent working hour was €35.07.[40]

Table 7.A9. Costs in 2016 euros (€).

Cost unit	Input	Sd*	Distribution*	Scenario	Source and additional details
Direct healthcare costs					
Selection and invitation	0			11.36	Assumption: Screening occurs during routine visits
Blood collection	10.71				Dutch cost-effectiveness guideline, 2016 [40]
ELISA	7.00				Assumption based on [41]
IFA screen	9.90				List price JBH (P.M. Schneeberger, pers comm.)
IFA titration	19.80				List price JBH (P.M. Schneeberger, pers comm.)
Initial diagnostic procedure after positive IFA	1,299				Blood collection, IFA titration, PCR, CRP/standard blood tests, PET scan, TTE (all once); TEE (half of the patients); specialist consultations (three times) (CP. Bleeker-Rovers, pers. comm)
Surgery	14,717			30,000	Based on 76% vascular surgeries, 19% heart valve surgeries and 5% other kind of surgeries (S.E. van Roeden, pers. comm.) with average cost of 10,639 [36], 16,124 [37] and 8,803 [36].
Antibiotic treatment (per year)					
First year	343				Based on treatment with doxycycline (1 dd 200mg) and hydroxychloroquine (3 dd 200mg) [42], pharmacy dispensing fee (6 times, at the assumption of delivery per 2 months) and additional fee for first delivery (2 times), serological antibiotic level determination (2 times) (CP. Bleeker-Rovers, pers. comm)
Consecutive years	297				Doxycycline and hydroxychloroquine, pharmacy dispensing fee (see first year)
Costs routine visits during treatment (per year)	1,440				PCR, IFA, specialist visit, CRP/standard blood tests (all 4 times per year). A PET scan in the first year for vascular infections (CP. Bleeker-Rovers, pers. comm)
Follow-up					
Year 1	912				PCR, IFA, specialist visit, CRP/standard blood tests (4 times per year) (CP. Bleeker-Rovers, pers. comm)
Year 2	864				PCR, IFA, specialist visit, CRP/standard blood tests (3 times per year) (CP. Bleeker-Rovers, pers. comm)
Year 3	456				PCR, IFA, specialist visit, CRP/standard blood tests (2 times per year) (CP. Bleeker-Rovers, pers. comm)
Year 4 and after	228				PCR, IFA, specialist visit, CRP/standard blood tests (1 times per year) (CP. Bleeker-Rovers, pers. comm)
Complications (per year)					
Heart failure	3,176				Van Giessen, 2016 [43]
Vascular prosthesis or aneurysm	2,430	358	Gamma		Prinssen, 2007 [44]
Embolic complication					
Year 1	12,352	1897	Gamma		Van Eeden, 2015 [45]
Year 2 and after	4,997	2038	Gamma		Van Eeden, 2015 [45], costs of the second half of the year extrapolated to a year
Other complications	0				Assumption
Indirect healthcare costs (lifelong)				Excluded	
Proven CQF	60,301				PAID toolkit [38], based on the difference between life expectancy of CQF-related death and non-CQF-related death. Costs of heart failure and vascular infections were excluded, because these costs could be related to CQF.
Probable CQF	47,183				
Direct non-healthcare costs					
Screening travel cost	0.42				Assumption travel costs to hospital
Initial diagnosis travel cost	11.42				Travel costs to hospital, including parking fee (2 times) [40]
Surgery travel cost	11.42				Travel costs to hospital, including parking fee (2 times) [40]

Table 7.A9 continued.

Cost unit	Input	Sd*	Distribution*	Scenario	Source and additional details
Direct non-healthcare costs					
Antibiotics travel cost (per year)	2.99				Travel costs to pharmacy (2 times) [40]
Travel cost of routine visits during treatment or follow-up	5.71				Travel costs to hospital, including parking fee [40]
Indirect non-healthcare costs					
Productivity loss screening	4.36-12.57				Half an hour of productivity loss (Assumption). Cost depends on age due to differences in net labor participation rates and average working hours per week.
Productivity loss initial diagnostics	105-302				1.5 day of lost productivity (Assumption). Cost depends on age due to differences in net labor participation rates and average working hours per week.
Productivity costs complication	5,936-17,089				We assumed that a CQF complication was leading to long-term work absence. Given that the friction method is the recommended approach in the Netherlands to value productivity losses, we limited the work absence of a complication to a standardized friction period of 85 days.[40] Cost depends on age due to differences in net labor participation rates and average working hours per week.

CQF: Chronic Q fever, CRP: C-reactive protein, ELISA: Enzyme-linked immunosorbent assay, IFA: Immunofluorescence assay, JBH: Jeroen Bosch hospital, PAID: Practical Application to Include future Disease costs, PCR: Polymerase chain reaction, PET: positron emission tomography, Sd: Standard deviation, TEE: transesophageal echocardiography, TTE: transthoracal echocardiography.

* Used for the multivariate probabilistic sensitivity analysis.

SUPPLEMENTAL RESULTS

Table 7.A10. Subdivision of the Dutch 2017 adult population (N= 13,678,496) to Q fever incidence area using 4-digit postal codes and 3-digit postal codes.

Area	4-digit postal codes		3-digit postal codes	
	N	%	N	%
High IA	1,650,873	12.07	2,135,169	15.61
Middle IA	2,637,196	19.28	3,637,843	26.60
Low IA	9,390,427	68.65	7,905,484	57.79

N: Number, IA: Incidence area

Table 7.A11. Subdivision of the Dutch 2017 adult population (N= 13,678,496) to specific risk groups

Population	Size	%
Persons with diagnosed risk factor	908,248	6.64
Cardiovascular risk factor	462,512	3.38
Heart valve disorder or –prosthesis	329,112	2.41
Aortic aneurysm or vascular prosthesis	77,323	0.57
Congenital heart anomaly	40,968	0.30
Endocarditis	15,109	0.11
Immunocompromised status	445,736	3.26
Underlying disease*	158,858	1.16
Medication use	286,878	2.10
Rheumatoid arthritis	217,764	1.59
Inflammatory bowel disease	69,115	0.51
Persons without diagnosed risk factor	12,770,248	93.36
≥60 years	3,633,184	26.56
Undiagnosed cardiovascular risk factor	141,221	1.03
Heart valve disorder	96,311	0.70
Aortic aneurysm	44,911	0.33
No risk factor †	3,491,963	25.53
18-59 years	9,137,064	66.80
Undiagnosed cardiovascular risk factor	2,379	0.02
Heart valve disorder	-	0.00
Aortic aneurysm	2,379	0.02
No risk factor †	9,134,685	66.78

* Includes HIV infection, asplenia, spleen dysfunction, malignancy (e.g. leukemia) or bone marrow transplant.
† No risk factor is defined here as patients without a cardiovascular risk factor or compromised immune system.

Table 7.A12. Estimation of the prevalence and number of *C. burnetii* infected individuals and CQF patients

Screening population	Prevalence scenario	Population size	Sero-prevalence	*C. burnetii* infections	CQF prevalence after epidemic	CQF patients after epidemic	CQF prevalence at screening	CQF patients at screening	Proven CQF patients at screening	Probable CQF patients at screening	Possible CQF patients at screening
High IA											
CVRF patients	Low	55,821	215	1,202	26.2	146	6.4	36	25	11	-
	High	72,197	1,070	7,725	130.1	939	62.4	451	311	140	-
IC patients	Low	53,796	215	1,159	14.8	80	3.6	20	13	6	-
	High	69,578	1,070	7,445	73.4	511	35.2	245	169	76	-
≥60y, unknown RF	Low	438,493	215	9,444	1.7	74	0.4	18	10	4	4
	High	567,128	1,070	60,683	8.4	477	4.0	229	119	54	56
18-59y, unknown RF	Low	1,102,763	215	23,750	0.4	49	0.1	12	0	0	12
	High	1,426,266	1,070	152,610	2.2	317	1.1	152	4	2	146
Middle IA											
CVRF patients	Low	89,172	15	135	1.8	16	0.5	4	3	1	-
	High	123,007	230	2,829	28.0	344	13.4	165	114	51	-
IC patients	Low	85,937	15	131	1.0	9	0.3	2	2	1	-
	High	118,545	230	2,727	15.8	187	7.6	90	62	28	-
≥60y, unknown RF	Low	700,473	15	1,064	0.1	8	0.0	2	1	0	1
	High	966,258	230	22,224	1.8	175	0.9	84	44	20	21
18-59y, unknown RF	Low	1,761,614	15	2,677	0.0	6	0.0	1	0	0	1
	High	2,430,034	230	55,891	0.5	116	0.2	56	1	1	54
Low IA											
CVRF patients	Low	317,519	2.7	87	0.3	11	0.1	3	2	1	-
	High	267,308	100	2,673	12.2	325	5.8	156	108	48	-
IC patients	Low	306,002	2.7	84	0.2	6	0.0	1	1	0	-
	High	257,613	100	2,576	6.9	177	3.3	85	58	26	-
≥60y, unknown RF	Low	2,494,218	2.7	685	0.0	5	0.0	1	1	0	0
	High	2,099,798	100	20,998	0.8	165	0.4	79	41	19	19
18-59y, unknown RF	Low	6,272,687	2.7	1,724	0.0	4	0.0	1	0	0	1
	High	5,280,764	100	52,808	0.2	110	0.1	53	1	1	51
Total	Low	13,678,496	31	42,143	0.3	414	0.1	102	57	26	19
	High	13,678,496	286	391,188	2.8	3,842	1.3	1,844	1,032	465	347

CVRF: Cardiovascular risk factor, CQF: chronic Q fever, IA: Incidence area, IC: Immunocompromised, RF: Risk factor, y: years of age.

