Chronic Q fever in the Netherlands

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Chapter 1

General introduction and outline of the thesis

GENERAL INTRODUCTION

History of Q fever

In 1935, Edward Holbrook Derrick was the first to describe an outbreak of febrile illness in abattoir workers in Queensland, Australia. As no underlying pathogen was known at that time, he called the complex of symptoms Q fever (for query fever). In the years thereafter, Macfarlet Burnet (Australia) and Herald Rae Cox (USA) independently isolated the causative pathogen. They demonstrated that the etiological agent displayed both properties of a virus and of rickettsiae. Therefore, it was first named Rickettsia burnetii. Shortly thereafter the classification of a new genus, called Coxiella, was proposed and the etiological agent was renamed *Coxiella burnetii*, honouring both Cox and Burnet.[1]

Coxiella burnetii

C. burnetii is a small, Gram-negative, intracellular bacterium. On the basis of the 16S rRNA coding gene, it falls into the gamma subdivision of the Proteobacteria. It has been demonstrated that *C. burnetii* strains display considerable genetic homogeneity with large intraregional differences.[2-4] The genome of the first isolate in the USA has been sequenced in 2003 and is called the Nine Mile strain.[2;5] Strain-specific differences seem to be associated with differences in virulence in regard to acute Q fever, but not in chronic Q fever.[2;6]

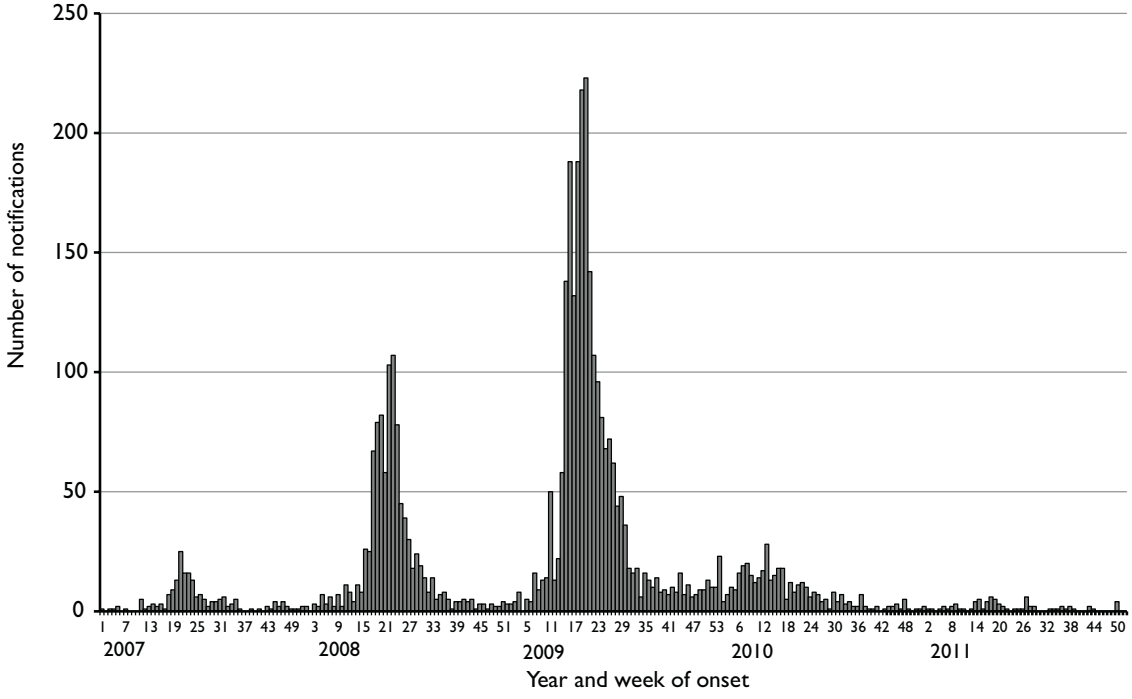
As *C. burnetii* is an obligate intracellular bacterium, it can be cultivated in embryonated eggs, laboratory animals and cell cultures. When cultured, *C. burnetii* displays antigenic variation: the less virulent antigenic phase II, was found to have characteristic differences in lipopolysaccharide (LPS) compared to the virulent wild-type form, called phase I.[5;7] Unexpectedly, during acute infection in humans, phase II antigen is first detected, later followed by phase I antigen, which is especially predominant in chronic Q fever.[5] This has important consequences for the diagnosis of Q fever, which will be discussed later.

C. burnetii exhibits two size variants, both possessing different densities and antigens.[5] The metabolically active large cell variant (LCV) corresponds to the intracellular form of *C. burnetii*. The metabolically inactive small cell variant (SCV) is the extracellular form, which is resistant to environmental stressors like chemical products, desiccation, low and high pH and UV radiation.1

Reservoir and human infection

Q fever is a zoonosis, caused by the bacterium *Coxiella burnetii*. It is prevalent in most countries in the world, and causes worldwide outbreaks of Q fever. *C. burnetii* has its main reservoir in small ruminants, mostly goats and sheep, but can also be present in pets, birds and presumably ticks.[1;5;8] Infected mammals shed large amounts of *C. burnetii* in urine, faeces, milk and especially birth products. Q fever causes abortions in goats and sheep: up to 109 microorganisms per gram of animal placental tissue can be found.[1;9] Due to the spore-like structure of SCV, which is extremely resistant to environmental factors, *C. burnetii* has the ability to survive for a long time outside its hosts. As *C. burnetii* can become airborne, it is able to spread over a large area by wind.[10;11] Living within five kilometres distance from an infected goat farm has proved to be an important risk factor for acquiring *C. burnetii* infection.[12] People get infected mainly from inhalation of contaminated dust or aerosols, and less commonly, from drinking or eating infected animal products like milk, cheese and eggs.[1;5] Sporadic human-to-human transmission has been described by blood transfusion, sexual intercourse, breast milk and transmission by amniotic and placental tissue.[13;14]

Figure 1. Number of notified acute Q fever patients in the Netherlands by week of onset of illness and week of notification (left axis) and number of small ruminant farms with abortion waves confirmed as *C. burnetii* positive by week of reporting (right axis), 1 January 2007–31 December 2010.



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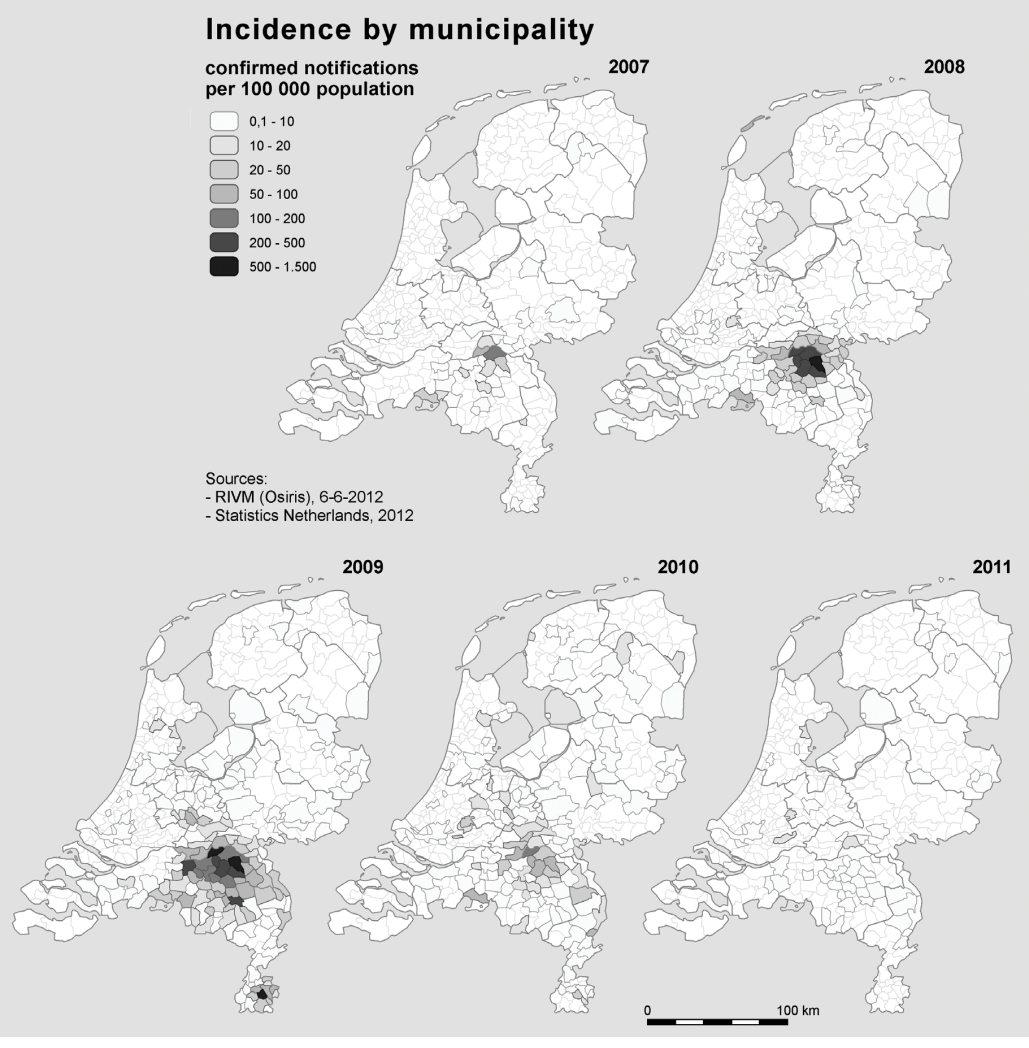
C. burnetii targets monocytes and macrophages, in which it exists in acidic vacuoles at pH 4.5. These phagosomes fuse with lysosomes to form phagolysosomes. The survival and multiplication of *C. burnetii* in acidic vacuoles, hampers antibiotics from killing the bacteria.[1;5;15;16]

Q fever in the Netherlands

In 2007, an outbreak of atypical pneumonia cases was reported by a general practitioner in a small Dutch village, Herpen, in the province Noord-Brabant. A substantial amount of patients needed to be hospitalised. In the same period, six cases of acute Q fever (for definition, see further this chapter) were reported by microbiological laboratories in the same province.[17;18] Q fever was a notifiable disease in the Netherlands since 1978, but until 2007 numbers of acute Q fever had been low (five to 20 cases a year, mostly work-related). This low number of cases was consistent with a low seroprevalence, 2.4%, of Q fever antibodies in the general population in 2006, just before the Q fever outbreak.[19] Ultimately, in 2007, 168 patients with acute Q fever were reported in the Netherlands. In the years thereafter, the epidemic expanded to 1000 cases in 2008 and 2354 cases in 2009 (figure 1). Moreover, the Q fever-afflicted area enlarged to adjacent regions in the south-east Netherlands (figure 2). Initially suggested in 2007, in 2008 a clear link was made between a cluster of acute Q fever cases and a diary goat farm with a Q fever-related abortion wave.[12;17;20]

In 2008, preventive regulations were instituted, consisting of making Q fever a notifiable disease in the veterinary section, vaccination of all non-pregnant goats in the Q fever-afflicted regions, stringent hygienic measures, and prohibition of the spread of manure from infected farms. The amount of vaccine was, however, insufficient to perform all the vaccinations necessary. As in 2009 the epidemic further expanded, the decision was made to take more radical measurements. Mandatory monitoring of bulk tank milk by polymerase chain reaction (PCR) for *C. burnetii* DNA was implemented, and pregnant goats on infected farms were culled.[21;22] After 2009, there was a fast decline in acute Q fever cases, as is illustrated in figures 1 and 2.

Figure 2. Incidence of notified Q fever patients by municipality in 2007, 2008, 2009, 2010 and 2011.



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However, as the acute Q fever cases subsided, new problems arose. A large number of patients with chronic sequalae of Q fever, namely chronic Q fever and Q fever fatigue syndrome, manifestations of Q fever which will be discussed later in this chapter, were increasingly observed during the aftermath of the acute Q fever epidemic.[22]

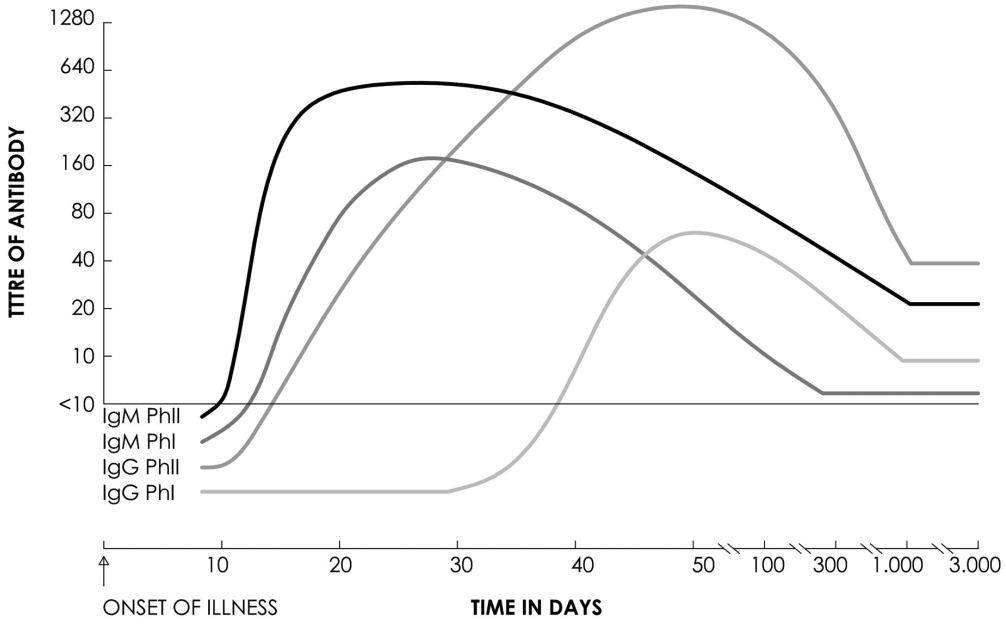
It is possible to prevent Q fever in humans by a vaccine that is produced and licensed in Australia, where it is used in occupational risk groups like abattoir workers and farmers. Vaccine can only be given to those who have not been infected with *C. burnetii* yet.[23] At the end of 2009, the Health Council of the Netherlands advised vaccination of high risk groups for the development of chronic Q fever in the high-incidence area. In total, 1781 patients enlisted for vaccination, of which 1366 were vaccinated. The others could not be vaccinated because of earlier infection with *C. burnetii*, proved by positive skin test or presence of antibodies (394 patients, 22%) or no-show at the vaccination session (21 patients, 1%).[22]

Manifestations of Q fever

Q fever has both acute and chronic manifestations. After infection, the majority of patients, a stated 50-60%, will not become symptomatic. Acute Q fever will develop in ~40% of all infected patients and is mostly a self-limiting disease. Most symptomatic patients report a sudden onset of a flu-like illness with fever, headache, myalgia, malaise, and respiratory symptoms. Atypical pneumonia is an important manifestation of acute Q fever, often accompanied by mild elevated transaminases. Severe acute Q fever hepatitis has been described, but has not been demonstrated in the Netherlands. More rare manifestations are pericarditis, myocarditis, meningeoencephalitis and meningitis. Symptoms can last up to 90 days and mostly resolve spontaneously.[1;8;13;24;25] Reportedly, hospitalisation is only needed in 2% of infected individuals.[1] However, during the Q fever epidemic in the Netherlands hospitalisation rates were up to 46% in 2007 and declined to 21% in 2009.[20] Antibiotic treatment, consisting of doxycycline, co-trimoxazole or quinolones, is warranted to shorten the duration of fever and to hasten recovery of pneumonia.[13] The estimated mortality of acute Q fever is approximately 1% in reports from France and the United Kingdom.[1;13;26;27] Mortality figures of (hospitalised) acute Q fever patients in the Netherlands are yet unknown. Risk factors for the development of acute Q fever are occupational exposure to *C. burnetii* (e.g. farmers, veterinarians, laboratory workers, abattoir workers), living within 5 km distance to a *C. burnetii*-infected goat or sheep farm, male sex, smoking and older age.[1;12;20] French research demonstrated that infection with Q fever during pregnancy is associated with adverse pregnancy outcome (e.g. miscarriages, premature delivery, stillbirth and intrauterine growth retardation).[14;28] Nevertheless, this association was not confirmed, at least in asymptomatic Q fever infections, in a randomised trial in the Netherlands (Munster J.M., et al., unpublished data, presented as oral presentation on ESCAIDE 2011)

After acute Q fever, a large amount of patients report persisting fatigue complaints, which can be accompanied by myalgia, arthralgia, sweats, breathlessness on exertion and blurring of vision. This complex of complaints, lasting >6 months after the acute episode, is called the (post-) Q fever fatigue syndrome (QFS) and incidence ranges from 20% up to 67% patients with resolved acute Q fever. QFS shares features with chronic fatigue syndrome (CFS) and with fatigue syndromes associated with other pathogens, such as Epstein-Barr virus. It can last up to 10 years after primary infection.[1;29-31] In the Netherlands, health status one year after the acute Q fever episode was significantly lower compared to age-, sex-, and geographically-matched controls. Clinically relevant severe fatigue levels were found in 52% of acute Q fever patients one year after infection.[29] QFS is a clinical diagnosis, and cannot be distinguished by laboratory or microbiological parameters

Figure 3. Idealised normal antibody response measured by immunofluorescence assaya

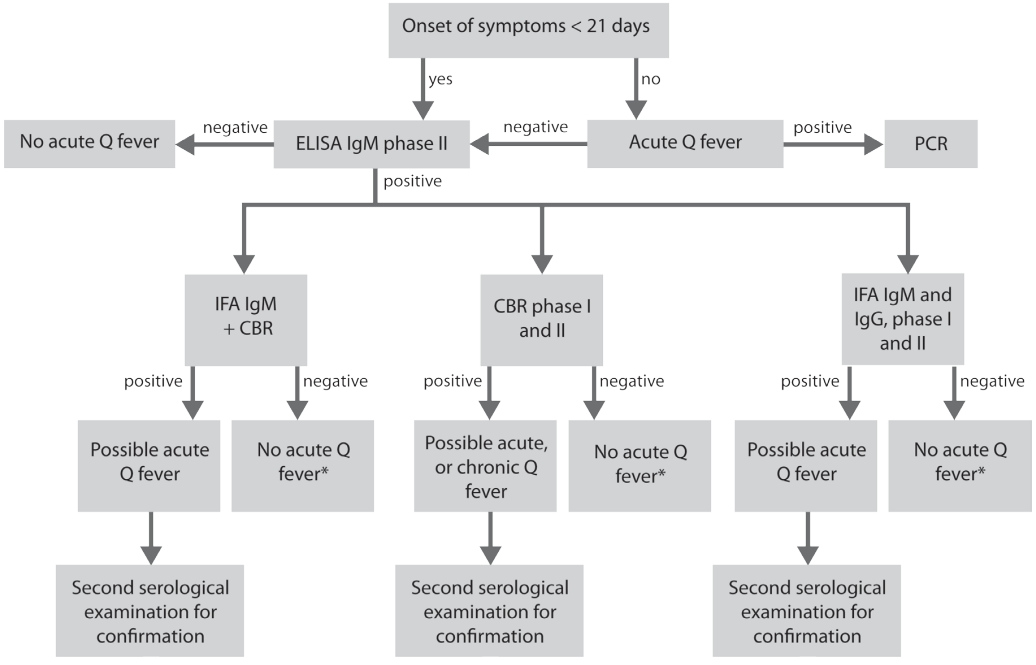


Adapted and with permission from Munster et al. Cost-effectiveness of a screening strategy for Q fever among pregnant women in risk areas: a clustered randomized controlled trial. BMC Womens Health 2010.
ain case of chronic Q fever antibody response is different.

from patients with a complete recovery of acute Q fever.[1;32] The pathophysiological mechanism is yet unknown. Genetic host factors seems to play an important role in CSF and QFS.[33;34] Recently, a theory of persistent non-infective *C. burnetii* antigenic complexes was proposed, which interact with immunogenetic polymorphisms in the host, thereby explaining chronic consequences of Q fever, including QFS.[35;36] Until now there is no evidence-based treatment available for QFS. At the Radboud University Nijmegen Medical Centre in the Netherlands, research into the development and treatment of QFS has been initiated. Results are expected in the years to come.

Primary infection with *C. burnetii*, both symptomatic and asymptomatic infection, will potentially lead to development of chronic Q fever in an estimated 1-5% of patients. Chronic Q fever can present even years after primary infection.[1;37] Until recently, endocarditis was the most frequently observed and described manifestation of chronic Q fever, accounting for 75% of all chronic Q fever cases.[1;38] In the course of the Dutch outbreak, *C. burnetii* infection of aortic aneurysms and vascular prosthesis has become increasingly identified as manifestation of chronic Q fever.[21;39;40] Other manifestations of chronic Q fever can include osteomyelitis, pericarditis, hepatitis, and placentitis in case of chronic Q fever during pregnancy.[1;41] Reported risk factors for the development of chronic Q fever are pre-existing cardiac valvulopathy, vascular grafts and aneurysms, immunosuppression and pregnancy.[40-45] Treatment consists of long-term antibiotic treatment for at least 18-24 months, preferably a combination of doxycycline and hydroxychloroquine. Chronic Q fever

Figure 4. Diagnostic algorithm acute Q fever



Adapted with permission from Wegdam-Blans et al [Laboratory diagnosis of acute Q fever]. Ned Tijdschr Geneeskd 2010. ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; IFA, immunofluorescence assay; CBR, complement binding reaction

has high morbidity and mortality of both up to 60% when left untreated.[38] Vascular infection, infection of prosthetic valves and male sex are associated with poor outcome.[38;40]

Notably, most previously published studies concerning chronic Q fever are descriptive, lack statistical quantification, or are limited to Q fever endocarditis. Whether these studies also apply to the Dutch epidemic is therefore unsure.

Diagnosis of Q fever

The diagnosis of Q fever is different for acute and chronic disease. Culture of *C. burnetii* is time-consuming, has low sensitivity and necessitates a biosafety level three lab.[46] Therefore, this technique is barely used in the Netherlands. *C. burnetii* PCR is more commonly used, although sensitivity has been demonstrated to be variable in different studies.[47-50] As described earlier in this chapter, Q fever exhibits antigenic variation with phase I and phase II antibodies, which is used in serological techniques for diagnosis. Phase II antibodies are first observed during infection, followed by phase I antibodies (figure 3).[5]

The most common used serological technique is immunofluorescence assay (IFA), but complement binding reaction (CBR) and enzyme-linked immunosorbent assay (ELISA) are also used.[51;52] The antibody response to *C. burnetii* needs seven to 15 days after onset of clinical symptoms to develop, which is an important drawback of serological diagnosis of acute Q fever.[1;47] PCR

is, therefore, an important additional diagnostic tool for acute Q fever as blood samples can test positive from one day up to approximately two weeks after onset of clinical symptoms. In a recent Dutch study, sensitivity of *C. burnetii* PCR on serum was proven to be above 90% at the early stage of acute Q fever.[47] A diagnostic algorithm was made for the microbiological diagnosis of acute Q fever, which is now used in the Netherlands (figure 4).[53]

The prognosis of chronic Q fever patients may improve if chronic Q fever cases are identified in an early stage. Yet, chronic Q fever starts insidiously and symptoms are often absent or non-specific.[1;37;38;40] A positive *C. burnetii* PCR on blood, in the absence of acute Q fever, proofs chronic Q fever, but lacks sensitivity.[50] Therefore, diagnosis of chronic Q fever relies strongly on serological techniques, primarily IFA. An elevated phase I IgG titre of >1:800, based on a French in-house IFA, or >1:1024, based on a commercially available IFA (Focus Diagnostics), has been used for the serological diagnosis of chronic Q fever.[54;55] Nevertheless, accuracy of IFA for the diagnosis of chronic Q fever is uncertain and will be further evaluated in this thesis.

OUTLINE AND AIMS OF THESIS

In this thesis, we aimed to describe the sequels of Q fever in the Netherlands, identify risk factors for the development of chronic Q fever and provide diagnostic algorithms to aid in an early diagnosis of this condition.

Part 1: Burden and magnitude of Q fever in the Netherlands
In ***Chapter 2*** in-hospital mortality related to acute Q fever in the Netherlands is assessed. Moreover, an overview of characteristics of the patients who died with acute Q fever is presented. In ***Chapter 3*** we investigated whether early diagnosis of acute Q fever and start of antibiotic treatment, before development of *C. burnetii* antibodies, affect the titres of IgG antibodies after the infection has resolved. In ***Chapter 4*** we assessed the magnitude of the Q fever outbreak in the catchment area of the Jeroen Bosch Hospital, located in the Q fever epidemic area, by extrapolating seroprevalence figures of two screening studies in high-risk groups: patients with a history of cardiac valve surgery (Chapter 9) and patients with a history of aortic aneurysm or vascular surgery.

Part 2: Diagnosis and classification of chronic Q fever
In ***Chapter 5***, a review of the available literature concerning the diagnosis of chronic Q fever is described. Based on the available evidence, we also present new diagnostic guidelines for chronic Q fever, which were constituted by the Dutch Q fever Consensus Group. This review led to controversy in the international literature about the diagnosis of chronic Q fever. In ***Chapter 6*** we evaluated the performance of these new diagnostic guidelines in comparison to a recently proposed French guideline. In ***Chapter 7*** we assessed serological profiles, using IFA and PCR on blood samples of patients with proven, probable or possible chronic Q fever.

Part 3: Chronic Q fever, risk groups, morbidity and mortality
Chapter 8 describes a case-control study in which risk factors for chronic Q fever were analyzed, comparing patients with proven chronic Q fever and patients with a PCR positive acute Q fever episode in 2009 who did not develop chronic Q fever within one year of follow- up. In ***Chapter 9*** seroprevalence of IgG antibodies against *C. burnetii* and chronic Q fever in patients with a history of cardiac valve surgery, an established high-risk group for the development of chronic Q fever, is

assessed. In ***Chapter 10*** we described all identified chronic Q fever cases, captured in the National Chronic Q Fever Database, five years after the start of the Dutch Q fever epidemic, in which we focus on mortality and morbidity

Part 4: Important clinical manifestations of Q fever endocarditis
In ***Chapter 11*** a case-series of three patients with so-called dual-pathogen endocarditis, caused by *C. burnetii* and another pathogen, are presented, which has important implications for patients with an endocarditis in Q fever epidemic areas. ***Chapter 12*** describes a case-series of three patients who underwent cardiac valve surgery without pre-operative assessment of Q fever status, but who appeared to have chronic Q fever after valve surgery.

Finally, in ***Chapter 13*** study results are summarized and recommendations for future research on the topic of chronic Q fever are formulated.

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Part 1

Burden and magnitude of Q fever in the Netherlands

Chapter 2

Acute Q fever related in-hospital mortality in the Netherlands

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ABSTRACT

A large outbreak of acute Q fever has been reported in the Netherlands with over 3500 cases from 2007 to 2009, during which 749 patients were hospitalized. In foreign cohorts, reported mortality rates in patients hospitalized with acute Q fever, ranged from 0.9 to 2.4%. We analyzed mortality among hospitalized patients with acute Q fever in the Netherlands. Physicians from hospitals in the afflicted region were asked to provide details about patients who died with a diagnosis of acute Q fever between 2007-2009. Nine patients (seven males, median age 72 years) from six hospitals were reported, who died within approximately one month following hospitalization with acute Q fever. Six definite acute Q fever cases and three probable cases were identified. Six patients presented with infiltrates on the chest X-ray and a median CURB-65 score of 3. Median time of hospitalization was 13 days (range 1-33). All patients had serious, often coinciding, underlying conditions including chronic cardiovascular disease, chronic lung disease, diabetes mellitus and malignancy. The mortality rate of patients hospitalized because of acute Q fever was estimated at approximately 1%. Patients who died with acute Q fever were often male, from older age, and had chronic coinciding underlying conditions, which gives an a priori higher risk of death.

INTRODUCTION

Q fever is a zoonotic infection, caused by *Coxiella burnetii*, an intracellular gram-negative coccobacillus. There is a large animal reservoir, with goats, sheep and cattle being the most common source of human infections, although infections of birds, pets and arthropods have also been described. When infected, mammals shed *C. burnetii* in urine, faeces, milk and especially birth products. In placental tissues of infected animals, up to 10^9 microorganisms per gram of tissue can be found. Humans get infected from direct contact with infected animals and/or inhalation of contaminated aerosols.[1-5] Most people infected with *C. burnetii* did not have close contact with infected animals, but were infected because of windborne spread of bacteria, which can travel over several kilometres.[3] Rarely, people get infected from drinking contaminated milk and sporadic human-to-human transmission has been described following contact with an infected parturient woman, blood transfusion or sexual intercourse.[2]

Q fever has both acute and chronic manifestations and the presentation of the disease is extremely variable. After infection, most patients (50-60%) remain asymptomatic. Typically, symptomatic patients report a flu-like illness with fever, myalgia, fatigue, headache and artralgia, often accompanied by respiratory signs of pneumonia. Mild elevations of transaminases can be present, and severe acute hepatitis may occur. More rarely, pericarditis, myocarditis, meningitis, peripheral neuropathy and haemolytic anaemia accompany an acute Q fever infection.[1-5] Symptoms can last from ten to 90 days, and usually resolve spontaneously. Antibiotic treatment with doxycycline or fluoroquinolones is only warranted in symptomatic patients to shorten duration of fever and to hasten recovery of pneumonia.[2;3] A significant part of acute Q fever patients subsequently develops a chronic fatigue syndrome, which can last five to ten years after the acute illness.[3;6;7]

Following an acute infection with *C. burnetii*, 1-5% of patients progress to chronic infection, which can even develop years after the primary infection. Endocarditis, vascular aneurysm and prosthesis infection are the most common manifestations. Most frequently affected are patients with pre-existent valvular disease and vascular defects (especially aortic aneurysms and aortic stents/prosthesis), immunocompromised patients and pregnant women.[2;3;8] Diagnosis of Q fever mandates notification to the municipal health authorities in the Netherlands. In the last three consecutive years, there has been a large expanding outbreak of Q fever in the south of the Netherlands: in 2007 a small outbreak of 168 cases was identified, in 2008 and 2009 the epidemic progressed to 1000 and 2357 cases respectively.[9]

According to the literature, rates of hospital admission in symptomatic patients with acute Q fever range from 2% to as high as 63%.[3] Reported overall mortality rates of acute Q fever range from 0.5 -2% in French and Australian populations.[2;3] Mortality data for hospitalized patients with acute Q fever range from 0.9 to 2.4% and are available from older reports from the United Kingdom (1979) and France (1992), respectively.[10;11]

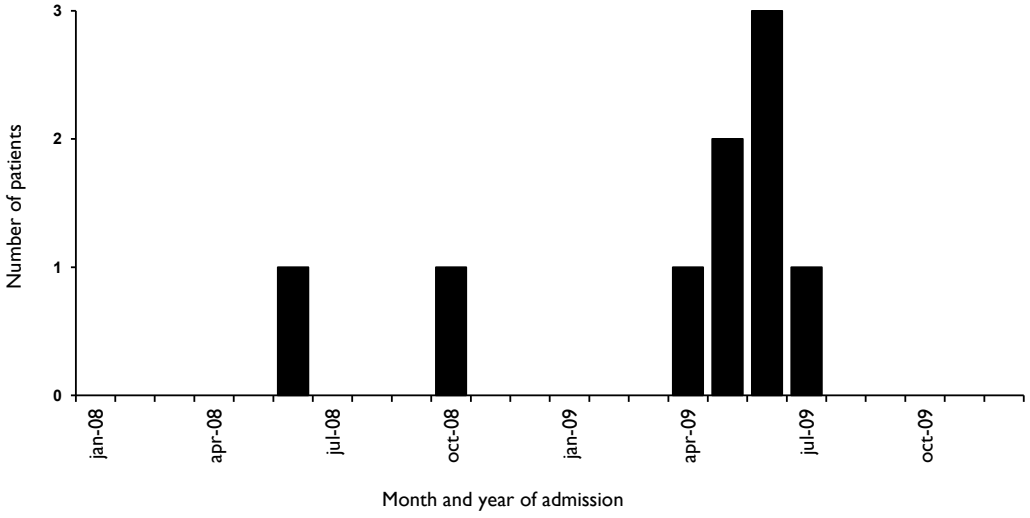
In the Netherlands, 749 patients are known to the national health services to have been hospitalized with acute Q fever from 2007 to 2009.[9] This number is presumably not completely accurate, as it is extracted from questionnaires send to the general practitioners of notified acute Q fever patients. Extrapolation of the previously published mortality rates for hospitalized patients with acute Q fever, allows for an estimation of seven to 18 deaths in this three-year period. However, although Q fever itself is a notifiable disease in the Netherlands since 1978, there is no requirement to notify deaths attributable to this disease. Therefore, there are no accurate data about mortality rates during the Q fever epidemic in the last three years in the Netherlands. In the present report, we assess the mortality rate among hospitalized patients with an acute Q fever infection and, in

Table 1. Overview of patient characteristics, clinical and microbiological features and antimicrobial treatment of nine fatal acute Q fever cases

	Sex	Age	Diagnosis	Days in hospital	CURB-65 score	Comorbidity	Antimicrobial treatment before diagnosis of Q fever	Antimicrobial treatment after diagnosis of acute Q fever	Other pathogens, besides *C. burnetii*
Patient 1	M	79	PCR	22	2	COPD with bronchiectasis, diabetes mellitus, alcohol abuse	penicillin and ciprofloxacin	doxycycline and flucloxacillin	Sputumculture: *Staphylococcus aureus*
Patient 2	F	64	PCR	19	3	Chronic heartfailure and left ventricular failure, heart valve disease (two valve prosthesis), diabetes mellitus, chronic renal disease	cefuroxime, after four days switch to doxyxcycline	moxifloxacin	-
Patient 3	M	82	serology (IFA)	13	3	COPD, peripheral vascular disease, myocardial infarction	amoxicillin with clavulanic acid and tobramycine, after two days switch to moxifloxacin	- (diagnosis post-mortem)	Bloodculture: *Staphylococcus hominis*
Patient 4	M	86	PCR and serology (CFT)	13	3	Lung fibrosis, CABG, diabetes mellitus	amoxicillin with clavulanic acid and fluconazole	- (diagnosis post-mortem)	Culture of bronchoalveolair lavage: *Candida lusitaniae*
Patient 5	M	83	PCR	1	1	Dementia, infrarenal aneurysm	amoxicillin with clavulanic acid	- (diagnosis (4 months) post-mortem)	-
Patient 6	M	55	PCR and serology (IFA)	33	-	Acute myeloid leukemia, lobectomy lung	vancomycin, meropenem, ceftazidime, co-trimoxazole, voriconazole and aciclovir	doxycycline, vancomycin, co-trimoxazole, voriconazole and aciclovir	Considered as colonization: Escherichia coli (ESBL), *Acinetobacter iwoffii, Acinetobacter baumannii, Candida glabrata*
Patient 7	M	72	PCR	9	3	Hypertrofic obstructive cardiomyopathy, mitralic-insufficiency, chronic atriumfibrillation, CVA, COPD	penicillin and ciprofloxacin, after one day switch to penicillin and doxyxycline	doxycycline	-
Patient 8	M	56	serology (CFT)	9	-	Metastastic lungcancer	doxycycline	doxycycline	-
Patient 9	F	69	serology (IFA)	9	-	COPD, diabetes mellitus	penicillin and ciprofloxacin	penicillin and ciprofloxacin	-

M, male; F, female; PCR, polymerase chain reaction; IFA, indirect immunofluorescence assay; CFT, complement fixation test; COPD, chronic obstructive pulmonary disease; CABG, coronary artery bypass graft; CVA, cerebral vascular accident; ESBL, extended-spectrum beta-lactamase

Figure 1. Month and year of admission of nine fatal acute Q fever cases



addition, evaluate epidemiologic characteristics of these patients. Death due to chronic Q fever was not evaluated yet as it can be expected that this condition still has to develop in a significant part of patients at risk.