Table 7.A13. Screening outcomes at a screening participation rate of 50%

Screening population	Prevalence scenario	Persons screened	ELISA positive	IFA positive	CQF patients detected	NNS CQF	Proven CQF patients detected	NNS proven CQF
High IA								
CVRF patients	Low	27,911	856	28	18	1,552	12	2,252
	High	36,098	2,689	288	225	160	155	232
IC patients	Low	26,898	821	20	10	2,750	7	3,990
	High	34,789	2,544	184	123	284	85	412
≥60y, unknown RF	Low	219,247	6,656	21	9	24,020	5	46,141
	High	283,564	20,292	190	86	3,281	60	4,760
18-59y, unknown RF	Low	551,381	16,731	29	6	90,913	0	3,585,959
	High	713,133	50,927	225	3	254,977	2	369,966
Middle IA								
CVRF patients	Low	44,586	925	3	2	22,002	1	31,924
	High	61,503	1,950	105	83	745	57	1,081
IC patients	Low	42,969	891	2	1	38,980	1	56,559
	High	59,273	1,862	67	45	1,320	31	1,915
≥60y, unknown RF	Low	350,237	7,261	2	1	340,477	1	654,042
	High	483,129	15,017	70	32	15,263	22	22,146
18-59y, unknown RF	Low	880,807	18,259	3	1	1,288,685	0	50,830,867
	High	1,215,017	37,728	82	1	1,186,195	1	1,721,146
Low IA								
CVRF patients	Low	158,759	3,197	2	1	121,642	1	176,499
	High	133,654	3,354	100	78	1,713	54	2,486
IC patients	Low	153,001	3,081	1	1	215,509	0	312,699
	High	128,807	3,216	64	42	3,036	29	4,405
≥60y, unknown RF	Low	1,247,109	25,107	2	1	1,882,392	0	3,615,996
	High	1,049,899	26,057	66	30	35,104	21	50,936
18-59y, unknown RF	Low	3,136,344	63,141	2	0	7,124,742	0	281,028,271
	High	2,640,382	65,495	78	1	2,728,249	1	3,958,636

CQF: chronic Q fever, CVRF: Cardiovascular risk factor, IA: Incidence area, IC: Immunocompromised, NNS: Number needed to screen, QALY: Quality-adjusted life year, RF: Risk factor, y: years of age.

Table 7.A14. Clinical and health impact of the analyzed screening strategies as compared to no screening at a screening participation rate of 50%

Screening population	Prevalence scenario	Additional antibiotic courses	Complications averted	Surgeries averted	CQF-related deaths averted	Life years saved	QALYs gained
High IA							
CVRF patients	Low	4.1	-8.4	-4.3	-2.1	15.2	17.1
	High	51.5	-104.7	-53.9	-25.8	190.2	214.9
IC patients	Low	2.2	-4.5	-2.3	-1.1	8.3	9.3
	High	28.0	-56.9	-29.3	-14.0	103.4	116.9
≥60y, unknown RF	Low	1.6	-3.2	-1.6	-0.8	5.8	6.6
	High	19.8	-40.1	-20.7	-9.9	72.9	82.4
18-59y, unknown RF	Low	0.1	-0.1	-0.1	-0.0	0.2	0.2
	High	0.6	-1.3	-0.7	-0.3	2.4	2.7
Middle IA							
CVRF patients	Low	0.5	-0.9	-0.5	-0.2	1.7	1.9
	High	18.9	-38.3	-19.7	-9.4	69.6	78.7
IC patients	Low	0.3	-0.5	-0.3	-0.1	0.9	1.1
	High	10.3	-20.9	-10.7	-5.1	37.9	42.8
≥60y, unknown RF	Low	0.2	-0.4	-0.2	-0.1	0.7	0.7
	High	7.2	-14.7	-7.6	-3.6	26.7	30.2
18-59y, unknown RF	Low	0.0	-0.0	-0.0	-0.0	0.0	0.0
	High	0.2	-0.5	-0.2	-0.1	0.9	1.0

Table 7.A14 continued.

Screening population	Prevalence scenario	Additional antibiotic courses	Complications averted	Surgeries averted	CQF-related deaths averted	Life years saved	QALYs gained
Low IA							
CVRF patients	Low	0.3	-0.6	-0.3	-0.1	1.1	1.2
	High	17.8	-36.2	-18.7	-8.9	65.8	74.4
IC patients	Low	0.2	-0.3	-0.2	-0.1	0.6	0.7
	High	9.7	-19.7	-10.1	-4.9	35.8	40.5
≥60y, unknown RF	Low	0.1	-0.2	-0.1	-0.1	0.4	0.5
	High	6.8	-13.9	-7.2	-3.4	25.2	28.5
18-59y, unknown RF	Low	0.0	-0.0	-0.0	-0.0	0.0	0.0
	High	0.2	-0.4	-0.2	-0.1	0.8	0.9

CQF: chronic Q fever, CVRF: Cardiovascular risk factor, IA: Incidence area, IC: Immunocompromised, QALY: Quality-adjusted life year, RF: Risk factor, y: years of age.

Table 7.A15. Incremental costs of the analyzed screening strategies as compared to no screening at a screening participation rate of 50%

Screening population	Prevalence scenario	Screening costs (€)	Direct healthcare costs (€)	Non-healthcare costs (direct and indirect) (€)	Total societal costs (excluding indirect healthcare costs) (€)	Indirect healthcare costs (€)	Total societal costs (including indirect healthcare costs) (€)
High IA							
CVRF patients	Low	503,270	-144,557	81,542	440,256	103,818	544,074
	High	671,548	-1,892,276	-155,227	-1,375,955	1,301,471	-74,484
IC patients	Low	484,832	-73,132	148,657	560,358	56,473	616,831
	High	644,881	-993,980	-88,602	-437,702	707,956	270,255
≥60y, unknown RF	Low	3,948,773	-52,244	527,743	4,424,273	39,806	4,464,079
	High	5,226,068	-679,387	657,153	5,203,834	499,016	5,702,850
18-59y, unknown RF	Low	9,930,185	9,116	6,290,432	16,229,733	1,288	16,231,021
	High	13,136,941	113,863	8,142,174	21,392,977	16,148	21,409,125
Middle IA							
CVRF patients	Low	798,757	-16,291	163,934	946,400	11,700	958,100
	High	1,110,506	-693,011	123,447	540,942	476,640	1,017,582
IC patients	Low	769,765	-8,242	273,324	1,034,847	6,364	1,041,211
	High	1,069,382	-364,027	266,486	971,841	259,276	1,231,117
≥60y, unknown RF	Low	6,273,987	-5,888	846,345	7,114,444	4,486	7,118,930
	High	8,705,394	-248,813	1,157,466	9,614,047	182,756	9,796,803
18-59y, unknown RF	Low	15,778,334	1,027	10,047,828	25,827,189	145	25,827,334
	High	21,890,903	41,700	13,862,862	35,795,465	5,914	35,801,379
Low IA							
CVRF patients	Low	2,843,033	-10,492	591,186	3,423,727	7,535	3,431,262
	High	2,401,947	-654,782	398,729	2,145,894	450,347	2,596,241
IC patients	Low	2,739,902	-5,308	981,177	3,715,771	4,099	3,719,870
	High	2,314,029	-343,946	719,500	2,689,583	244,973	2,934,557
≥60y, unknown RF	Low	22,332,648	-3,792	3,014,362	25,343,218	2,889	25,346,107
	High	18,851,093	-235,088	2,528,039	21,144,045	172,674	21,316,719
18-59y, unknown RF	Low	56,164,141	662	35,777,732	91,942,535	93	91,942,628
	High	47,406,356	39,400	30,122,503	77,568,258	5,588	77,573,846

CVRF: Cardiovascular risk factor, IA: Incidence area, IC: Immunocompromised, QALY: Quality-adjusted life year, RF: Risk factor, y: years of age.

Table 7.A16. Costs of screening of 50% of all adults in the Netherlands as compared to no screening at all

Cost component	Without screening (€, million)	Screening (€, million)	Difference (€, million)
Direct healthcare costs			
Screening	-	123.43	123.43
Blood sampling	-	73.24	73.24
ELISA	-	47.87	47.87
IFA	-	2.32	2.32
Treatment of CQF	33.43	31.84	-1.59
Diagnostic procedures	1.39	2.18	0.79
Surgeries	8.80	6.17	-2.64
Antibiotics	0.51	0.61	0.09
Follow-up during treatment	1.78	2.10	0.32
Follow-up after treatment	2.57	3.26	0.69
Complications	18.37	13.20	-5.17
Indirect healthcare costs	-	4.32	4.32
Direct non-healthcare costs	0.15	3.07	2.92
Travel costs screening	-	2.89	2.89
Travel costs treatment of CQF	0.15	0.18	0.03
Indirect non-healthcare costs	4.20	59.01	54.82
Productivity loss screening	-	55.92	55.92
Productivity loss treatment of CQF	4.20	3.09	-1.10
Total societal costs	37.77	217.35	179.58

CQF: Chronic Q fever, ELISA: Enzyme-Linked Immuno Sorbent Assay, IFA: Immunofluorescence assay.

Table 7.A17. Cost-effectiveness of screening strategies as compared to no screening at a screening participation rate of 50%

		Screening		No Screening		Difference		
Screening population	Prevalence scenario	Costs (€, million)*	QALYs*	Costs (€, million)*	QALYs*	Costs (€, million)*	Total QALYs*	ICER (€ per QALY gained)
High IA								
CVRF patients	Low	1.44	174.9	0.89	157.8	0.54	17.1	31,737
	High	11.11	2,192.7	11.19	1,977.8	-0.07	214.9	Cost-saving
IC patients	Low	1.15	95.1	0.53	85.8	0.62	9.3	66,145
	High	6.94	1,192.8	6.67	1,075.9	0.27	116.9	2,312
≥60y, unknown RF	Low	4.78	165.5	0.32	158.9	4.46	6.6	679,136
	High	9.70	2,074.8	4.00	1,992.4	5.70	82.4	69,208
18-59y, unknown RF	Low	16.25	259.7	0.02	259.5	16.23	0.2	76,308,665
	High	21.62	3,255.4	0.21	3,252.8	21.41	2.7	8,029,064
Middle IA								
CVRF patients	Low	1.06	19.7	0.10	17.8	0.96	1.9	495,918
	High	5.11	803.0	4.10	724.3	1.02	78.7	12,929
IC patients	Low	1.10	10.7	0.06	9.7	1.04	1.1	990,755
	High	3.67	436.8	2.44	394.0	1.23	42.8	28,755
≥60y, unknown RF	Low	7.15	18.7	0.04	17.9	7.12	0.7	9,610,222
	High	11.26	759.9	1.47	729.7	9.80	30.2	324,632
18-59y, unknown RF	Low	25.83	29.3	0.00	29.2	25.83	0.0	1,077,459,984
	High	35.88	1,192.2	0.08	1,191.3	35.80	1.0	36,661,479
Low IA								
CVRF patients	Low	3.50	12.7	0.06	11.5	3.43	1.2	2,757,608
	High	6.47	758.7	3.87	684.4	2.60	74.4	34,912
IC patients	Low	3.76	6.9	0.04	6.2	3.72	0.7	5,495,846
	High	5.24	412.7	2.31	372.3	2.93	40.5	72,544
≥60y, unknown RF	Low	25.37	12.0	0.02	11.5	25.35	0.5	53,126,291
	High	22.70	717.9	1.38	689.4	21.32	28.5	747,603
18-59y, unknown RF	Low	91.94	18.8	0.00	18.8	91.94	0.0	5,955,497,518
	High	77.65	1,126.5	0.07	1,125.6	77.57	0.9	84,075,394

CQF: Chronic Q fever, CVRF: Cardiovascular risk factor, IA: incidence area, IC: Immunocompromised, ICER: Incremental cost-effectiveness ratio, QALY: Quality-adjusted life year, RF: Risk factor, y: years of age.

* In CQF patients only, except costs of screening.

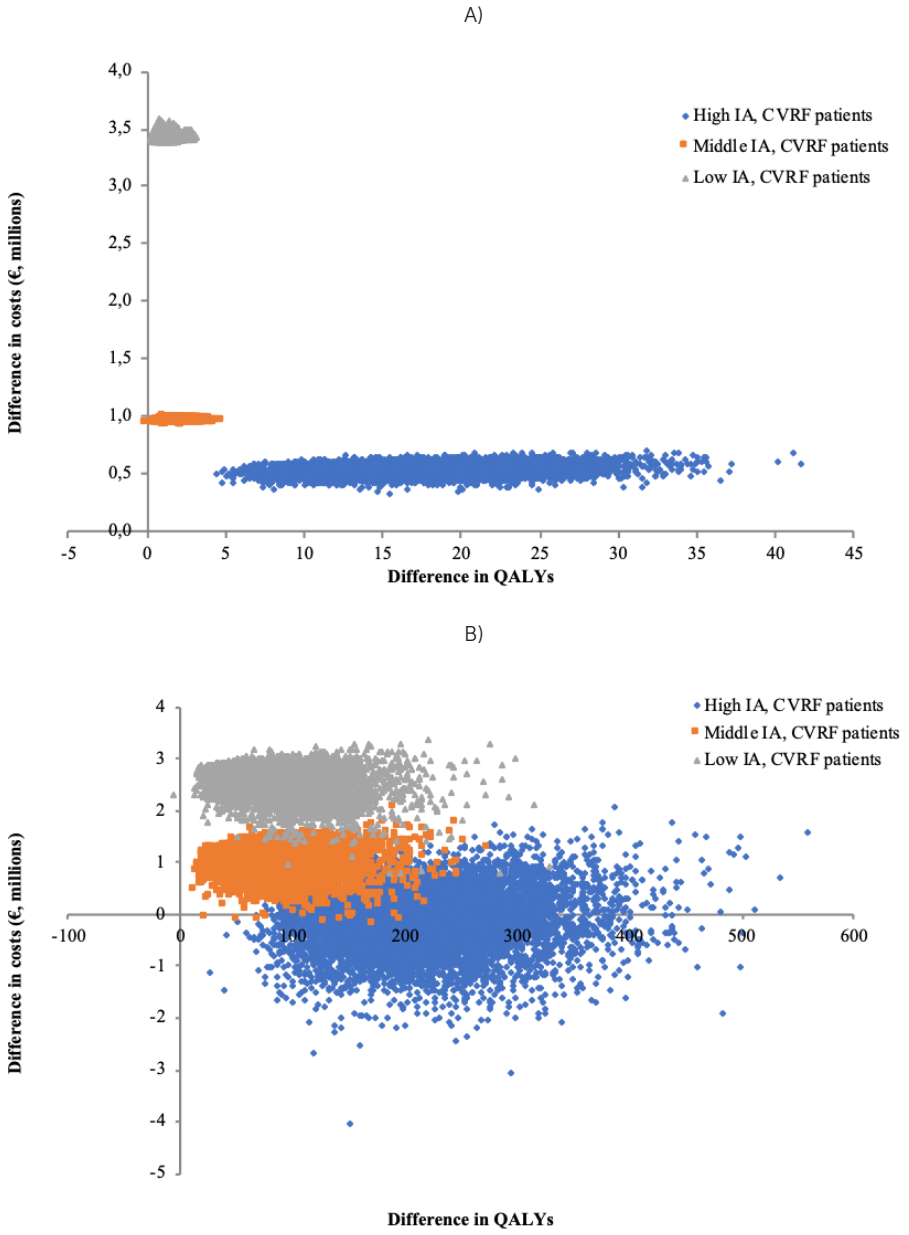

Figure 7.A2. Results of the multivariate sensitivity analysis using 10,000 simulations for screening of patients with a cardiovascular risk factor in high, middle and low incidence areas for the (A) low CQF prevalence scenario and (B) high CQF prevalence scenario.

CQF: chronic Q fever, CVRF: Cardiovascular risk factor, IA: Incidence area, QALY: Quality-adjusted life year.