METHODS

Q fever in the Netherlands is mostly restricted to the middle and southern areas of the country. By October 2009, clinicians and microbiologists from twelve hospitals in the afflicted regions were asked to provide details about patients who were admitted at their hospital and died with a diagnosis of acute Q fever. If an acute Q fever-related death was reported, we requested information about patient characteristics, co-morbidity, performed diagnostic procedures (chest X-ray, polymerase chain reaction (PCR), serology), severity of pneumonia and antimicrobial treatment.

Until 2008, laboratory diagnosis of acute Q fever in the Netherlands was mainly established by serologic testing for antibodies to phase I and phase II antigens of *C. burnetii*. The most common used tests are the indirect immunofluorescence assay (IFA; Focus Diagnostics, Inc., Cypress, CA, USA), complement fixation test (CFT; Siemens Healthcare Diagnostics GmbH, Eschborn, Germany) and enzyme-linked immunosorbent assay (ELISA; Institut Virion\Serion GmbH, Würzburg, Germany). Appearance of phase II IgM and IgG antibodies indicates an acute Q fever infection. Seroconversion usually takes place 7-15 days after onset of clinical symptoms.[12]

Since 2009, PCR on serum has become an important tool in the diagnosis of Q fever. PCR for *C. burnetii* allows for diagnosis of acute Q fever early after onset of disease, before seroconversion has taken place.[13] Adhering to recently published Dutch guidelines, the diagnosis acute Q fever was considered definitive on the basis of either a positive serum PCR or a seroconversion or fourfold increase in antibody titres to *C. burnetii* as detected by either IFA or CFT in two consecutive serum samples. The diagnosis was considered possible when there were clinical signs compatible with an acute Q fever infection in concordance with the presence of antibodies to *C. burnetii* as detected by either IFA or CFT in a single serum sample.[14]

When pneumonia was suspected and confirmed on chest X-ray, the CURB-65 score was used as an index for the severity of pneumonia. The CURB-65 score is a clinical method of predicting the mortality of community-acquired pneumonia (CAP). It consists of 5 criteria, scoring 1 point each: confusion of new onset, urea > 7 mmol/l, respiratory rate = 30 breaths, blood pressure systolic < 90 mmHg or diastolic = 60 mmHg and age = 65 years. A CURB-65 score of 0 gives a less than 1% 30-day mortality risk, score of 1 a 3% risk, score of 2 a 13% risk, score of 3 a 17% risk, score of 4 a 42% risk and score of 5 a 57% risk.[15]

RESULTS

Survey results

Nine patients who had died following hospitalization with acute Q fever were identified; seven males and two females (78 versus 22%), admitted in six different hospitals. All patients were at least 55 years or older. Median age at time of death was 72 years (range 55-86). All patients had serious, often coinciding, underlying conditions including chronic cardiovascular disease (five patients; 56%), chronic lung disease (seven patients; 78%), diabetes mellitus (four patients; 44%) or malignancy (two patients; 22%) (*table 1*). Median time of hospitalization before death was 13 days (range 1-33). There were no reported deaths due to acute Q fever in 2007. Two patients died in 2008 and seven patients in 2009 (*figure 1*).

Clinical and microbiological diagnosis of acute Q fever

An overview of the clinical and microbiological features is presented in *table 1*. Six patients presented with evident infiltrates on chest X-ray and a median CURB-65 score on admission of 3 (range 1-3). The other three patients had no evident infiltrative changes on the chest X-ray, and as a result no CURB-65 score was calculated. One of them suffered from acute myeloid leukaemia and chemotherapy-induced neutropenia, which could explain the absence of infiltrative changes. Another patient suffered from metastastic lung carcinoma hindering detection of any infiltrative changes. Median C-reactive protein (CRP) level and white blood cell count (WBC) at hospital admission were respectively 100 mg/l (range 43-267) and 10.2 x 10e9/l (range 4.0-14.0, after exclusion of the patient with chemotherapy-induced neutropenia).

A definite laboratory diagnosis of acute Q fever was made by positive serum PCR for *C. burnetii* in four seronegative patients and two seropositive patients. In one patient, who died one day after hospital admission, the definite diagnosis of acute Q fever was established four months post-mortem through PCR on a stored seronegative serum sample. In three patients with clinical signs compatible with an acute Q fever infection, a laboratory diagnosis of possible acute Q fever was made on the basis of positive serology in a single serum sample. As these patients died nine to 13 days after hospital admission, a second serum sample to confirm the diagnosis could not be obtained. Both patients with a definite and a possible diagnosis were included in the overall analysis.

Antimicrobial treatment

Table 1 gives an overview of the prescribed antimicrobials and co-pathogens for the nine patients who died with an acute Q fever infection. Five patients were initially treated with antibiotics with proven activity against *C. burnetii*. The sixth patient switched after two days and the seventh patient after four days of admission to an antibiotic with proven activity against *C. burnetii*, before the actual diagnosis was made. After diagnosis of acute Q fever, antibiotic-treatment was switched from ciprofloxacin to doxycycline in one patient and from doxycycline to moxifloxacin in a sec-

ond patient, while in a third patient doxycycline was added to co-trimoxazole. In the two patients who were never treated with an adequate antibiotic regime for *C. burnetii,* the diagnosis of acute Q fever was made post-mortem.

In cultures of four patients, other pathogens were detected, which influenced the choice of antimicrobial treatment.

DISCUSSION

We identified nine patients who died, within approximately one month following hospital admission, with definite or possible acute Q fever in the period of 2007 to October 2009. With 749 known hospital admissions due to acute Q fever in the Netherlands from 2007 to 2009, the in-hospital mortality rate is approximately 1%, which is relatively low and illustrative of the relatively mild nature of the acute form of this disease. In comparison, the reported overall in-hospital mortality rate of CAP in the Netherlands is 8%.[16] Seven out of the nine patients were males. This is in line with the fact that male sex is a risk factor for symptomatic acute Q fever and the reported incidence of acute Q fever in males and females. In surveys from Australia and France, males are respectively fivefold and 2,5-fold more likely to develop symptomatic acute Q fever.[1-3;5]

Doxycycline and fluoroquinolones are the antibiotics of choice for acute Q fever. Treatment lessens the duration of fever and hastens recovery of pneumonia, but the effect on mortality has not been investigated. Initiation of treatment three days after symptom onset is reported to be less effective, however good clinical responses have been observed with treatment up to a week from start of symptoms.[1-3] Five out of nine patients were initially treated with adequate antibiotic regimes for acute Q fever. Two patients started adequate therapy at admission-day two and four, respectively.

All nine patients had serious, often coinciding, underlying conditions including chronic cardiovascular disease, chronic lung disease, diabetes mellitus and malignancy. In four patients, co-pathogens, besides *C. burnetii*, were detected. It is feasible that these pathogens contributed to some extent to the patients' death and influenced the choice of antimicrobial therapy. In addition, six patients were older than 65 years, which is one of the risk factors that stratifies patients with CAP to a higher risk class in the CURB-65 score.15 With a score of 3, the median CURB-65 score in the six patients who presented with pneumonia was relatively high, representing a 30-day mortality risk of 17%.

In comparison, median age at time of hospitalization of 28 patients with non-lethal acute Q fever that had been included in 2008-2009 in a prospective observational study on the aetiology of CAP at the Jeroen Bosch Hospital was 55 (range 23-96; 25% of patients older than 65 years). This cohort consisted of 19 males and 9 females (68 vs 32%). Median CURB-65 score on admission was 0 (range 0-4) and median time of hospitalization was six days (range 2-14). Overall, patients in this cohort had far less relevant underlying conditions (three patients (11%) with chronic cardiovascular disease, five patients (18%) with diabetes mellitus, one patient with chronic hepatitis C and one patient with chronic use of methotrexate for chronic arthritis, while no patients with chronic lung disease or malignancy were identified.(unpublished data) Likewise, an earlier Dutch report by de Wit et al. of 25 non-lethal, hospitalized, acute Q fever cases in the Netherlands, described a CURB-65 score of 0 ± 1 (mean ± SD).[17] These observations indicate that hospitalized patients, who eventually died with an acute Q fever infection, were at time of presentation already more severely ill than patients who survived.

The attempt of this report was to make an estimation of in-hospital acute Q fever related-deaths in the Netherlands and to describe the patient characteristics of the fatalities. It is feasible that death as a result of acute Q fever is underreported. There are no adequate databases of hospitalized acute Q fever patients in the Netherlands yet and the exact number of patients hospitalized due to acute Q fever is not known. Also, there is no requirement to notify deaths attributable to this disease. Furthermore, it is more than likely that due to its design, our survey was not all-comprehensive. PCR for *C. burnetii* was, in most hospitals, not introduced until early 2009. It is, therefore, possible that in 2007 and 2008, seronegative patients may have died from pneumonia or febrile disease caused by *C. burnetii*, in whom the diagnosis could not be established with PCR. These patients are missed in this survey. This is illustrated by the fact that in one seronegative case, diagnosis was made through PCR four months post-mortem. However, even if death to acute Q fever is underreported, this also holds true for non-fatal cases of the disease. For example, Schneeberger et al have previously shown that retrospective PCR analysis on stored serum samples from patients in whom the diagnosis acute Q fever had not been made using serologic techniques allowed for this diagnosis to be made in 5/50 (10%) cases.[13] Moreover, especially in 2007 and 2008, many clinicians were still unaware of the existence of a Q fever epidemic. Since there is an evident overlap in symptoms with other febrile diseases, the possibility of acute Q fever could easily be overlooked and, subsequently, no diagnostic tests to detect *C. burnetii* were ordered. Thus, death to acute Q fever as well as acute Q fever itself might be underreported, warranting some caution towards the in-hospital mortality rate reported in this survey.

In conclusion, the in-hospital mortality rate of acute Q fever in the Netherlands can be estimated around 1%, which is relatively low compared to the overall in-hospital mortality rate of CAP and illustrates the relatively mild nature of the acute form of this disease. Patients who died with acute Q fever, were often male, from older age, and had chronic coinciding underlying conditions, which gives an a priori higher risk of death. The rate of death cannot be accurately defined, because there is no obligation to register Q fever-related admissions and fatalities in the Netherlands. Better registration is necessary to provide a detailed estimation of mortality because of acute Q fever.

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Chapter 3

Early diagnosis and treatment of symptomatic acute Q fever patients does not prohibit IgG antibody responses to *Coxiella burnetii*

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ABSTRACT

Little is known about the effect of timing of antibiotic treatment on development of IgG antibodies following acute Q fever. We studied IgG antibody responses in symptomatic patients diagnosed either before, or during development of the serologic response to *Coxiella burnetii*. Between May 15th and 31st, 2009, 186 patients presented with acute Q fever, of which 181 were included in this retrospective study: 91 early diagnosed (ED) acute Q fever patients, defined as negative IgM phase II ELISA and positive PCR, and 90 late diagnosed (LD) acute Q fever patients, defined as positive/dubious IgM phase II ELISA and positive immunofluorescence assay (IFA). Follow-up serology at three, six, and 12 months was performed using IFA (IgG phase I and II). High IgG antibody titers were defined as IgG phase II titers = 1:1024 together with IgG phase I = 1:256. At 12 months, 28.6% of ED patients and 19.5% of LD patients had high IgG antibody titers (p=0.17). No statistically significant differences were found in frequencies of IgG phase I and IgG phase II antibody titers at all follow-up appointments for adequately and inadequately treated patients overall, as well as for ED and LD patients analyzed separately. Additionally, no significant difference was found in frequencies of high antibody titers and between early (treatment started within seven days after seeking medical attention) and late timing of treatment. This study indicates that early diagnosis and antibiotic treatment of acute Q fever do not prohibit development of the IgG antibody response.

INTRODUCTION

Q fever is a zoonosis, caused by the intracellular bacterium *Coxiella burnetii*.[1] The presentation of the disease is extremely variable; most individuals (60%) remain asymptomatic after infection.[1;2] In symptomatic acute Q fever patients, the most common presentations range from a (self-limiting) flu-like illness to pneumonia or hepatitis. Chronic Q fever, which mainly presents as endocarditis or vascular infection, develops in approximately 2% of infected patients.[3;4] Until 2007, roughly 5-20 cases of acute Q fever were notified in the Netherlands each year and the seroprevalence was low (2.4% in 2006-2007).[5] However, between 2007 and 2010, large Q fever outbreaks occurred in a previously non-endemic area in the south of the Netherlands with over 4,000 notified symptomatic cases.[6]

Laboratory diagnosis of Q fever is mainly based on serologic testing for antibodies against phase I and phase II antigens.[1] *C. burnetii* has two antigenic states: during acute Q fever infection, antibodies against phase II antigens predominate, whereas high phase I antibodies titers are more prevalent in cases of chronic Q fever.[2;7;8] The most commonly used serologic test is the immunofluorescence assay (IFA). Seroconversion usually takes place 10-15 days after onset of acute disease1 with the appearance of IgM antibodies against phase II antigens (IgM phase II), followed by IgG antibodies against phase II antigens (IgG phase II), IgM antibodies against phase I antigens (IgM phase I), and finally IgG antibodies against phase I antigens (IgG phase I).[7] Since 2009, polymerase chain reaction (PCR) for detection of *C. burnetii* DNA has become an important tool in the diagnosis of acute Q fever in our laboratory. PCR enables diagnosis of acute Q fever early after onset of disease (the first three weeks after onset of symptoms), often before seroconversion has taken place. As the serological response develops, PCR becomes negative in patients who do not develop chronic Q fever.[9] Current international recommendations advise routine follow-ups to detect patients that develop a chronic Q fever infection, consisting of at least three consecutive serologic tests in the first year after diagnosis of acute Q fever.[10;11] Most infected individuals are asymptomatic but in the case of symptomatic individuals, symptoms of acute Q fever can last from 10-90 days, and usually resolve spontaneously. Antibiotic treatment with doxycycline or fluoroquinolones is only warranted in symptomatic patients to shorten duration of fever and to hasten recovery of pneumonia if present.[12]

For infections caused by Borrelia spp, e.g. borreliosis, which is also treated with doxycycline,[13;14] it has been reported that early antibiotic treatment may prohibit development of the IgG antibody response in patients with erythema migrans15-18 or neuroborreliosis16. As Q fever and borreliosis can both develop into a chronic disease which can be difficult to diagnose, there may be more similarities between the two than previously thought. Little is known about the development of IgG antibodies following antibiotic treatment of acute Q fever. The purpose of this study is to investigate the IgG antibody response in symptomatic patients diagnosed and treated either before or during development of the serologic response to *C. burnetii*.

MATERIALS AND METHODS

Patients

Those eligible for this retrospective study were all symptomatic patients diagnosed with acute Q fever between May 15th and May 31st, 2009, at the Department of Medical Microbiology and Infection Control of the Jeroen Bosch Hospital, 's-Hertogenbosch. Patients excluded were those who

were younger than 18 years of age, who were pregnant, who had submitted an earlier sample for Q fever testing, or who had positive serology at diagnosis that was later found to be negative when paired with a subsequent negative tested sample.

Diagnostic work-ups in these acute Q fever patients were performed according to a diagnostic algorithm for acute Q fever introduced on May 1st, 2009.[4] In short, serum samples were screened with an enzyme-linked immunosorbent assay (ELISA) for IgM phase II (Institut Virion\Serion GmbH, Würzburg, Germany). Depending on outcome of ELISA IgM phase II, date of onset of disease, and inpatient or outpatient setting, either an in-house PCR for *C. burnetii* DNA[9] or IFA for IgM and IgG antibodies against phase I and phase II antigens (Focus Diagnostics, Inc., Cypress, CA, USA) was performed (for more details see Jager et al.[4]). IgG antibody responses during follow-ups were evaluated by comparing two groups of patients, the early diagnosed (ED) group (negative ELISA IgM phase II and positive PCR at time seeking medical attention), and the late diagnosed (LD) group (positive or dubious ELISA IgM phase II confirmed by positive IFA (IgG phase II and/or IgM phase II = 1:32) at time seeking medical attention).

Serological follow-up

In line with the internationally recommended routine follow-up of acute Q fever, patients in both groups were asked to provide a serum sample at 3, 6, and 12 months after diagnosis.[10;11] Follow-up serology was performed using IFA for IgG phase II and phase I antibodies. Samples were titrated up to 1:4096 and, if still positive, were categorized as > 1:4096. Samples of patients with an IgG phase I titer = 1:1024 at month 12 were analyzed using PCR, to check whether *C. burnetii* DNA was still present. High IgG antibody titers in follow-up samples were arbitrarily defined as IgG phase II titers = 1:1024 in combination with IgG phase I titers = 1:256. This IgG phase I titer is two dilutions lower than the cut-off titer for chronic Q fever of = 1:1024 set by the Dutch Q Fever Consensus Group.[19]

Data collection

Outcome of serologic tests, date of onset of disease and date of seeking medical attention were collected from the laboratory information system (LIS). If unavailable in the LIS, date of onset of disease was obtained by contacting the general practitioners (GP). Date of onset of disease extracted from the LIS was considered more pertinent than those obtained retrospectively from the GP, as the former were written on the laboratory form at the moment the patient visited the GP. In addition, data on treatment (type, dosage, start date, and duration of antibiotic treatment prescribed) were obtained from the GP. For patients diagnosed during hospitalization, data were obtained from medical records. Included patients were assigned with a unique code and identifying information was deleted from the database. Adequate treatment was defined as treatment with a *C. burnetii* covering antibiotic at the appropriate dosage for at least ten days (e.g. doxycycline 200mg/day, moxifloxacin 400mg/day, ciprofloxacin 1000mg/day or co-trimoxazole 1920mg/day). Other dosage schemes or antibiotics were considered inadequate for treating symptomatic acute Q fever patients. Early antibiotic treatment was defined as starting treatment before diagnosis or within 7 days after seeking medical attention.

Data analysis

Descriptive characteristics of the ED and LD groups were investigated by calculating the mean and standard deviation, median and interquartile range [IQR], and relative frequencies. Depending on the variable, Student's t test (age), Mann-Whitney U tests (non-parametric data; e.g. total number

of days between: onset of disease to seeking medical attention for diagnosis, seeking medical attention for diagnosis and sample at 12-month follow-up, seeking medical attention for diagnosis and start of adequate antibiotic treatment; comparing the frequencies of IgG antibody titers), and Chi-square tests (gender, frequencies of high antibody titers, and adequate, inadequate, and early treatment) were performed to assess significant differences. Patients without at least one follow-up sample were included in the descriptive characteristics but were excluded from further analysis. Data were analyzed using Microsoft Excel (Microsoft Corp., USA) and IBM SPSS Statistics version 19.0.0 (SPSS Inc., USA). Additionally, a separate analysis was performed excluding LD patients without IgG phase I antibodies at 3, 6, and 12 months. This was done because it cannot be ignored that patients in the LD group, for whom no PCR was performed at time of seeking medical attention and who did not develop IgG phase I antibodies during follow-up, are in fact patients with a resolved Q fever infection and persisting IgG phase II antibodies.

RESULTS

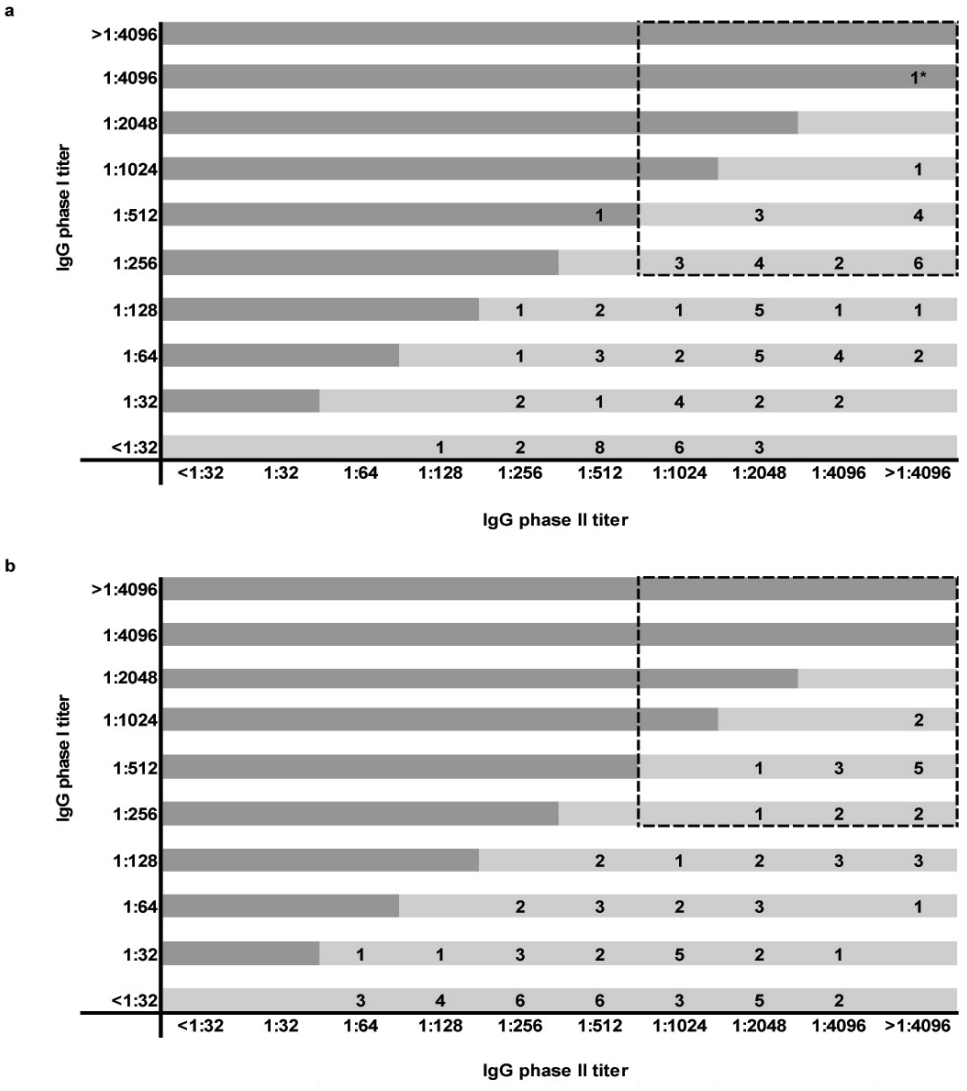
Between May 15th and May 31, 2009, 92 ED patients and 94 LD patients were diagnosed. One ED patient (<18 years of age) and four LD patients (<18 years of age, n=1; pregnant, n=1; submitted an earlier sample for Q fever testing, n=1; positive serology at diagnosis that was tested negative in a paired retest with a consecutive negative tested sample, n=1) did not meet the inclusion criteria. Therefore, 91 acute Q fever patients were included in the ED group and 90 patients in the LD group. Characteristics of both groups are presented in Table 1. Date of onset of disease was available for 92.3% (84/91) of patients in the ED group and for 85.6% (77/90) in the LD group. Both groups showed comparable results, except for time between onset of disease to seeking medical attention (median number of days [interquartile range]: ED 4.5 [3–5], LD: 12 [7.5–23.5]; p=0.00), which is in line with the definition of both groups. In 79.0% of the patients, the GP applied for the laboratory test, while in 21.0% a hospital physician requested the test.

Table 1. Characteristics of acute Q fever patients in the early and late diagnosed group

	Early diagnosed[a] (n=91)	**Late diagnosed[b] (n=90)**
Age (mean ± SD)	48 ± 15	51 ± 16
Male gender (n (%))	57 (62.6)	56 (62.2)
Number of days from onset of disease to seeking medical attention for diagnosis (median [IQR])[c]	4.5 [3–5][c]	12 [7.5–23.5][c]
Total number of days between seeking medical attention for diagnosis and sample at 12 months of follow-up (median [IQR])	364 [360–371]	364 [359–369]

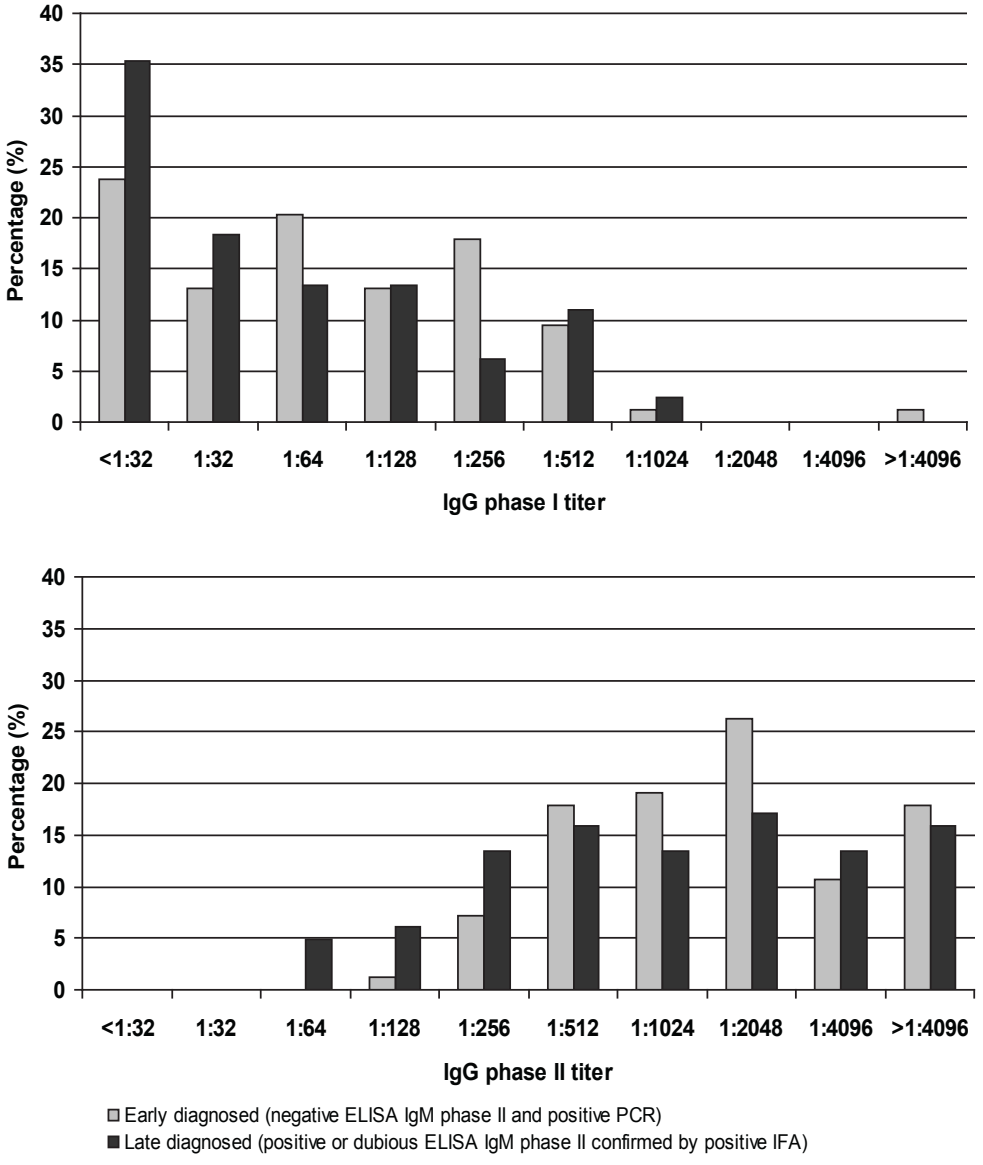
IQR: Interquartile range. [a]Early diagnosed group: negative ELISA IgM phase II and positive PCR, [b]late diagnosed group: positive or dubious ELISA IgM phase II confirmed by positive IFA (IgG phase II and/or IgM phase II ≥1:32), [c]based on n=84 patients in the early diagnosed and n=77 in the late diagnosed group, cstatistical significant difference (p=0.00): in line with the definition of the groups

Figure 1. Distribution of IgG phase I and phase II antibody titers as determined by immunofluorescence assay at 12 months of follow-up in acute Q fever patients.



a: Early diagnosed group (negative ELISA IgM phase II and positive PCR), n=84 samples. b: Late diagnosed group (positive or dubious ELISA IgM phase II confirmed by positive IFA (IgG phase II and/or IgM phase II ≥ 1:32), n=82 samples. Dash pattern box indicates high antibody titers. * Patient with proven chronic Q fever infection

Figure 2. Percentage of acute Q fever patients with IgG phase I and IgG phase II antibody titers as determined by immunofluorescence assay at 12 months of follow-up in the early diagnosed and late diagnosed group



In the diagnostic sera of the LD group, IgM phase II ELISA was positive in 95.6% (86/90); the remaining four samples showed a dubious result. However, these four samples showed positive results for IFA IgM phase II and IgG phase II. IFA IgM phase I and phase II was performed for 70 LD samples: all samples showed positive results for IgM phase II, and 65.7% (46/70), 1.4% (1/70), and 32.9% (23/70) were IgM phase I positive, dubious, and negative, respectively. IgG phase II was positive for 98.9% (89/90) of samples, and one showed a dubious result, while IgG phase I was positive in 35.6% (32/90), dubious in 1.1% (1/90), and negative in 63.3% (57/90) of the LD patients. Six patients (three ED patients and three in the LD group) did not provide any follow-up sample and thus were excluded from further analysis.

In the ED group, 26.4% (23/87) had high IgG antibody titers (IgG phase II titers = 1:1024 in combination with IgG phase I titers = 1:256) at month 3, 24.4% (21/86) at month 6, and 28.6% (24/84) at month 12. In the LD group, 16.7% (14/84) had high IgG antibody titers at month three, 18.5% (15/81) at month six, and 19.5% (16/82) at month 12. Differences between both groups were not statistically significant. There were seven patients (five ED and two LD patients) that had high titers at all three follow-up appointments. In total, 71 (40 ED and 31 LD) of the 181 patients (39.2%) had a high titer in at least one of their follow-up appointments. Figure 1 presents the distribution of IgG phase I and phase II titers in both groups at 12-month follow-up. PCR was negative for all 12-month samples with an IgG phase I titer = 1:1024, except for the one patient in this study that developed chronic Q fever (this will be discussed later).

Figure 2 shows the percentage of patients with IgG phase I and phase II antibody titers at 12 months after initial diagnosis in the ED and LD group. Although higher frequencies of the lower antibody titers were observed in the LD group, the differences between both groups were not statistically significant (IgG phase I: p=0.07; IgG phase II: p=0.11). There was a statistically significant difference between the IgG phase II antibody titers of the groups, with the LD group having lower titers than the ED group at three months after initial infection (p=0.02). IgG phase I titers however showed a borderline significant difference (p=0.05). At 6 months, a borderline significant

Table 2. Treatment of acute Q fever patients with at least one follow-up sample in the early and late diagnosed group

	Early diagnosed[a] (n=85)	**Late diagnosed[b] (n=80)**
Adequate treatment[c] (n (%))	71 (83.5)	66 (82.5)
Inadequate treatment (n (%))	12 (14.1)	12 (15.0)
No treatment (n (%))	2 (2.4)	2 (2.5)
Early treatment[d] (n (%))	75 (88.2)[e]	61 (76.3)[e]
Number of days from seeking medical attention for diagnosis to adequate treatment (median [IQR])[f]	0 [0–1]	0 [-3–7]

IQR: Interquartile range. [a]early diagnosed group: negative ELISA IgM phase II and positive PCR, [b]late diagnosed group: positive or dubious ELISA IgM phase II confirmed by positive IFA (IgG phase II and/or IgM phase II), [c]defined by treatment with a *C. burnetii* covering antibiotic at the appropriate dosage for at least 10 days (e.g. doxycycline 200mg/day, moxifloxacin 400mg/day, ciprofloxacin 1000mg/day, co-trimoxazole 1920mg/day), [d]defined as starting treatment before diagnosis or within 7 days after seeking medical attention, regardless of treatment being adequate or not, [e]statistical significant difference: p=0.04, [f] based on n=70 patients in the early diagnosed and n=65 in the late diagnosed group, the start date of treatment was unknown for one early and one late diagnosed patient

difference was observed for IgG phase II titers (p=0.05), while IgG phase I titers showed no statistically significant difference (p=0.08) (data not shown).

Data on antibiotic treatment for patients with at least one follow-up sample (n=175) could be obtained for 165 patients: 85 ED and 80 LD patients (two GPs did not participate in the study, n=3 LD patients; treatment unknown, n=4 patients (1 ED, 3 LD); dosage or duration of antibiotic unknown, n=3 patients (2 ED, 1 LD)). Adequate treatment was prescribed in 83.0% (137/165) of the patients, 14.5% (24/165) received inadequate treatment, and four patients (2.4%) did not receive any treatment (Table 2). The median number of days between seeking medical attention for microbiological diagnosis and start of any treatment as well as adequate treatment was zero for both groups, though the range between both groups differed (ED= -4–13, LD=-19–109 days; the minus sign means treatment started before the diagnostic sample was submitted). A statistically significant difference was found for early (treatment started within 7 days after seeking medical attention) or late timing of treatment between the ED and LD groups; ED patients received treatment earlier, regardless of being treated adequate or not (p=0.04) (Table 2). Among adequately treated patients only, we also found a statistically significant difference for early treatment timing (ED: 91.5%, LD: 75.8% received treatment within 7 days after seeking medical attention; p=0.01). No statistically significant differences were observed in frequencies of IgG phase I and IgG phase II antibody titers at all follow-up appointments for adequate and inadequate (including no treatment) treated patients overall, as well as for ED and LD patients analyzed separately. One borderline significant result (p=0.06) was observed for IgG phase I at 12-month follow-up for patients treated adequately, where LD patients showed lower antibody titers than ED patients (median IgG phase I titer: ED: 1:64, LD: 1:32). Additionally, no significant difference was found in frequencies of high antibody titers and in early or late timing of treatment (data not shown).

Among those who provided a sample at all follow-up appointments, absence of IgG phase I antibody titers in all three follow-up samples was observed in 2.4% (2/82) of the ED patients and 13.0% (10/77) of the LD patients (p=0.01). Treatment data were available for nine of these twelve patients; all nine received adequate treatment. When the ten LD patients without detectable IgG phase I antibodies at any time point were excluded from analysis, 22.2% (16/72) of the remaining cases in the LD group had high IgG antibody titers at month 12 (p=0.37). IgG phase II antibodies were present in all ED and LD patients.

One patient with an aorta-femoral bypass (in the ED group and treated adequately) showed a chronic Q fever serologic profile at month six (IgG phase II titer > 1:4096 and IgG phase I > 1:4096). A positive serum PCR (Ct value 33.6) confirmed a chronic Q fever infection, and titers remained high at month 12 (both IgG phase II and phase I titer > 1:4096) (Figure 1a).

DISCUSSION

In this study, the development of IgG antibodies in an early and a late diagnosed and treated groups of patients with symptomatic acute Q fever infection were examined. The results show that IgG phase I and phase II responses are not inhibited by early diagnosis and subsequent treatment of the acute Q fever infection. No significant differences in high antibody titers were observed between the ED and LD groups at three-, six-, and 12-month follow-up. Additionally, timing and adequateness of treatment did not result in differences in the IgG phase I and phase II antibody responses. We did find a statistically significant difference for earlier timing of treatment in ED patients compared to LD patients.