Table 7.A18. Cost-effectiveness of screening without the inclusion of indirect medical costs

Screening population	CQF prevalence scenario	Difference in QALYs	Difference in costs (without indirect healthcare costs	ICER (€ per QALY gained) (without indirect healthcare costs)
High IA				
CVRF patients	Low	17.1	440,256	25,681
	High	214.9	-1,375,955	-6,402
IC patients	Low	9.3	560,358	60,090
	High	116.9	-437,702	-3,744
≥60y, unknown RF	Low	6.6	4,424,273	673,080
	High	82.4	5,203,834	63,152
18-59y, unknown RF	Low	0.2	16,229,733	76,302,609
	High	2.7	21,392,977	8,023,009
Middle IA				
CVRF patients	Low	1.9	946,400	489,862
	High	78.7	540,942	6,873
IC patients	Low	1.1	1,034,847	984,699
	High	42.8	971,841	22,699
≥60y, unknown RF	Low	0.7	7,114,444	9,604,166
	High	30.2	9,614,047	318,576
18-59y, unknown RF	Low	0.0	25,827,189	1,077,453,928
	High	1.0	35,795,465	36,655,423
Low IA				
CVRF patients	Low	1.2	3,423,727	2,751,552
	High	74.4	2,145,894	28,856
IC patients	Low	0.7	3,715,771	5,489,790
	High	40.5	2,689,583	66,488
≥60y, unknown RF	Low	0.5	25,343,218	53,120,236
	High	28.5	21,144,045	741,547
18-59y, unknown RF	Low	0.0	91,942,535	5,955,491,462
	High	0.9	77,568,258	84,069,338

CQF: Chronic Q fever, CVRF: Cardiovascular risk factor, IC: Immunocompromised, ICER: Incremental cost-effectiveness ratio, QALY: Quality-adjusted life year, RF: Risk factor, y: years of age.

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CHAPTER 8

GENERAL DISCUSSION

OCCUPATIONALLY EXPOSED PEOPLE (CHAPTER 2 AND 3)

WHAT IS ALREADY KNOWN ON THIS TOPIC

Ruminants are the primary source for human *C. burnetii* infection (1). Occupationally exposed people, like veterinarians, culling workers, and farmers, are at particular risk for such an infection.[1] The seroprevalence in farmers and farm residents of dairy goat and cattle farms has previously been investigated in the Netherlands and was found to be 69% (2009-2010) and 72% (2010-2011) respectively.[2, 3] However, the seroprevalence was unknown for sheep farm residents in this country. It is likely that risk for infection differs between dairy and non-dairy sheep farm residents because the seroprevalence is higher among dairy sheep than among non-dairy sheep (19% vs. 2% respectively).[4] Veterinarians in the Netherlands were also found to be at increased risk for a *C. burnetii* infection, as 65% of Dutch veterinarians had antibodies against the bacterium in 2009.[5] In a cross sectional study in 2006, 19% of veterinary students had antibodies against *C. burnetii*, with a higher prevalence among students in an advanced stage of the study.[6] However, the seroconversion rate during the study and associated risk factors during veterinary training were still unknown.

WHAT THESE STUDIES ADDS

Sheep farm residents

The *C. burnetii* seroprevalence with associated risk factors in both dairy and non-dairy sheep farm residents across the Netherlands were investigated, i.e. the farmer and a maximum of two family members aged ≥12 years residing at the farm. In 2009-2010, 18/27 (67%) of the dairy sheep farm residents, and 139/271 (51%) of the non-dairy sheep farm residents were found to be seropositive; however, this was not a significant difference. None of the seropositive dairy sheep farm residents, and only six of the 139 seropositive non-dairy sheep farm residents (4%) had been diagnosed with acute Q fever. Additionally, one seropositive dairy sheep farm resident out of 18 (6%) had a serological profile indicative for a chronic infection.

Veterinary students

From 2006 through 2010, two cohorts of students of the only veterinary medicine school in the Netherlands at Utrecht University were followed, to estimate the *C. burnetii* seroconversion rate during their study and associated risk factors. Of the participants with a blood sample at baseline, 13 of the 131 (10%) participants were seropositive. Of the 118 seronegative participants at baseline, 23 seroconverted during the follow-up period of 362 person-years, which resulted in an incidence of 0.06 per person-year. Three non-study related factors were associated with seroconversion. Contact with

sheep and living on a goat or sheep farm were risk factors for seroconversion. Next, contact with hay, straw, silage grass, or animal feed during their study, was a risk factor for seroconversion. None of the seroconverted participants reported that they had been diagnosed with acute Q fever, and none of the participants had a serological indication for a chronic infection.

RECOMMENDATIONS ARISING FROM THESE STUDIES

A high *C. burnetii* seroprevalence was found among sheep farm residents and a high seroconversion rate among veterinary students, of whom only few had been diagnosed with acute Q fever. This is supported by data from the national infectious diseases registration system, in which only 4% of the notified acute Q fever patients were working in the agricultural sector or animal care in the period 1-1-2007 through 8-12-2009.[7] A possible explanation for the low burden in this group is that infection with *C. burnetii* may be more often symptomatic in older individuals, as notified acute Q fever patients had a median age of 50 years.[7] Farmers and veterinary students are likely to be exposed at young age and may therefore be more frequently infected asymptomatically.

Next, in the two described studies, only one dairy sheep farm resident had a serological profile indicating a chronic infection. However, in veterinarians, which is the studied group in an advanced stage of their career, a high IgG phase I seroprevalence was found.[5, 8] It remains debatable whether presence of antibodies in occupationally exposed people with frequent boosting is of clinical significance, and needs further investigation.[8] A full assessment of the role of occupational exposure in chronic Q fever is not possible as the Dutch national chronic Q fever database contains anonymous information from medical files, and occupation is generally not recorded in medical files. To be able to investigate whether occupation is a risk factor for chronic disease, occupation should be added to the chronic Q fever database retrospectively, and if necessary, the database should be adapted to a non-anonymous database to add the occupation to the database.

Screening for chronic Q fever in occupationally exposed people might be considered. As chronic Q fever is a severe disease, possibly resulting in death, it should be diagnosed as soon as possible.[9] As the burden of chronic Q fever unknown in occupationally exposed people in the Netherlands, it is not advisable to screen all people at this moment. However, occupationally exposed people who are at higher risk for chronic Q fever (such as people with an aneurysm, vascular prosthesis, or heart valve defect) should be annually screened for IgG phase I antibodies.[9] If an IgG phase I titer of $\geq$1:512 is found, these people should be referred to an internal medicine specialist for further medical examination and for possible treatment, to be able to prevent further complications of chronic Q fever.

Additionally, vaccination of the occupationally exposed groups might be considered. Since 2002, occupational groups are offered vaccination with the Q-VAX vaccine in Australia.[10] Vaccination is not straightforward, as any person who had previous exposure to *C. burnetii* should not receive the vaccination due to an increased risk of adverse events following immunization.[11] In the Netherlands, the vaccine is not registered, but was offered in 2011 to people at risk for chronic Q fever in the high incidence area.[12] The Health Council of the Netherlands does not advise vaccination for occupationally all exposed people at this moment, because there is little information available about the burden of disease in this group, and because the veterinary measures taken are sufficient to protect most workers[13, 14], this is in line with the results of this thesis. Burden is an important factor according to the Wilson and Jungner criteria whether vaccination should be introduced.[15] However, vaccination might be considered in individual cases, for example for occupationally exposed people with a higher risk of a chronic infection. If more information becomes available about the burden of disease in this group of people, then the advice of screening and vaccination should be reconsidered.

PREGNANCY OUTCOMES IN AREAS AFFECTED BY Q FEVER (CHAPTER 4)

WHAT IS ALREADY KNOWN ON THIS TOPIC

Case series reports described that pregnant women with untreated acute or chronic Q fever infection may result in adverse pregnancy outcomes in up to 81% of the cases, including spontaneous abortion, perinatal death, preterm delivery, and low birth weight.[16-19] Additionally, in some of these studies, the *C. burnetii* bacterium was detected in placental tissue, where it sometimes also caused necrosis.[16, 18, 19] The risk was highest when infection occurred during the first trimester.[16] These results were supported by community-based studies among pregnant women in Canada and Spain.[20, 21] However, studies in Denmark, and the Netherlands found no elevated risk for pregnant women with a serological indication of a *C. burnetii* infection.[22-24] Additionally, in case reports from Denmark and the Netherlands, the *C. burnetii* bacterium was not detected in placental tissue.[25, 26] Last, a randomized controlled clinical trial conducted during the large outbreak in the Netherlands showed no benefit of screening for antibodies against *C. burnetii* during pregnancy.[27]

WHAT THIS STUDY ADDS

Because of these inconsistent findings, an ecological study was performed in which pregnancy outcomes from women residing in Q fever-affected areas were compared with outcomes from women in areas without Q fever notifications. Information on the

pregnancy outcome at the individual level was obtained from the Netherlands Perinatal Registry. Residential area of the pregnant woman was categorized as 'affected' or 'not-affected' by the Q fever outbreak in 2008-2010, based on number of notifications in the residential area. Pregnancy outcomes of 2003-2004 were used as a baseline, to correct for any existing differences in pregnancy outcomes between the areas before the large outbreak. In total, 58,737 pregnancy outcomes of women residing in Q fever-affected areas were evaluated, with 310,635 outcomes from women in areas without Q fever notifications. In this study, no association between residing in a Q fever-affected area and both preterm delivery and perinatal mortality was found. In contrast, a weak association with small for gestational age was found, with a population-attributable fraction of 0.7%. This means that if Q fever had not occurred, 0.7% of the children small for gestational age in the Q fever-affected areas could have been prevented, if in fact there is a causal relationship, for which the present ecological study design can provide no evidence. In the case of a causal relationship it was estimated that 38 children small for gestational age would be attributable to residing in a Q fever affected area. Other known risk factors, like heavy smoking and young maternal age, had a stronger association with adverse pregnancy outcomes. Furthermore, no dose-response relation between a higher incidence of Q fever notifications and all three studied pregnancy outcomes was found, and no stronger association for women who were in the first trimester of the pregnancy during the months of highest acute Q fever incidence was found.