We observed statistically significant lower IgG phase II antibody titers in the LD group than in the ED group at the three-month follow-up, and borderline or non-significant differences in IgG phase I and phase II at the other follow-ups. This observation contradicts the hypothesis that, as observed in borreliosis, early diagnosis and treatment would prohibit the IgG antibody response.

Moreover, we found a statistically significant difference between both groups in the percentage of patients without a detectable IgG phase I antibody response: 2.4% in the ED group and 13.0% in the LD group. A possible explanation for this increased proportion in the LD group is that these patients may in fact have a resolved Q fever infection with persisting IgG phase II antibodies. Transient reappearance or persistence of IgM antibodies in such patients might result from non-specific polyclonal activation due to another (undiagnosed) infection as has been observed in at least one case of legionellosis (unpublished). Omitting the LD patients without detectable IgG phase I antibody response from the analysis did not influence the outcome of our study as the percentage of remaining patients with high IgG antibody titers at month 12 (22.2%) in the LD group remained lower compared to ED patients (28.6%). Another possible explanation might be that in these patients, IgG phase I antibodies were produced only for a short time and therefore were not detected at the three-, six-, and 12-month follow-ups. There were indeed two patients in the ED group with PCR proven acute Q fever in whom IgG phase I antibodies were not detected during follow-ups.

The hypothesis that early antibiotic treatment could hamper the development of the IgG antibody response was started by the observation of this phenomenon in several studies of patients with erythema migrans in the course of a *Borrelia burgdorferi* infection.[15-18] As Q fever and borreliosis can both develop into a chronic disease which can be difficult to diagnose, and both are treated with doxycycline, there may be more similarities. Hammers-Berggren et al. found similar results for neuroborreliosis.[16] It was observed that antibodies against *B. burgdorferi* develop gradually and slowly during the course of infection before treatment.[16;20-23] Early on in infection, patients show high IgM and low IgG antibody levels, while those in the later stages have low IgM and high IgG antibody levels. Seeing that patients that had early treatment had low or no IgG antibody levels, it was suggested that antibiotic treatment prevents the development of further IgG antibody response,[16;17] but it is less likely that the IgM response is also inhibited.[15] A similar observation that early antibiotic treatment affected the antibody response to cytoplasmic proteins was also observed in mice infected with *Brucella melitensis*.[24]

As it was the third year of the 2007-2010 Dutch Q fever epidemic, we expected that physicians in the high-risk area were already aware of the symptoms of the acute infection. Lassche et al. performed a questionnaire study among GPs in the high-risk area with at least five acute Q fever patients in the first six months of 2009. In this study, 95% of the GPs (N=20) indicated that they already started treatment when a Q fever infection was suspected or when a patient had signs of pneumonia, without awaiting the confirmation by microbiological tests.[25] However, in our study, we found a lower rate of GPs prescribing antibiotic treatment before laboratory results were available (60.0% of patients received treatment on the day of sampling or before, regardless of treatment being adequate or inadequate). As we included samples submitted to the laboratory between May 15th and May 31st, 2009, without selecting GPs with a minimum number of diagnosed patients, the rate of starting treatment at time of seeking medical attention of 60.0% found in this study is probably a more accurate estimate than the 95% from the questionnaire study.

Although our analysis did not show that early diagnosis and treatment of acute Q fever diminishes the development of the IgG antibody response, it is of interest to evaluate if these findings can be extrapolated to clinical outcome. The development of chronic Q fever and Q fever fatigue

syndrome after an acute Q fever infection are of particular interest. Up to May 1st, 2012, no additional chronic Q fever patients were diagnosed among the 181 patients included, except the one adequately treated ED patient with a known vascular risk factor who was diagnosed with chronic disease at their month six follow-up appointment. Another interesting possibility for future research is to assess the effect of early antibiotic treatment on the time interval in which IFA IgG titers reach maximum levels (in general four to eight weeks after the onset of disease)[26;27] but this would require more frequent acquisition of follow-up samples.

In conclusion, our observations indicate that early diagnosis and antibiotic treatment of acute Q fever do not prohibit development of the IgG antibody response after follow-up appointments at three, six, and 12 months after initial diagnosis.

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Chapter 4

Screening for *Coxiella burnetii* seroprevalence in chronic Q fever high-risk groups reveals magnitude of Dutch Q fever outbreak

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ABSTRACT

The Netherlands experienced an unprecedented outbreak of Q fever between 2007 and 2010. The Jeroen Bosch Hospital (JBH) in 's-Hertogenbosch is located in the centre of the epidemic area. Based on Q fever screening programmes, seroprevalence of IgG phase II antibodies to *Coxiella burnetii* in the JBH catchment area was 10.7% (785 tested, 84 seropositive, 95% confidence interval (CI) = 8.5–12.9%). Seroprevalence seemed not influenced by age, gender or area of residence. Extrapolating these data, an estimated 40,600 persons (95% CI = 32,200–48,900) in the JBH catchment area have been infected by *C. burnetii* and are, therefore, potentially at risk for chronic Q fever. This figure by far exceeds the nationwide number of notified symptomatic acute Q fever patients and illustrates the magnitude of the past Dutch Q fever outbreak. Clinicians in epidemic Q fever areas should be alert for chronic Q fever, even if no acute Q fever episode was reported.

INTRODUCTION

Q fever is a zoonosis, which occurs in worldwide outbreaks, and is caused by the intracellular Gram-negative bacterium *Coxiella burnetii*. Most important animal reservoirs are goats, sheep and cattle, although infections of birds, pets and arthropods have also been described. When infected, mammals shed *C. burnetii* in urine, faeces, milk and especially birth products. Humans get infected from inhalation of contaminated aerosols. Most people become infected with *C. burnetii* because of windborne spread of bacteria, which can travel over several kilometres.[1-4] Initial infection results in 50-60% of patients in asymptomatic seroconversion. Acute Q fever, a mild flu-like illness sometimes complicated by pneumonia or hepatitis, develops in 40-50% of infections.[1;2] Reportedly, 1-5% of patients develop chronic Q fever, with endocarditis and vascular infection of an aortic aneurysm or central vascular reconstruction as most common manifestations. Risk factors predisposing to chronic Q fever are pre-existent cardiac valvulopathy, vascular grafts and aneurysms, immunosuppression and pregnancy.[2;5;6]

The Netherlands experienced an unprecedented outbreak of acute Q fever between 2007 and 2010 with over 4,000 notified symptomatic cases (168 in 2007, 1,000 in 2008, 2,354 in 2009, 506 in 2010 and 81 in 2011; data National Institute for Public Health and the Environment). Although, the acute Q fever epidemic has subsided following government measures at the end of 2009, a rising number of chronic Q fever cases is currently seen.[3;7] Since initial infection is often asymptomatic, this figure is probably an underestimation. As municipal screening for *C. burnetii* antibodies has not been performed, the magnitude of the Dutch Q fever outbreak, and the number of patients potentially at risk for chronic Q fever, remains unknown.

In May 2009, amidst the epidemic, *C. burnetii* IgG seroprevalence among blood donors in the area with the highest reported Q fever incidence in the Netherlands was assessed. This survey showed that 12.2% of blood donors were seropositive for *C. burnetii* IgG phase II antibodies.[7] Another study assessed *C. burnetii* IgG phase II seroprevalence at 9.0% among pregnant women in serum samples obtained between June 2007 and May 2009.[8] Here, we set out to estimate the number of *C. burnetii* infected persons in the catchment area of the Jeroen Bosch Hospital (JBH), which is located in the centre of the epidemic region, using seroprevalence rates obtained after the epidemic had ceased. These rates were extracted from two programmes for early detection of unnoticed chronic Q fever in high-risk patients offering subsequent appropriate medical intervention to identified patients.

MATERIALS AND METHODS

First, a call/recall screening programme among high-risk patients with an aortic aneurysm or central vascular reconstruction was initiated in November 2009 in the catchment areas of the JBH and the neighbouring Bernhoven Hospital. Second, a screening programme among high-risk patients with a history of cardiac valve surgery was conducted between November 2010 and January 2011 in the JBH catchment area. A regional medical ethics committee waived the need for informed consent as far as testing of high-risk groups for chronic Q fever is concerned. The JBH catchment area comprises 11 municipalities with a total of 379,100 inhabitants as of 31 December 2010. Of these, 27.4% resided in the city of 's-Hertogenbosch, and the others in rural areas.(data Statistics Netherlands)

Screening was performed on sera obtained by venapuncture. Regardless of patients having past Q fever or chronic Q fever, we defined seropositivity as any IgG titre against *C. burnetii* phase

II antigens (IgG phase II) of = 1:128 as measured by immunofluorescence assay (IFA; Focus Diagnostics, Cypress, CA, USA). We have observed that IgG phase II can be detected during acute Q fever, chronic Q fever and past Q fever and are the longest circulating antibodies during the immune response to *C. burnetii*. These antibodies are absent only during the very early stage of acute Q fever.[9] Although the manufacturer defines seropositivity as a titre of = 1:16, we choose for a higher cut-off to prevent overestimation of the number of *C. burnetii*-infected persons as a result of false positivity or cross-reactivity in the serologic assay.[2;10]

We additionally gathered information about sex, age, and residence in urban or rural areas. To study the influence of age on seroprevalence, the screened population was divided in two groups with a 35-year span. One group consisted of patients with year of birth from 1915 to 1949, while the other group consisted of patients with year of birth from 1950 to 1984. We compared prevalence of seropositivity among the different groups and used x^2-tests to assess significance of findings. Significance level was set at p-value =0.05.

RESULTS

On 31 May 2011, a total of 932 patients had entered the two screening programmes, of which 785 patients lived in the JBH catchment area. Of these, 84 patients had an IgG phase II titre of = 1:128, resulting in a seroprevalence rate of 10.7% (95% confidence interval (CI): 8.5–12.9%). There was no significant difference in seroprevalence between the two screening programmes (Table 1). Extrapolating these figures, the population estimate for *C. burnetii* antibody prevalence in the JBH catchment area is 40,600 persons (95% CI = 32,200–48,900 persons). In the years 2007 to 2010, only 644 patients with symptomatic acute Q fever living in the JBH catchment area were notified (data Municipal Health Services). There was no significant difference between seroprevalence in the groups with year of birth from 1915-1949 and 1950-1984. Likewise, there was no significant difference in seroprevalence between males and females. With regard to geographical distribution, there was no significant difference in seroprevalence between patients living in the city and patients residing in more rural areas (Table 1).

DISCUSSION

In the Netherlands, the estimated seroprevalence of *C. burnetii* phase II IgG in 2006-2007, just before the outbreak, was 2.4% (using IFA with an IgG phase II cut-off titre of = 1:32), although this study was largely conducted in municipalities which were not affected by the recent Q fever outbreak.[11] Using a more conservative cut-off titre of = 1:128 for *C. burnetii* phase II IgG, we currently found a seroprevalence of 10.7% in the JBH catchment area, indicating the epidemic nature of the recent outbreak. We calculate that the estimated number of *C. burnetii*-infected persons in the JBH catchment area is 40,600 persons (95% CI = 32,200–48,900 persons), which is 50-75 folds higher than the number of notified patients. Since our catchment area comprises only 11 of the 57 municipalities recognised as high incidence Q fever areas by the National Institute for Public Health and the Environment, the nationwide number of *C. burnetii*-infected persons in the Netherlands will be even far higher. This suggests also that the percentage of asymptomatic cases might be considerably higher than the 50-60% reported previously.[1;2] These figures indicate that a large group of patients is potentially at risk for development of chronic Q fever. Clinicians in

Table 1. Seroprevalence rates of IgG antibodies against *C. burnetii* phase II antigens in the catchment area of the Jeroen Bosch Hospital in the screening program for patients with aortic aneurysm or central vascular reconstruction (vascular screening), for patients with a history of cardiac valve surgery (valvular screening) and the two screening programmes combined (all patients).

Characteristic	Vascular screening	Seroprevalence (%, 95% CI)	Valvular screening	Seroprevalence (%, 95% CI)	All patients	Seroprevalence (%, 95% CI)	*p*-value[b]
Screened population	276	11.2 (7.5–14.9)	509	10.4 (7.8–13.1)	785	10.7 (8.5–12.9)	0.723[c]
Year of birth							0.265
1915-49 (1924-47)[a]	261	11.1 (7.3–14.9)	415	9.6 (6.8–12.5)	676	10.2 (7.9–12.5)	
1950-84 (1950-71)[a]	15	13.3 (0.0–30.5)	94	13.8 (6.9–20.8)	109	13.8 (7.3–20.2)	
Gender							0.694
Male	227	9.3 (5.5–13.1)	265	11.3 (7.5–15.1)	492	10.4 (7.7–13.1)	
Female	49	20.4 (9.1–31.7)	244	9.4 (5.7–13.1)	293	11.3 (7.6–14.9)	
Geographical location							0.483
Urban	92	16.3 (8.8–23.9)	180	9.4 (5.2–13.7)	272	11.8 (7.9–15.6)	
Rural	184	8.7 (4.6–12.8)	329	10.9 (7.6–14.3)	513	10.1 (7.5–12.7)	

CI, confidence interval, [a]range (2.5th -97.5th percentiles of all patients), [b]*p*-values calculated for each characteristic of all patients, unless otherwise indicated, [c]*p*-value comparing seroprevalence in vascular screening and valvular screening

endemic areas should be aware of chronic Q fever in patients with risk factors, like pre-existent cardiac valve disease, aortic aneurysm or vascular prosthesis, also when there is no history of acute Q fever.[2;5]

As of yet there is no standard cut-off titre for use in seroprevalence studies of *C. burnetii* antibodies. We choose a high IgG phase II cut-off titre to prevent overestimation of the number of *C. burnetii* infected persons. Yet, it seems more likely that this conservative cut-off resulted in underestimation. IgG phase II titres < 1:128 and even negative titres have been observed after one-year follow-up of acute Q fever patients in the Dutch outbreak.[12] This makes it feasible that IgG phase II titres < 1:128 in persons living in an (previously) epidemic area might well reflect past Q fever. If our cut-off titre was set at = 1:64, 117 out of 785 (14.9%) patients would have been considered seropositive indicating an even larger magnitude of the Dutch Q fever outbreak. This figure is in line with the reported seroprevalence of 12.2% in Dutch blood donors, and 9.0% among pregnant women, using an IgG phase II cut-off titre of = 1:64.7;13 In our opinion, use of a high cut-off titre also allowed for selection of relatively recent infections since IgG phase II titres decrease with time following acute infection.

It could be argued that the patient groups that were screened are not representative of the normal population and our seroprevalence rate should, therefore, not be extrapolated to the whole JBH catchment area. However, *C. burnetii* is an extremely infectious pathogen.[1] Therefore, seroprevalence merely reflects exposure to this pathogen, which is not expected to differ between high-risk groups for chronic Q fever development and the normal population, as is illustrated by the seroprevalence rate among Dutch blood donors. In contrast, the risk of complications of *C. burnetii* infection, namely chronic Q fever, is indeed increased in our screened populations.

There are conflicting reports on age- and gender-related differences in seroprevalence rates of *C. burnetii* antibodies. In Zimbabwe, a seroprevalence rate of 37% was noted without age- or gender-related differences.[14] In the United States, seroprevalence for persons = 20 years was 3.1%, increased with age and was higher for men.[15] We explain the absence of age- and gender-related differences in our survey as a consequence of emergence of Q fever in an epidemic situation. This differs from endemic situations like in Zimbabwe and the United States in which infection might be more related to occupational exposure. However, especially in the younger age group, our sample size was relatively small and, therefore, the absence of age-related differences in seroprevalence needs to be regarded with caution. We found no difference in seroprevalence between rural and urban populations. In this context, it is notable that the only city in our catchment area, 's-Hertogenbosch, is relatively small (39.98km^2) and that windborne spread reportedly can transport *C. burnetii* up to 18 km into metropolitan areas.[4]

In conclusion, using a conservative cut-off, we estimate that 32,200–48,900 persons in the JBH catchment area have been infected by *C. burnetii* in the Dutch Q fever outbreak. This number exceeds the number of notified patients with symptomatic acute Q fever in this region 50-75 fold. Seroprevalence in the JBH catchment area seems not influenced by age, gender or area of residence. Clinicians in areas recognised as high incidence Q fever regions should be alert for chronic Q fever in high risk patients, even if no acute Q fever episode was reported.

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Part 2

Diagnosis and classification of chronic Q fever

Chapter 5

Chronic Q fever: review of the literature and a proposal of new diagnostic criteria

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ABSTRACT

A review was performed to determine clinical aspects and diagnostic tools for chronic Q fever. We present a Dutch guideline based on literature and clinical experience with chronic Q fever patients in The Netherlands so far. In this guideline diagnosis is categorized as proven, possible or probable chronic infection based on serology, PCR, clinical symptoms, risk factors and diagnostic imaging.

INTRODUCTION

Q fever is a zoonosis, caused by the Gram-negative coccobacillus *Coxiella burnetii*. There is a large animal reservoir, with goats, sheep and cattle being the most common source of human infections.[1-3] After primary infection, an estimated 1-5% of patients progress to chronic Q fever, which can become manifest years after initial infection. Endocarditis and infections of aneurysms or vascular prostheses are the most common manifestations.[4;5] Arthritis, osteomyelitis or hepatitis are rare manifestations of chronic Q fever. In a small number of patients no focus can be found.[6-8] Pre-existent cardiac valvular disease, aortic aneurysm, vascular grafts, immunocompromised state, and pregnancy are reported risk factors for the development of chronic Q fever.[7;9;10]

If left untreated, chronic Q fever leads to considerable morbidity and a mortality up to 60%.[5] Long-term antibiotic treatment, preferably consisting of hydroxychloroquine and doxycycline, and sometimes aggressive surgery are required in patients with established chronic Q fever.[4;5;11] The consequences of an adequate diagnosis are therefore of major impact for suspected chronic Q fever patients. However, the diagnosis of chronic Q fever is challenging: culture of *C. burnetti* is difficult and time-consuming, requires a level 3 biosafety laboratory and lacks sensitivity.[12] The diagnosis of chronic Q fever currently relies on serology and detection of DNA in blood or tissue with polymerase chain reaction (PCR). Chronic Q fever is proven when *C. burnetti* DNA is detected by PCR in blood or tissue in the absence of acute infection, although sensitivity of these techniques is low.[13] Serological diagnosis is based on antigenic variation of *C. burnetti*, in which after culture in cells, a virulent variant phase I antigen, shifts to an avirulent phase II variant. During acute infection, antibodies to phase II antigens are detected first, followed by phase I antibodies. Persisting high levels of antibodies to phase I and phase II antigens, are thought to be caused by continuous antigenic stimulation and considered indicative for chronic Q fever.[2;14] A cutoff for phase I IgG of = 1:800 has been internationally accepted for the serological diagnosis of chronic Q fever, which is based on an in house produced immunofluorescence assay (IFA).[15] Due to a high number of false-positive tests with a cut-off of 1:800 and development of other (commercial) IFA-assays, discussions about the optimal cut-off values and suggestions of the incorporation of clinical characteristics in the diagnosis of chronic Q fever have emerged.[8;16;17]

Since 2007, the southern parts of the Netherlands suffered from a major Q fever outbreak, with more than 4000 reported symptomatic cases. As the majority of patients have mild or asymptomatic acute infection, the actual incidence is probably much higher.[18] Due to hygienic measures, vaccination of goats and culling of pregnant goats, the acute Q fever epidemic in the Netherlands has subsided. Nevertheless, chronic Q fever is seen in a rising number of patients.[19] Uniformity in the assays and interpretation of test results is necessary for good patient care. Given the uncertainties in diagnostic algorithms for chronic Q fever, the impact of an adequate diagnosis on the prognosis and treatment, and the increasing number of patients, we performed a review of the available literature on the clinical aspects and diagnostic tools for chronic Q fever. Based on this available evidence, a new proposal for the diagnosis of chronic Q fever was formulated in a consensus meeting of clinicians and microbiologists which combines clinical, radiological and microbiological factors.

MATERIALS AND METHODS

Search strategy

A literature search was conducted in Pubmed to identify publications on the diagnostic features of chronic Q fever. We used the MESH-terms "Q fever" and "*Coxiella burnetii*" in combination with the subheading "diagnosis" and "microbiology", and the MESH terms "radiography" OR "ultrasonography" OR "echocardiography" OR "Positron-Emission Tomography" OR "Immunology" OR "PCR" OR "fluorescent antibody technique" OR "serologic test" OR "diagnostic imaging". Language was restricted to "English" only. The reference lists of the identified publications were screened for relevant additional publications. No restrictions were set for the clinical reference standard method applied. Publications were eligible for inclusion if: (i) clinical parameters were described in well defined cohorts of chronic Q fever patients or (ii) diagnostic tools for chronic Q fever such as IFA, PCR, immunohistochemistry, or culture, were evaluated against the golden standard used at the time of publication. Case-reports and case series with less than five patients and reviews were excluded. Papers concerning other topics than human chronic Q fever were excluded, such as studies about acute Q fever and animal studies. Features solely associated with the localisation and complications of chronic Q fever infection (for example heart failure and embolism in case of endocarditis, abdominal pain in case of infected aortic abdominal aneurysm) were not reviewed. If studies described the same patient cohorts, we only included the study which used the final and complete patient data.

Data extraction

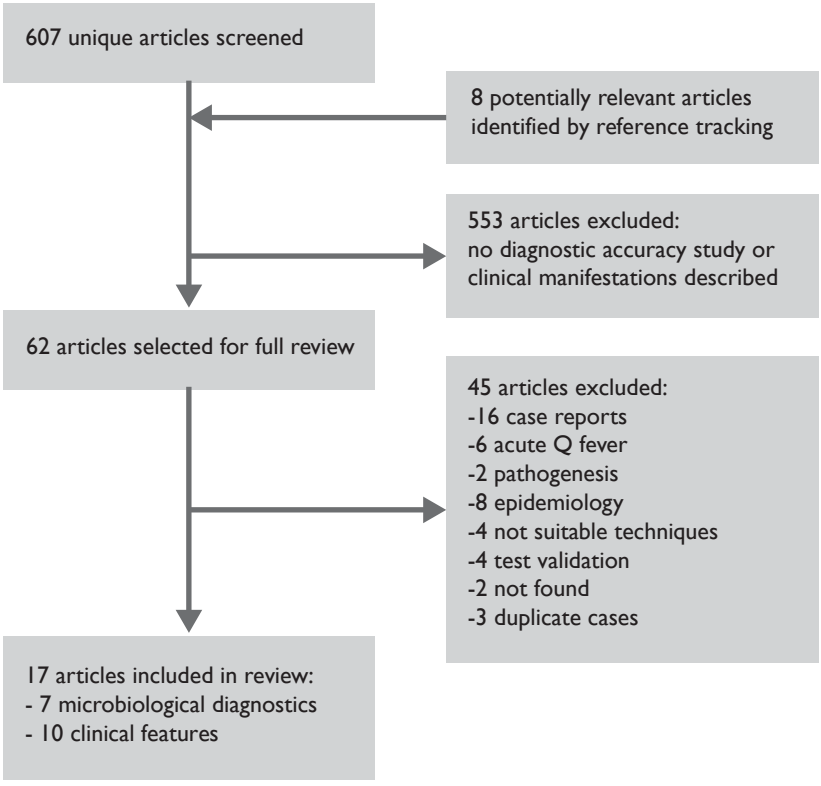
Titles and abstracts were screened by two reviewers (MW and LK). If the title or abstract suggested a publication meeting the inclusion criteria, full text was obtained. Selected full text articles were read by both reviewers and included in agreement. All included publications were critically reviewed and summarized. The diagnostic and clinical data were extracted from each included publication and drawbacks and biases were noted. Microbiological, clinical, laboratory and radiological features were considered separately.

RESULTS

Selection

We initially identified 607 unique publications describing features of chronic Q fever. Based on title and abstract, 553 articles were excluded because they did not describe diagnostic accuracy or clinical manifestations. An additional eight publications were detected through the reference lists. After reviewing the 62 remaining papers, 45 articles were excluded because they were case reports or series describing less than five patients (16), they described acute Q fever only (6), pathogenesis only (2), epidemiology only(8), diagnostic microbiological parameters not suitable in clinical practice (4), tests validation without a clinical diagnosis (4), full text was not available in accessible libraries (2) or because the report presented a cohort of patients that was also included in a subsequent study (3). The remaining 17 articles met the inclusion criteria. Seven manuscripts described the microbiological diagnosis and 10 manuscripts described clinical symptoms and features. Of the latter, 8 articles described imaging techniques and 9 described laboratory parameters in patients with chronic Q fever (Figure 1).

Figure 1. Flow-diagram of selection and revision of articles



Clinical features of patients diagnosed with chronic Q fever

We found 10 studies describing clinical features of at least five chronic Q fever patients.[4;5;7;10;20-25] Their results are summarized in Table 1. Table 2 includes comments on these studies and descriptions of the definition of chronic Q fever used. One article dealt with vascular chronic Q fever only.[4] One article described endocarditis, vascular infections, as well as more rare manifestations of chronic Q fever.[7] The remaining eight studies presented cases with Q fever endocarditis only [5;10;20-25]. In case of Q fever endocarditis, a diagnosis based on the modified Duke criteria, including phase I IgG =1:800, was most frequently used. Vascular chronic Q fever was diagnosed when phase I IgG =1:800 was present in combination with a clinical aneurysm or vascular infection, or positive *C. burnetti* PCR or culture of vascular tissue. Fever was the most common presenting symptom of chronic Q fever in 23-100% of the cases (data available in nine reports), followed by weight loss (data in eight reports), which was present in 11-100% of patients. Hepato- and/or splenomegaly were described in five reports, and were present in 11-90% of cases. Five reports mentioned fatigue in 9-100% cases. In three studies, night sweats were reported as presenting symptom in 26-100% of cases. One study also evaluated the absence of symptoms, which was observed in 6% of patients.

Biochemical laboratory features of patients presenting with chronic Q fever

Biochemical laboratory features were scarcely described (Table 1). Elevated liver enzymes were reported in four articles and present in 14-90% of cases, although exact quantification of the elevation was lacking.[7;22;24;25] Thrombocytopenia was mentioned in three studies and present in 29-88% of cases.[7;24;25] Elevated erythrocyte sedimentation rate (ESR) was reported in two studies and present in 80-83% of cases.20;25 Table 2 includes comments on these studies and descriptions of the definition of chronic Q fever used.

Table 1. Summary of publications describing the clinical characteristics of chronic Q fever

Reference, location and year of publication	Number patients	Clinical parameters	
		fever	night sweats
Million et al.[a] France, 2010[5]	Endocarditis (104)	76% (79/104)[b]	nd
Wiener-Well et al. Israel, 2009[20]	Endocarditis (9)	67% (6/9)	33% (3/9)
Kokkini et al. Greece, 2008[21]	Endocarditis (5)	100% (5/5)	100% (5/5)
Scott et al. USA, 2008[22]	Endocarditis (7)	100% (7/7)	nd
Botelho-Nevers et al. France, 2007[4]	Vascular infections (40)	83% (33/40)	nd
Landais et al.[a] France 2007[10]	Endocarditis (22)	23% (5/22)	nd
Salamand et al. France, 2002[23]	Endocarditis 19	68% (13/19)	26% sweats and chills (5/19)
Raoult et al.[a] France, 2000[7]	Endocarditis (225), vascular infection (25), pregnancy (15), hepatitis (8), osteomyelitis (7), unusual presentations(10)	endocarditis: 81% (158/194), hepatitis: 100% (8/8), osteomyelitis: 57%[b] (4/7)	nd
Boyle et al. Ireland, 1999[24]	Endocarditis (7)	nd	nd
Tobin et al. Ireland, 1982[25]	Endocarditis (10)	60% (6/10)	nd

nd, not described; ESR, erythrocyte sedimentation rate. [a]partial identical cases described, [b]percentages do not match with stated amount of patients in the text and tables, [c]only known in 66% of cases

Microbiological diagnosis of patients with chronic Q fever

We found seven publications that evaluated microbiological techniques for the diagnosis of chronic Q fever(Table 3).[8;12;13;15;26-28] The reference standards for the definition of chronic Q fever in these publications are summarized in Table 2. Five of the reviewed studies included patients with Q fever endocarditis only. In one study, both patients with Q fever endocarditis and patients with vascular infection caused by Q fever were included.[13] In one study, no clinical specifications were mentioned.[15] Five studies included a control group. Composition of the control group varied from patients with bacterial blood culture positive endocarditis without serological evidence of chronic Q fever, blood donors, acute Q fever disease, or serologically diagnosed non-endocardtitis related viral infection or a combination of these patients. In two studies, the control group was

Clinical parameters				Laboratory results
weight loss	fatigue/ weakness	spleno- hepatomegaly	no symptoms	
48% (50/104)	nd	splenomegaly 22% (23/104) hepatomegaly 12% (12/104)	6% (6/104)	nd
11% (1/9)	33% (3/9)	nd	nd	ESR >40 83% (5/6)[c]
100% (5/5)	100% (5/5)	nd	0%	nd
43% (3/7)	nd	no	0%	elevated liver enzymes 14% (1/7)
53% (21/40)	28% (11/40)	nd	nd	nd
nd	asthenia 9% (2/22)	nd	nd	nd
26% (5/19)	57% (11/19)	hepatomegaly 11% (2/19)	nd	nd
hepatitis: 25% (2/8)	nd	endocarditis: hepato- and/or splenomegaly 42% (81/194) osteomyelitis: splenomegaly 14% (1/7)	nd	thrombocytopenia 35% (68/194), elevated liver enzymes 17% (32/194)
Most common symptom	nd	hepatomegaly 57% (4/7)	nd	thrombocytopenia 29% (2/7), elevated liver enzymes 71% (5/7)
nd	nd	hepato-splenomegaly 90% (9/10)	nd	thrombocytopenia 88% (7/8), elevated liver enzymes 90% (9/10), elevated ESR 80% (8/10)

Table 2. Definitions of chronic Q fever and comments on studies in Table 1, Table 3 and Table 4

	Definitions of chronic Q fever and comments on studies
Frankel et al France, 2011[8]	Definition of chronic Q fever is object of study. Patients with positive *C. burnetii* serology and echocardiographic imaging, which was classified according to the modified Duke criteria in no, possible or definite endocarditis were compared .
Million et al. France, 2010[5]	Q fever endocarditis diagnosed (1) with modified Duke criteria which includes IFA, phase I IgG ≥ 1:800 and positive *C. burnetii* blood culture, and echocardiographic imaging, or (2) positive PCR on blood, serum, or valve samples. Unknown amount of patients with positive PCR or culture. Valvular insufficiency was not described according to revised Duke-criteria. Prosthetic valves not separately described.
Wiener-Well et al. Israel, 2009[20]	Q fever endocarditis diagnosed with modified Duke criteria which include IFA, phase I IgG ≥ 1:800 and positive *C. burnetii* blood culture, and transesophageal echocardiographic and/or histopathological findings. No confirmation of diagnosis with culture and/or PCR. ESR only in 66% of patients available.
Kokkini et al. Greece, 2008[21]	Q fever endocarditis diagnosed with serology (type and cut-off not defined, but IFA phase I IgG in all cases >1:1024) and echocardiographic imaging. No confirmation with culture and/or PCR.
Scott et al. USA, 2008[22]	Q fever endocarditis diagnosed with IFA and CBR (cut-off not defined, but IFA phase I IgG all >1:1024) and echocardiographic imaging according to modified Duke criteria. No confirmation with culture and/or PCR.
Botelho-Nevers et al. France, 2007[4]	Vascular chronic Q fever infection diagnosed by (1) positive culture or PCR in vascular biopsy or (2) IFA phase I IgG ≥1: 800, or positive blood PCR and a clinical suspicion of aortic aneurysm or graft infection. Diagnosis chronic Q fever only confirmed in 12/40 patients with positive *C. burnetii* culture or PCR on blood or tissue.
Landais et al. France, 2007[10]	Q fever endocarditis diagnosed with modified Duke criteria which include IFA, phase I IgG ≥ 1:800, and echocardiographic imaging. No confirmation with culture and/or PCR.
Fenollar et al. France, 2004[8]	Q fever endocarditis diagnosed (1) with modified Duke criteria which includes IFA, phase I IgG ≥ 1:800 and positive and echocardiographic imaging. Vascular infections were diagnosed on the basis of the presence of a vascular aneurysm or prosthesis associated with vascular symptoms, fever or phase I IgG ≥ 1:800 and no evidence of other infections
Rolain et al France, 2003[26]	Q fever endocarditis diagnosed with endocarditis according to the modified Duke criteria and positive PCR or culture for *C. burnetii* from valve samples.
Lepidi et al. France, 2003[27]	IFA IgG phase I ≥ 1:800. Control-group exists of culture positive endocarditis (non-Q fever, defined by negative IFA); no PCR and culture for Q fever of this group.
Salamand et al. France, 2002[23]	Q fever endocarditis diagnosed with modified Duke criteria which include IFA, phase I IgG ≥ 1:800, and echocardiographic imaging. No confirmation with culture and/or PCR, two cases confirmed with histology of the removed valve.
Raoult et al. France, 2000[7]	Q fever endocarditis diagnosed with IFA, phase I IgG ≥ 1:800, and clinical findings, defined in all subgroups as follows: endocarditis, modified Duke criteria; vascular infection, vascular aneurysm and prosthesis, osteoarticular infection, bone lesion; hepatitis prolonged disease with liver transaminase increase; pregnancy, all pregnant women and babys with antibodies (also <1:800); other, long evolution and no other etiologies. No or unknown confirmation with culture and/or PCR. Valvular insufficiency not described according to Duke-criteria. No description of location or clinical characteristics of vascular infections. Definition of pregnancy related chronic Q fever unclear, therefore not to determine which amount of pregnant patients only had an acute infection during pregnancy.
Boyle et al. Ireland, 1999[24]	Q fever endocarditis diagnosed with CFT, phase I IgG ≥ 1:200. No confirmation with culture and/or PCR. 1 patient with phase I IgG <1:200. Echocardiographic imaging only performed in 4 patients.
Musso et al. France, 1995[12]	IFA IgG phase I ≥ 1:800. No control group. Unclear which amount of patients had vascular chronic Q fever or Q fever endocarditis
Dupont et al France, 1994[15]	IFA IgG phase I ≥ 1:800 and or positive culture *C. burnetii* and clinical chronic Q syndrome. Evaluated method and the inclusion definition of chronic Q fever were approximately the same.
Brouqui et al. France, 1994[28]	Endocarditis (not defined) and IFA IgG phase I ≥ 1:800 and IgA phase I ≥ 1:50. No control group. Not all techniques were performed in allsamples.
Tobin et al. Ireland, 1982[25]	Definition of Q fever endocarditis based on phase I IgG≥ 1:200 (unclear if CFT or IFA is used) and clear valvular lesion on echocardiography and existence of. 4/10 patients with phase I IgG <1:1024 and 2/10 phase I IgG <1:200. No confirmation with culture and/or PCR Echocardiographic results not described

IFA, immunofluorescence assay; PCR, polymerase chain reaction; ESR, erythrocyte sedimentation rate; CFT, complement fixation test

Table 3. Summary of publications describing the microbiological diagnosis for chronic Q fever

Reference, location and year of publication	Methods evaluated	Specimen	Number of patients	Microbiological techniques					
				IFA IgG I titer	≥ 1:400	≥ 1:800	≥ 1:1600	≥ 1:3200	≥ 1:6400
Dupont et al France, 1994[15]	cut off IFA IgG phase I	serum	chronic Q fever (148) acute Q fever (486) controls (1960)	Se (%) Sp (%) PPV (%) NPV (%)	nd nd nd nd	100 99.6 98.1 100	80.4 99.8 100 99.6	nd nd nd nd	nd nd nd nd
Rolain et al France, 2003[26]	cut off IFA IgG phase I	serum	Q fever endocarditis (30) controls (194)	Se (%) Sp (%) PPV (%) NPV (%)	100 93.3 71.4 100	100 99.5 96.8 100	100 100 100 100	nd nd nd nd	nd nd nd nd
Frankel et al France, 201[18]	cut off IFA IgG phase I	serum	Q fever endocarditis (172)	PPV %	nd	37	47[a]	57	75
				Technique	**Valve culture**	**Valve PCR**	**IH**	**Blood culture**	**Blood PCR**
Brouqui et al France, 1994[28]	immunohistochemistry culture *C. burnetii*	Valve tissue and serum	Q endocarditis (17)	Se (%)	73.3	-	66.7	40.0	
Musso et al France, 1995[12]	culture *C. burnetii*	heparinized blood	Q fever endocarditis and vascular infection (17)	Se (%)	-	-	-	52.9	
Lepidi et al France, 2003[27]	PCR *C. burnetii* culture *C. burnetii* immunohistochemistry	valve tissue	Q fever endocarditis (9) controls (35)	Se (%) Sp (%) PPV (%) NPV (%)	- - - -	100 - - -	55.5 100 100 89.7	- - - -	- - - -
Fenollar et al France, 2004[8]	PCR *C. burnetii*	serum	Q fever endocarditis (44) and Q fever vascular infection (4) controls (100)	Se (%) Sp (%) PPV (%) NPV (%)	- - - -	- - - -	- - - -	- - - -	22.9[a] 100 100 73.0

IFA, immunofluorescence assay (IFA); nd, not defined; T, treatment; PPV, positive prospective value; NPV, negative prospective value; Se, sensitivity; Sp, specificity. IH, immunohistochemistry; PCR, polymerase chain reaction. [a]do not match with stated amount of patients in the text and tables

not specified. Three studies described sensitivity, specificity, positive predictive value(PPV) and negative predictive value(NPV) of different IgG phase I titres in chronic Q fever patients.[8;15;26] Although studies of Dupont and Rolain are not completely comparable due to the different chronic Q fever definitions used; sensitivity, specificity, positive predictive and negative predictive values were within the same range.[15;26] Markedly different were the positive predictive values of Frankels' study. It was however not possible to collect other statistical characteristics from the reported data.[8]

Four studies described the sensitivity of *C. burnetti* culture and detection of *C. burnetti* DNA by PCR in valve tissue and blood. Both culture and PCR were more sensitive, when performed on tissue compared to blood. Sensitivity of *C. burnetti* culture from cardiac valves was 88.9 % (8/9) and 73.3% (11/15) in two studies.[27;28] Sensitivity of *C. burnetti* culture in blood specimens was only 40.0% (6/15) and 52.9% (9/17).[12;28] Comparable differences were found for DNA detection by PCR in blood and tissue with a sensitivity of 22.9% in blood specimens and 100% using cardiac valves.[8;27] Reported specificity was 100%. Sensitivity of immunohistochemical staining using mouse anti-*C. burnetti* monoclonal antibody on valves from patients was 55.5% (5/9) and 66.7% (10/15).[27;28]

Table 4. Results of review on reports dealing with echocardiography in chronic Q fever endocarditis

Reference, location and year of publication	Echocardiogram			
	TEE or TTE	**vegetation**	**abscess**	**worsening of valvular function**
Million et al.[a] France, 2010[5]	TEE if TTE was negative[b]	28% (29/102)	7% (7/102)	75% (77/102) discovery or worsening of valvular insufficiency, 1%(1/102) mitral valve cordae tendinae rupture, 1% (1/102) large mitral valve perforation)
Wiener-Well et al. Israel, 2009[20]	nd	nd	nd	nd
Kokkini et al. Greece, 2008[21]	nd	40% (2/5)	20% (1/5)	nd
Scott et al. USA, 2008[22]	both performed in all patients	43% (3/7)	nd	57% (4/7) perivalvular regurgitation or prosthetic valve dehiscence
Landais et al.[a] France 2007[10]	TEE in all patients	18% (4/22)	nd	64% (14/22) acute valvular insufficiency or paraprosthetic leakage
Salamand et al. France, 2002[23]	all TTE, 53% (10/19) TEE[c]	21% (4/19)	11% (2/19)	nd
Raoult et al.[a] France, 20007	Not mentioned	50% (97/194)	10% (19/194)	21% (40/194) valvular regurgitation
Boyle et al. Ireland, 1999[24]	57% (4/7) TEE performed	50% (2/4)	nd	nd

TEE, transesophageal echocardiogram; TTE, transthoracal echocardiogram; nd, not described. [a]partial identical cases, [b]different performance between TTE and TTE not described, [c]valvular abscess in one patient not seen on TTE, but seen on TEE

Results of imaging techniques of patients diagnosed with chronic Q fever

Eight reports described results of echocardiographic imaging in patients diagnosed with Q fever endocarditis (Table 4).[5;7;10;20-24] Definitions of chronic Q fever in these articles are described in Table 2. Reports dealing with other imaging techniques, or imaging in non-endocarditis chronic Q fever were not found. In the reviewed articles, the used echocardiographic technique was either not described, or it was unclear what percentage of patients underwent transthoracic echocardiography (TTE) and what percentage transesophageal echocardiography (TEE). Echocardiographic findings lacked description according to the content of the revised Duke criteria in most reports.[29] All major Duke-criteria were described in only two reports. Six reports described vegetations on echocardiography, which were present in 21-50% of cases. Prosthetic dysfunction was described in six reports (3-57% of cases). Worsening of valvular insufficiency was reported in 3 reports (21-75% of cases). Cardiac (valve) abscesses were described in 4 reports (7-21% of cases). In only two reports, valvulopathies not meeting the major Duke criteria, like calcifications, prolaps and stenosis were mentioned, although the exact cumulative percentage of these valvulopathies were not separately described. In addition, one manuscript reported that 4% of chronic Q fever endocarditis patients had a prosthetic valve without echocardiographic abnormalities and found an incidence of pericardial effusion in 3% of chronic Q fever endocarditis.

	Echocardiogram		
	prosthetic dysfunction	**valvulopathy not meeting the major Duke criteria**	**no valvular ,or other pathology**
	3%(3/102) paraprosthetic leakage, 3% (3/102) prosthetic avulsion	7% (7/102) calcification, 3% (3/102) discovery or worsening of stenosis, 2% (2/102) valvular minimal alteration	4% (4/102) no echocardiographic abnormalities except presence of prosthetic valve, 3% pericardial effusion
	nd	nd	nd
	nd	nd	nd
	57% (4/7) perivalvular regurgitation or prosthetic valve dehiscence	nd	nd
	9% (2/22)	9% (2/22) bicuspid aortic valve, 9% (2/22) new mitral valve insufficiency or new mitral valve prolaps	nd
	26% (5/19)	nd	nd
	20% (38/194) prosthetic dehiscence	nd	nd
	25% (1/4) leak	nd	nd

DISCUSSION

With this review, we aimed to summarize the available knowledge on clinical presentation, microbiological diagnosis, and imaging techniques for the diagnosis of chronic Q fever and to provide a basis for uniformity in the work-up of patients suspected of having this infection. Due to the relative rarity of the disease, studies that have evaluated the diagnostic value of clinical, laboratory, imaging and microbiological parameters for chronic Q fever are limited to case reports and are mainly retrospective, descriptive analyses of a limited number of patients. As there were no randomized trials available, meta-analysis and/or systematic review of the selected articles was not possible. Instead, we described the major outcomes of these studies. When evaluating the available evidence, the microbiological diagnosis of chronic Q fever mainly relies on serology. In older papers differences in serological profiles between patients with acute, past and chronic infections were already notified, using ELISA, CFT as well as IFA: IgG phase I and to a lesser extent IgA phase I were markedly elevated in patients with chronic Q fever.[14;30-34] In some studies, chronic Q fever used to be based on phase I IgG =1:200 defined by complement fixation test (CFT). However, clinical validation of the CFT cut off was missing and its use in case of chronic Q fever has been abandoned since. Also, the value of IgA antibodies in the diagnostics of chronic Q fever has become less prominent. New microbiological tests and techniques for the diagnosis of (chronic) Q fever are in development, but still need clinical evaluation and incorporation in clinical practice.[35-38] Dupont et al. were the first in 1994 to propose an IFA IgG phase I cut off = 1:800 based on a retrospective serological study of 53 patients with chronic infections.[15] Drawback of this study is that the evaluated method and the definition of chronic Q fever were approximately the same. Nevertheless, the Duke criteria for the diagnosis of endocarditis were modified in 2000, including the proposed IFA IgG phase I titre =1:800 as a major criterion.[29] Subsequently, there have been publications in which the limitations of these this cut-off value were described.17;39-41 High phase I IgG antibodies, for example, found in patients with cardiovascular risk factors, but without clinical symptoms, may pose a therapeutic dilemma. After all, when a diagnosis of chronic Q fever is made, prolonged and intensive antibiotic therapy with a minimum of 18 months is warranted. Recently, a cut-off value of 1:1600 is suggested, based on re-evaluation of serological data and a high amount of false-positive tests with a cut-off of 1:800.[8] The effect on the negative predictive value of a cut-off titre if 1:1600 was not mentioned. Conversely, chronic Q fever cannot be excluded when phase I IgG antibody titre < 1:800 is detected.[42] Furthermore, inter and intra laboratory variations of immunofluorescence methods and results are probably of significant importance. Recently, Healy et al. showed that the concordance in result interpretation from three different centres was only 35%.[16] Interpretation of serological results in the light of clinical information was therefore advised, although definition of these parameters were lacking in Healy's article. In the Netherlands most laboratories use the commercial IFA of Focus Diagnostics, with titration based on a multiple of 16. An IgG phase I titer = 1:1024 is currently used as a cut-off value where above chronic Q fever is conceivable.[40]

Although collected from small patient cohorts, review of the test characteristics of culture and nucleic acid amplification techniques showed that sensitivity of *C. burnetti* DNA detection by PCR is markedly dependent on the type of specimen sampled. Sensitivity ranges from almost 100%, when performed on (valve) tissue, to 47% and 23% respectively when culture and PCR are performed on peripheral blood. It is not known why yields are low when detecting *C. burnetti* in blood from chronic Q fever patients, but as *C. burnetti* is an intracellular micro-organism it is conceivable that the cellular compartment (buffy coat) of peripheral blood is more sensitive for DNA

detection. Fenollar et al. also hypothesized that circulating antibodies and immune complexes act as DNA scavengers.[13]

Clinical features of patients with chronic Q fever are only scarcely described in cohorts of five patients or more. Most important presenting features are non-specific findings such as fatigue, fever, weight loss, night sweats and hepato-splenomegaly, although the prevalence of all of these symptoms showed great variation between the different study cohorts. For example, one report described fever in 23% of cases, while another report mentioned fever in 100% of cases. One study found that 6% of chronic Q fever cases remained without symptoms. Fatigue as a presenting symptom is often described, although in daily practice this is not a useful parameter, considering the overlap with Q fever related fatigue syndrome which occurs in approximately 20% of patients after acute Q fever infection.19;43 Interpretation of the diagnostic value of the presenting symptoms currently described is hampered by the wide range in definitions of chronic Q fever. In most definitions, diagnosis of chronic Q fever was based on serological techniques; confirmation with PCR or culture was often not possible or the percentage of patients with a confirmed chronic Q fever infection was not reported. It is interesting to define if different genotypes of *C. burnetti* are responsible for variation in clinical presentation and serological results. However, in the reviewed literature, these data were not provided.

Internationally accepted diagnostic criteria for infectious endocarditis are the revised Duke criteria in which echocardiography plays an important role. Transesophageal imaging is superior to transthoracic echocardiography in detecting vegetations, particularly in patients with prosthetic valves.[29] However, in our reviewed reports, the used echocardiographic technique was commonly unclear or not described. Superiority of TEE was only described in one report in which a valvular abscess in one patient was not seen on TTE, but seen on TEE.[23] Our review shows that large pathologic findings demonstrating infective endocarditis (IE) are only found in a quarter of chronic Q fever patients. Valvular insufficiency was the most common found feature on echocardiography in case of chronic Q fever endocarditis, although strict definition and quantification of this finding were not reported. In a large proportion of patients with Q fever endocarditis, echocardiographic findings are non-specific or even absent. These minor echocardiographic criteria, not being part of the revised Duke criteria, were only rarely confirmed by histology, *C. burnetti* culture or PCR in the available literature. Non-specific findings on pathologic examination of cardiac valves infected with *C. burnetti* have been described, although in those reports, comparison with echocardiographic results were missing.5;28 Quantification of echocardiographic findings in case of chronic Q fever endocarditis is not possible from our data. For that purpose, a systematic descriptive analysis of echocardiographic findings in patients with chronic Q fever is obligatory.

For vascular Q fever infections, no well described cohorts of patients that underwent radiological imaging are available. The only information is available from case reports.[42;44;45] Historically, computed tomography (CT) has been the standard for diagnostic imaging of vascular (graft) infections. MRI and duplex ultrasound are other options. However the positive predictive value of these diagnostic instruments is not optimal.[46] On CT scans, air bubbles can be detected around an infected graft in about half of the cases but are highly unspecific as in 50% of grafts these bubbles are present for weeks or even months after surgery. Moreover, it is difficult to differentiate between infection, hematoma, and lymphocele on CT scans. In chronic low-grade infections, CT is often false-negative.[47] In comparison to CT, fluorodeoxyglucose positron emission tomography combined with CT (FDG-PET/CT) has shown a superior diagnostic performance in (low-grade) vascular infections. PET has shown a sensitivity exceeding 90% in comparison to 64% for CT.[48]

Table 5. Dutch consensus guideline on chronic Q fever diagnosis

Proven chronic Q fever	Probable chronic Q fever	Possible chronic Q fever
Positive *C. burnetii* PCR in blood or tissue[a] OR 2. IFA ≥ 1:800 or 1:1024 for *C. burnetii* phase I IgG[b] AND - definite endocarditis according to the modified Duke criteria[29] OR - proven large vessel or prosthetic infection by imaging studies (^{18}FDG-PET, CT, MRI or AUS)	IFA ≥ 1:800 or 1:1024 for *C. burnetii* phase I IgG[b] AND - Valvulopathy not meeting the major criteria of the modified Duke criteria[29] - Known aneurysm and/or vascular or cardiac valve prosthesis without signs of infection by imaging studies (TEE/ TTE, ^{18}FDG-PET, CT, MRI or AUS) - 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, hepatosplenomegaly, persistent raised ESR and CRP - Granulomatous tissue inflammation, proven by histological examination - Immunocompromised state	IFA ≥ 1:800 or 1:1024 for *C. burnetii* phase I IgG[b] without manifestations meeting the criteria for proven or probable chronic Q fever

IFA, immunofluorescence assay; FDG-PET, fluorodeoxyglucose positron emission tomography; CT, computer tomography; MRI, magnetic resonance imaging; AUS, abdominal ultrasound; TEE, transesophageal echocardiography; TTE, transthoracic echocardiography.[a] In absence of acute infection, [b]cut-off is depending on the IFA technique used, respectively, in house developed or commercial IFA technique.

Specificity exceeds 90% with the adoption of clear criteria for image interpretation (only focal, homogeneous, abnormal uptake interpreted as positive for infection).[47-50] In order to avoid false-positive results, FDG-PET/CT should be performed at least 2 months after vascular surgery.[48;51;52] However, the role of PET/CT in detection of infected grafts or aneurysms by *C. burnetti*, which gives a low grade infection, has not been studied systematically yet. Two case reports in which PET/CT demonstrated superior diagnostic imaging compared to CT-scan in *C. burnetti* vascular graft and aneurysm infections have been described.44;45 FDG-PET/CT scanning has also been evaluated in patients with osteomyelitis, spondylitis and spondylodiscitis (not caused by chronic Q fever). It has been shown that PET scanning offers high sensitivity and specificity.[53-57] Gratz et al. demonstrated that PET was superior in comparison to MRI imaging in spondylodiscitis.[57] Major advantage of PET/CT is that not only inflammation of vascular structures can be visualised, but also possible infectious foci in other parts of the body. It is used in the work-up of "fever of unknown origin" as an instrument to identify the origin of fever if other diagnostics have failed.[58] Therefore, it seems to be suitable for the assessment in case of a suspected chronic Q fever infection where no cardiac or vascular focus is found.

In light of the issues addressed in this review, the diagnosis of chronic Q fever is complex. PCR and culture are very specific, but lack sensitivity. Diagnosis based on phase I IgG alone is not sufficient to prove or exclude chronic Q fever. Based on the results of the review, we believe that combining risk factors, clinical parameters, microbiology, imaging and laboratory results is obligatory to make a well considered diagnosis of chronic Q fever. Although many questions have remained unanswered, with the current available literature our Dutch consensus group has composed a guideline on chronic Q fever, in which chronic Q fever is categorized as proven, probable or possible chronic infection. (Table 5) Proven chronic Q fever cases are classified as positive *C. burnetti* PCR (or culture) in blood or tissue, or patients with phase I IgG = 1:800 or phase I IgG = 1024 (depending on in house IFA technique or commercial IFA technique respectively), in combination with definite endocarditis according to the revised Duke criteria, or evident infection of aneurysm or vascular graft on CT, PET-CT, duplex ultrasound or MRI. In addition: diagnosis of proven chronic Q fever by positive PCR can only be stated in absence of an acute infection. Positive PCR can be observed during the first two weeks of clinical symptoms of acute Q fever.[59] Although most patients with chronic Q fever have no known episode of acute Q fever, diagnosis of chronic Q fever based on phase I IgG = 1:1024 in patients with a documented episode of acute Q fever, can only be made at least six months after this episode.[8;9;42] Long-term antibiotic treatment, preferably consisting of hydroxychloroquine and doxycycline, should always be initiated in case of proven chronic Q fever. Frequent clinical and serological monitoring is warranted, whenever improvement stagnates, surgical intervention must be considered. Probable chronic Q fever is diagnosed in those patients with phase I IgG = 1:1024 who have established risk factors for chronic Q fever, show echocardiographic abnormalities not meeting the revised Duke criteria, probable rare manifestations of Q fever (e.g. hepatitis, osteomyelitis) or signs of systemic inflammation. Frequent monitoring is warranted, consisting of three monthly clinical and microbiological follow-up. Radiographical imaging (echocardiogram, PET/CT) should be performed whenever clinical improvement stagnates or worsens. Whether to start antibiotic treatment should be discussed in a multidisciplinary team, consisting of clinicians and microbiologists with experience in chronic Q fever. Possible chronic Q fever is diagnosed in patients with solely a phase I IgG = 1:1024, without any of the manifestations mentioned in the categories proven and probable. Frequent monitoring is warranted, consisting of three monthly clinical and microbiological follow-up. Radiographical imaging (echocardiogram, PET/CT) should be performed whenever clinical improvement stagnates or worsens. In general, no antibiotic treatment should be initiated in patients with possible chronic Q fever. Consequence of this guideline is that in every patient with suspected chronic Q

fever, an echocardiography and preferably a PET-CT has to be performed to differentiate between these categories. Our classification might need adjustment when prospective clinical data become available from the Dutch national chronic Q fever monitoring system including all patients with chronic Q fever diagnosed in the Netherlands (expected in 2012). For now, it is a practical tool for the diagnosis of chronic Q fever and determination of an individualized treatment plan. This classification is also very important for an adequate comparison of results of future research, which proved to be very difficult in the presently available literature.

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Chapter 6

Chronic Q fever diagnosis: consensus guideline versus expert opinion

Submitted

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ABSTRACT

Chronic Q fever, caused by *Coxiella burnetii*, is an infection with high mortality and morbidity if left untreated. Controversy about the diagnosis of the complex disease has emerged recently. We applied both the Dutch consensus guideline from the Dutch Q Fever Consensus Group and the diagnostic criteria proposed by the leading expert in Q fever, professor Raoult from France, to all chronic Q fever patients included in the Dutch National Chronic Q Fever Database. Of the cases with proven chronic Q fever according to the Dutch guideline, 31% would be left undiagnosed with Raoult's guideline. Four patients with chronic Q fever based on the Dutch consensus guideline but without chronic Q fever according to the guideline proposed by Raoult died of chronic Q fever-related causes. High sensitivity is of major importance in the diagnosis of chronic Q fever because mortality of untreated chronic Q fever is high. Until results from future studies are available by which current guidelines can be modified to definite evidence-based diagnostic criteria for this complex disease, it is our opinion that the Dutch literature-based consensus guideline is safer and easier to use in clinical practice than Raoult's expert-based guideline.

INTRODUCTION

Coxiella burnetii is the causative agent of Q fever, a zoonosis occurring worldwide.[1] Recently there has been a large epidemic in the Netherlands with over 4000 cases of acute Q fever notified between 2007 and 2010.[2;3] Chronic Q fever develops in an estimated 1-5% of all infected humans and can become manifest even years after primary infection.[1;4] Endocarditis and infection of aneurysms or vascular prostheses are the most common manifestations.[1;5;6]

Untreated chronic Q fever has a poor prognosis, with a reported mortality rate of up to 60%.[1;7] Adequate antibiotic treatment reduces mortality of Q fever endocarditis to less than 5%.[7] Treatment preferably consists of a combination of doxycycline and hydroxychloroquine for at least 18 months (non-prosthetic infection) to 24 months (prosthetic infection) and is advised to be continued in case of unfavourable clinical or serologic response.[7;8] Antibiotic guidelines for vascular chronic Q fever are not available yet, but antibiotic regimes for Q fever endocarditis have been applied to this disease entity as well. Early surgical intervention, with removal of infected material might improve the diagnosis of vascular chronic Q fever.[6;9] In the early course of chronic Q fever, most patients are asymptomatic or experience non-specific symptoms such as low-grade fever, night sweats, and weight loss.[1;4;6;7] Findings on echocardiography are often non-specific or absent in case of endocarditis, which makes the diagnosis of chronic Q fever challenging.[7]

A positive polymerase chain reaction (PCR) or culture of *C. burnetii* in blood or tissue, in the absence of acute Q fever, is a strong indicator for chronic Q fever. However, sensitivity on blood samples is only 50-60% for both PCR and culture in patients with chronic Q fever.[10;11] Therefore, serology is also important for the diagnosis of chronic Q fever. A phase I IgG cut-off titre of 1:800, which is based on an in-house developed immunofluorescence assay (IFA), has been internationally accepted for the diagnosis of chronic Q fever and is included in the modified Duke criteria for diagnosis of endocarditis.[12;13] In the Netherlands, a commercial IFA (Focus Diagnostics) is mostly used, with a proposed cut-off value of 1:1024 for chronic Q fever.[14] Yet, recent studies show that serology results alone are not sufficient for the diagnosis of chronic Q fever, but should be combined with clinical data.[15]

Faced with a large Q fever outbreak in the Netherlands and a rising number of (presumed) chronic Q fever patients, we were not able to find answers to all our questions about this complex disease in the literature. Moreover, randomized trials on diagnosis and treatment of this disease were lacking and available data were not all applicable to the Dutch situation. For example, we found far more vascular localizations of chronic Q fever, with often severe complications, than described previously. Therefore, the Dutch Q Fever Consensus Group was initiated, in which diagnosis and subsequent treatment consequences for suspected chronic Q fever were discussed. We performed a thorough literature review and constructed a new guideline for the diagnosis of chronic Q fever, differentiating between proven, probable and possible chronic Q fever (Chapter 5: table 5). We added advice for treatment and follow-up regimes for these three groups of patients. Antibiotic treatment and, if indicated, surgical treatment are recommended for all patients with proven chronic Q fever. The decision to start antibiotic treatment in patients with probable chronic Q fever depends on clinical characteristics and the condition of the patient, and should be discussed in a multidisciplinary team. In possible chronic Q fever patients, antibiotic treatment should not be initiated, but follow-up is indicated.

The Dutch consensus guideline was recently published.[14] Subsequently a reaction of professor Raoult was published, opposing our proposed guideline and formulating another guideline based on his expert opinion (Table 1).[16] Professor Raoult is the undisputed leading expert in Q fever

Table 1. Diagnostic guideline for chronic Q fever proposed by Raoult[16]

1[a]: Q fever endocarditis

A. Definite Criteria

Positive culture, PCR, or immunochemistry of a cardiac valve

B. Major Criteria

Microbiology: positive culture or PCR of the blood or an emboli or serology with IgG I antibodies ≥6400

Evidence of endocardial involvement:

-Echocardiogram positive for IE: oscillating intra-cardiac mass on valve or supporting structure, in the path of regurgitant jets, or on implanted material in the absence of an alternative anatomic explanation; or abscess; or new partial dehiscence of prosthetic vale; or new valvular regurgitation (worsening or changing of pre-existent murmur not sufficient)

-PET-scan showing a specific valve fixation and mycotic aneurysm

C. Minor criteria

Predisposing heart condition (know or found on echocardiography)
Fever, temperature >38°C
Vascular phenomena, major arterial emboli, septic pulmonary infarcts, mycotic aneurysm (see at PET-scan), intracranial hemorrhage, conjunctival hemorrhages, and Janeway's lesions
Immunological phenomena: glomerulonephritis, Osler's nodes, Roth spots, or rheumatoid factor
Serological evidence: IgGI antibodies ≥800 <6400

Diagnosis definite

1. 1A criterion
2. 2B criterion
3. 1B, and 3C criterion

Diagnosis possible

1. 1B criterion, 2C criteria (including microbiology evidence, and cardiac predisposition)
2. 3C criteria (including positive serology, and cardiac predisposition)

2[b]: Q fever vascular infection

A. Definite Criteria

Positive culture, PCR, or immunochemistry of an arterial sample (prosthesis or aneurysm) or a periarterial abscess or a spondylodiscitis linked to aorta

B. Major Criteria

Microbiology: positive culture or PCR of the blood or an emboli or serology with IgG I antibodies ≥6400

Evidence vascular involvement

-CT-scan: aneurysm or vascular prosthesis + periarterial abscess, fistula, or spondylodiscitis

-PET-scan: specific fixation on an aneurysm or vascular prosthesis

C. Minor criteria

Serological IgGI ≥800 <6400
Fever, temperature >38°C
Emboli
Underlying vascular predisposition (aneurysm or vascular prosthesis)

Diagnosis definite

1. 1A criterion
2. 2B criterion
3. 1B, and 2C criterion (including microbiology and vascular predisposition)

Diagnosis possible

Vascular predisposition, serological evidence and fever or emboli

and his expert opinion and scientific publications from his research group should be considered by anyone working in the field of Q fever. Here, we reflect on this controversy by applying both guidelines to cases from the Dutch National Chronic Q Fever Database.

MATERIALS AND METHODS

After the recent outbreak of Q fever in the Netherlands we initiated the "Dutch National Chronic Q Fever Database", which is a joint effort of multiple hospitals in the Q fever afflicted areas to monitor all chronic Q fever cases in the Netherlands. All hospitals with chronic Q fever patients, also outside the notified Q fever epidemic areas, were actively approached. Design of the database and use of the collected information for analysis and scientific publications were approved by the Medical Research Ethics Committee of the University Medical Center Utrecht in Utrecht, the Netherlands. Part of these data were already published in a report discussing serologic profiles of patients with chronic Q fever.[15] In Dutch National Chronic Q Fever Database, patients are included as proven, probable and possible chronic Q fever according to the Dutch consensus guideline. We re-evaluated these chronic Q fever cases with the diagnostic criteria proposed by Raoult (Table 1).

RESULTS

Until the end of May 2012, 284 patients were included in our database: 151 patients (53.7%) had proven chronic Q fever, while 64 patients (22.5%) and 69 patients (24.3%) had probable and possible chronic Q fever according to the Dutch consensus guideline, respectively. Of the cases with proven chronic Q fever according to the Dutch guideline, 46 patients (30.5%) would be left undiagnosed with Raoult's guideline. For probable chronic Q fever this would be 58 cases (90.6%) and for possible chronic Q fever cases all patients. Eight patients with proven chronic Q fever based on PCR-positivity in blood and suspicion of endocarditis would be diagnosed with possible Q fever endocarditis only by Raoult's guideline (Table 2) and 18 patients with proven chronic Q fever would not be diagnosed with Q fever endocarditis at all, because echocardiography results did not

Table 2. Comparison of chronic Q fever diagnosis according to the Dutch consensus guideline[14] and the guideline proposed by Raoult[16]

	Dutch consensus guideline		
	Proven chronic Q fever n=151 (%)	**Probable chronic Q fever n=64 (%)**	**Possible chronic Q fever n=69 (%)**
Raoult's guideline			
Definite Q fever endocarditis	21 (13.9)	0	0
Possible Q fever endocarditis	8 (5.3)	4 (6.3)	0
Definite Q fever vascular infection	76 (50.3)	0	0
Possible Q fever vascular infection	0	2 (3.1)	0
No diagnosis of chronic Q fever	46 (30.5)	58 (90.6)	69 (100.0)

Table 3. Characteristics and outcome of patients diagnosed with chronic Q fever using the Dutch consensus guideline[14] but without (definite) chronic Q fever according to the guideline proposed by Raoult[16]

	Raoult's guideline:	
	Possible Q fever endocarditis or vascular infection n=14 (%)	**No diagnosis n=173 (%)**
Dutch consensus guide line:		
Proven Q fever	8 (57.1)	46 (26.6)
Endocarditis	8 (57.1)	18 (10.4)[a]
PCR positive in blood	6 (42.9)	18 (10.4)
Evidence of endocardial involvement	2 (14.3)	0
Vascular infection	0	24 (13.9)[a]
PCR positive in blood	0	7 (4.0)
Vascular focus on imaging	0	17 (9.8)
Other or no focus (all PCR in blood positive)	0	7 (4.1)
Deceased	2 (14.3)	8 (4.6)
Death probably due to Q fever	2 (14.3)	4 (2.3)[b]
Probable Q fever	6 (42.9)	58 (33.5)
Endocarditis	4 (28.6)	22 (12.7)
Vascular infection	2 (14.3)	16 (9.3)
Other or no focus	0	20 (11.6)
Deceased	2 (14.3)	4 (2.3)
Death probably due to Q fever	1 (7.1)	0
Possible Q fever	0	69 (39.9)

[a] in three proven chronic Q fever patients imaging studies revealed that the focus of infection was both on the heart valves and vascular structures; [b]two patients with positive *C. burnetii* PCR on vascular and heart valve tissue obtained at autopsy

match any major clinical Duke criterion as is often observed in Q fever endocarditis.[7] Of the eight proven chronic Q fever endocarditis patients (Dutch consensus guideline) who were diagnosed with possible endocarditis according to Raoult's guideline, two would be defined as definite endocarditis by the modified Duke criteria.[13]

Twenty-four patients with a vascular Q fever infection (Dutch consensus guideline) would not be diagnosed with chronic Q fever using Raoult's criteria (Table 3). Seventeen of these patients had a fluorodeoxyglucose positron emission tomography-computer tomography (FDG-PET/CT) positive vascular lesion with phase I IgG =1:800 and <1:6400. Seven patients had a positive PCR in blood in combination with an aneurysm or vascular prosthesis but no signs of infection on FDG-PET/CT. There were five proven chronic Q fever patients according to the Dutch consensus guideline with no known focus and two patients with another focus than endocarditis or vascular infection who would have been missed with Raoult's criteria: five of them (repeatedly) had a positive PCR in blood but no clear infectious focus on echocardiography and FDG-PET/CT. One

patient had a positive PCR in blood with clinical pericarditis, and one patient had a positive PCR in blood during pregnancy with phase I IgG >1:1024 and a positive PCR of placental tissue.

Ten proven chronic Q fever patients who were not diagnosed with definite chronic Q fever using Raoult's criteria died (two with possible chronic Q fever and eight without chronic Q fever according to Raoult's guideline). Six of these patients died due to clear chronic Q fever related manifestations (two with possible chronic Q fever and four without chronic Q fever according to Raoult's guideline, Table 3). The two patients with possible chronic Q fever died of complications caused by endocarditis, one of them had a double-pathogen endocarditis with Staphylococcus aureus. Two of the four patients without chronic Q fever according to Raoult's guideline died due to aorta-duodenal fistula, both with a phase I IgG >1:1024, but <1:6400, negative PCR on blood, and a clear FDG-positive vascular focus on PET/CT. In one of these two patients, Q fever vascular infection was confirmed post-mortem with a positive PCR of the abdominal aortic aneurysm. No autopsy was performed on the other patient. The third patient with a history of a biological heart valve replacement, an FDG-PET/CT negative aortic aneurysm, and a positive PCR on blood eventually died of heart failure. Post-mortem analysis demonstrated a positive PCR of the heart valve confirming Q fever endocarditis. Another chronic Q fever patient with positive blood PCR and minor valve lesions according to the Duke criteria died of gastro-intestinal bleeding probably due to aorta-intestinal fistula.

DICUSSION

Raoult opposes to the term "chronic Q fever", but makes a distinction in two manifestations: Q fever endocarditis and Q fever vascular infection. This distinction is not accompanied by therapeutic consequences for each of these manifestations. Moreover, more rare manifestations, like pericarditis, hepatitis, and osteomyelitis are left undefined.

An important difference in the diagnostic criteria proposed by Raoult and the Dutch Q Fever Consensus Group is the diagnostic value attributed to *C. burnetii* PCR positivity of blood samples. Being unaware of other clinical entities presenting with a positive *C. burnetii* PCR in blood than acute and chronic Q fever, we believe that a positive blood PCR, in the absence of acute Q fever, proves chronic Q fever. Raoult on the other hand states that a positive PCR in blood should be accompanied by a clear endocarditis focus on echocardiography, a clear vascular focus on imaging studies, or at least two or three "minor criteria". Moreover, great value is attributed by Raoult to the phase I IgG titre proposing a phase I IgG =1:6400 as major criterion for Q fever endocarditis and Q fever vascular infection, opposed to a phase I IgG =1:800 and <1:6400 being proposed as a minor criterion. This is in contradiction to the internationally accepted modified Duke criteria, in which a phase I IgG =1:800 is stated as a major criterion for infective (Q fever) endocarditis.[13]

The sensitivity of the Dutch guideline is higher. With Raoult's criteria approximately 30% of proven chronic Q fever cases would be missed and almost all probable and possible cases, including at least four patients that eventually died due to chronic Q fever related causes. The number of deaths might even have been higher if fewer patients with chronic Q fever were treated as would be the case when Raoult's guidelines would have been applied to the Dutch chronic Q fever patients. Specificity of the Dutch consensus guideline is probably lower, but as untreated chronic Q fever has high mortality and high morbidity, we believe sensitivity is of greater importance in clinical practice. Missing and therefore not adequately treating proven chronic Q fever possesses a high risk for severe complications and death in these patients as was illustrated with our data.