RECOMMENDATIONS ARISING FROM THIS STUDY

As most pregnant women with a *C. burnetii* infection remain asymptomatic [28], routine screening and treatment is an intervention to be considered in this group. No association between residing in a Q fever affected area and preterm delivery and perinatal mortality was found, and there might only be a marginal effect on having a child small for gestational age. However, it cannot be concluded from this study whether there is a causal relationship between residing in a Q fever affected area and having a child small for gestational age. Because no dose-response relation was found, and no stronger association was found for women who were in the first trimester of the pregnancy during the months of highest Q fever incidence, the causal relationship is less likely. Additionally, the evidence on effectiveness of antibiotic treatment in pregnant women is scarce, and can possibly lead to complications. In conclusion, there might be a small risk for an adverse pregnancy outcome due to a *C. burnetii* infection, but screening of all pregnant women and possible antibiotic treatment in an area experiencing a Q fever outbreak is not justified, which is in line with the recommendations of Munster et al.[27, 29]

CHRONIC Q FEVER SCREENING (CHAPTER 5, 6 AND 7)

WHAT IS ALREADY KNOWN ON THIS TOPIC

Chronic Q fever is a severe disease, which can lead to death.[9] Therefore, it is important to diagnose chronically infected persons as soon as possible, to start long-term antibiotic treatment to prevent complications. There are various strategies for detecting or possibly preventing chronic Q fever. The first possible strategy is to follow-up diagnosed acute Q fever patients for detection of chronic Q fever.[30] Having a valvulopathy, like a leaking heart valve, is an important risk factor for developing chronic Q fever endocarditis.[31, 32] Progression to endocarditis has been reported in patients with undiagnosed and clinically silent valvulopathy.[33] French researchers therefore recommend offering acute Q fever patients an echocardiography and prescribing long-term antibiotics for those with a valvulopathy, to prevent chronic Q fever.[31, 33] In 2007, at the start of the Dutch outbreak, all treating physicians were advised to refer acute Q fever patients to a cardiologist for an echocardiography. However, when the number of acute Q fever notifications sharply increased in 2008, cardiologists became increasingly reluctant to continue routine screening because it drained at lot of resources and mostly "minor" valvulopathies were diagnosed, which have no clinical relevance. Additionally, none of the patients with mainly minor valvulopathies were diagnosed with chronic Q fever, and screening was stopped.[34, 35] However, these patients might have been diagnosed with chronic Q fever after a longer period.

Another strategy to diagnose chronic Q fever as soon as possible is screening of high-risk patients. Other risk factors for chronic Q fever next to a valvulopathy are a vascular prosthesis, an aneurysm, and immunosuppression.[31, 36-38] During the outbreak, these patients groups were not routinely screened for chronic Q fever in the Netherlands, but only in small-scale study context.[39-41] Chronic Q fever may be diagnosed years after the acute infection, but whether screening seven years after the large outbreak will uncover new patients is unknown. If this is the case, then a cost-effectiveness study is important to investigate whether the costs outweigh the benefits.

WHAT THESE STUDIES ADDS

Screening of acute Q fever patients for valvulopathy

To evaluate if patients with newly detected valvulopathies had a higher risk to develop chronic Q fever, patients who were diagnosed with acute Q fever in 2007 or 2008 were invited, for a four-year follow-up study. Later, the chronic Q fever database in 2016 was checked, which is approximately eight years after the acute Q fever diagnosis, to see whether even more patients with chronic Q fever had been diagnosed. A total

of 509 patients were included in the study, of which 306 patients (60%) underwent echocardiography screening at time of diagnosis. Of these screened participants, 20 had already been diagnosed with one or more valvulopathies before the acute Q fever episode. Of the remaining 286 screened participants, 84 had a newly detected valvulopathy, of which two (2%) developed chronic Q fever, and of those with no valvulopathy, 12 of the 202 (6%) were diagnosed having chronic Q fever. The difference between the groups was not significant. The two patients with newly detected valvulopathy did not receive antibiotic prophylaxis to prevent chronic Q fever, as no professional guidelines existed. Last, no new chronic Q fever patients were diagnosed after the initial four-year follow-up period.

Screening of patients with valvulopathy for chronic Q fever

Seven years after the Q fever outbreak, in 2016-2017, a cross-sectional study in a hospital in the epicenter of the Q fever outbreak was performed, to investigate how many chronic Q fever patients can be detected by routine screening of high-risk patients with valvulopathy and to establish whether the existing policy of not screening should be changed. Patients of 18 years and older were eligible for inclusion if they had a newly diagnosed or already known valvulopathy at the cardiology outpatient clinic, or were admitted to the cardiology ward. Patients with insufficiency or stenosis of aortic or mitral valves that were natural or artificial were invited. Of the 904 included patients, 133 (15%) had evidence of a previous *C. burnetii* infection, of whom 6 (5%) had a chronic infection. Even such a long time after the outbreak, new chronic Q fever patients were identified. Therefore, a cost-effectiveness study was performed to provide more insight into costs and benefits of screening.

Cost-effectiveness study for screening of chronic Q fever

With a health-economic decision model, the cost-effectiveness of a one-of screening program for chronic Q fever seven years after the outbreak was evaluated. In the model, spatial data on acute Q fever, results from targeted screening programs, and clinical data from the Dutch national chronic Q fever database was used to parameterize it. The adult Dutch population was divided in residing in three acute Q fever incidence areas during the outbreak in 2007-2010, namely low, middle, and high incidence area. Then, these three groups were further subdivided based on presence of a medically-diagnosed cardiovascular risk factor (such as an aneurysm or heart valve disease), an immunocompromised risk status (immunocompromised due to an underlying disease or due to immunosuppressive medication use), or an unknown risk status. Last, the six groups of patients were divided in groups of ≥60 years and <60 years, as the prevalence of the cardiovascular risk factors increases with age. In total, twelve different sub-groups were considered for screening. Because of the uncertainties of the chronic Q fever

prevalence, two scenarios were considered; a low and a high chronic Q fever prevalence scenario. The cost-effectiveness varied largely between sub-populations and region. Mass screening was not cost-effective in any region and any prevalence scenario. However, targeted screening of cardiovascular risk patients in high Q fever incidence areas was estimated cost saving in the high prevalence scenario and €31,373 per quality adjusted life year (QALY) gained in the low prevalence scenario. The next most cost-effective strategy would be screening of immunocompromised patients residing in high incidence areas, €2,312 per QALY gained for the high prevalence scenario, and €66,145 per QALY gained for the low prevalence scenario.

RECOMMENDATIONS ARISING FROM THESE STUDIES

To decide whether a new screening approach should be implemented, often the Wilson and Jungner criteria are used.[15] For echocardiographic screening of acute Q fever patients, some criteria are supportive for screening. First, chronic Q fever is a severe disease that may lead to death. Next, Dutch hospitals have the health infrastructure to implement such an echocardiographic screening. Last, this type of screening is not invasive and is therefore acceptable as screening test. However, some criteria are not supportive. First, if screening is implemented, many acute Q fever patients will receive antibiotic prophylaxis when a heart valve disorder is found, even if chronic Q fever is unlikely to develop without prophylaxis. Second, as in an earlier study performed in the Netherlands, mostly minor valvulopathies were diagnosed, without any clinical significance.[35] Last, no cost-effectiveness study was performed on this topic. Therefore, it can be concluded that echocardiographic screening of acute Q fever patients would lead to unnecessary large-scale and long-term use of antibiotics and screening is not advisable at this moment. More research is needed into what type and grades of valvulopathy are actually risk factors for the development of chronic Q fever. On the other hand, as more chronic Q fever infections with a vascular focus were diagnosed in the Netherlands, screening of acute Q fever patients for the presence of an aneurysm might be an alternative screening option in a future outbreak.[9]