In our opinion, patients without endocarditis or vascular infection on imaging studies, but with a positive PCR in blood should also be treated for chronic Q fever, as they may suffer from not yet clinically visible endocarditis or vascular infection, which was confirmed by the post-mortem results of two of our patients described above. We agree with the statement that part of the patients with probable chronic Q fever and most patients with possible chronic Q fever will eventually not have chronic Q fever. We therefore do not advocate treating all of these patients with long-term antibiotics. Nevertheless, we do think that these patients should all be analysed for a chronic Q fever focus and should remain under close follow-up, at least until further research offers more clarity to the prognosis of these patients. If these patients are not diagnosed with possible or probable chronic Q fever, they might not receive such close follow-up. Moreover, the Dutch consensus guideline is easier to use, adds treatment advice, and also applies to patients with rarer chronic Q fever manifestations other than endocarditis and vascular infection. We hope that with the future results of the Dutch National Chronic Q Fever Database and joint efforts of international researchers and experts in the field of Q fever these guidelines can be modified to definite evidence-based criteria for this complex disease. In the meantime the Dutch consensus guideline based on the available literature is in our opinion safer and easier to use in clinical practice than Raoult's expert-based guideline.

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Chapter 7

Microbiological challenges in the diagnosis of chronic Q fever

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ABSTRACT

Diagnosis of chronic Q fever is difficult: PCR and culture lack sensitivity, hence diagnosis relies mainly on serologic tests using immunofluorescence assay (IFA). Optimal phase I IgG cut-off titers are debated, but are estimated between 1:800 and 1:1600. In patients with proven, probable or possible chronic Q fever, we studied phase I IgG antibody titers at time of positive blood PCR, at diagnosis, and at peak levels during chronic Q fever.

We evaluated 200 patients, of whom 93 (46.5%) had proven, 51 (25.5%) probable, and 56 (28.0%) had possible chronic Q fever. Sixty-five percent of proven cases had positive *C. burnetii* PCR on blood, which was associated with high phase I IgG. Median phase I IgG titers at diagnosis and peak titers in patients with proven chronic Q fever were significantly higher compared to patients with probable and possible chronic Q fever. The positive predictive value for proven chronic Q fever, compared to possible chronic Q fever at titers 1:1024, 1:2048, 1:4096, =1:8192, was 62.2%, 66.7%, 76.5%, =86.2% respectively. However, sensitivity drops to <60% when cut-off titers =1:8192 were used. Although our study demonstrated a strong association between high phase I IgG titers and proven chronic Q fever, increasing the current diagnostic phase I IgG cut-off to 1:1024 is not recommended due to increased false negative findings (sensitivity <60%) and high morbidity and mortality of untreated chronic Q fever. Our study emphasizes that serologic results are not diagnostic on their own, but should always be interpreted in combination with clinical parameters.

INTRODUCTION

Q fever is caused by *Coxiella burnetii*, which is a worldwide prevalent intracellular Gram-negative bacterium leading to outbreaks.[1;2] Q fever has both acute and chronic manifestations. Acute Q fever is characterized by a mild, self-limiting influenza-like illness, sometimes complicated by pneumonia or hepatitis.[2;3] In 1-5% of all infected patients, chronic Q fever develops, which can occur even years after primary infection.[2;4;5] Established risk factors that predispose to chronic Q fever are pre-existent cardiac valvulopathy, vascular grafts and aneurysms, immunosuppression and pregnancy.[4;6;7] Most commonly described forms of chronic Q fever are endocarditis (~75%), vascular infection (~10%) or pregnancy-related disorders(~6%).[7] However, in the Netherlands, half of the chronic Q fever cases consist of patients with infected aneurysms and/or vascular prostheses.[8] Untreated chronic disease can lead to severe complications such as cardiac failure, ruptured aneurysms or death.[2;9] Between 2007 and 2010 a large outbreak of Q fever occurred in the Netherlands with over 4000 identified cases of acute Q fever and an increasing number of chronic Q fever cases.[8;10-12] Before complications occur, most patients report no or unspecific symptoms such as low-grade fever, night sweats and weight loss.[2;5;9;13] In cases of Q fever endocarditis, pathologic findings on echocardiogram can be non-specific or even absent.[7;9]

Due to the non-specific clinical presentation, diagnosis of chronic Q fever relies on additional microbiological analysis. A positive polymerase chain reaction (PCR) or culture of *C. burnetii* in blood or tissue, in absence of acute Q fever, is diagnostic for chronic Q fever. Nevertheless, in blood, sensitivity is only 50-60% for both PCR and culture in patients with chronic Q fever.[14;15] The sensitivity of PCR is reported to diminish when titers of IgG against *C. burnetii* phase I antigens (phase I IgG) reach 1:25600 and higher.[14] Therefore, serological analysis is pivotal for the diagnosis of chronic Q fever. An avirulent form of *C. burnetii* (Nine Mile strain phase II) has been produced in vitro that differs antigenically from the infectious agent (Nine Mile phase I) and is useful for serologic diagnostics.[1] During acute infection, IgG antibodies against *C. burnetii* phase II antigens (phase II IgG) are predominantly produced, whereas chronic Q fever is usually characterized by persistent high level of phase I IgG, often in the presence of high phase II IgG titers.[1;2;16] A phase I IgG cut-off titer of 1:800, which is based on an in-house developed immunofluorescence assay (IFA), has been proposed and internationally accepted for the serological diagnosis of chronic Q fever.[17] In the Netherlands, the IFA test from Focus Diagnostics is most widely used, with a proposed cut-off value of 1:1024 for possible chronic Q fever.[12] However, discussions about the optimal cut-off value have emerged, based on additional clinical data showing a high number of false-positive tests with a cut-off titer of 1:800 or 1:1024.[3;18-20]

Recently, the Dutch consensus group for the diagnosis of (chronic) Q fever proposed to categorize patients into proven, probable or possible chronic Q fever. This classification ranks the probability of having chronic Q fever based on PCR, serology, clinical parameters, imaging studies and pathology (see Materials and Methods section for details). In a Dutch cohort of proven, probable and possible chronic Q fever patients, we studied the predictive value of serologic profiles on the diagnosis of chronic Q fever.

MATERIALS AND METHODS

Patients

In order to monitor chronic Q fever cases in the Netherlands after the recent outbreak, a nationwide database has been constructed in which information about all chronic Q fever cases is being collected. Patients are classified as proven, probable or possible chronic Q fever according to the recent Dutch consensus guideline.[21] The first group (= proven chronic Q fever) consists of patients with chronic Q fever either confirmed with positive *C. burnetii* PCR on plasma, serum or tissue or with a phase I IgG of =1024 in combination with a proven vascular infection on positron emission tomography (PET), computed tomography (CT) or magnetic resonance imaging (MRI), or endocardial involvement according to the major criteria of the modified Duke criteria. The second group (= probable chronic Q fever) concerns patients with phase I IgG of =1:1024 with known risk factors for chronic Q fever, non-major valvulopathy according to the modified Duke criteria, suspected non-vascular or non-cardial localization of chronic Q fever infection or aspecific signs of chronic infection. The third group (= possible chronic Q fever) consists of patients with solitary phase I IgG of =1:1024, without other indications for probable or proven chronic Q fever.[21] The proven chronic Q fever group includes patients with the most definite form of chronic Q fever, in contrast to the possible chronic Q fever group which represents patients with solitary phase I IgG titers = 1:1024, consistent with chronic Q fever, but without other manifestations of chronic disease. The probable chronic Q fever group includes patients with either actual chronic Q fever not (yet) fulfilling the criteria for proven chronic Q fever, but also patients without chronic Q fever but serologic findings consistent with this condition and a known risk factor for chronic Q fever. In the current study, we used information about patients included in this database until September 2011. Patients were included in the analysis when they fulfilled the criteria of proven, probable or possible chronic Q fever and were aged =18 years. Patients in which IFA was not performed, or titration was not complete for at least one serological examination, were excluded. We also excluded patients who received blood transfusions before determining serology, because this could have caused significant dilution of the immunoglobulin count.

Microbiological analysis

The routine microbiological work-up for chronic Q fever patients consisted of serology and PCR. Serology, as well as PCR, were previously performed at local laboratories from the different hospitals. Serological analysis was performed using IFA (Focus Diagnostics, Inc., Cypress, CA, USA) on serum samples according to the manufacturer's instructions. Titration was carried out with dilutions according to a binary scale and a detection cut-off titer of 1:32. PCR for *C. burnetii* DNA on serum, plasma and, if available, tissue samples was performed using an in-house assay as previously described. An input volume of 500 µl serum or plasma was used for DNA isolation 22.

Data collection and storage

Information on patient characteristics, imaging results, clinical chemistry, haematology and microbiology results and antibiotic therapy were collected from the hospital records and were interpreted by two researchers (LK and AK) to avoid information bias. To examine the association of serologic profiles in different subgroups of chronic Q fever (proven, probable, possible), we evaluated the height of IgG phase I titers at three different time points, which could coincide: time of chronic Q fever diagnosis, time of the highest IgG phase I titer per individual (peak value) and time that PCR for *C. burnetii* in plasma or serum became first positive. Time of diagnosis was

defined as the moment when a patient had clinical evaluation and was classified as proven, probable or possible according to the outcome of this evaluation. All data were processed and stored anonymously in SPSS version 18 (SPSS inc., Chicago, Illinois, USA).

Statistical methods

We used the binary dilution scales (e.g. 1:1024 = 1:(210) is therefore 10 on a binary scale, 1:2048 corresponds to 11, etcetera), to perform all analyses. We analyzed the distribution of phase I IgG titers in proven chronic Q fever patients with negative or positive *C. burnetii* PCR in blood. We used logistic regression analysis to estimate the association between the height of phase I IgG titers and a positive PCR in blood. To determine the difference in distribution of phase I IgG titers, at time of diagnosis and at time of peak titer between patients with proven, probable and possible chronic Q fever we used chi-square (X2) tests or Mann-Whitney U test as appropriate. To determine whether IFA can differentiate between "true" chronic Q fever and patients with elevated phase I IgG only, we chose to compare patients with proven and possible chronic Q fever, as these two groups represent cases at both ends of the spectrum. We calculated phase I IgG titer test characteristics, sensitivity and specificity, and prediction scores, positive predictive value (PPV) and negative predictive value (NPV), for proven chronic Q fever versus possible chronic Q fever. For this analysis, we chose to evaluate peak titers only, as those where, in contrast to titers at time of diagnosis, independent of time of referral to the clinic. To assess the discriminative qualities of the model, a receiver operator characteristics (ROC) curve was constructed and the area-under-the-ROC curve (AUC) was estimated. In all analyses, the significance level was set at p=0.05.

Ethical consideration

Design of the database and the use of the collected information for analysis and scientific publications were approved by the Medical Research Ethics Committee of the University Medical Center Utrecht in Utrecht, the Netherlands.

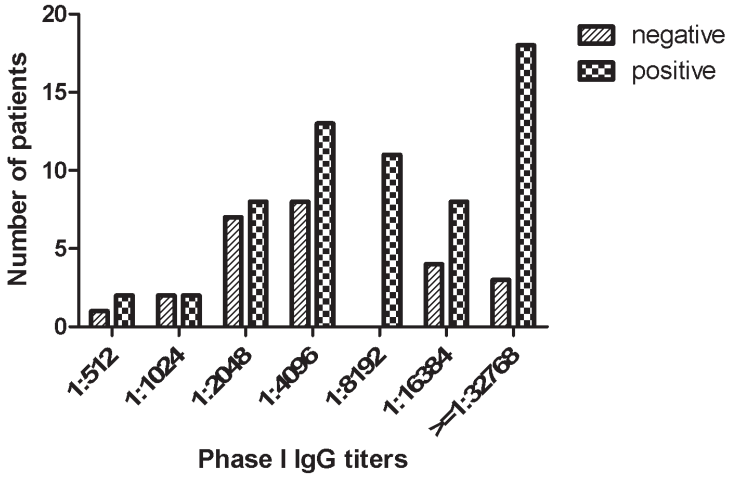
RESULTS

In total, 200 patients were included in our study. Ninety-three patients (46.5%) had proven chronic Q fever, 51 patients (25.5%) probable chronic Q fever, and 56 patients (28.0%) possible chronic Q fever. In all, 138 patients (69.0%) were male, 69 males had proven chronic Q fever (74.2% of proven chronic Q fever cases), 33 males had probable chronic Q fever (64.7%), and 36 males had possible chronic Q fever (64.3%). Mean age at diagnosis was 64.4 years with a standard deviation (SD) of ± 14.5 years (proven 69.0 ± 12.4 years, probable 63.1 ± 14.1 years, possible 57.3 ± 15.4 years).

Of the 93 patients with proven chronic Q fever, 52 patients (55.9%) had a positive *C. burnetii* PCR in blood only (plasma or serum), 10 (10.8%) in tissue only and 13 (14.0 %) in both blood and tissue. In 18 patients (19.4%) *C. burnetii* DNA could not be detected in either blood or tissue, but were defined as proven chronic Q fever based on evidence of endovascular or endocardial infection.

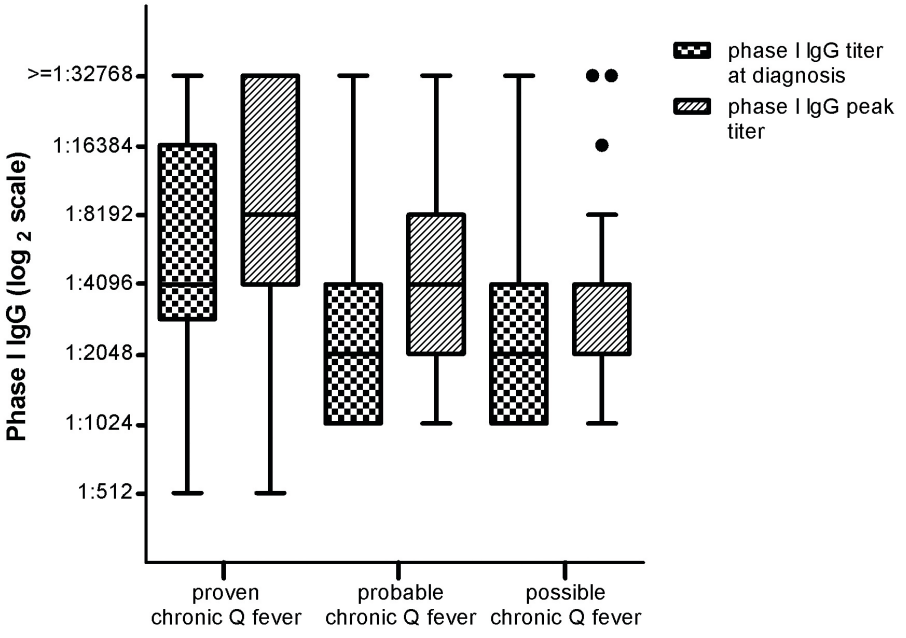
Figure 1 demonstrates the distribution of phase I IgG titers at time of positive or negative *C. burnetii* PCR in blood, irrespective of outcome of PCR on tissue, in 87 proven chronic Q fever patients (in four patients no PCR on blood was performed, in two patients phase I IgG was not available at time of PCR analysis). Logistic regression analysis demonstrated a significant rise in

Figure 1. Phase I IgG titers at time of *C. burnetii* PCR positive (n=62) and negative (n=25) findings in blood (plasma or serum) of proven chronic Q fever cases.



PCR positive patients include patients with PCR positive findings in blood with or without positive tissue PCR; PCR negative patients include patients with negative PCR findings in blood with or without positive tissue PCR. Logistic regression analysis demonstrated a significant rise in probability of positive PCR in case of increasing phase I IgG titers using a binary dilution scale (Odds Ratio (OR) 1.35, 95% confidence interval 1.02-1.77, p = 0.033).

Figure 2. Box plot demonstrating the distribution of titers of IgG antibodies against *C. burnetii* phase I antigens (phase I IgG) at time of diagnosis and at time of peak titers in proven, probable and possible Q fever cases.



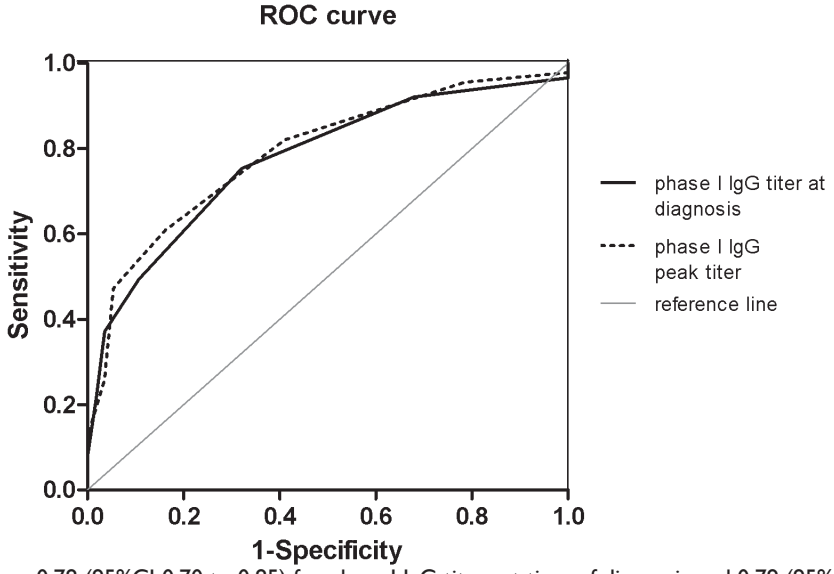
The black bars indicate the median IgG titers. Titers on the y-axis are represented on a binary scale. Median phase I IgG titers were significantly higher in proven chronic Q fever cases (p < 0.05).

probability of positive PCR in case of each increasing phase I IgG titre, using a binary dilution scale (Odds Ratio (OR) 1.35, 95% confidence interval 1.02-1.77, p = 0.033).

Peak IFA values were available for all 200 patients. Titers at time of diagnosis was available in 196 patients: in one proven chronic Q fever patient no IFA titration at time of diagnosis was performed, and in three patients blood and fluid transfusion was administered before blood sampling for microbiological analysis. In 93 cases (46.5%) the IFA result at time of diagnosis was at the same time as the peak IFA value.

A box plot presenting the distribution of phase I IgG titer, at time of diagnosis and time of peak titer amongst patients with proven, probable and possible chronic Q fever is shown in Figure 2. Median phase I IgG titer at time of diagnosis and peak titer in proven chronic Q fever were 1:4096 and 1:8192 respectively, which was for both significantly higher (p <0.01) compared to 1:2048

Figure 3. Receiver operator characteristics (ROC) curve of titers of IgG antibodies against *C. burnetii* phase I antigens at time of diagnosis and peak titers to differentiate between proven and possible chronic Q fever.



The AUC was 0.78 (95%CI 0.70 to 0.85) for phase I IgG titers at time of diagnosis and 0.79 (95%CI 0.72 to 0.86) for phase I IgG peak titers.

Table 1. Sensitivity and specificity of cut-off titers of IgG antibodies against *C. burnetii* phase I antigens for proven chronic Q fever compared to possible chronic Q fever at time of phase I IgG peak titers

IgG phase I titer	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
1:1024	97.8	Nd	62.2	Nd
1:2048	94.6	21.4	66.7	70.6
1:4096	80.6	58.9	76.5	64.7
1:8192	60.2	83.9	86.2	55.9
1:16384	47.3	94.6	93.6	52.0
≥1:32786	25.8	96.4	94.4	47.8

PPV, positive predictive value; NPV, negative predictive value; nd, not determinable.

and 1:4096 respectively for probable chronic Q fever and 1:2048 at both time points for possible chronic Q fever. Table 1 displays the test characteristics and prediction scores for the different cut-off levels of peak phase I IgG titers when proven chronic Q fever cases are compared with possible chronic Q fever cases. The positive predictive value (PPV) for proven chronic Q fever ranged from 62.2% till 94.3% at titers of 1:1024 to >1:16384. Test characteristics of titers at time of diagnosis and peak titers are displayed in a ROC curve in Figure 3. The AUC was 0.78 (95%CI 0.70-0.85) for phase I IgG titers at time of diagnosis and 0.79 (95%CI 0.72-0.86) for phase I IgG peak titers for differentiating proven chronic Q fever, from possible chronic Q fever.

DISCUSSION

We have shown that the height of phase I IgG titers is strongly associated with the chance of proven chronic Q fever in patients with a serological suspicion of chronic Q fever. This relation was most clear when peak phase I IgG titers were used. IFA titers of =1:8192 had a very high PPV (>85%) for proven chronic Q fever, thereby urging the need for an extensive search for an infectious focus and a low threshold for the initiation of antibiotic treatment, in patients presenting with such titers. On the other hand, the sensitivity using this cut-off titer is less than 60%, leading to unacceptable high percentages of missed cases. Cut-off titers of 1:1024, 1:2048 and 1:4096 of phase I IgG have high sensitivity for diagnosis of proven chronic Q fever (97.8%, 94.6% and 80.6%, respectively), but also a low PPV (62.2%, 66.7% and 76.5%, respectively) (Table 1). With our data, test characteristics of phase I IgG titers <1:1024 could not be defined, as we used a phase I IgG cut-off titer of =1:1024 for the diagnosis of suspected chronic Q fever. However, there were three PCR positive proven chronic Q fever patients with a phase I IgG titer of 1:512 at time of diagnosis. In one of these patients, this could tentatively be explained by the use of several immunosuppressive agents impairing the serological response to *C. burnetii*. In the other patients we found no explanation. This illustrates that a phase I IgG titer of <1:1024 does not exclude chronic Q fever, presumably especially in immunocompromised hosts.

We confirmed that *C. burnetii* PCR on blood samples does not exhibit enough sensitivity for the diagnosis of chronic Q fever, as illustrated by the fact that no circulating *C. burnetii* DNA was detectable in 69.9% of proven chronic Q fever cases, the most definite cases of chronic Q fever. In contrast to the report of Fenollar et al.14, we were not able to demonstrate that sensitivity of *C. burnetii* PCR on blood samples is negatively influenced by phase I IgG titers. Instead, we found a positive association between height of phase I IgG titer and the likelihood of a positive PCR in blood samples.

The discriminative performance of IgG titers for differentiation between proven and possible chronic Q fever (areas under the ROC-curve of 0.78 for titers at time of diagnosis and 0.79 for peak titers) suggest that serology alone is no more than a reasonable predictor to identify chronic Q fever patients. In our opinion, increasing the currently used cut-off titers of phase I IgG to >1:1024 or >1:800 (depending on which IFA test is used) is not preferable because of unacceptable high number of undetected cases and the high morbidity and mortality of untreated chronic Q fever. We recommend maintaining the cut-off of phase I IgG of 1:1024 (in case the IFA from Focus Diagnostics, Inc is used) as a screening tool for chronic Q fever. To prevent unnecessary long-term antibiotic treatment, it is therefore important to define accompanying clinical parameters to secure the diagnosis of chronic Q fever. An attempt to define these clinical parameters was formulated in the recent Dutch classification for the diagnosis of chronic Q fever.21

Our study has several weaknesses. First, our patients were classified in recently defined subgroups, namely proven, probable and possible chronic Q fever. Confirmation of this classification is still lacking. Especially patients classified as probable chronic Q fever might include both patients with actual chronic Q fever not (yet) fulfilling the criteria for proven chronic Q fever, as well as patients with a high phase I IgG titer who happen to have a known risk factor for chronic Q fever in the absence of the disease. Because of this heterogeneity, we chose to focus on the difference between the groups of proven and possible chronic Q fever patients, as these two groups represent both ends of the spectrum of probability of actual chronic Q fever. Second, in the beginning of the Dutch Q fever epidemic, diagnostic tests were not yet executed following a standard diagnostic work up. Therefore, particularly in patients classified as possible chronic Q fever, further radiological investigations are sometimes lacking. As a consequence, this might have underestimated the number of proven or probable chronic Q fever patients. On the other hand, most untreated possible chronic Q fever cases have shown a spontaneous decline of phase I IgG to <1:1024 indicating that indeed no chronic Q fever infection was present in these patients. Third, the Dutch classification for the diagnosis of chronic Q fever only includes patients with phase I IgG =1:1024 and/or positive *C. burnetii* PCR. Therefore, no analysis of titers beneath the cut-off of 1:1024 was performed. This would, however, be interesting, as we found three patients with PCR positive proven chronic Q fever who presented with a phase I IgG titer of 1:512.

In conclusion, we demonstrated that high phase I IgG titers are strongly associated with proven chronic Q fever, especially if titers exceeded 1:4096. We confirmed low sensitivity of PCR on blood for the diagnosis of chronic Q fever, but in contrast to previous reports, we found a positive association between heights of phase I IgG and positive blood PCR. Due to low sensitivity of high phase I IgG titers and high morbidity and mortality of untreated chronic Q fever, we would not advocate increasing of cut-off values of phase I IgG titers for the diagnosis of chronic Q fever. Above all, this study emphasizes that serology is not a diagnostic tool on its own, but should be interpreted in regard to the clinical background.

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Part 3

Chronic Q fever: Risk groups, morbidity and mortality

Chapter 8

Identification of risk factors for chronic Q fever, the Netherlands

Emerg Infect Dis. 2012; 18: 563–570

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ABSTRACT

Since 2007 the Netherlands have experienced a large Q fever outbreak. To identify and quantify risk factors for development of chronic Q fever after *Coxiella burnetii* infection, we performed a case control study. Co-morbidity, cardiovascular risk factors, medication and demographic characteristics from patients with proven, probable or possible chronic Q fever (cases), were compared with patients who had acute Q fever in 2009, but did not develop chronic Q fever (controls). 105 cases (44 proven, 28 probable and 33 possible chronic Q fever cases) and 201 controls were selected. Independent risk factors for development of proven chronic Q fever were valvular surgery (OR 43.6; 95%CI 4.70-405), vascular prosthesis (OR 26.8; 95%CI 4.88-147), aneurysm (OR 25.9; 95%CI 4.55-147), renal insufficiency (OR 16.0; 95%CI 2.06-123) and increasing age (OR 1.06; 95%CI 1.02-1.11 per year). An area under the ROC curve of 0.91 (95%CI 0.85-0.97) showed the goodness-of-fit of this risk factor model.

INTRODUCTION

Q fever, a zoonosis caused by the intracellular Gram-negative bacterium *C. burnetii*, is a worldwide prevalent disease causing outbreaks.[1;2] Q fever has various presentations, with acute and chronic manifestations. Acute Q fever is mostly a self-limiting mild flu-like disease sometimes complicated by severe pneumonia or hepatitis. Asymptomatic acute infection occurs in 50-60% of patients.[3-5] Of patients infected by *C. burnetii*, 1-5% progress to chronic Q fever, which can manifest months to years after primary infection.[2;4;6] Previous data, mainly from France, show that endocarditis is by far the most common manifestation (±75%), followed by vascular infections of aortic aneurysms and vascular prostheses (±10%).[5;7-9] In the Netherlands, however, an equal distribution of endocarditis and vascular infections is seen.[10]

Chronic Q fever has high morbidity and mortality if untreated, which makes early case finding and preventive measures for patients at high risk of great importance. It requires long-term antibiotic treatment, preferably a combination of doxycycline and hydroxychloroquine for duration of at least 18-24 months. Formerly identified risk factors that predispose to chronic Q fever are preexisting cardiac valvulopathy, vascular grafts and aneurysms, immunosuppression and pregnancy. However, most published studies are descriptive, lack statistical quantification or included specific high risk groups only.[6-9;11;12]

Since 2007, the Netherlands have been confronted with a large Q fever outbreak, with over 4000 acute Q fever cases reported.[13] Due to asymptomatic disease and overlap with other febrile diseases, the actual number of Q fever infections is probably much higher. Although the acute Q fever epidemic in the Netherlands has subsided, the number of patients with chronic Q fever is rising.[10;14] In this unique population, we aimed to identify and quantify risk factors for development of chronic Q fever after *C. burnetii* infection.

MATERIALS AND METHODS

Study design and setting

A case control study was performed to identify risk factors for the development of chronic Q fever. Cases and controls were recruited from the Jeroen Bosch Hospital (JBH) in 's-Hertogenbosch, and Bernhoven Hospital (BH) in Oss and Veghel: regional hospitals located in the centre of the Dutch Q fever epidemic area. The study design was approved by the Medical Research Ethics Committee of the University Medical Centre Utrecht.

Patients

Cases were defined as all patients known in JBH or BH, above the age of 18, which were diagnosed from January 1st 2007 to May 1st 2011 with chronic Q fever. For selection of cases we used existing datasets and spontaneous notifications from both hospitals. Classically, the diagnosis of chronic Q fever relied on serology and PCR. Chronic Q fever is considered proven if *C. burnetii* is detected by PCR in blood or tissue in the absence of acute infection. However, sensitivity of this technique is only ± 50%.[15;16] Persisting high levels of IgG antibodies to phase I antigens (phase I IgG), and to a lesser extent phase II antigens (phase II IgG), are indicative of chronic Q fever.[1] The optimal cutoff value for phase I IgG with immunofluorescence assay (IFA) to diagnose chronic Q fever is still matter of debate, and is dependent of the test used, but probably lies between titers of 1:800 and 1:1600.[7;17-19] Recently, a new diagnostic proposal was made by the Dutch Q fever consensus

group, which combines PCR, serology and clinical data and categorizes cases in proven, probable and possible chronic Q fever. The first group consists of patients with chronic Q fever, confirmed with positive *C. burnetii* PCR on blood or tissue, or a phase I IgG of = 1:1024 in combination with a proven vascular infection on PET, CT-scan or MRI, or endocardial involvement according to the major criteria of the modified Duke criteria on echocardiogram.[20] The second group concerns patients with phase I IgG of = 1:1024 with known risk factors, non-major valvulopathy according to the modified Duke criteria20, suspected non-vascular or non-cardial localisation of chronic Q fever infection or aspecific signs of chronic infection. The last group consists of patients with solely raised phase I IgG titers = 1:1024 without other suggestions for probable or proven chronic Q fever. In contrast to the other two subgroups, in general, these patients do not receive long-term antibiotic treatment but enter a follow-up program. Many of them already demonstrated spontaneous decline in phase I IgG titers. Here, we defined cases according to these definitions (Chapter 5: table 5).21

Controls were selected from an existing cohort of patients seen by general practitioners with acute Q fever in 2009, with a positive *C. burnetii* PCR on serum samples. Controls were included if they were aged 18 years or older at time of diagnosis of acute Q fever, and if the serologic profile was not suggestive of chronic Q fever during at least one year of serological follow-up (i.e. decreasing antibody titers and phase I IgG <1:1024). Patients with serological follow-up of less than one year after the episode of acute Q fever were also excluded.

All cases, except one, and all controls lived in the same postal code area (5000-5400) in the Netherlands.

Microbiological analyses

Microbiologic diagnostics for chronic Q fever patients (cases) consisted of IFA (Focus Diagnostics, Inc., Cypress, CA, USA) on serum samples and PCR for *C. burnetii* DNA on serum, plasma and tissue samples.

The diagnostic work-up to evaluate *C. burnetii* infection in control patients with documented acute Q fever had been performed according to a diagnostic algorithm for acute Q fever introduced in May 2009. Primarily, sera samples are screened with an enzyme-linked immunosorbent assay for IgM antibodies to *C. burnetii* phase II antigens (MII-screen; Institut Virion\Serion GmbH, Würzburg, Germany). Depending on date of onset of disease, and inpatient or outpatient setting, PCR for *C. burnetii* DNA is performed if MII-screen is negative).[22-24] In patients with confirmed acute Q fever, serological follow-up was performed at 3, 6 and 12 months consisting of IFA for IgM and IgG antibodies to *C. burnetii* phase I and phase II antigens.

Data collection and storage

We collected patient characteristics including demographic variables, medical history, medication, pathology, microbiology results, imaging records, therapy and outcome of both cases and controls. The collected information of the cases was already available in the hospital registration systems and was interpreted by two researchers. All controls were sent a questionnaire and an informed consent form in which we asked permission to request patient's data from their general practitioner and at the hospital registration system. Although debatable, routine echocardiographic screening following diagnosis of acute Q fever is not standard care in the Netherlands, as no benefit was observed in an earlier Dutch evaluation.[25;26] Therefore, both for chronic Q fever cases and acute Q fever controls, details about cardiac valvulopathy were retrieved by review of medical records. The obtained information was processed and stored anonymously with the use

of coded data. SPSS version 18.0 was used for storage and analysis of the collected data. (SPSS inc., Chicago, Illinois, USA)

Statistical analysis

Within this study we conducted three analyses: an overall analysis including "all" chronic Q fever cases (i.e. proven, probable and possible chronic Q fever cases), an analysis including "proven and probable" chronic Q fever cases and an analysis including "proven" chronic Q fever cases only. We performed these analyses to determine whether exclusion of possible chronic Q fever, and to a lesser extent probable chronic Q fever, the groups in which disease status is doubtful, influenced the overall results. Univariate and subsequent multivariate logistic regression analyses were performed to calculate odds ratios (OR), corresponding 95% confidence intervals (CI) and p-values of the included variables for the development of chronic Q fever. In univariate analysis, missing values were excluded. Variables with zero observations among cases and <2 observations in the control group (or vice versa) were excluded (hematological malignancies, bone marrow transplantation, dialysis, renal transplant, non-renal organ transplant, congenital cardiac deviation, pulmonary diseases and auto immune disorder). For potential dichotomous risk factors with zero observations in one cell amongst either the cases or controls, but with >2 observations in the other cell, we applied a Fisher's exact test to calculate p-values. Variables with one or more observations and =25% missing values in cases and controls, a p-value of <0.10 in univariate analysis or known association in previous reports with the development of chronic Q fever were subsequently analyzed in a multivariable model. The variables vascular history and valvular pathology were not included in multivariate analysis, as they covered more specified variables, which were separately included in multivariable analysis, namely vascular prosthesis, aneurysm, other vascular surgery, peripheral arterial disease, cerebrovascular disease, and valvular surgery, valvular disease (non surgery). Eighteen cases and zero controls had a history of valvular surgery. Because of the expected importance of this risk factor and the high incidence within the cases, we considered inclusion in multivariate analysis viable. Moreover, excluding this variable caused that the logistic regression model could not be fitted. Therefore, we randomly changed one of the observations of the control group from zero into one, which artificially reduced the association, yet allowed us to fit the regression model. The variables age, vascular history, vascular prosthesis, aneurysm, other vascular surgery, cerebrovascular disease, peripheral vascular disease, valvular pathology, valvular surgery, valvular deviation, ischemic heart disease, other cardiovascular diseases, hypertension, dyslipidemia, diabetes, malignancy (non-hematological) and renal insufficiency could be included in multivariate analysis of all groups. The variable immune disorder was also included in multivariate analysis for the "probable and proven" and "proven" sub groups. The variable pacemaker was also included in multivariate analysis for the "proven" group. Statin, clopidogrel, acenocoumarol and proton pump inhibitor usage, and hospitalization and adequate treatment during acute Q fever showed differences between cases and controls with a p-value of <0.10. However, these variables could not be included in multivariate analyses because of >25% missing values, almost exclusively in cases, due to an unrecognized acute Q fever episode. After selecting predictors for our final multivariable model, we evaluated their possible interactions between by including two-way interactions in consecutive models. Interactions were not significant and therefore not included in the model. To assess the goodness-of-fit of the final model, an ROC curve was constructed and the area under the ROC curve (AUC) was estimated. Significance lever was set at p-value =0.05.

Table 1. Results of the univariate analysis of risk factors for chronic Q fever cases compared to acute Q fever cases[a]

Risk factor	Acute Q fever n=201 (%)	All chronic Q fever n=105 (%)	OR (95% CI)	*p*
Male	129 (64.2)	70 (66.7)	1.12 (0.68-1.84)	0.665
Age, mean (SD)	52.5 (± 13.7)	63.9 (± 13.5)	1.06 (1.04-1.09)[b]	0.000
Smoking	85 (42.5)	43 (44.3)	1.08 (0.66-1.76)	0.765
Medical history				
Vascular history	9 (4.5)	33 (31.4)	9.78 (4.46-21.4)	0.000
Vascular prosthesis	2 (1.0)	15 (14.3)	16.5 (3.71-74.0)	0.000
Aneurysm	2 (1.0)	12 (11.4)	12.8 (2.82-58.5)	0.001
Other vascular surgery	3 (1.5)	7 (6.7)	4.71 (1.19-18.6)	0.027
Peripheral arterial disease	6 (3.0)	11 (10.5)	3.80 (1.37-10.6)	0.011
Cerebrovascular disease[c]	8 (4.0)	11 (10.5)	2.82 (1.10-7.25)	0.031
Valvular pathology	10 (5.0)	25 (23.8)	5.97 (2.47-13.0)	0.000
Valvular disease (ns)[d]	10 (5.0)	17 (16.2)	3.69 (1.62-8.39)	0.002
Valvular surgery	1 (0.5)[e]	18 (17.1)	41.4 (5.44-315)	0.000
Congenital cardiac disease	1 (0.5)	1 (1.0)	1.92 (0.12-31.1)	0.645
Ischemic cardiac disease[f]	17 (8.5)	28 (26.7)	3.94 (2.04-7.61)	0.000
Pacemaker	2 (1.0)	3 (2.9)	2.93 (0.48-17.8)	0.244
Other cardiac history[g]	12 (6.0)	26 (24.8)	5.18 (2.49-10.8)	0.000
Hypertension	56 (27.9)	44 (41.9)	1.87 (1.14-3.07)	0.013
Dyslipidemia	39 (19.4)	32 (30.5)	1.82 (1.06-3.13)	0.031
Diabetes mellitus (type 1& 2)	13 (6.5)	15 (14.3)	2.41 (1.10-5.28)	0.028
Non-haematologic malignancy	6 (3.0)	16 (15.2)	5.84 (2.21-15.4)	0.000
Immune disorder[h]	2 (1.0)	4 (3.8)	3.94 (0.71-21.9)	0.117
COPD	14 (7.0)	13 (12.4)	1.89 (0.85-4.18)	0.117
Other pulmonary disease[i]	6 (3.0)	3 (2.9)	0.96 (0.23-3.90)	0.950
Liver disease	1 (0.5)	3 (2.9)	5.88 (0.60-57.3)	0.127
Renal insufficiency	2 (1.0)	12 (11.4)	12.8 (2.82-58.5)	0.001
Auto immune disease[j]	2 (1.0)	1 (1.0)	0.96 (0.09-10.7)	0.971
Pregnancy[k]	0 (0.0)	3 (2.9)	--	<0.005
Medication at time of acute Q fever				
Proton pump inhibitors[l]	15 (7.5)	7 (11.7)	1.63 (0.63-4.20)	0.313
Statin[l]	29 (14.5)	19 (31.7)	2.73 (1.40-5.35)	0.003
Carbasalate calcium[l]	6 (3.0)	2 (3.3)	1.12 (0.22-5.67)	0.896
Acenocoumarol[l]	6 (3.0)	7 (11.7)	4.27 (1.38-13.3)	0.012
Clopidogrel[l]	2 (1.0)	3 (5.0)	5.21 (0.85-31.9)	0.074
Acute Q fever				
Adequate treatment[l,m]	157 (89.7)	37 (84.1)	0.61 (0.24-1.56)	0.298
Hospitalization[l]	36 (18.0)	26 (35.1)	2.47 (1.36-4.49)	0.003

ns, non surgery; COPD, chronic obstructive pulmonary disease; OR, Odds Ratio; CI, Confidence Interval. [a]n indicates no. patients with information available for that category, [b]OR per year of increasing age, ccerebrovascular disease and transient ischemic attack, [d]cases: 10 aortic valve defects (no bicuspid valves), 9 mitral valve defects (no prolaps), 4 tricuspid valve defects, controls: 6 aortic valve defects (no bicuspid valves), 3 mitral valve defects (1 prolaps), [e]n=0 in reality, [f]angina pectoris and myocardial infarction, [g]atrial fibrillation, congestive

Proven and probable chronic Q fever			Proven chronic Q fever		
n=72 (%)	OR (95% CI)	*p*	n=44 (%)	OR (95% CI)	*p*
50 (69.4)	1.27 (0.71-2.26)	0.420	32 (72.7)	1.49 (0.72-3.07)	0.281
67.3 (± 11.8)	1.09 (1.07-1.12)[b]	0.000	68.4 (± 10.8)	1.11 (1.07-1.15)b	0.000
33 (49.3)	1.31 (0.75-2.29)	0.336	22 (55.0)	1.65 (0.84-3.27)	0.149
29 (40.3)	14.4 (6.35-32.6)	0.000	23 (52.3)	23.4 (9.57-57.1)	0.000
15 (20.8)	26.2 (5.82-118)	0.000	14 (31.8)	46.4 (10.0-215)	0.000
12 (16.7)	19.9 (4.33-91.4)	0.000	9 (20.5)	25.6 (5.30-123)	0.000
5 (6.9)	4.93 (1.15-21.2)	0.032	4 (9.1)	6.60 (1.42-30.6)	0.016
8 (11.1)	4.06 (1.36-12.2)	0.012	6 (13.6)	5.13 (1.57-16.8)	0.007
9 (12.5)	3.45 (1.28-9.31)	0.015	5 (11.4)	3.09 (0.96-9.96)	0.058
23 (31.9)	8.97 (4.00-20.1)	0.000	13 (29.5)	8.01 (3.23-19.8)	0.000
14 (19.4)	4.61 (1.95-10.9)	0.001	9 (20.5)	4.91 (1.86-13.0)	0.001
18 (25.0)	66.7 (8.70-511)	0.000	10 (22.7)	58.8 (7.29-474)	0.000
--	--	--	--	--	--
23 (31.9)	5.08 (2.52-10.2)	0.000	17 (38.6)	6.82 (3.11-14.9)	0.000
3 (4.2)	4.33 (0.71-26.4)	0.113	3 (6.8)	7.28 (1.18-45.0)	0.033
23 (31.9)	7.39 (3.44-15.9)	0.000	15 (34.1)	8.15 (3.47-19.1)	0.000
35 (48.6)	2.45 (1.41-4.27)	0.002	24 (54.5)	3.11 (1.59-6.06)	0.001
23 (31.9)	1.95 (1.06-3.58)	0.031	16 (36.4)	2.37 (1.17-4.81)	0.017
10 (13.9)	2.33 (0.97-5.58)	0.057	7 (15.9)	2.74 (1.02-7.32)	0.045
10 (13.9)	5.24 (1.83-15.0)	0.002	6 (13.6)	5.13 (1.57-16.8)	0.007
4 (5.6)	5.85 (1.05-32.7)	0.044	3 (6.8)	7.28 (1.18-45.0)	0.033
9 (12.5)	1.91 (0.79-4.62)	0.152	6 (13.6)	2.11 (0.76-5.84)	0.151
2 (2.8)	0.93 (0.18-4.71)	0.929	--	--	--
2 (2.8)	5.71 (0.51-64.0)	0.157	1 (2.3)	4.65 (0.29-75.8)	0.280
12 (16.7)	19.9 (4.33-91.4)	0.000	9 (20.5)	25.6 (5.30-123)	0.000
1 (1.4)	1.40 (0.13-15.7)	0.784	--	--	--
2 (2.8)	--	<0.005	1 (2.3)	--	<0.005
5 (14.7)	2.13 (0.72-6.29)	0.173	5 (23.8)	3.85 (1.24-12.0)	0.020
15 (44.1)	4.66 (2.13-10.2)	0.000	13 (61.9)	9.58 (3.65-25.1)	0.000
2 (5.9)	2.02 (0.39-10.5)	0.401	2 (9.5)	3.40 (0.64-18.0)	0.150
5 (14.7)	5.58 (1.60-19.4)	0.007	2 (9.5)	3.40 (0.64-18.0)	0.150
2 (5.9)	6.19 (0.84-45.5)	0.073	2 (9.5)	10.4 (1.39-78.2)	0.023
22 (78.6)	0.42 (0.15-1.17)	0.098	12 (70.6)	0.28 (0.09-0.87)	0.028
16 (38.1)	2.80 (1.37-5.76)	0.005	9 (34.6)	2.41 (0.99-5.84)	0.051

heart failure, pericarditis, bradycardia, ischemic cardiomyopathy, and left ventricular hypertrophy, [h]prednisone cumulative dose >750mg, TNFα-blocker usage, methotrexate usage, mycofenolate mofetil usage, splenectomy,[i]asthma, recurrent pneumonia, [j]rheumatoid arthritis, kp-value based on calculated $\chi 2$ from manual continuity correction, [l]>25% missings in case groups, [m]defined as 10-14 days doxycycline.

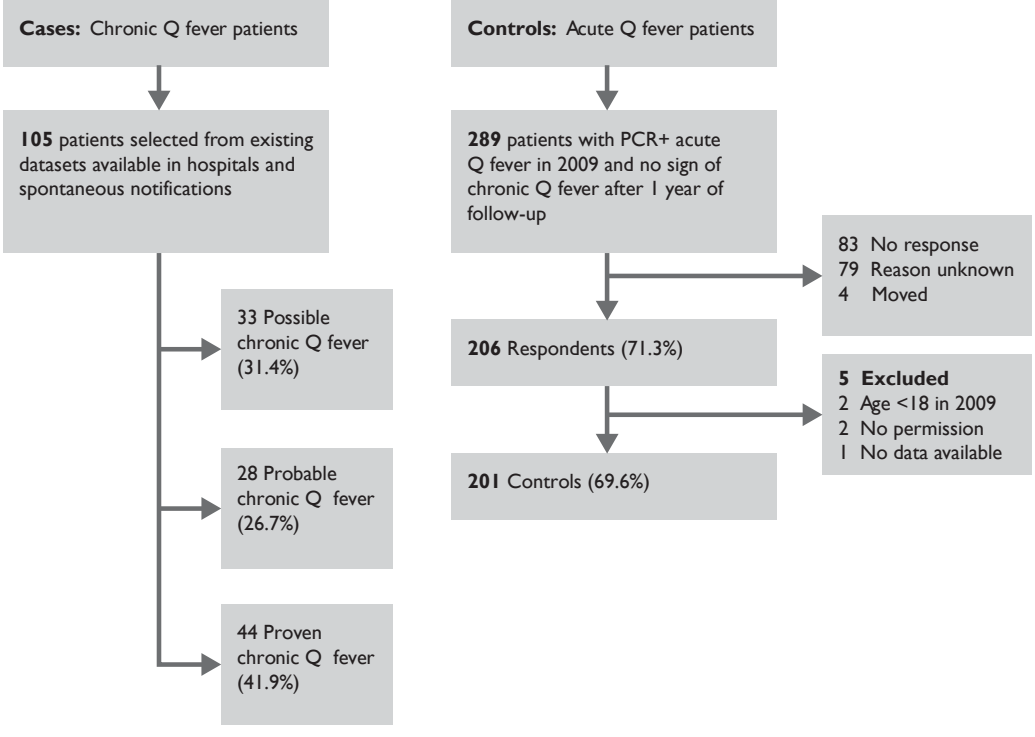
RESULTS

We identified 105 patients with proven, probable or possible chronic Q fever of which 44 (42%) had proven, 28 (27%) probable, and 33 (31%) possible disease. Of the patients with proven chronic Q fever, 27 (61%) had positive *C. burnetii* PCR in blood only, 5 (11%) had positive PCR in tissue only, 8 (18%) had positive PCR in tissue and blood, and 4 (9%) had no positive PCR in blood or tissue. Focus of infection in case of proven chronic Q fever was endocarditis in 12 patients (27%), 26 patients had an endovascular infection (59%), while 6 patients (14%) had no clear infection focus. Of the patients with probable chronic Q fever, suspected foci were cardiac valves in 12 patients (43%), endovascular lesions in 1 patient (4%) or another focus (e.g. pregnancy, or clinical symptoms of infection like weight loss, night sweats, and fever) in 15 patients (54%). Long-term antibiotic treatment was started in 40/44 patients (91%) with proven chronic Q fever, in 18/28patients (64%) with probable chronic Q fever, and in 5/32 patients (15%) with possible chronic Q fever. Three proven chronic Q fever patients died before diagnosis of chronic Q fever, one patient with proven chronic Q fever refused therapy.

In all, 289 controls patients with PCR proven acute Q fever in 2009 were sent a questionnaire; 201 of them (69.6%) responded, signed the informed consent form and fulfilled the inclusion criteria. (Figure 1)

Results of the univariate analysis are listed in table 1. The variables age, vascular history, vascular prosthesis, aneurysm, other vascular surgery, cerebrovascular disease, peripheral vascular disease, valvular pathology, valvular surgery, valvular deviation, ischemic heart disease, other car-

Figure 1. Flow diagram: enrollment, selection and inclusion of controls and cases.
Abbreviations: PCR, polymerase chain reaction.



diovascular diseases, hypertension, dyslipidemia, diabetes, malignancy (non-hematological) renal insufficiency and pregnancy showed significant differences between cases and controls.

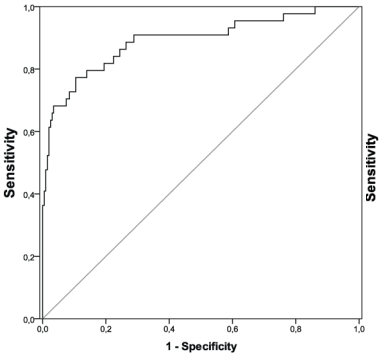
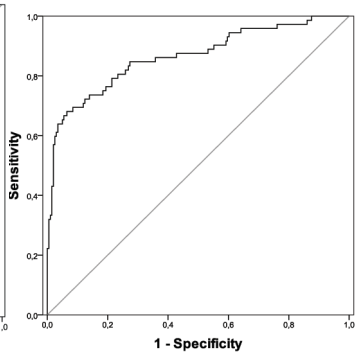
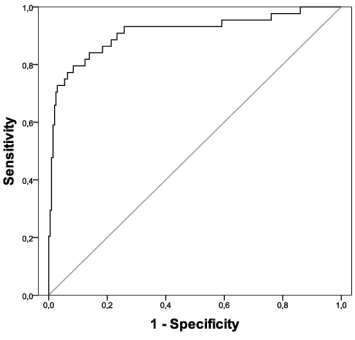
Results of the multivariate analyses are presented in table 2. Valvular surgery (OR 31.5; 95% (CI) 3.99-249), vascular prosthesis (OR 10.4; 95%CI 2.17-50.0), aneurysm (OR 8.65; 95%CI 1.74-42.9), malignancy (non-hematological) defined as several kinds of solid tumors (OR 3.90; 95%CI 1.33-11.5), and age (OR 1.03; 95%CI 1.01-1.06) were independently associated with the development of chronic Q fever in all cases. The final discriminative performance was good, with an AUC under the ROC curve (c-statistic) of 0.77 (95%CI 0.71-0.83). (Figure 2) Risk factors identified in the analysis of the "proven" cases, representing the most definite chronic Q fever cases, were valvular surgery (OR 43.6; 95%CI 4.70-405), vascular prosthesis (OR 26.8; 95%CI 4.88-147), aneurysm (OR 25.9; 95%CI 4.55-147), renal insufficiency (OR 16.0; 95%CI 2.06-123), and age (OR 1.06; 95%CI 1.02-1.11). The discriminative performance of this model for the development of proven chronic Q fever was c-statistic 0.91 (95%CI 0.85-0.97) (Figure 2).

Table 2. Results multivariate analyses: risk factors for development of chronic Q fever

Risk factors[a]	All chronic Q fever		Proven and probable chronic Q fever		Proven chronic Q fever	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Valvular surgery[b]	31.5 (3.99-249)	0.001	47.7 (5.87-387)	0.000	43.6 (4.70-405)	0.001
Vascular prosthesis[c]	10.4 (2.17-50.0)	0.003	14.9 (2.96-75.2)	0.001	26.8 (4.88-147)	0.000
Aneurysm[d]	8.65 (1.74-42.9)	0.008	13.5 (2.60-70.4)	0.002	25.9 (4.55-147)	0.000
Renal insufficiency[e]	--	--	9.08 (1.44-57.2)	0.019	16.0 (2.06-123)	0.008
Non-haematologic malignancy	3.90 (1.33-11.5)	0.013	--	--	--	--
Age (continuous)	1.03 (1.01-1.06)[f]	0.005	1.06 (1.03-1.09)[f]	0.000	1.06 (1.02-1.11)[f]	0.005

[a]Possible risk factors entered in all analyses are: age, vascular prosthesis, aortic aneurysm, other vascular surgeries, peripheral arterial disease, cerebrovascular disease, valvular surgery, valvular disease (non-surgical), ischemic cardiac disease, other cardiac history, hypertension, dyslipidemia, diabetes, non haematologic malignancy, renal insufficiency. Immune disorder was also entered in the analyses of "proven and probable chronic Q fever" and "proven chronic Q fever". Pacemaker was also entered in the analysis of "proven chronic Q fever". [b]Valvular surgery in the "proven" group are subdivided into biological valve (n=6), prosthetic valve (n=3), and valve repair (n=1) all located in the aortic valve (n=10). Within the controls there were no patients with history of valvular surgery. [c]Locations of vascular prostheses in "proven" group were infrarenal and iliac (n=6), infrarenal (n=4), thoracic (n=2), and unknown (n=2). Types of vascular prosthesis were Y-prosthesis (n=7), endovascular aneurysm repair (EVAR) (n=2), stent graft (n=2), Bentall (n=1) and unknown (n=2). For the two control patients specifications of the prostheses were unknown. [d]Locations of aneurysms in "proven" group were infrarenal (n=6), infrarenal and iliac (n=2), and suprarenal, infrarenal and iliac (n=1). Within the control group aneurysms were located both infrarenal and iliac (n=2). [e]Observed stages of chronic kidney disease according to the KDOQI-guidelines[27] in the "proven chronic Q fever", were stage 3 (n=6), stage 4 (n=2), and stage 5 (n=1) and in the controls solely stage 3 (n=2). [f]OR per year of increasing age.

Figure 2. ROC curves illustrating goodness-of-fit of the risk factor models for chronic Q fever development. Abbreviations: AUC, area under the curve; CI, confidence interval.

All chronic Q fever cases (n=105)	Proven and probable chronic Q fever cases (n=72)	Proven chronic Q fever cases (n=44)
		
AUC 0.77; (95%CI 0.71-0.83) *p* <0.001	AUC 0.86; (95%CI 0.81-0.92) *p* <0.001	AUC 0.91; (95%CI 0.85-0.97) *p* <0.001
Risk factors included in the model and number of observations within cases	Risk factors included in the model and number of observations within cases	Risk factors included in the model and number of observations within cases
-Valvular surgery (n=18)	-Valvular surgery (n=18)	-Valvular surgery (n=10)
-Vascular prosthesis (n=15)	-Vascular prosthesis (n=15)	-Vascular prosthesis (n=14)
-Aneurysm (n=12)	-Aneurysm (n=12)	-Aneurysm (n=9)
-Malignancy (non-hematological) (n=16)	-Renal insufficiency (n=12)	-Renal insufficiency (n=9)
-Age (continuous) (Mean=63.9)	-Age (continuous) (Mean=67.3)	-Age (continuous) (Mean=68.4)

AUC, area under the curve; CI, confidence interval.

DISCUSSION

To our knowledge, this is the first study that analyzed a large number of potential risk factors for chronic Q fever in a large number of patients. Most former studies have been limited by a low number of cases and evaluation of few risk factors. Moreover, quantification of these risk factors was lacking.[6-9;11;12]

In our study we have focused mainly on cases with proven chronic Q fever, as this group included patients with the most definite form of chronic Q fever. Cases with proven chronic Q fever also showed the strongest correlation with the identified risk factors. In multivariable analysis, valvular surgery, vascular prosthesis, aneurysms, renal insufficiency, and age were the most important risk factors for the development of chronic Q fever in proven cases. In the analysis of all chronic Q fever cases, malignancy (non-hematological) appeared also to be a risk factor. However, this could not be reproduced in the sub analyses of the more definite cases (e.g. proven and probable cases). Hence, malignancy (non-hematological) as a risk factor remains uncertain. Valvular

surgery, vascular prostheses and aneurysms, were the strongest predictors in this study, which confirms observational findings from earlier studies. Explanation lies in the association with the preferred localization of chronic Q fever infection. A novel finding is the association between mild renal insufficiency and chronic Q fever. The majority of patients with chronic Q fever and renal disease in our study had stage three renal insufficiency according to the KDOQI guidelines.27 Although terminal renal insufficiency can decrease the immune response, this association was not found for mild renal disease.[28] Renal insufficiency is associated with vascular disease, which may explain the elevated incidence of chronic Q fever in these patients.29 Increasing age also predisposes significantly for the development of chronic Q fever. This was also illustrated in a recent report of van der Hoek et al.[23] Explanation probably lies in the increased prevalence of cardiovascular diseases and the decrease of cellular immunity during aging.[30;31] Age >60 appeared the best cut-off above which the risk for chronic Q fever is increased significantly.

Pre-existing cardiac valvulopathy has been stated to give an estimated risk of 39% for the development of chronic Q fever after infection with *C. burnetii*.[6;32;33] In contrast, recent reports showed no elevated risk in case of (mild) valvulopathy in the Dutch outbreak.[25;26] Although in our study, univariate analyses showed that (non-surgical) cardiac valvulopathy increased the risk for the development of chronic Q fever, this was not confirmed in the multivariable analysis. This can be explained by the fact that 9/17 cases (53%) with non-surgical valvulopathy also had a history of valvular surgery of one of the other valves. Location and type of valvular defects did not differ significantly between cases and controls. (footnote Table 1) A possible explanation for the discrepancy with previous observations lies in the fact that our study was conducted four years after start of the Q fever epidemic, while chronic Q fever endocarditis in case of (non-surgical) cardiac valvulopathy might become manifest later.[6;8] Furthermore, strain specific differences in clinical presentation might also be of importance.[4;26] It must be taken into account that presence of valvulopathy in both cases and controls could have been missed, as this was assessed only through review of medical records. However, echocardiography, which was standard care in all suspected cases of chronic Q fever, revealed no additional congenital or bicuspid valve defects in cases, in comparison to assessment of valvulopathy through review of medical records. From other than the above mentioned defects, it could not be determined if these were pre-existent or caused by chronic Q fever at these echocardiograms.

Immunosuppression, although not well defined, was indicated as a risk factor in former reports. Yet, clear definition and statistical empowerment is lacking.[8] Although univariate analyses did show an elevated risk for immunosuppression, especially for proven chronic Q fever cases, this was not confirmed in our multivariable analysis. Immunocompromised patients may be underrepresented in our study as it was conducted in a peripheral hospital setting. Further evaluation of this risk factor should be performed in future studies. Pregnancy, another formerly reported risk factor, showed an association with the development of chronic Q fever in univariate analysis. Due to zero pregnant women in the control group and only three pregnant women in all cases, evaluation of pregnancy in multivariate analyses was not possible. Currently, a study specifically designed to evaluate associations between pregnancy and Q fever is ongoing in the Netherlands, which could elucidate the role of this probable risk factor pregnancy in the development of chronic Q fever.

In our opinion we gathered representative study data from this large Q fever outbreak, due to well-documented data and the willingness of patients to participate. The fact that cases and controls were living in the same area increases the comparability of these groups and strengthens the results. Our study has potential weaknesses though. First, all controls had an acute episode

in 2009 and information about their presenting symptoms was obtained in 2011, introducing a possible recall bias. We tried to reduce recall bias by requesting additional information from the general practitioner and by reviewing clinical test results and doctor's reports in the hospital registration systems. Second, information bias could be caused by the subjective interpretation of doctor's reports of both cases and controls. We tried to reduce this bias by interpreting doctor's reports by two research members independently (LK and SD). Third, serological follow-up of the controls after the acute Q fever episode was only one year, which is the normal follow-up period in The Netherlands. Since chronic Q fever can become manifest years after initial infection, development of chronic Q fever after this follow-up period is still possible.[6;34] However, 75% of chronic Q fever develops within six months after primary infection.[34] Moreover, according to the observed decrease in antibody titers of these patients, progression into chronic Q fever is not very likely. Fourth, as a consequence of the inclusion of patients with symptomatic acute Q fever as control group, the results can only be generalized to patients with symptomatic acute Q fever. Although, it probably provides an adequate indication of risks factors for patients with mild or asymptomatic primary Q fever. Fifth, notably, almost all controls received antibiotic treatment at time of acute Q fever, in contrast to the cases in which only a minority had symptomatic acute Q fever. This might influence the chance of developing chronic Q fever, although there is no quantitative evidence that treatment for acute Q fever reduces the chance for chronic Q fever.[4] Sixth, chronic Q fever cases were selected and classified according to the definitions of the Dutch Q fever consensus group, which still need confirmation. The definition of probable chronic Q fever contains several criteria, which we also included as potential risk factors in our study (e.g. valvular disease, vascular prosthesis, aneurysm, immunosuppressive state). Nevertheless, proven chronic Q fever, for which these criteria are not part of the definition, was also predicted with the identified risk factors in multivariate analysis, thereby confirming the independent risk association of these variables. Seventh, some chronic Q fever cases were identified during screening programs of patients with valvular surgery, aneurysms or vascular prostheses. Patients with these risk factors can therefore be overrepresented within the cases, although all proven cases had symptomatic disease. Eight, the results of this study have to be considered in view of a predominant *C. burnetii* strain, which is responsible for the majority of the Dutch human Q fever infections.[35] Worldwide, geographical differences are observed in Q fever manifestations, which might be due to differences in *C. burnetii* strains.[4]

In conclusion, previous valvular surgery, vascular prosthesis, aneurysms, renal insufficiency, and age were identified as major risk factors for the development of chronic Q fever in this study. As untreated chronic Q fever comes with serious morbidity and mortality, awareness is required in people with acute Q fever possessing the identified risk factors. This may indicate close follow-up or even prophylactic treatment in high-risk groups. Moreover, in case of large Q fever outbreaks, screening is advisable for patients with these identified risk factors.

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Chapter 9

Prevalence of chronic Q fever in patients with a history of cardiac valve surgery in a *Coxiella burnetii* epidemic area

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ABSTRACT

Chronic Q fever develops in 1-5% of patients infected with *Coxiella burnetii*. The risk for chronic Q fever endocarditis has been estimated at ~39% in case of preexisting valvulopathy and is potentially even higher for valvular prostheses. Since 2007, The Netherlands has faced the largest Q fever outbreak ever reported, allowing for more precise risk estimation of chronic Q fever in high-risk groups. Patients with a history of cardiac valve surgery were selected for microbiological screening through a cardiology outpatient clinic in the Q fever epidemic area. Blood samples were analyzed for phase I and II IgG against *C. burnetii* and, if titers were above a defined cut-off level, *C. burnetii* PCR was performed. Chronic Q fever was considered proven if *C. burnetii* PCR was positive, and probable if phase I IgG titer was =1:1024. Among 568 patients, seroprevalence of *C. burnetii* antibodies (IgG titer greater than or equal to 1:32) was 20.4% (n=116). Proven or probable chronic Q fever was identified among 7.8% of seropositive patients (n=9). Valve characteristics did not influence the risk for chronic Q fever. Patients with chronic Q fever were significantly older than patients with past Q fever infection. In conclusion, screening of high-risk groups is a proper instrument for early case-finding of chronic Q fever. Estimated prevalence of chronic Q fever is 7.8% among seropositive patients with a history of cardiac valve surgery, which is substantially higher than in non-selected populations, but lower than previously reported. Older age seems to increase vulnerability to chronic Q fever in this population.

INTRODUCTION

Q fever is a zoonosis, caused by *Coxiella burnetii*, an intracellular Gram-negative coccobacillus. Q fever has acute and chronic manifestations. Acute Q fever mostly presents as a self-limiting flu-like illness, sometimes complicated by pneumonia or hepatitis. However, most patients, 50-60%, remain asymptomatic, which makes *C. burnetii* infections often undetected.1;2 After acute Q fever, 10-20% of patients have persisting fatigue complaints, also known as Q fever Fatigue Syndrome (QFS).[3] Chronic Q fever develops in 1-5% of patients with *C. burnetii* infection, and can become manifest even years after primary infection. The most common manifestations are endocarditis, mycotic vascular aneurysm and vascular prosthesis infection.[2;4-6] Chronic Q fever mostly affects patients with pre-existent valvular disease, vascular prosthesis and aortic aneurysm, immunocompromised patients and pregnant women.[1;4;7;8] Diagnosis of chronic Q fever relies on serology, polymerase chain reaction (PCR) and culture. Chronic Q fever is considered proven if *C. burnetii* is detected by PCR or culture in blood or tissue in combination with a corresponding serologic profile in the absence of acute infection. However, PCR and culture on blood specimens both have low sensitivity for the diagnosis of chronic Q fever.[9;10] Serological diagnosis is based on the antigenic variation of *C. burnetii*.[11] During acute infection, IgM and IgG antibodies against phase II antigens (phase II IgM and IgG) are detected first, followed by IgM and IgG antibodies against phase I antigens (phase I IgM and IgG). Persisting high titers of phase I IgG, and to a lesser extent phase II IgG, are indicative of chronic Q fever infection.[11-13]

The reported estimated risk of progression from acute Q fever infection to endocarditis in patients with any cardiac valvulopathy is ~39%, and is thought to be even higher for patients with cardiac valve prosthesis.[4;14] In contrast, a recent Dutch report showed a very low risk of progression to chronic Q fever endocarditis in case of clinically insignificant valvular disease.[15] Chronic Q fever endocarditis has high morbidity and mortality, up to 60%, if left untreated. Long-term antibiotic treatment can reduce mortality to less than 5%.[14] An early diagnosis and subsequent initiation of adequate treatment is therefore mandatory. The recommended treatment for chronic Q fever endocarditis is a combination of doxycycline and hydroxychloroquine for at least 18 months for native valves and 24 months for prosthetic valves.[14;16]

From 2007 on, there has been an expanding outbreak of Q fever in the south of the Netherlands with over 4000 notified cases of acute Q fever.[17] As the majority of patients have mild or asymptomatic acute infection, the actual incidence is probably much higher. In 2010 the epidemic has dampened, although a rising amount of chronic Q fever patients is seen.[17;18] This large outbreak allows for a more precise risk estimation of chronic Q fever and evaluation of a screening program in patients with cardiac valve disease. We therefore studied the prevalence of chronic Q fever in this epidemic area in patients with a history of cardiac valve surgery.

MATERIALS AND METHODS

Patient's enrollment

Patients with a history of cardiac valve surgery were selected from the cardiology outpatient clinic of the Jeroen Bosch Hospital in 's-Hertogenbosch, The Netherlands, which is located in the centre of the Q fever epidemic area. Our screening was approved by a local medical ethics review committee. We included all patients, age =18 years, listed under the registration code "follow-up after

cardiac valve surgery" on 1 November 2010. We excluded one patient who had received valve prosthesis because of valvular damage due to chronic Q fever endocarditis.

All patients identified as alive at the start of our screening were sent an information letter and an invitation for microbiological screening from November 2010 until January 2011. Patients with probable or proven chronic Q fever (see Definitions paragraph below) were further evaluated at the internal medicine outpatient clinic. The extent of this evaluation was individually assessed but consisted at least of anamnesis, physical examination and echocardiography. All patients with antibodies against *C. burnetii*, as a result of either chronic Q fever or past infection (see Definitions paragraph below), were approached for follow-up microbiological analysis after three months, to examine the development of antibody titers. After this time-period, follow-up was individually determined depending on outcome of microbiological analysis.

Microbiological screening

Screening was performed on serum and EDTA plasma samples obtained by venapuncture. First, sera were screened for phase I and II IgG with immunofluorescence assay (IFA; Focus Diagnostics, Inc., Cypress, CA, USA) according to the manufacturer's instructions using a detection cut-off titer of =1:32. If one or both antibodies were present at or above this cut-off, exact titers of phase I and II IgM and IgG were determined. Real-time PCR for *C. burnetii* DNA was performed on EDTA plasma if phase I IgG titer =1:512.[9;19] This cut-off, which is below the chronic Q fever definition cut-off titer of 1:1024 (see Definitions paragraph below), was chosen to increase the probability of capturing all cases of chronic Q fever. As the acute Q fever epidemic had virtually subsided on 1 November 2010, our screening program was not designed to identify cases of acute Q fever or asymptomatic primary infection.

Definitions

A titer of greater than or equal to 1:800 for IgG antibodies specific for phase I antigen using an in-house IFA, has been internationally accepted for the serological diagnosis of probable chronic Q fever.[13;14] Recently, an increase of this cut-off to a titer of greater than or equal to 1:1600 has been proposed, based on new clinical data suggesting considerable overdiagnosis with the cut-off titer of greater than or equal to 1:800.[20] In the Netherlands, essentially all laboratories use the commercial IFA from Focus Diagnostics, Inc.. Chronic Q fever was considered probable in this high-risk group if the phase I IgG titer was =1:1024 using the commercial IFA and proven in case of positive *C. burnetii* PCR on blood or tissue in combination with a corresponding serologic profile.[21] Patients with a phase I IgG titer <1:1024 and a negative *C. burnetii* PCR (if performed) are considered to have past Q fever infection.

Statistics

Clinical and microbiological data of all patients with *C. burnetii* antibodies were collected, stored and analyzed in a SPPS 18.0 based database. Qualitative data were compared by use of the Fisher's Exact test. Mean values were compared by use of the independent samples t-test. Results were expressed as means or percentages, with respectively standard deviations (SD) or p values. The significance level was set at p value = 0.05.