Seven years after the large outbreak, still new high-risk patients were diagnosed with chronic Q fever. Again, the Wilson and Jungner criteria can be used whether this type of screening should be implemented.[15] Some criteria are not supportive for screening. Although screening among general population in a village in high incidence area and targeted screening programs have been carried out, it is unknown for the majority of the Netherlands how many people have actually experienced a Q fever infection in the past and how many developed a chronic infection, especially in the various types of incidence areas.[39-42] Next, also the effectiveness of screening in the prevention of complications and premature death is not widely investigated. Surgery, however, is known to have a

positive impact on survival of chronic Q fever patients with a vascular infection.[43] Last, a standard for treatment of chronic Q fever patients is missing.[44] First-line treatment is a combination of doxycycline with hydroxychloroquine, but may cause significant toxicity such as photosensitivity, retinopathy, and black hyperpigmentation.[45, 46] On the other hand, there are also criteria that are supportive for screening. Important, after establishing that chronic Q fever is a severe disease, is whether the facilities for diagnosis and treatment are available. The Medical Microbiology Laboratories in the Q fever-affected areas have developed a lot of expertise in Q fever diagnostics. Additionally, they are able to process large number of samples, especially if screening would be introduced in phases. Next, there are at least eight hospitals across the country that have an infectious disease consultant with particular experience in treating chronic Q fever patients. Last, screening of cardiovascular risk patients was found to be cost-saving in the high prevalence scenario and €31.373/QALY gained in a low prevalence scenario. To indicate whether a screening is cost-effective, this incremental cost-effectiveness ratio (ICER) is often compared to a cost-effectiveness threshold. The Netherlands has no official threshold, but a threshold of €20.000/QALY is conventionally used for preventive measures, and €50.000/QALY for therapeutic measures.[47] The higher threshold should be used for chronic Q fever in high-risk patients, because there are therapeutic measures available, like surgery, to prevent further complications. Next to the Wilson and Jungner criteria, other factors could also play a role in deciding whether a screening program should be implemented. For example, there was great societal pressure from the Q fever patient organization, provincial politicians and some public health and clinical specialists for a national screening program. This all taken together, a one-of screening program for people with a known risk factor in high incidence areas should be advised, which are areas with a high Q fever incidence or areas with a farm with Q fever abortion waves during the outbreak. In March 2019, a pilot screening of cardiovascular risk patients and patients with an immunocompromised risk status in a few general practices in a high incidence area was started. Of the first tested 769 persons, 183 (24%) experienced Q fever in the past. Five were considered suspect for chronic Q fever (3% of the seropositive patients), of which four were confirmed as proven chronic Q fever and one as false positive (personal communication Daphne Reukers, 25-11-2019). The number of people who have experienced an infection in low incidence areas might be underestimated in these areas based on the notifications, as doctors in these areas might have been less aware of Q fever. Therefore, the risk of chronic Q fever might also be underestimated in these areas. In 2016/2017, the RIVM performed a nationwide serosurvey (Pienter III), primarily aimed at assessing the protection of the general population against vaccine-preventable diseases, but in which also will be investigated how many people have experienced a Q fever infection in the past in low Q fever incidence areas. This could then be compared with a similar survey (Pienter II) that was performed in 2006/2007, so

before the large outbreak.[48] The nationwide serosurvey might point to possible Q fever 'hot spots' outside the already known affected areas, in which also a one-of screening program should be performed in high-risk patients.

CONCLUSIONS

In the first part of this thesis, a high *C. burnetii* seroprevalence among sheep farm residents and a high *C. burnetii* seroconversion rate among veterinary students was found, mostly without clinical symptoms of acute Q fever. The risk for chronic Q fever is still unknown in these occupational groups. Therefore, occupation should be added to the chronic Q fever database, to be able to estimate the burden of disease of chronic Q fever in occupationally exposed people. As this burden is still unknown for this group, screening or vaccinating of all occupationally exposed people for chronic Q fever is not advisable. However, annually screening for IgG phase I antibodies in people who are at higher risk for chronic Q fever should be advised, and if an IgG phase I titer of $\geq$1:512 is found, they should be referred to an internal medicine specialist for further medical examination and for possible treatment, to prevent further complications.

In the next part of this thesis, no association between residing in a Q fever affected area and preterm delivery and perinatal mortality was found, and there might only be a marginal effect on having a child small for gestational age. Therefore, mass screening of all pregnant women and possible antibiotic treatment in case of an outbreak is not justified.

Last, the identification of chronic Q fever patients. Echocardiographic screening of acute Q fever patients would lead to unnecessary large-scale and long-term use of antibiotics and therefore such a screening is not advisable. On the other hand, a one-of screening of high-risk patients (cardiovascular patients and patients with an immunocompromised risk status) in high incidence areas for chronic Q fever should be performed, as we estimated that screening was cost-effective. Additionally, even ten years after the large outbreak still new chronic Q fever patients have been diagnosed in a high-risk group of patients with a valvulopathy. If screening will be cost-effective in low incidence areas depends on the seroprevalence, which is largely unknown in these areas after the outbreak. This seroprevalence will be investigated in a new nationwide serosurvey, performed in 2016-2017, will show if any unknown 'hot spots' were missed. In these possible new 'hot spots', the one-of chronic Q fever screening should also be performed in high-risk patients.

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APPENDICES

SUMMARY

This thesis has three aims. The first aim is to investigate how often farm residents and veterinary students are infected with *C. burnetii*. The second aim is to investigate whether living in a Q fever-affected area is a risk factor for an adverse pregnancy outcome. The last aim is to study two strategies to diagnose chronic Q fever infections. First, echocardiographic screening of all acute Q fever patients for detecting a heart valve defect is investigated, as antibiotic prophylaxis for patients with cardiac valvulopathy is considered an important approach to prevent chronic Q fever-related endocarditis. Second, a one-of screening of high-risk patients is researched, namely in patients with a valvulopathy. In addition, a cost-effectiveness study of a one-of screening program is performed, in order to identify not yet diagnosed chronic Q fever patients, and to investigate which scenario is the most cost-effective.

Chapter 1 provides a general introduction to Q fever and describes the content and aims of this thesis.

The *C. burnetii* seroprevalence was already known for goat and cattle farm residents, but not yet for sheep farm residents. Additionally, the seroprevalence was known to be higher for dairy sheep, than for non-dairy sheep. The *C. burnetii* seroprevalence among dairy and non-dairy sheep farm residents is investigated in **Chapter 2**. In 2009-2010, 18/27 (67%) of the dairy sheep farm residents, and 139/271 (51%) of the non-dairy sheep farm residents were *C. burnetii* seropositive, which was not significantly different. Only 4% of the seropositive non-dairy sheep farm residents reported to be diagnosed with acute Q fever in the past, and only 1/18 (6%) of the seropositive dairy sheep farm residents had a serological profile indicative for a chronic Q fever infection.

The *C. burnetii* seroprevalence in Dutch veterinarians is high. However, the seroconversion rate during the veterinary study period is not yet known. In **Chapter 3**, two cohorts of in total 118 *C. burnetii* seronegative patients were followed during their veterinary training from 2006 through 2010. Twenty-three students seroconverted during the follow-up period of 362 person-years, which resulted in an incidence of 0.06 per person-year. None of the seropositive participants were diagnosed with acute Q fever in the past and none had a serological profile indicative for a chronic infection.

Case series reports described that pregnant women with an untreated Q fever infection may result in adverse pregnancy outcome in up to 81% of the cases. However, some large community-based studies found no elevated risk for pregnant women with a serological indication of a *C. burnetii* infection. Because of the inconsistent findings, an

ecological study is described in **Chapter 4**, in which pregnancy outcomes from women residing in Q fever-affected areas were compared with the pregnancy outcomes from women in areas without any Q fever notifications, in 2003-2004 and 2008-2010. No association was found between residing in a Q fever-affected area and both preterm delivery and perinatal mortality. In contrast, a marginal higher risk for having a child small for gestational age was found.

In the last part of this thesis, two strategies are investigated for chronic Q fever screening. Having a valvulopathy, like a leaking heart valve, is an important risk factor for developing chronic Q fever endocarditis. French researchers recommend offering acute Q fever patients an echocardiography and prescribing long-term antibiotics for those with a valvulopathy, as progression to endocarditis has also been reported in patients with undiagnosed and clinical silent valvulopathy. In **Chapter 5**, for 509 acute Q fever patients who were diagnosed in 2007 or 2008 and participated in a four-year follow-up study, it was evaluated whether they received echocardiographic screening. Of the 286 screened patients who were not previously diagnosed with a valvulopathy, 84 had a newly detected valvulopathy, of which two (2%) were diagnosed with chronic Q fever, and of those with no valvulopathy 12 of the 202 (6%) were diagnosed with chronic Q fever, which was no statistical significant difference. The two patients with a newly detected valvulopathy did not receive antibiotic prophylaxis to prevent chronic Q fever, as no professional guidelines existed. Last, no new chronic Q fever patients were diagnosed after the initial four-year follow-up period.

The second strategy that is investigated in this thesis was to screen high-risk patients, namely people with a valvulopathy, for having a chronic Q fever infection. Seven years after the large outbreak in the Netherlands, a cross-sectional study is performed in a hospital in the epicenter of the outbreak, as described in **Chapter 6**. Of 904 included patients who had a valvulopathy at the cardiology outpatient clinic or were admitted to the cardiology ward, 133 (15%) had evidence of a previous *C. burnetii* infection, and of whom 6 (5%) had a chronic infection. Even such a long time after the outbreak, new chronic Q fever patients were identified. Therefore, a cost-effectiveness study was performed to get more insight into costs and benefits of screening.

In **Chapter 7**, a health-economic decision model is used to evaluate the cost-effectiveness of a one-of screening program for chronic Q fever seven years after the outbreak. The cost-effectiveness is calculated for several subgroups of Q fever incidence areas (low, middle, high), presence of risk factor (cardiovascular risk factor, immunocompromised risk factor, or an unknown risk status), and age (<60 years and ≥60 years). In total, twelve different subgroups are considered for screening. Because

of the uncertainties of the chronic Q fever prevalence, two scenarios are considered; a low and a high chronic Q fever prevalence scenario. The cost-effectiveness varied largely between the several sub-populations and regions. Mass screening was not cost-effective in any region nor in any prevalence scenario. However, targeted screening of cardiovascular risk patients living in high Q fever incidence areas was estimated to be cost saving in the high prevalence scenario and €31,373 per quality adjusted life year (QALY) gained in a low prevalence scenario. The next most cost-effective strategy would be screening of immunocompromised patients living in high incidence areas, €2,312 per QALY gained for the high prevalence scenario, and €66,145 per QALY gained for the low prevalence scenario.

Finally, a general discussion of the findings of the present thesis is given in **Chapter 8**, including information on what was already known, what the studies reported in this thesis add, and recommendations arising from these studies. The most important recommendations are:

- The burden of Q fever is low among sheep farm residents and veterinary students. Vaccination of these occupationally exposed people is not justified, which is in line with the Health Council report.
- A high number of sheep farm residents and veterinary students are found to be *C. burnetii* seropositive, as was also found in other occupational groups. However, the clinical relevance of the presence of *C. burnetii* antibodies is not yet known. Therefore, occupation should be added to the National Chronic Q fever Database, to be able to investigate this possible relationship further.
- Until more is known, occupationally exposed people with an additional risk factor, should be annually screened for *C. burnetii* IgG phase I antibodies, which is indicative for a chronic infection. People with a *C. burnetii* IgG phase I titer of ≥1:512 should be medically examined and possibly receive antibiotic treatment, to prevent further complications of chronic Q fever.
- No risk for preterm delivery or perinatal death was found, but it cannot be excluded that there is a marginal increased risk for a having child small for gestational age due to residing in a Q fever-affected area. Therefore, screening of all pregnant women and possible antibiotic treatment in an area experiencing a Q fever outbreak is not justified.
- Echocardiographic screening of all acute Q fever patients would lead to unnecessary large-scale screening and long-term use of antibiotics and is therefore not advisable.
- Seven years after the Dutch Q fever outbreak, new chronic Q fever patients are still detected among patients with a heart valve defect in a hospital in the epicenter of the outbreak.
- A one-of screening program for chronic Q fever is advisable for high-risk patients,

such as cardiovascular and immunocompromised patients, in areas with a high acute Q fever incidence during the outbreak several years ago.

- The number of people who experienced a *C. burnetii* infection in low incidence areas might be underestimated based on notifications, as doctors in these areas might have been less aware of Q fever. A nationwide serosurvey, performed in 2016-2017, will show if any unknown 'hot spots' were missed. In these possible new hot spots, the one-of chronic Q fever screening should also be performed in high-risk patients.

SAMENVATTING

Dit proefschrift heeft drie doelen. Het eerste doel is om te onderzoeken hoe vaak veehouders en diergeneeskundestudenten geïnfecteerd zijn of zijn geweest met de *C. burnetii* bacterie, welke de ziekte Q-koorts veroorzaakt. Het tweede doel is om te onderzoeken of zwangeren die in een gebied wonen waar Q-koorts voorkwam een verhoogd risico hebben op een negatieve zwangerschapsuitkomst. Het laatste doel is om twee strategieën te bestuderen om chronische Q-koortsinfecties op te sporen. De eerste strategie is echocardiografische screening voor het aantonen van een hartklepaandoening bij alle patiënten met acute Q-koorts. Deze patiënten zouden dan volgens eerdere studies antibiotische profylaxe moeten krijgen om aan chronische Q-koorts gerelateerde endocarditis te voorkomen. In de tweede strategie wordt bekeken of met een eenmalige screening van risicopatiënten - patiënten met een hartklepaandoening - chronische Q koorts kan worden geïdentificeerd. Bovendien wordt de kosteneffectiviteit van een eenmalig screeningsprogramma onderzocht om nog niet-gediagnosticeerde chronische Q-koorts patiënten te identificeren en te bepalen welk scenario het meest kosteneffectief is.

Hoofdstuk 1 is een algemene inleiding over Q-koorts en beschrijft de inhoud en doelstellingen van dit proefschrift.

Het was al bekend hoe vaak bewoners van geiten- en rundveeboerderijen geïnfecteerd zijn geweest met de *C. burnetii* bacterie, maar nog niet voor bewoners van schapenboerderijen. Wel was het bekend dat melkschapen vaker geïnfecteerd zijn geweest dan niet-melkschapen. Hoe vaak bewoners van melk- en niet-melkschapenboerderijen *C. burnetii* geïnfecteerd zijn geweest is onderzocht in **hoofdstuk 2**. In 2009-2010 hadden 18/27 (67%) van de bewoners van melkschapenboerderijen en 139/271 (51%) van de bewoners van niet-melkschapenboerderijen een *C. burnetii* infectie doorgemaakt, wat niet significant verschillend was. Zes van de 139 (4%) bewoners van niet-melkschapenboerderijen met een doorgemaakte infectie gaven aan in het verleden te zijn gediagnosticeerd met acute Q-koorts en 1 van de 18 (6%) van de bewoners van melkschapenboerderijen met een doorgemaakte infectie had een serologisch profiel dat aanwijzingen gaf voor een chronische Q-koortsinfectie.

Ook Nederlandse dierenartsen hebben vaak een *C. burnetii* infectie doorgemaakt. Hoe hoog de kans is om Q-koorts op te lopen tijdens de studie diergeneeskunde is echter nog niet bekend. In **hoofdstuk 3** werden twee cohorten van in totaal 118 *C. burnetii* seronegatieve studenten gevolgd tijdens hun veterinaire studie van 2006 tot 2010. Drieëntwintig studenten liepen tijdens de follow-up periode van 362 persoonsjaren een

Q-koorts infectie op, een incidentie van 6 per 100 persoonsjaren. Geen van de studenten die een infectie tijdens hun studie doormaakten werd in het verleden gediagnosticeerd met acute Q-koorts en niemand had een serologisch profiel dat aanwijzingen gaf voor een chronische Q-koortsinfectie.

Het is duidelijk dat veel veehouders en diergeneeskunde studenten een infectie met *C. burnetii* hebben doorgemaakt. De ziektelast door deze infecties lijkt in deze beroepsmatig blootgestelde groepen beperkt.

In eerdere casus studies is beschreven dat zwangere vrouwen met een onbehandelde Q-koortsinfectie in 81% van de gevallen een negatieve zwangerschapsuitkomsten kunnen hebben. In enkele grootschalige onderzoeken werd echter geen verhoogd risico gevonden voor een ongunstige uitkomst bij zwangere vrouwen met een *C. burnetii* infectie. Vanwege de inconsistente bevindingen deden wij een ecologisch onderzoek wat beschreven is in **hoofdstuk 4**, waarin zwangerschapsuitkomsten van vrouwen die in door Q-koorts getroffen gebieden woonden, werden vergeleken met de zwangerschapsuitkomsten van vrouwen in gebieden zonder Q-koortsmeldingen, in de jaren 2003-2004 en 2008 -2010. Er werd geen verband gevonden tussen wonen in een Q-koorts gebied en vroeggeboorte of perinatale sterfte. Er werd wel een marginaal verhoogd risico gevonden voor het krijgen van een kleiner kind voor de zwangerschapsduur. Dit betekent dat er mogelijk wel een relatie is tussen wonen in een door Q-koorts getroffen gebied en het hebben van een negatieve zwangerschapsuitkomst, maar dat de kans heel erg klein is.