RESULTS

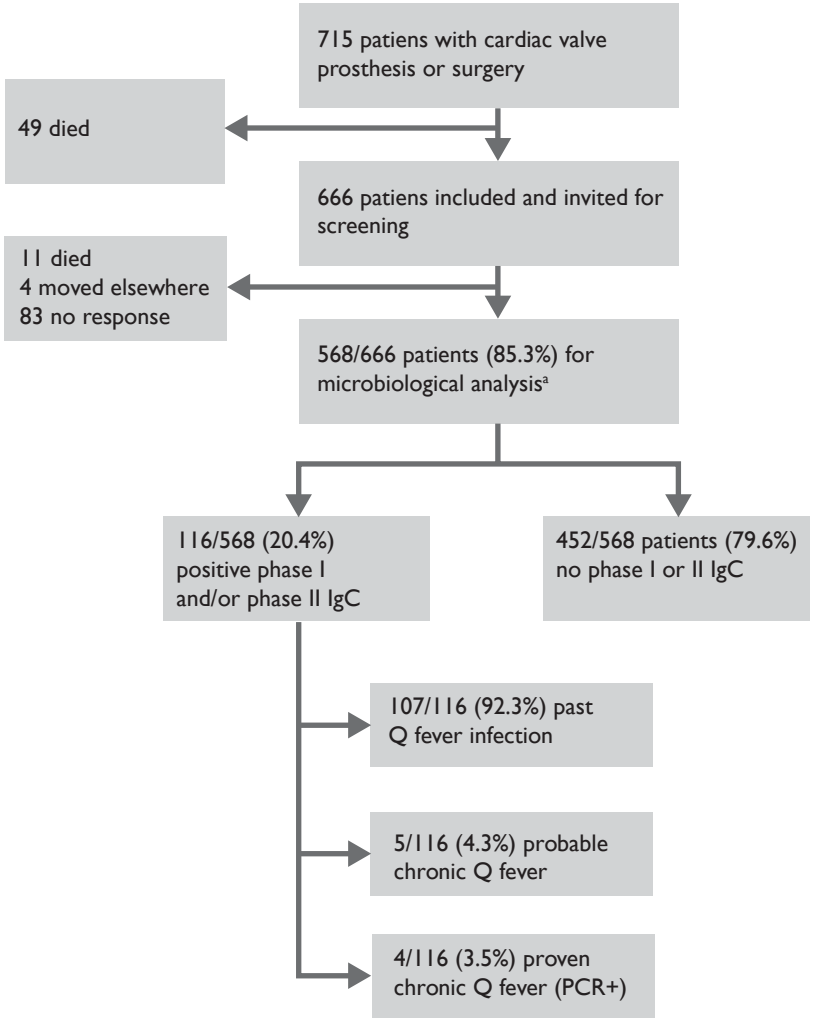
Assessment of the registration code "follow-up after cardiac valve surgery" on 1 November 2010 revealed 715 patients. Patients had been under follow-up after valve surgery for a maximum duration 33 years. Of the 715 patients, 49 had deceased in the last three years. The 666 remaining patients were invited for screening by letter. In all, 568 patients (85.3%) responded and supplied blood samples for microbiological analysis. Eighty-three patients (12.5%) did not participate, four patients (0.6%) moved elsewhere and 11 patients (1.7%) had died during enrollment of the study. Phase I and/or phase II IgG (IgG titer greater than or equal to 1:32) were detected in 116/568 patients (20.4%), indicating chronic or past *C. burnetii* infection. Only five seropositive patients (4.3%) had a notified acute Q fever in the preceding years. Nine/116 patients (7.8%) had proven or probable chronic Q fever. (Fig. 1) These nine patients represent 1.6% of the total screened population. Results of their microbiological analysis are presented in Table 1. Four/116 patients (3.4%) had proven chronic Q fever based on a positive *C. burnetii* PCR in plasma and 5/116 patients (4.3%) had probable chronic Q fever based on a phase I IgG titer =1:1024. In six patients, long-term antibiotic treatment, consisting of a combination of doxycycline and hydroxychloroquine, was initiated. Three patients refused antibiotic treatment, because of high age and fear of side effects. One patient, who had probable chronic Q fever at initial screening, progressed to PCR positive proven chronic Q fever during follow-up, after refusal of antibiotic therapy. All probable and proven chronic Q fever patients were invited at the outpatient clinic for individual further evaluation and work-up. One patient complained of fatigue and exertional dyspnoea, and another patient of fatigue only. The remaining seven patients (77.8%) had no complaints. All patients were offered trans-esophageal echocardiogram (TEE). Two patients refused; they underwent transthoracic echocardiogram (TTE). Echocardiogram showed slight deterioration of mitral valve prosthetic function in one patient, who also showed increased intensity around this valve on positron emission tomography-computed tomography (PET-CT). Echocardiogram in the remaining eight chronic Q fever patients showed no abnormalities. PET-CT revealed an aneurysm, without increased intensity, in one patient, but was unremarkable in all others.

Table 1. Microbiological results in nine patients with a history of cardiac valve surgery and proven or probable chronic Q fever at initial screening, and after three months of follow-up[a]

	Initial results				After 3 months follow-up		
Patient	Phase II IgG titer	Phase I IgG titer	PCR	Antibiotic treatment	Phase II IgG titer	Phase I IgG titer	PCR
1	1:65536	1:32768	+	yes	1:32768	1:16384	-
2	1:2048	1:1024	-	refused	1:4096	1:4096	-
3	1:16384	1:32768	-	refused	1:16384	1:16384	-
4	1:4096	1:2048	-	refused	1:4096	1:4096	-
5	1:16384	1:16384	-	yes	1:16384	1:16384	-
6	1:32768	1:32768	-	refused	1:8192	1:4096	+
7	1:32768	1:65536	+	yes	1:32768	1:16384	+
8	1:16384	1:16384	-	yes	1:16384	1:16384	-
9	1:4096	1:512	+	yes	1:2048	1:2048	+

[a] Antibody titers determined by immunofluorescence assay. PCR, polymerase chain reaction; - = negative; + = positive

Figure 1. Flow diagram showing patient inclusion and Q fever screening results.



PCR, polymerase chain reaction. [a]IgM and IgG antibodies against phase II antigens determined by immunofluorescence assay (IFA)

Table 2. *C. burnetii* phase I and phase II IgG titers[a] in patients with a history of cardiac valve surgery and past Q fever infection (n=107)

	Phase I IgG	Phase II IgG
Titer	**No. of patients (%)**	**No. of patients (%)**
negative	85 (79.4)	0
1:32	6 (5.6)	29 (27.1)
1:64	7 (6.5)	24 (22.4)
1:128	3 (2.8)	19 (17.8)
1:256	3 (2.8)	13 (12.1)
1:512	3 (2.8)	9 (8.4)
1:1024	0	5 (4.7)
1:2048	0	5 (4.7)
≥1:4096	0	3 (2.8)

No., number, [a]Antibody titers determined by immunofluorescence assay.

Past Q fever infection with no indication of chronic infection was detected in 107/116 (92.2%) patients. All 107 patients had phase II IgG antibodies. Results of phase I IgG titrations are presented in Table 2. Microbiological analysis of 101 follow-up blood samples after three months did not reveal development of chronic Q fever in any of these patients. Phase II IgG titers declined in 53 patients (52.5%), remained the same in 34 patients (33.7%) and increased in 14 patients (13.9%, maximum titer 1:4096). Among the 14 patients with increasing phase II IgG titers, two had a low positive phase II IgM titer at initial screening. This could theoretically indicate a recent *C. burnetii* infection but this seems unlikely since the Q fever outbreak had virtually subsided at time of initial screening (24). Phase I IgG titers declined in 7 patients (6.9%), remained the same in 74 patients (73.3%), and increased in 21 patients (20.1%, maximum titer 1:256). Most of the increased phase I titers at follow-up were titers of 1:32 or 1:64, which had been negative at first screening. One patient had died in the three months period (outcome initial screening: phase II IgG 1:512; phase I IgG negative), while four other patients did not participate in follow-up. All patients with increased phase I or phase II IgG titers at first follow-up were subsequently screened again six months after initial screening. No progression to chronic Q fever was observed in any of these patients.

Clinical data, characteristics of valve surgery and cardiovascular (risk) factors of all patients with probable or proven chronic Q fever infection and past Q fever infection are listed in Table 3. There were no significant differences in clinical features and location, year and type of valve surgery between these two groups. Patients with chronic Q fever were significantly older than patients with past Q fever infection.

Table 3. Clinical characteristics, characteristics of valve surgery, and cardiovascular (risk) factors in patients with a history of cardiac valve surgery and chronic Q fever, or past Q fever infection[a]

	Chronic Q fever (%)[c] n=9	Past Q fever (%)[d] n=107	Pe
Male	6 (66.7%)	66 (61.7%)	0.534
Age, mean ± SD	76.9 ± 10.8	67.4 ± 12.1	0.025[f]
Location of valve surgery[b]			0.611
aortic valve	6 (66.7%)	83 (77.6%)	
mitral valve	4 (44.4%)	29 (27.1%)	
tricuspid valve	0	4 (3.7%)	
Year of valve surgery			0.978
2010	0	8 (7.5%)	
2006-2009	4 (44.4%)	42 (39.3%)	
2001-2005	3 (33.3%)	31 (29.0%)	
1996-2000	0	9 (8.4%)	
before 1996	2 (22.2%)	17 (15.9%)	
Type of valve surgery[a]			0.795
biological valve(s)	4 (44.4%)	30 (28.0%)	
mechanic valve(s)	4 (44.4)	61 (57.0%)	
Bentall-procedure	0	5 (4.7%)	
valve repair(s)	1 (11.1%)	13 (12.1%)	
Coronary disease	5 (55.6%)	34 (31.8%)	0.140
History of CABG	4 (44.4%)	23 (21.5%)	0.126
History of PTCA	1 (11.1%)	9 (8.4%)	0.569
Hypertension	4 (44.4%)	46 (43.0%)	0.599
Diabetes	0	12 (11.2%)	0.595
Dyslipidemia	4 (44.4%)	30 (28.0%)	0.248
Immunity disorder	0	0	-

Data are number (%) of patients unless otherwise indicated. [a]SD, standard deviation; CABG, coronary artery bypass graft; PTCA, percutaneous transluminal coronary angioplasty. [b]percentages add up to >100%, due to multiple valve surgeries in some patients (1 chronic Q fever patient (11.1%) and 9 past Q fever patients (8.4%), [c]probable and proven chronic Q fever, [d]patients with positive phase I and/or phase II IgG (titer greater than or equal to 1:32) without evidence of chronic Q fever, [e]Fisher's Exact Test unless otherwise indicated. [f]independent samples t-test, 95% Confidence Interval 1.225-17.698

DISCUSSION

Using a targeted screening program in a high-risk population for development of Q fever endocarditis in an epidemic area, we found a seroprevalence of antibodies against *C. burnetii* antigens of 20.4%. In comparison; in May 2009, amidst the Q fever epidemic, *C. burnetii* IgG seroprevalence was assessed among blood donors in the area with the highest reported Q fever incidence in the Netherlands , showing a seroprevalence of 12.2% for *C. burnetii* IgG phase II antibodies using a one dilution higher cut-off titer of =1:64.[22] Of the seropositive patients, 7.8% had evidence of probable or proven chronic Q fever. A recent report stated that in a group of 686 unselected patients diagnosed with acute Q fever in 2007 and 2008 in the Netherlands, 1.6% progressed to chronic Q fever, which is much lower than observed in our high-risk population.[23] This confirms earlier findings that a history of previous cardiac valve surgery gives a highly increased risk for development of chronic Q fever. Yet, the formerly estimated risk of ~39% for the development of chronic Q fever in patients with cardiac valvulopathies could not be reproduced. This estimation resulted from a retrospective analysis of patients identified by a Q fever reference centre over a 16-year time span and may therefore differ from our results.[4] Strain specific clinical features might however also be responsible for the discrepancy.

A response rate of 85.3%, seroprevalence rate of 20.4%, of which only 4.3% had a notified acute Q fever episode in the past, and chronic Q fever prevalence rate of 1.6% in the total screened population indicates that our screening program is a proper instrument for early case-finding of chronic Q fever patients in this high-risk group. Mild damage of the prosthetic valves was seen in only one patient. Eight patients (88.9%) did not show any sign of valvular damage or vegetation on echocardiogram, and seven patients (77.8%) with proven chronic Q fever had no symptoms. One patient who had refused treatment progressed however from probable chronic Q fever to proven disease during follow-up. We think, therefore, that targeted screening and subsequent long-term antibiotic treatment allows for prevention of serious morbidity and mortality. As sensitivity of blood PCR for detection of chronic Q fever is far from optimal,9 both probable and proven chronic Q fever cases were managed similarly and offered long-term antibiotic treatment.

To identify if screening could be limited to subgroups of patients with a history of valve surgery, we compared valve properties, e.g. year of surgery, location and type, between the group of chronic Q fever patients and patients with past Q fever infection. No significant differences were seen. The same results were obtained for cardiovascular (risk) factors. However, patients with suspected chronic Q fever were significantly older than patients with past Q fever infection. Although the number of chronic Q fever patients in our study was small, this suggests that older age does raise the risk for development of chronic Q fever in patients with cardiac valve abnormalities, which could be explained by the fact that older age is associated with a diminishing immune response.[24] Remarkably, the study population of Fenollar et al,4 in which the risk for chronic Q fever was estimated at ~39%, had a lower mean age (59.6 years, range 45-74) compared to our study population.

The risk of chronic Q fever in our study could have been overestimated. Only four patients had proven chronic Q fever with a positive *C. burnetii* PCR. In the other five patients only serologic evidence for chronic Q fever (phase I IgG titer =1:1024) was found. These patients could have been wrongly diagnosed as having chronic Q fever, thus causing an overestimation of this condition.[25] As some patients underwent valve surgery during the Q fever outbreak in the Netherlands, we cannot be sure that patients who had surgery in the time period 2007- 2010, actually did not have chronic Q fever already before surgery. Nevertheless, as these patients did have severe valvu-

lopathy requiring surgical intervention at that time, they still had a reported important risk factor for chronic Q fever development.[4;8] Unfortunately, screening for Q fever antibodies before valve surgery in patients from epidemic areas is not standard care in the Netherlands yet.

There are also several reasons why the estimated prevalence of chronic Q fever in patients with a history of valvular surgery may be an underestimation. First, chronic Q fever can develop even years after primary infection, while our screening program was executed within two years after the peak of the epidemic in 2009. Yet, it is known that 75% of chronic Q fever develops within six months after primary infection.[6] In addition, follow-up of seropositive patients after three months, and again after six months in cases with increased phase I or II IgG titers at first follow-up, revealed no additional chronic cases, although this follow-up period is relatively short. Moreover, most patients with past Q fever already had undetectable phase I IgG titers at initial screening (79.4%), which makes development of chronic Q fever in our opinion less likely. Nevertheless, we cannot exclude that some patients might develop chronic Q fever in the future, which could cause a (small) difference in the prevalence rates of chronic Q fever reported in our study. Second, we identified 60 patients with a history of cardiac valve surgery who had died in the last three years with unknown *C. burnetii* serostatus. After analysis of their medical records, chronic Q fever related disease could not be excluded as having contributed to their death in nine cases (15.0%). If among the deceased patients, these nine patients had actual chronic Q fever, Q fever antibody seroprevalence would decrease to 19.5% with a chronic Q fever prevalence of 14.6%. A third reason for underestimation of chronic Q fever cases is the possibility that we overestimated the number of past Q fever infections by using a detection cut-off titer of =1:32 for phase I and II IgG. These titers can also be caused by cross-reacting antibodies to other pathogens or by aspecific reactions.[26] Yet, it is also known that, following Q fever infection, antibodies to *C. burnetii* can disappear over time or titers can become very low, leading to an underestimation of the infection rate.[23;27] This was illustrated by one case in our study, who had a confirmed acute Q fever episode in 2009, and demonstrated a phase II IgG titer of 1:32 in our screening. Nevertheless, if a cut-off titer for phase II IgG of =1:128 would have been used in our study, still 64/568 patients (11.3%) would be seropositive. Prevalence of chronic Q fever in the seropositive group would then be 14.1% (9/64), which is still markedly lower than the previously reported 39%.[4]

In conclusion, targeted screening in a high risk population seems a proper instrument for early case-finding of chronic Q fever, which potentially allows for prevention of serious morbidity and mortality. Therefore, screening for *C. burnetii* antibodies in patients with a history of valve surgery could also be considered in other outbreak settings. We have found a seroprevalence of Q fever antibodies of 20.4% in patients with a history of valvular surgery. Prevalence of chronic Q fever in seropositive patients was 7.8%, which is substantially lower than stated in previous reports of patients with cardiac valve disease. In this study, patients with older age and a history of valve surgery seem to be more vulnerable for the development of chronic Q fever.

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Chapter 10

Chronic Q fever in the Netherlands five years after the start of the Q fever epidemic: results from the Dutch Chronic Q Fever Database

Submitted

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ABSTRACT

From 2007 to 2010 the Netherlands experienced a large Q fever outbreak. To gain more insight in the chronic consequences of infection with *C. burnetii* in the Netherlands, an initiative called the Dutch National Chronic Q Fever Database was started. Five years after the start of the Q fever epidemic, we present data generated from this initiative. We characterize chronic Q fever in the Netherlands and identify predictors of mortality. We included 284 chronic Q fever patients, of which 151 (53.7%) had proven, 64 (22.5%) probable and 69 (24.3%) possible chronic Q fever. Among proven and probable chronic Q fever patients, vascular focus of infection (56.7%) was more prevalent than endocarditis (34.9%). An acute Q fever episode could be recalled in 27.0%. All-cause mortality was 19.1%, while chronic Q fever-related mortality was 13.0%; 9.3% among endocarditis patients and 18.0% among vascular chronic Q fever patients. Increasing age (*p*-values 0.004, 0.010), proven chronic Q fever (*p*-values 0.020, 0.002), vascular chronic Q fever (*p*-values 0.024, 0.005), acute presentation with chronic Q fever (*p*-values 0.002, <0.001) and surgical treatment of chronic Q fever (*p*-values 0.025, <0.001) were significantly associated with all-cause mortality and chronic Q fever-related mortality, respectively. In conclusion, the majority of chronic Q fever patients had a vascular infection. Most chronic Q fever cases were not preceded by an apparent acute Q fever episode. In our population, mortality related to chronic Q fever was 13.0%. Older patients, and presentation with vascular complications necessitating acute surgical intervention, had highest risk for chronic Q fever mortality.

INTRODUCTION

Q fever is a zoonosis caused by the bacterium *Coxiella burnetii*, which has its main reservoir in small ruminants. Outbreaks of the disease occur worldwide.[1] After primary infection, 50-60% of patients remain asymptomatic, while others develop symptomatic acute Q fever, a flu-like illness which is mostly self-limiting.[1-3] Between 2007 and 2010, a large outbreak of acute Q fever was observed in the Netherlands with over 4000 reported cases.[4] Probably, these figures are rather conservative as seroprevalence studies show that at least over 40.000 people were infected by *C. burnetii*.[5] The epidemic subsided after hygienic and veterinary measures, consisting of nationwide vaccination of goats and culling of pregnant goats on infected farms.[4;6]

From previous observations, it is know that chronic Q fever can develop in approximately 1-5% of all infected patients and mostly becomes manifest within the first year after infection, but can also present several years later.[1;7] Most common manifestations are endocarditis, according to literature accounting for approximately 75% of all chronic Q fever cases, and infections of vascular prosthesis and aortic aneurysms.[1;8-10] Less common manifestations are osteomyelitis, pericarditis and hepatitis.[9] Important risk factors are heart valve pathology, especially valve prosthesis, vascular prosthesis and aneurysms.[10-13] Immunosuppression also seems to be associated with an elevated risk, as are older age, pregnancy and (mild) renal insufficiency.[3;12;13] Before severe complications occur, most patients are asymptomatic or report only non-specific symptoms such as low-grade fever, night sweats and weight loss.[1;7;10;14] In case of endocarditis pathologic findings on echocardiography are often non-specific or absent.[14] An early diagnosis of chronic Q fever has major clinical implications, as chronic Q fever causes high morbidity and mortality up to 60% when left untreated.[1;14] Long-term antibiotic treatment, consisting of doxycycline and hydroxychloroquine, and resection of infected vascular and valvular tissue is thought to improve prognosis.[10;14;15]

Unfortunately, diagnosing chronic Q fever is challenging. A positive polymerase chain reaction (PCR) or culture of *C. burnetii* in blood or tissue, in the absence of a serologic profile for acute Q fever, is considered diagnostic for chronic Q fever, but has a limited sensitivity of only 50-60%.[16-18] Therefore, serological analysis has an essential role in the diagnosis of chronic Q fever. Serology relies on antigenic variation that *C. burnetii* exhibits when it is cultured in cells, in which the virulent phase I antigen shifts to the avirulent phase II antigen. Chronic Q fever is characterized by persisting high titers of IgG antibodies against *C. burnetii* phase I antigens (phase I IgG).[3] A phase I IgG cut-off titer of 1:800, based on an in-house developed immunofluorescence assay (IFA), as well as a cut-off titer of 1:1024 based on a commercially available IFA (Focus Diagnostics) have been used for the serological diagnosis of chronic Q fever.[16;19-21] Recent studies show that serology results alone, in the absence of PCR positivity, are also not sufficient for the diagnosis of chronic Q fever, but should be combined with clinical data.[22]

The Dutch consensus group for the diagnosis of Q fever proposed to categorize patients into proven, probable or possible chronic Q fever. This classification ranks the probability of having chronic Q fever based on PCR, serology, clinical parameters, imaging studies and pathology (Table 1).16

To gain more insight in the chronic consequences and long-term prognosis of infection with *C. burnetii* after the recent outbreak in the Netherlands, an initiative called the "Dutch National Chronic Q Fever Database" was started. This is a joint effort of multiple hospitals in the Q fever afflicted areas to monitor and asses all chronic Q fever cases in the Netherlands. Five years after the start of the Q fever epidemic, we present data generated from this initiative, which is one of the largest cohorts of chronic Q fever patients in the world, with special emphasis on mortality due to chronic Q fever.

METHODS

Dutch National Chronic Q Fever Database

All Dutch hospitals that treat chronic Q fever patients, also outside the notified Q fever epidemic areas, were actively approached to include patients in the database. Design of the database and the use of the collected information for analysis and scientific publications were approved by the Medical Research Ethics Committee of the University Medical Center Utrecht in Utrecht, the Netherlands. In the current study, we used information about patients included in this database until the end of May 2012. Part of the serological data were already published in a paper discussing serologic profiles of patients with chronic Q fever.22

Patients

Patients were included in the database when they were aged 18 years or older and when they fulfilled the criteria of proven, probable or possible chronic Q fever according to the recent Dutch consensus guideline (Chapter 5: table 5).[16] In this guideline the proven chronic Q fever group represents patients with the most definite form of chronic Q fever, in contrast to the possible chronic Q fever group, which represents patients with only phase I IgG titers = 1:1024, serologically indicating chronic Q fever, but without other manifestations of chronic disease.

For the analysis of mortality of chronic Q fever patients, we chose to include only the patients with proven and probable chronic Q fever, as probably a certain number of possible chronic Q fever patients does not have actual chronic Q fever. For deceased patients we assessed the cause of death and whether mortality was due to chronic Q fever: some patients did not die of chronic Q fever-related diseases (e.g. malignancies) or it could not be defined whether death was due or related to chronic Q fever (e.g. malaise, sepsis). Death associated with chronic Q fever was defined as definitely (caused by vascular complications, endocarditis), probably (heart failure in case of suspected endocarditis, gastro-intestinal bleeding in case of suspected arterio-intestinal fistula, clinical deterioration in case of antibiotic refusal, severe side effects of medication), possibly (coinciding severe comorbidities, unknown cause of mortality) or not associated (other cause of mortality like cancer) by one researcher (LK).

Data collection and storage

Information on patient characteristics, imaging results, laboratory results, antibiotic therapy and outcome were collected by one researcher (LK) from patient records provided by the hospitals that participated in the database. All data were stored and processed anonymously in SPSS version 18 (SPSS inc., Chicago, Illinois, USA).

Microbiological analysis

In the Netherlands, routine microbiological work-up for the diagnosis of chronic Q fever consists of serology, using IFA (Focus Diagnostics, Inc., Cypress, CA, USA), and PCR for *C. burnetii* DNA on plasma or serum and, if available, tissue. Titration of antibody levels was carried out at the different hospital sites with dilutions according to a binary scale and a detection cut-off titer of 1:32. Some hospitals performed complement binding reaction (CBR) tests for the diagnosis and follow-up of chronic Q fever. However, in all but three patients CBR results were at least at one time point confirmed with IFA. PCR for *C. burnetii* DNA was performed using an in-house assay as previously described.[23] An input volume of 500 µl plasma or serum was used for DNA isolation.

Statistical methods

We used descriptive statistics to present data on baseline and clinical characteristics. In univariate analyses, patient characteristics were related to mortality using the log-rank test. Analyses were conducted separately for all-cause mortality and chronic Q fever-related mortality defined as death definitely or probably caused by chronic Q fever. Risk factors that were significantly and mostly associated with mortality in univariate analysis were additionally evaluated using multivariable Cox regression analysis. Due to multicollinearity and the limited number of deceased subjects, we could not include all risk factors simultaneously. The multivariable analyses were also conducted for all-cause mortality and chronic Q fever related mortality separately in two models (Table 4). A Kaplan-Meier survival curve based on the multivariable model was constructed for all-cause mortality. Given the limited number of deceased subjects, variables that apply only to part of the population (e.g., characteristics acute Q fever episode and surgical treatment) were only evaluated univariately. Significance level was set at *p*-value =0.05.

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RESULTS

Until the end of May 2012 284 patients were included in the chronic Q fever database. Of these, 151 patients (53.7%) had proven chronic Q fever, 64 patients (22.5%) probable chronic Q fever, and 69 patients (24.3%) possible chronic Q fever. Baseline characteristics and details of chronic and acute Q fever episodes of these patients are shown in Table 1. Microbiology results of all chronic Q fever patients are presented in Table 2.

Overall mortality in chronic Q fever patients was 15.8%: 23.2% in proven chronic Q fever patients (23.2%), 9.4% in probable chronic Q fever patients, and 5.8% in possible chronic Q fever patients. Of the 215 patients with proven or probable Q fever, 41 (19.1%) died during a median follow-up of 14.3 months (interquartile range 25-75%: 10.1-23.1 months). Mortality definitely or probably associated with chronic Q fever among patients with proven or probable chronic Q fever was 13.0% (28/215 patients), with a 9.3% mortality rate among endocarditis patients and 18.0% in vascular chronic Q fever cases. Figure 1 shows that 17/41 (41.5%) deceased patients with probable or proven chronic Q fever died of vascular complications, 6/41 (14.6%) of endocarditis-related complications, 3/41 (7.3%) of side effects of antibiotic treatment of chronic Q fever (mostly renal failure), 7/41 (17.1%) of gradual clinical deterioration and malaise, including one patient who refused treatment for chronic Q fever and one patient who died of gastro-intestinal bleeding while an arterio-intestinal fistula was suspected, 7/41 (17.1%) died of other causes (e.g. malignancies, sepsis), while in 1/41 (2.4%) patients the cause of death is unknown.

Figure 2 presents a Kaplan-Meier survival curve based on the multivariable model. Of the deceased patients with chronic Q fever, 50% died upon presentation or within the first four months after diagnosis.

In Table 3 clinical characteristics of all proven and probable chronic Q fever patients who died (n=41) are compared with those of patients who did not die (n=174). The group of deceased patients was analysed as an all-cause mortality group (n=41) and as a group with chronic Q fever-related mortality (n=28). Increasing age (*p*-value 0.004 and 0.010), proven chronic Q fever (*p*-value

Table 1. Baseline and clinical characteristics of chronic Q fever cases in the Netherlands

	All chronic Q fever n=284(%)	Proven chronic Q fever n=151(%)	Probable chronic Q fever n=64(%)	Possible chronic Q fever n=69(%)
Male	204 (71.6)	115 (76)	48 (75)	41 (59)
Age in years, mean (SD)	64.9 (14.1)	68.6 (12)	65.2 (15)	56.6 (15)
Focus of infection[a]				
Endocarditis	75 (26.4)	49 (32)	26 (41)	-
Infection of valve prosthesis	38 (13.3)	31 (21)	7 (11)	-
Vascular infection	122 (42.0)	104 (69)	18 (28)	-
Infection of vascular prosthesis	57 (20.1)	45 (30)	12 (19)	-
Other focus	6 (2.1)	2 (1)[h]	4 (76)[k]	-
No focus	92 (33.1)	7 (5)[i]	16 (25)[l]	69 (100)
Known acute Q fever episode	106 (37.3)	28 (19)[j]	30 (47)	48 (70)
Adequate treatment[b,c]	81/106 (76)	19/28 (68)	24/30 (80)	38/48 (79)
Echocardiogram (TEE or TTE) performed[†]	21/106 (20)	8/28 (29)	3/30 (10)	10/48 (21)
Imaging of abdominal aorta by AUS[c]	14/106 (13)	5/28 (18)	4/30 (13)	5/48 (10)
Risk factor for chronic Q fever[c]	34/106 (32)	17/28 (61)	15/30 (50)	2/48 (4)
Chronic Q fever prophylaxisc,[d]	4/106 (4)	4/28 (14)	-	-
Risk factors for chronic Q fever	152 (53.5)	107 (71)	42 (66)	3 (4)
History of valvulopathy	68 (23.9)	48 (32)	19 (30)	1 (1)
Valve prosthesis or plasty	45 (15.8)	36 (24)	9 (14)	-
History of aneurysm	28 (9.9)	22 (15)	6 (9)	-
History of vascular prosthesis	61 (21.5)	49 (32)	12 (19)	-
Immunosuppressione	19 (6.7)	11 (7)	8 (13)	-
Pregnancy during acute episode	5 (1.8)	1 (1)	2 (3)	2 (3)
Surgical treatment due to Q fever	65 (22.9)	64 (42)	1 (2)	-
Valve surgery[f]	13 (4.6)	12 (8)	1 (2)	-
Vascular surgery[f]	53 (19.0)	54 (36)	-	-
Deceased[g]	45 (15.8)	35 (23)	6 (9)	4 (6)
Deceased patients with endocarditis	12/75 (16)	10/49 (20)	2/24 (8)	NA
Deceased patients with vascular infection	28/122(23)	26/104(25)	2/18 (11)	NA
Definitely or probably deceased due chronic Q fever	28 (9.8)	27 (18)	1 (2)	-

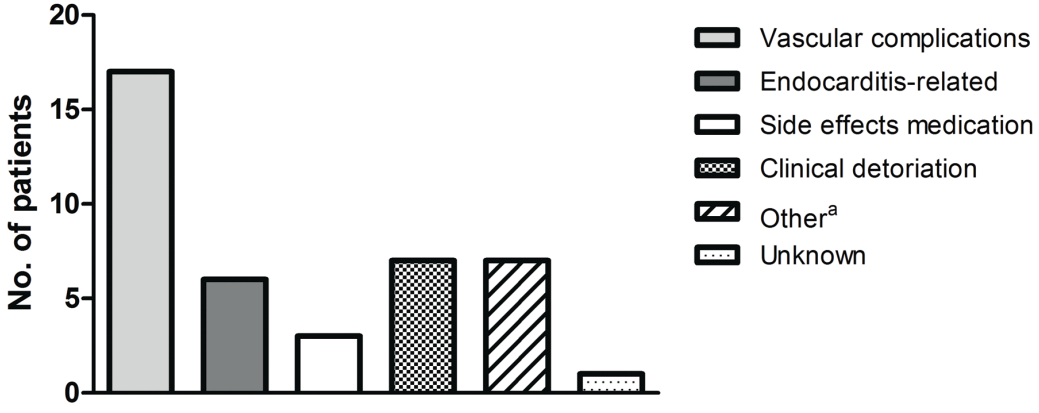
SD, standard deviation; TEE, transesophageal echocardiography; TTE, transthoracic echocardiography; AUS, abdominal ultrasound; NA, not applicable; PCR, polymerase chain reaction. [a]in eleven proven chronic Q fever patients imaging studies revealed that the focus of infection could be both on the heart valves and vascular structures, [b]proven effective antibiotic treatment regime for at least 10 days, [c]percentages estimated on all chronic cases with an acute Q fever episode only, [d]doxycycline and hydroxychloroquine for at least 6 months, [e]prednisone cumulative dose >750mg, TNFα-blocker usage, methotrexate usage, mycofenolate mofetil usage, splenectomy, haematologic malignancies [f]two patients underwent both vascular and heart valve surgery: one patient underwent replacement of thoracic aneurysm and aortic valve, one patient underwent replacement of aortic valve and vascular surgery of an aneurysm of the left arteria iliaca, [g]in two deceased proven chronic Q fever patients focus of infection could be both on heart valves and vascular structures, [h]one patient with pericarditis and one patient with infected placenta (confirmed by PCR), [i]all had positive PCR in blood: six patients had cardiovascular risk factors, two were immunocompromised, [j]data missing in seven patients, [k]one patient with pericarditis, two patients with chronic serologic profile during pregnancy, and one patient with spondylodiscitis, [l]six patients with clinical signs of systemic infection, ten patients were immunocompromised

Table 2. Results of microbiology tests (PCR and IFA) of all proven, probable and possible chronic Q fever patients

	All chronic Q fever n=284(%)	Proven chronic Q fever n=151(%)[a]	Probable chronic Q fever n=64(%)[c]	Possible chronic Q fever n=69(%)
PCR positive in blood only	60 (21.1)	60 (40)	-	-
PCR positive in tissue only	25 (8.8)	25 (17)	-	-
PCR positive in blood and tissue	35 (12.3)	35 (23)	-	-
Phase I IgG titer				
1:512	2 (0.7)	2[b] (1)	-	-
1:1024	35 (12.3)	6 (4)	13 (20)	16 (23)
1:2048	61 (21.5)	24 (16)	13 (20)	24 (35)
1:4096	62 (21.8)	28 (19)	17 (27)	17 (25)
1:8192	36 (12.7)	17 (11)	10 (16)	9 (13)
>1:8192	79 (27.8)	66 (44)	10 (6)	3 (4)

PCR, polymerase chain reaction; IFA, immunofluorescence assay; CBR, complement binding reaction. [a]for two patients no titration of serology above 1:4096 performed, for three patients no reliable IFA results due to blood transfusion or fluid suppletion, for three patients no IFA performed (all PCR in blood or tissue positive), but CBR (all PCR positive in blood or tissue positive), [b]one patient with positive PCR in blood, one patient with positive PCR in vascular tissue, [c]in one patient no titration of serology above 1:4096 performed.

Figure 1. Causes of death of deceased proven and probable chronic Q fever patients



[a]Other causes of death were malignancies, renal failure and sepsis due to another pathogen which was unrelated to endocarditis or vascular infection

Table 3. Deceased and non-deceased patients with proven and probable chronic Q fever

	Non-deceased n=174 (%)	Deceased n=41 (%)	p^e	Definitely or probably deceased due to chronic Q fever n=28 (%)	p^e
Male	133 (76)	30 (73)	0.67	21 (75)	0.83
Age, mean (SD)	66.5 (13)	72.1 (12)	0.004	72.5 (13)	0.010
Risk factor for chronic Q fever	119 (68)	30 (73)	0.40	20 (71)	0.62
Proven chronic Q fever	116 (67)	35 (85)	0.020	27 (96)	0.002
Endocarditis	63 (36)	12 (29)	0.28	7 (25)	0.21
Valve prosthesis or plasty	31 (18)	7 (17)	0.92	4 (14)	0.70
Vascular infection	94 (54)	28 (68)	0.024	22 (79)	0.005
Vascular prosthesis	50 (29)	7 (17)	0.36	4 (14)	0.25
Other focus	6 (3)	0	0.19	0	0.28
No focus	20 (11)	3 (10)	0.31	0	0.05
Acute presentation with chronic Q fever	35 (20)	18 (44)	0.002	16 (57)	<0.001
Acute Q fever episode	45 (26)	13 (32)	0.77	8 (29)	0.66
Adequately treated with antibiotics[a,b]	34/45 (76)	9/13 (69)	0.91	4/8 (50)	0.27
Risk factor for chronic Q fevera	22/45 (50)	10/13 (77)	0.076	6/8 (75)	0.18
Prophylactic therapy[a,c]	2/22 (9)	1/10 (10)	0.79	0/8	0.47
Adequate antibiotic treatment of chronic Q fever	145 (83)	35 (85)	0.96	23 (82)	0.69
Surgical treatment of chronic Q fever	49 (28)	19 (46)	0.025	17 (61)	<0.001
Emergency surgical treatment[d]	21/49 (43)	15/19 (79)	0.006	13/17 (76)	0.013

SD, standard deviation. [a]proven effective antibiotic treatment regime for at least 10 days, [b]percentages and p-value estimated on all chronic cases with an acute Q fever episode only, [c]doxycycline and hydroxychloroquine for at least 6 months, [d]percentages and *p*-value estimated on all chronic cases who underwent surgical treatment only, [e]*p*-value estimated with log-rank test

0.020 and 0.002), vascular infections as focus of chronic Q fever (*p*-value 0.024 and 0.005), acute presentation with chronic Q fever (*p*-value 0.002 and <0.001), and surgical treatment of chronic Q fever (*p*-value 0.025 and <0.001) were significantly associated with all-cause mortality and chronic Q fever-related mortality respectively. Subanalysis of patients who underwent surgical intervention, demonstrated that especially emergency surgical treatment, is associated with mortality (*p*-values 0.007 for all-cause mortality, 0.017 for chronic Q fever-related mortality).