In het laatste deel van dit proefschrift worden twee strategieën onderzocht voor screening op chronische Q-koorts. Het hebben van een hartklepaandoening, zoals een lekkende hartklep, is een belangrijke risicofactor voor het ontwikkelen van chronische Q-koorts endocarditis. Franse onderzoekers bevelen aan om alle acute Q-koorts patiënten een echocardiografie aan te bieden. Voor de patiënten met een hartklepaandoening adviseren ze vervolgens langdurige antibiotica voor te schrijven, aangezien progressie naar endocarditis ook is gemeld bij patiënten met een nog niet-gediagnosticeerde en een klinische niet relevante hartklepaandoening. In **hoofdstuk 5** werd van 509 patiënten die in 2007 of 2008 werden gediagnosticeerd met acute Q-koorts en die deelnamen aan een follow-up onderzoek van vier jaar, nagegaan of zij echocardiografisch gescreend werden. Van de 286 gescreende patiënten bij wie nog geen hartklepaandoening eerder was gediagnosticeerd, hadden 84 een nieuw ontdekte hartklepaandoening, waarvan er twee (2%) werden gediagnosticeerd met chronische Q-koorts en van degenen zonder hartklepaandoening werden 12 van de 202 (6%) gediagnosticeerd met chronische Q-koorts, wat geen statistisch significant verschil was. De twee patiënten met een nieuw

ontdekte hartklepaandoening ontvingen ook geen antibiotische profylaxe om chronische Q-koorts te voorkomen, omdat er geen professionele richtlijnen over bestaan. Tenslotte werden na de eerste follow-up periode van vier jaar geen nieuwe patiënten met chronische Q-koorts gediagnosticeerd. Onze resultaten laten zien dat de Franse aanpak van het echocardiografisch screenen van acute Q-koorts patiënten en degenen met een hartklepaandoening langdurig antibiotica voorschrijven niet noodzakelijk is.

De tweede strategie die in dit proefschrift wordt onderzocht, is het screenen van patiënten met een hartklepaandoening op een chronische Q-koortsinfectie. Zeven jaar na de grote uitbraak in Nederland is een dwarsdoorsnede onderzoek uitgevoerd in een ziekenhuis in het epicentrum van de uitbraak, zoals is beschreven in **hoofdstuk 6**. Van 904 geïncludeerde patiënten met een hartklepaandoening die de polikliniek cardiologie hadden bezocht of werden opgenomen op de afdeling cardiologie, hadden 133 (15%) aanwijzingen voor een doorgemaakte *C. burnetii* infectie en 6 (5%) daarvan hadden een chronische infectie. Lang na de uitbraak werden nog nieuwe patiënten met chronische Q-koorts geïdentificeerd. Daarom is een kosteneffectiviteitonderzoek uitgevoerd om meer inzicht te krijgen in de kosten en baten van screening op chronische Q-koorts.

In **hoofdstuk 7** is een gezondheidseconomisch beslismodel gebruikt om de kosteneffectiviteit van een screeningprogramma voor chronische Q-koorts zeven jaar na de uitbraak te evalueren. De kosteneffectiviteit is berekend voor verschillende subgroepen: hoe vaak kwam Q-koorts voor in een gebied (weinig, middel, veel), aanwezigheid van een risicofactor (cardiovasculaire risicofactor, verzwakt immuunsysteem, of een onbekende risicostatus), en leeftijd (<60 jaar en ≥60 jaar). Vanwege de onzekerheden over hoe vaak chronische Q-koorts voorkomt zijn twee scenario's overwogen; een scenario met weinig en veel chronische Q-koorts. De kosteneffectiviteit variatie was groot tussen de verschillende subpopulaties en regio's. Massascreening was in geen enkele regio of scenario rendabel. Een gerichte screening van patiënten met een hart- of vaataandoening die in een gebied wonen waar veel Q-koorts voorkwam werd echter geschat als kostenbesparend in het scenario met veel chronische Q-koorts, en €31.373 per levensjaar in goede gezondheid (QALY) in een scenario met weinig chronische Q-koorts. Screening van patiënten met een verzwakt immuunsysteem die in gebieden wonen waar veel Q-koorts voorkwam zou de volgende meest kosteneffectieve strategie zijn, met €2.312 per gewonnen QALY in het scenario met veel chronische Q-koorts en €66.145 per gewonnen QALY voor in het scenario met weinig chronische Q-koorts.

Ten slotte worden in **hoofdstuk 8** de bevindingen van dit proefschrift bediscussieerd, inclusief informatie over wat al bekend was, wat de onderzoeken in dit proefschrift toevoegen en aanbevelingen die uit deze onderzoeken voortvloeien. De belangrijkste aanbevelingen zijn:

- De ziektelast van Q-koorts is laag bij bewoners van schapenboerderijen en diergeneeskunde studenten. Vaccinatie van deze beroepsmatig blootgestelde mensen is niet gerechtvaardigd, wat in overeenstemming is met het rapport van de Gezondheidsraad.
- Een hoog percentage bewoners van schapenboerderijen en diergeneeskunde studenten blijken een *C. burnetii* infectie te hebben doorgemaakt, zoals ook werd aangetoond voor andere beroepsgroepen. De klinische relevantie van de aanwezigheid van *C. burnetii* antilichamen is echter nog onduidelijk. Daarom zou het nuttig zijn om het beroep toe te voegen aan de Nationale Chronische Q-koorts Database om deze mogelijke relatie verder te kunnen onderzoeken.
- Tot meer bekend is, zouden beroepsmatig blootgestelde mensen met een extra risicofactor jaarlijks moeten worden gescreend op *C. burnetii* IgG fase I-antilichamen, om een chronische infectie tijdig op te sporen. Mensen met een *C. burnetii* IgG fase I-titer van ≥1: 512 moeten vervolgens medisch worden onderzocht en mogelijk worden behandeld met antibiotica om verdere complicaties van chronische Q-koorts te voorkomen.
- Er zijn geen aanwijzingen gevonden voor een verhoogd risico op vroeggeboorte of perinatale sterfte voor zwangeren die wonen in een Q-koorts gebied, maar het kan niet worden uitgesloten dat er een marginaal verhoogd risico is voor krijgen van een kind dat klein is voor de zwangerschapsduur. Daarom is screening van alle zwangere vrouwen en mogelijke behandeling met antibiotica in een gebied met een Q-koorts uitbraak niet gerechtvaardigd.
- Echocardiografische screening van alle patiënten met acute Q-koorts en degenen met een hartklepaandoening antibiotica profylaxe voor te schrijven zou leiden tot een onnodig grootschalige screening en langdurig gebruik van antibiotica en is daarom niet aan te raden.
- Een eenmalig screeningsprogramma voor chronische Q-koorts is aan te raden voor risicopatiënten in gebieden waar acute Q-koorts veel voorkwam tijdens de uitbraak enkele jaren geleden, zoals patiënten met een hart- of vaataandoening en patiënten met een verzwakt immuunsysteem.
- Het aantal mensen dat een *C. burnetii* infectie heeft doorgemaakt in gebieden waar weinig acute Q-koorts is gemeld kan op basis van meldingen zijn onderschat, omdat artsen in deze gebieden mogelijk minder op de hoogte waren van Q-koorts. In het bloed wat is afgenomen bij een groot nationaal serologisch (Pienter onderzoek), welke is uitgevoerd in 2016-2017, kunnen we onderzoeken of onbekende 'hot spots'

zijn gemist. In deze mogelijke nieuwe hotspots zou de eenmalige screening op chronische Q-koorts ook moeten worden uitgevoerd bij risicopatiënten.

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Marit (Maria Margaretha Alida) de Lange was born on 20 June 1985 in 's-Hertogenbosch, the Netherlands. After Secondary School, she started her Bachelor Life Sciences with Microbiology as specialization, at the University of Applied Sciences Utrecht. She performed her graduation internship at the Department of Medical Microbiology and Infection Control of the Jeroen Bosch Hospital, where she studied the rapid susceptibility testing and microcolony analysis of *Candida* spp. which was cultured and imaged on porous aluminum oxide. After graduation, she worked for one year as a microbiological laboratory technician at the Medical Microbiology Department of the University Medical Centre Utrecht. In 2007, she continued her studies with the Biomedical Sciences Master Epidemiology at the Radboud University Nijmegen. During her master she performed an internship at the Municipal Health Service Hart voor Brabant, where she evaluated the communication of the Municipal Health Service with municipalities during the large Q fever outbreak. A second internship was performed at the Academic Collaborative Centre for Public Health (AMPHI) of Radboud University Nijmegen, where she studied the *C. burnetii* seroprevalence of sheep farm residents, which resulted in the first publication of this thesis.

After obtaining her Master's degree in 2011, she started as a junior researcher at the Epidemiology and Surveillance Unit of the Centre of Infectious Disease Control, National Institute for Public Health and the Environment (RIVM) in Bilthoven. She was appointed at the Department for Respiratory Infections and in the first years of her appointment she mainly focused on the influenza surveillance and Q fever. In 2017, she started her PhD project, in which she was supervised by prof. dr. R.A. Coutinho, dr. W. van der Hoek, and dr. P.M. Schneeberger. Besides her research project, she continued working on other respiratory pathogens like influenza virus, Legionella, Enterovirus D68, and SARS-CoV-2.

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