In multivariable Cox regression analysis, age (hazard ratio (HR) 1.57; 95% confidence interval (95%CI) 1.13-2.19 per 10 year increase of age) and acute presentation with chronic Q fever (HR 1.96; 95%CI 1.02-3.78) were independent predictors of all-cause mortality (Table 4). Age was also an independent predictor of chronic Q fever related-mortality (HR 1.59; 95%CI 1.14-2.21 per 10 year increase of age).

Figure 2. Kaplan-meier survival curve for proven and probable chronic Q fever patients

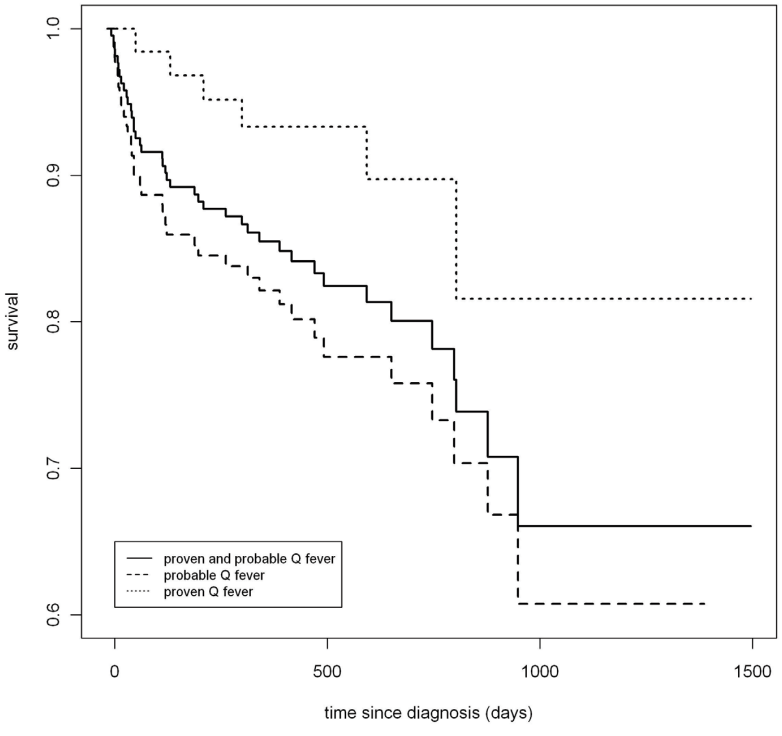


Table 4. Adjusted risk factors for mortality among patients with proven and probable chronic Q fever

Multivariable model - Risk factors included[a]	**All-cause mortality HR (95%CI)**	**Mortality definitely or probably due to chronic Q fever HR (95%CI)**
Model 1		
Age in 10 years (continuous)	1.57 (1.13-2.19)	1.68 (1.09-2.59)
Proven chronic Q fever	1.63 (0.62-4.26)	5.65 (0.71-44.82)
Vascular infection	1.48 (0.73-2.99)	1.81(0.71-4.59)
Acute presentation with chronic Q fever	1.96 (1.02-3.78)	2.75 (1.27-5.95)
Model 2		
Age in 10 years (continuous)	1.59 (1.14-2.21)	1.71 (1.11-2.62)
Proven Q fever	1.84 (0.71-4.76)	6.66 (0.84-52.59)
Vascular infection	1.34 (0.63-2.85)	1.46 (0.53-3.99)
Surgical treatment of chronic Q fever	1.51 (0.75-3.04)	2.09 (0.89-4.89)

HR, hazard ration; 95%CI, 95% confidence interval. [a]two multivariable models were constructed because of multicollinearity between acute presentation with chronic Q fever and surgical treatment of chronic Q fever: model 1 includes acute presentation with chronic Q fever and model 2 surgical treatment of chronic Q fever.

DISCUSSION

We have presented the first results of long term follow-up of chronic Q fever patients from the Dutch National Chronic Q Fever Database five years after the start of the Q fever outbreak in the Netherlands. Until the end of May 2012, 284 patients with proven, probable or possible chronic Q fever were identified. During the Q fever epidemic over 4000 cases of acute Q fever were notified.[4] Of all chronic Q fever cases, 108 patients (38.0%) recalled an acute Q fever episode, while among proven and probable cases, only 58 patients (27.0%) had a known acute Q fever episode. Therefore, the risk for chronic Q fever development after a known acute Q fever episode is approximately 2.5% if all chronic Q fever cases are taken into account, and 1.5% if limited to proven and probable chronic Q fever cases. These figures are in line with the previously reported rate of 1-5%.[1;7] However, based on seroprevalence studies estimates of the total number of *C. burnetii* infections in the Dutch outbreak are up to 10-fold higher than the number of notified cases.[5;24] Extrapolating these data, the number of identified chronic Q fever patients in the Netherlands does not reach the rate of 1-5% of *C. burnetii* infections by far.[1] This could indicate that the majority of chronic Q fever cases is still undetected. However, this is less likely as it is more than two years after the end of the Q fever epidemic. It could also indicate that in the Dutch outbreak the percentage of *C. burnetii*-infected patients who eventually develop chronic Q fever is lower than previously reported.

Most patients with proven or probable chronic Q fever did not recall an episode of acute Q fever (27.0%), which is in contrast to patients with possible chronic Q fever (69.6%), who were mostly detected in follow-up programmes after a recognized acute Q fever episode. This indicates that asymptomatic primary infections also bring about the risk for development of chronic Q fever, maybe even more than symptomatic infections. Therefore, early detection of chronic Q fever might not be accomplished only by follow-up programmes of recognized acute Q fever cases; screening of high risk-groups after an acute Q fever outbreak should also be prioritised.

Remarkably, among the proven and probable chronic Q fever patients in the Netherlands, we found more patients with a vascular focus of infection (56.7%) than patients with endocarditis (35.3%). This is in contrast with earlier reports from France where endocarditis predominates.[1] It might be that this disparity is caused by strain-specific differences, leading to distinct clinical presentations.[25] Another explanation might be increased awareness in the Netherlands of this previously relatively unacknowledged manifestation of chronic Q fever. In eleven patients, imaging studies revealed that the focus of infection could be both on the heart valves and vascular structures, which was confirmed in one deceased patient at autopsy, where both the aortic valve as well as an aneurysm were found *C. burnetii* DNA positive by PCR. We found nine proven chronic Q fever patients with a positive PCR in blood, who did not have a clear vascular infection or endocarditis. One of these patients had signs of a pericarditis, while another patient had serologic results consistent with chronic Q fever during pregnancy and positive *C. burnetii* PCR of placental tissue. In seven patients no infection focus was found despite thorough work-up with echocardiography and fluorodeoxyglucose positron emission tomography-computer tomography. Six of these patients had risk factors for cardiovascular disease and two were immunocompromised (one patient with acute lymphatic leukaemia and one patient on long term prednisone therapy).

Risk factors for chronic Q fever development were present in the majority of patients (53.5% of all cases, 69.3% in proven and probable cases), except for patients with possible chronic Q fever infection (4.3%). Risk factors in possible chronic Q fever cases constituted of minor valvulopathies only, for example sporadic aortic valve insufficiency. Nevertheless, in a significant number

of patients these risk factors were not recognized before presentation. More than half of patients with proven vascular chronic Q fever had no history of vascular anomalies beforehand. These patients presented at the hospital with aneurysms for which in various instances emergency vascular surgery was needed. Although the role of echocardiography after an acute Q fever episode remains controversial and needs further evaluation,[14;26;26;27] screening for an aortic aneurysm at time of acute Q fever with abdominal ultrasound, could result in detection of patients at risk for vascular chronic Q fever at an early stage. Therefore, we advise to screen for aortic aneurysms in case of acute Q fever, especially when risk factors for vascular disease are present like diabetes mellitus, hypertension, smoking, dyslipidemia or positive family history for vascular disease. In our current analysis, minor heart valve lesions (for example grade I aortic valve insufficiency) were also included as risk factor for chronic Q fever, although the influence of minor valvulopathies on chronic Q fever development is debated.[14;25;26] This could have lead to overestimation of the amount of risk factors in all chronic Q fever cases.

Overall mortality in chronic Q fever patients was 15.8%. Mortality was highest in patients with proven chronic Q fever (23.2%), compared to patients with probable chronic Q fever (9.4%), and possible chronic Q fever (5.8%). As possible chronic Q fever cases most likely represent at least in part patients who do not have actual chronic Q fever, we omitted them from the analysis of chronic Q fever mortality. In total, we identified 215 patients with proven or probable chronic Q fever among whom all-cause mortality was 19.1%, during a median follow-up of 14.3 months (interquartile range 25-75%: 10.1-23.1 months). If only patients with chronic Q fever-related mortality were analysed, mortality among patients with proven and probable chronic Q fever was 13.0%, with 9.3% mortality rate among endocarditis patients and 18.0% in vascular chronic Q fever cases. These figures confirm previous studies reporting differences in mortality rates of both disease entities.[10;14;15] Most patients died due to vascular complications of vascular chronic Q fever. Mortality was highest in the first months after the diagnosis of chronic Q fever. Not unexpectedly, in multivariable analysis, older age was significantly associated with death in both the all-cause mortality and chronic Q fever-related mortality groups. Moreover, acute presentation with chronic Q fever (e.g. symptomatic aneurysm, severe endocarditis) was significantly associated with all-cause mortality. Although in univariate analysis proven chronic Q fever, surgical treatment of chronic Q fever, and vascular focus of infection were also significantly associated with all-cause and Q fever related mortality, this was not confirmed in multivariable analysis. However, the number of deceased patients was relatively small to perform reliable multivariable analysis. Moreover, there was correlation between the included variables. Analysis of the data showed that the elevated mortality risk in case of surgical intervention was caused by the necessity of emergency surgical procedures for patients who presented with acute complications of chronic Q fever, especially in case of vascular infections and to a lesser amount of endocarditis.

In conclusion, the data of the Dutch National Chronic Q Fever Database demonstrate that vascular infection is a very common manifestation of chronic Q fever in the Netherlands where it is more prevalent than Q fever endocarditis. Most patients with chronic Q fever do not recall an episode of acute Q fever. Chronic Q fever is a severe infection with overall high mortality. Especially older patients with a vascular infection, who present with acute (vascular) complications needing urgent surgical intervention, are at risk of death due to chronic Q fever. Measures to reduce mortality and morbidity of chronic Q fever should be directed at early case finding. This can be achieved by screening of high risk groups in case of a Q fever epidemic and surveillance for risk factors of chronic Q fever.

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Part 4

Important clinical manifestations of Q fever endocarditis

Chapter 11

Chronic Q fever-related dual pathogen endocarditis: a case series of three patients

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ABSTRACT

Following *Coxiella burnetii* infection, there is 1-5% risk of chronic Q fever. Endocarditis, mycotic aneurysm and vascular prosthesis infection are common manifestations. We present three patients with endocarditis by *C. burnetii* concomitant with another bacterial pathogen. Chronic Q fever should, therefore, be considered in all endocarditis patients in endemic regions.

CASE REPORTS

Case 1.
The first patient was a 69-year-old man with a history of acute rheumatoid disease, congenital combined mitral valve insufficiency and stenosis and severe aortic valve insufficiency, for which he received aortic valve prosthesis 31 years ago. Two months before presentation, he had been hospitalized because of suspected sepsis and congestive heart failure. At that time, *Coxiella burnetii* serology (immunofluorescence assay; Focus Diagnostics, Inc., Cypress, CA, USA) was found positive, PCR was not yet available. A diagnosis of acute Q fever was suspected (table 1). Blood, sputum and urine cultures revealed no other pathogens. He was treated with moxifloxacin for two weeks, after which he recovered and was discharged. Two months later, he presented with fever, chills and dizziness. He also complained of shortness of breath and paroxysmal nocturnal dyspnoea. Upon physical examination, he had a systolic murmur at the left fifth intercostal space that had not been reported before. Laboratory results are displayed in table 2. Under suspicion of endocarditis, treatment with amoxicillin and gentamicin was initiated. Transthoracic echocardiography (TTE) showed progression of mitral valve insufficiency, but no vegetations. Multiple blood cultures grew Streptococcus salivarius. In addition, *C. burnetii* serology was suggestive of chronic Q fever. PCR for *C. burnetii* was positive on serum of this episode. In retrospect, PCR on serum drawn during the first admission also proved to be PCR positive. (table 1) A diagnosis of dual pathogen endocarditis with S. salivarius and *C. burnetii* was made. Doxycycline and hydroxychloroquine with a planned duration of 24 months were added to the antibiotic regimen. Throughout 21 months follow-up, medication levels have been adequate. No worsening of mitral valve function was detected with repeated echocardiography. Serology revealed a gradual fourfold decrease of phase I IgG and disappearance of phase II IgM. PCR for *C. burnetii* proved already negative after 6 months of treatment (table 1).

Case 2.
This was a 71-year-old man with a history of hypertension. Two months before presentation, he had been treated with penicillin for culture-proven meningitis and bacteraemia caused by Streptococcus mitis. To exclude endocarditis as a cause for S. mitis bacteraemia, a TTE had been performed which showed mild mitral valve regurgitation, but no other abnormalities and no vegetations. He was discharged in fairly good condition. Two months later, he presented with chest pain

Table 1. *C. burnetii* serology (IFA) and PCR

	Phase I IgM	Phase I IgG	Phase II IgM	Phase II IgG	Serum PCR
Case 1					
first admission	1:1024	1:2048	1:2048	1:2048	positive
second admission[a]	1:8192	1:32768	1:8192	1:16384	positive
Case 2	1:512	1:65536	1:256	1:65536	positive
Case 3	1:64	1:4096	1:64	1:2048	negative

IFA, immunofluorescence assay; PCR, polymerase chain reaction; phase I IgM, IgM antibodies to *C. burnetii* phase I antigens; phase II IgM, IgM antibodies to *C. burnetii* phase II antigens; phase I IgG, IgG antibodies to *C. burnetii* phase I antigens; phase II IgG, IgG antibodies to *C. burnetii* phase II antigens. [a]2 months after first admission

and a fever. He reported that he suffered from episodes of feeling warm and cold, malaise and loss of energy. On examination, there was a new systolic murmur on the apex. Laboratory results are shown in table 2. Serology for *C. burnetii*, requested by the general practitioner one week before presentation, revealed titers suggestive of chronic Q fever, which was confirmed by a positive serum PCR for *C. burnetii* (table 1). TTE showed severe eccentric mitral valve insufficiency with a vegetation on the anterior mitral valve leaflet. Thoracic CT-angiography to rule out aortitis was without abnormalities. PET-scan revealed no other localization for chronic Q fever. Under suspicion of chronic Q fever endocarditis, treatment with doxycycline and hydroxychloroquine was initiated with a planned duration of 18 months. However, after several days, multiple blood cultures again grew S. mitis. A diagnosis of dual pathogen endocarditis with S. mitis and *C. burnetii* was made and treatment with penicillin and gentamicin for 6 weeks was added. He responded well and six months later he performed physical activities without complaints. Phase I IgG antibody titer had dropped to 1:4096, while phase II IgM titer dropped to 1:32. Serum PCR became negative at 6 months of treatment (table 1). Repeated TTE showed no progression of mitral valve insufficiency, regression of vegetations and good left ventricular function.

Case 3.

This was an 88-year-old woman with a history of hypertension and cholecystectomy. Two days before presentation, she had experienced chills, vomiting and collapse, which recovered spontaneously. At presentation, she had chills, fever, a productive cough and exertional dyspnoea. Physical examination revealed a new systolic murmur at the second intercostal space left. Laboratory results are shown in table 2. Under the suspicion of acute endocarditis, treatment with flucloxacillin and gentamicin was initiated. Multiple blood cultures grew Staphylococcus aureus. TTE showed mitral valve insufficiency with possible vegetations under the posterior mitral valve leaflet. Despite antibiotic treatment, her clinical situation rapidly deteriorated and she died of septic shock 7 days after admission. Autopsy was denied. Serological examination performed on the day of death showed evidence of chronic Q fever, while *C. burnetii* PCR on serum was negative (table 1). Based on the serological profile, a post-mortem diagnosis of dual pathogen endocarditis with S. aureus and *C. burnetii* was suspected.

Table 2. Results of laboratory tests at presentation

Measurement	Reference value	Case 1	Case 2	Case 3
WBC (10^9/L)	4,0-10,0	5,5	8,5	18,3
CRP (mg/l)	<6	15	37	322
Creatinin (µmol/l)	♂? 60-110; ♀ 50-100	104	85	114
GFR (MDRD) (ml/min/1.73m²)	>60	61	77	39
AST (U/l)	♂ < 35; ♀ < 30	32	26	83
ALT (U/l)	♂ < 45; ♀ < 35	18	44	93
Troponin T (µg/l)	<0,1	-	<0,05	<0.05
Troponin I (µg/l)	<0,2	<0,2	-	-
Creatinin kinase (U/l)	♂ < 170; ♀ < 145	17	27	62

WBC, white blood cell count; CRP, C-reactive protein; GFR (MRDR), estimated glomerular filtration rate using modification of diet in renal disease formula; AST, aspartate aminotransferase ; ALT, alanine aminotransferase.

DISCUSSION

Q fever is a zoonotic infection, caused by *C. burnetii*, an intracellular Gram-negative coccobacillus. The presentation of the disease is variable with both acute and chronic manifestations. After acute infection, 40-60% of patients remain asymptomatic, while others develop symptoms ranging from a flu-like illness to severe presentations including pneumonia and hepatitis. Following acute infection, 1-5% of patients progress to chronic infection, which can develop even years after the primary infection. Endocarditis, mycotic aneurysm and vascular prosthesis infection are the most common manifestations.[1;2] Patients with pre-existent valvular disease or vascular defects, especially aortic aneurysms and aortic stents and prosthesis, immunocompromised patients and pregnant women are most frequently affected.[1-4] The estimated risk of transformation from acute infection to Q fever endocarditis in patients with pre-existing valvulopathy is approximately 40%.[3;4]

Laboratory diagnosis of chronic Q fever relies on serology and PCR. When cultured in cells, *C. burnetii* exhibits antigenic variation. The virulent variant named phase I, shifts to an avirulent variant, phase II. During acute infection, antibodies predominantly to phase II antigens are detected first, whereas persisting high levels of antibodies to phase I antigens are indicative of chronic Q fever infection.[5-7] Chronic Q fever is suspected if titers of phase I IgG antibodies are > 800, and is evident if culture of *C. burnetii* is positive or *C. burnetii* DNA is detected by PCR in blood or tissue in the absence of acute infection.[5;7] A phase I IgG antibody titer > 1:800 or positive culture for *C. burnetii* is included as a major criterium in the modified Duke criteria for diagnosis of infective endocarditis[8;9]

The most effective antibiotic treatment for chronic Q fever endocarditis consists of a combination of doxycycline and hydroxychloroquine for 18 months for native valves and 24 months for prosthetic valves, until a four-fold decrease of phase I IgG titers and a complete clearance of phase II IgM is reached. If phase I IgG titers remain higher or phase II IgM detectable, treatment should be extended.[5]

Q fever endocarditis is an indolent disease and symptoms are nonspecific. Often, the diagnosis is made when significant valvular damage has already occurred.[3;4] A diagnosis of chronic Q fever endocarditis might be missed if other pathogens are first isolated, in case of a so-called 'dual pathogen endocarditis'. Endocarditis due to a concomitant infection with *C. burnetii* and another causative agent has thus far incidentally been described in seven cases only, all in France, in a time period of at least 13 years.[8;10;11] Here we have presented three patients with a *C. burnetii* dual pathogen endocarditis in a two-year period. This observation is related to one large outbreak of acute Q fever in the southern part of the Netherlands with approximately 4000 cases from 2007 onwards.[12] As a result, chronic Q fever, Q fever endocarditis and associated Q fever dual pathogen endocarditis are increasingly diagnosed. Although the epidemic seems to subside, this is expected to progress in the years to come. Although *C. burnetii* diagnostics are included in the modified Duke criteria for diagnosis of infective endocarditis, systematic serological testing for *C. burnetii* is not common practice, especially when another etiological pathogen has already been identified. An adequate diagnosis of Q fever endocarditis has important clinical implications, as this condition requires long-term treatment and follow-up and has poor prognosis if untreated, with mortality approaching 100% and the need for surgery rising up to 60%.[5] It is therefore, advisable to perform microbiological analysis for *C. burnetii* in patients with suspected endocarditis, especially in (previously) endemic areas of Q fever.

Chronic Q fever is thought to develop after an acute episode due to an ineffective immune response, which results in continuous multiplication of *C. burnetii* in macrophages.[3;6] The exact sequence of events leading to dual pathogen endocarditis is unclear. Presumably, initial presence of Q fever endocarditis and associated indolent progression of valvular damage, makes the patients more susceptible to endocarditis with other pathogens. On the other hand, pre-existing valvular lesions put patients at risk for both Q fever endocarditis and bacterial endocarditis due to other pathogens. Both micro-organisms require adequate and sufficiently long treatment.

In conclusion, we report three patients who presented with chronic Q fever-related dual pathogen endocarditis. Q fever endocarditis needs long-term antibiotic treatment and has poor prognosis if left untreated. It is, therefore, recommended to perform microbiological analysis for *C. burnetii* through serology and serum PCR and, if possible, tissue PCR, in patients presenting with an endocarditis, especially for those residing in a region with high seroprevalence of Q fever, regardless of other identified pathogens.

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Chapter 12

Delayed diagnosis of chronic Q fever in patients requiring cardiac valve surgery

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ABSTRACT

Untreated chronic Q fever has high morbidity and mortality. We present three cases of chronic Q fever, in which diagnosis was delayed until after cardiac valve surgery. Screening for Q fever of patients requiring valve surgery in epidemic areas secures early initiation of treatment and can prevent morbidity and mortality.

CASE REPORTS

Case 1.
A 73-year-old man was diagnosed with aortic stenosis seven years ago. Additional medical history included atrial fibrillation and transient ischemic attacks. He underwent aortic valve replacement with a bioprosthesis (type Medtronic mosaic) in May 2011 because of progressive aortic stenosis. As there were no macroscopic signs of endocarditis, microbiological or pathological examination of the removed valve was not requested. Four months after valve replacement, the patient developed paravalvular insufficiency of the bioprosthesis requiring a second valve replacement. No vegetations were observed on transesophageal echocardiography. Again no macroscopic signs of endocarditis were noticed and therefore no microbiological or pathological examination of the removed valve was requested. At this point, serology for *Coxiella burnetii* was performed, revealing chronic infection (table 1). PCR on plasma for *C. burnetii* DNA was also positive. The patient had not been aware of a previous acute Q fever infection, nor had he suffered from fever, night sweats, weight loss or malaise. Further examination by FDG-Positron Emission Tomography (FDG-PET) combined with low dose Computed Tomography (CT) demonstrated no other chronic Q fever focus or vascular abnormalities. He started antimicrobial therapy with doxycyclin and hydroxychloroquine and is doing well after 3 months follow-up. *C. burnetii* serology performed in retrospect on serum drawn at time of the first valve replacement already demonstrated a serologic profile consistent with chronic Q fever. PCR on serum for *C. burnetii* DNA was negative (table 1).

Case 2.
A 78-year-old man had a medical history of aortic valve stenosis, abdominal aortic aneurysm with an endovascular aneurysm repair in 2005. Because of this endoprosthesis, he was tested for chronic Q fever in July 2011 in a screening programme for high-risk groups.[1] Screening demonstrated chronic Q fever infection, although he had not been aware of an acute Q fever episode, nor had he complaints of night sweats, weight loss, malaise or fever. Due to progressive aortic valve stenosis, he was already on a waiting list for elective valve replacement at an academic cardiovascular centre. This centre, located outside the Q fever epidemic area and unaware of the Q fever status of the patient, placed a bioprosthesis in August 2011. The patient recovered well. The native valve was not further examined because there were no macroscopic signs of endocarditis. Post-

Table 1. *C. burnetii* serology and PCR results

Patient	Phase I IgG	Phase 2 IgG	*C. burnetii* PCR	C-reactive protein
Case 1.				
Before valve surgery[a]	1:32768	1:65536	negative	<6
After valve surgery	1:8192	1:8192	positive	52
Case 2.				
Before valve surgery	1:16384	1:16384	positive	22
After valve surgery	1:16384	1:16384	positive	26
Case 3.				
After valve surgery	1:8192	1:8192	positive	11

[a]Q fever serology was performed in retrospect

surgery, the screenings results were acted upon. Combined FDG-PET/CT scan showed no signs of infection at the abdominal aortic prosthesis, nor elsewhere. In September 2011, the patient started antimicrobial therapy with doxycycline and hydroxychloroquine and he is doing well up to three months later.

Case 3.
A 70-year-old woman had a longstanding history of rheumatoid arthritis, treated with infliximab and consecutively etanercept, as well as corticosteroids and azathioprine. In 2009, she was admitted to hospital because of heart failure due to mitral valve insufficiency, possibly resulting from chordal rupture, combined with an atrial septal defect and left ventricular systolic dysfunction. October 2010, a mitral valve reconstruction was performed combined with a coronary bypass procedure and atrial septal defect closure. April 2011, screening for Q fever was performed as she registered for vaccination against *C. burnetii*, which was offered by the government to high-risk patients with aortic (endo)vascular prostheses or cardiac valve abnormalities. A chronic Q fever infection was diagnosed (table 1). She did not remember any symptoms fitting a previous acute Q fever episode, nor did she suffer from fever, night sweats, malaise or weight loss. FDG-PET/CT-scan showed no uptake in the large vessels. On transesophageal echocardiography, an insufficiency of the mitral valve repair was observed. Antimicrobial treatment with doxycycline and hydroxychloroquine was started, under which PCR for *C. burnetii* DNA in blood became negative. However, as she developed elevated liver enzymes and severe nausea and vomiting possibly resulting from hydroxychloroquine, therapy was switched to moxifloxacine monotherapy.

DISCUSSION

We present three patients with chronic Q fever and valvular disease requiring valve surgery. In these patients, diagnosis and treatment were delayed until after elective valve surgery, which was performed because of progressive valvular dysfunction. Early diagnosis and antimicrobial treatment of Q fever endocarditis might have prevented secondary surgery. Q fever is a zoonosis caused by the intracellular Gram-negative bacterium *C. burnetii*. It is a worldwide prevalent disease, occurring in outbreaks, with both acute and chronic stages.[2] Recently, the Netherlands were confronted with an outbreak of over 4000 acute Q fever cases between 2007 and 2010.[3] Acute Q fever is a self-limiting febrile disease, but only occurs in 40-50% off all infected patients.[2] Chronic Q fever occurs in 1-5% of all patients with a *C. burnetii* infection and can develop even years after primary infection.[2;4] Most important manifestations of chronic Q fever are endocarditis, and infection of vascular prosthesis and aortic aneurysms.[5] Patients with pre-existing valvular disease have a reported 40% risk of Q fever endocarditis when infected with *C. burnetii*.[4;6] Symptoms of Q fever endocarditis can be non-specific and vegetations are usually absent or small. As also observed in the presented cases (table 1), C-reactive protein and erythrocyte-sedimentation rate can be normal or only mildly elevated.[7] The most frequent presentations of Q fever endocarditis are a new valvular insufficiency or worsening of pre-existing valvular insufficiency.[7-9] Valves infected with *C. burnetii* can appear normal overall on visual inspection – as also demonstrated in the presented cases – and even on histological evaluation.[10] Diagnosis of chronic Q fever is challenging and consists of serology and PCR on blood and, if available, tissue. Although a positive PCR or culture of *C. burnetii* in blood or tissue, in the absence of acute Q fever, proves chronic infection, sensitivity is only 50-60% in patients with chronic Q fever.[11] When cultured in cells, *C. burnetii*

exhibits antigenic variation in which the virulent variant, called phase I, shifts to an avirulent variant, phase II. During acute infection, antibodies predominantly to phase II antigens are detected first, whereas persisting high levels of antibodies to phase II, and especially phase I antigens, are indicative of chronic Q fever.[12] A phase I IgG titer of >1:800 or >1:1024, depending on the type of immunofluorescence assay (IFA) used, has been internationally accepted for the serological diagnosis of chronic Q fever.[13;14] Long-term antibiotic treatment, preferably a combination of doxycycline and hydroxychloroquine is the treatment of choice for chronic Q fever and should be continued 18 months for native valves and 24 months for prosthetic valves, until a four-fold decrease of phase I IgG titers and a complete clearance of phase II IgM is reached. If phase I IgG titers remain high or phase II IgM detectable, treatment duration should be extended. Chronic Q fever has a high morbidity and mortality, rising up to >60% if treatment is delayed or not initiated. With adequate treatment, mortality of Q fever endocarditis has declined to 5%. Chronic Q fever involving prosthetic valves is associated with higher mortality, longer treatment, and elevated chance of complications.[8] In our cases, preoperative diagnosis of chronic Q fever might have prevented the second valve replacement in case one and the delay in initiation of treatment in case two and three. We advise preoperative screening for chronic Q fever in all patients undergoing elective valve surgery in a Q fever epidemic area.

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Chapter 13

Summary, general discussion and perspectives

SUMMARY

Q fever is a complex disease, with both acute and chronic manifestations. Detected in 1935, its name was derived from the phrase 'query'. Nowadays, this name is still relevant: many aspects of this disease remain unsolved and disputed. With new insights from the recent outbreak of Q fever in the Netherlands, we tried to fill some of the gaps in the knowledge about Q fever.

Part 1. Burden and magnitude of Q fever in the Netherlands
From 2007 on there has been a large outbreak of Q fever in the Netherlands, which subsided in 2010 only after interventions from the government in the veterinary sector.

In ***chapter 2*** we have estimated the acute Q fever-associated in-hospital mortality rate. Available information from six hospitals in the Q fever affected areas revealed 749 patients admitted with acute Q fever, of which nine patients (1%) died. The patients who succumbed from, or with, Q fever were of older age (age >65 years), were male, and had serious underlying diseases, which already raised the a-priori risk of death. This mortality rate is comparable to earlier studies in other countries. As there is no obligation for registration of hospitalized patients with acute Q fever and patients who died of acute Q fever, these numbers could even be overestimated. Moreover, in the beginning of the Q fever epidemic many clinicians were not aware of this disease entity and Polymerase Chain Reaction (PCR) as a diagnostic tool for acute Q fever was not introduced yet. This may have caused some acute Q fever cases to have been left undiagnosed. This study illustrates the low mortality rate of acute Q fever.

To assess whether early antibiotic treatment inhibits antibody responses after an acute Q fever episode, we have studied IgG antibody responses in symptomatic patients diagnosed and treated either before, or during the development of serologic response to *C. burnetii* in ***chapter 3.*** Serological profiles of 181 acute Q fever patients of whom 91 were diagnosed early (ED) (defined as negative IgM phase II with enzyme-linked immunosorbent assay (ELISA) and positive PCR) and 90 were diagnosed late (LD) (defined as positive/dubious IgM phase II with ELISA and positive immunofluorescence assay (IFA)), were analysed. Serologic profiles consisting of IgG phase I and II titers, were determined using IFA at three, six, and 12 months. At 12 months, 28.6% of ED patients and 19.5% of LD patients had high IgG antibody titers (defined as IgG phase II titers = 1:1024 and IgG phase I = 1:256), which was not statistically significant. In addition, we also found no significant differences in frequencies of IgG phase I and IgG phase II antibody titres at all follow-up for adequately and inadequately, and early- and late-treated patients. This study indicates that early diagnosis and antibiotic treatment of acute Q fever do not preclude development of the IgG antibody response.

In ***chapter 4***, we have estimated the magnitude of the Q fever outbreak in the Netherlands by extrapolating the prevalence of *C. burnetii* antibodies in two high-risk groups for development of chronic Q fever in 2010 and 2011. The 785 patients in these populations were all living in the catchment area of the Jeroen Bosch Hospital (JBH) in 's-Hertogenbosch, which is located in the centre of the epidemic area. Seropositivity for IgG phase II antibodies to *C. burnetii* was present in 10.7% of patients (95% confidence interval (CI) = 8.5–12.9%). Seroprevalence was not influenced by age, gender or area of residence. By extrapolation of these data, we estimated that 40,600 persons (95% CI = 32,200–48,900) in the JBH catchment area have been infected with *C. burnetii*. This figure by far exceeds the number of notified symptomatic acute Q fever patients and illustrates the magnitude of the past Dutch Q fever epidemic.

Part 2. Diagnosis and classification of chronic Q fever

Faced with a large Q fever epidemic in the Netherlands, the Dutch Q fever Consensus Group was constituted. First, a guideline for the diagnosis of acute Q fever was formulated (see introduction). However, after 2009 an increasing number of chronic Q fever patients was observed, with a significant amount of morbidity and mortality. Diagnosis of chronic Q fever proved to be complex and uniformity in diagnosis of chronic Q fever was lacking. Therefore, a review of the available literature, which was limited to case reports and mainly retrospective and descriptive analyses of a small number of patients, was performed. A proposal for a new guideline for the diagnosis of chronic Q fever was formulated, which combines clinical, radiological and microbiological factors. This guideline, in which chronic Q fever is categorized as proven, probable or possible, and the literature it is based on is described in ***chapter 5***. Proven chronic Q fever is classified as either positive *C. burnetii* PCR (or culture) in blood or tissue, in absence of an acute infection, or patients with phase I IgG = 1:800 or phase I IgG = 1024 (depending on in house IFA technique or commercial IFA technique, respectively), in combination with definite endocarditis according to the revised Duke criteria, or evident aneurysm or vascular graft infection on computed tomography (CT), positron emission tomography (PET)-CT, duplex ultrasound or magnetic resonance imaging (MRI). Long-term antibiotic treatment should always be initiated in case of proven chronic Q fever and frequent clinical and serological monitoring, at least three-monthly, is warranted. Surgical intervention should be considered in absence of favourable response to treatment. Probable chronic Q fever is diagnosed in those patients with phase I IgG = 1:1024 who have established risk factors for chronic Q fever, show echocardiographic abnormalities not meeting the revised Duke criteria, probable rare manifestations of Q fever (e.g. hepatitis, osteomyelitis) or signs of systemic inflammation. It is advised to discuss whether to start antibiotic treatment in a multidisciplinary team. Three-monthly clinical and microbiological follow-up, and radiographic imaging (echocardiogram, PET/CT) whenever clinical improvement stagnates or worsens, is advised. Possible chronic Q fever is diagnosed in patients with solely a phase I IgG = 1:1024, without any of the manifestations mentioned in the categories proven and probable, and largely reflects patients with no chronic Q fever at all, but only elevated antibodies against *C. burnetii*. Antibiotic treatment is not warranted. The follow-up regime is equal to patients with probable chronic Q fever.

Publication of this manuscript led to discussion in the international medical literature. Subsequently, Professor Raoult from France proposed another diagnostic guideline for chronic Q fever. In ***chapter 6*** a comparison is made between this diagnostic guideline and that of the Dutch Q fever Consensus Group. An important difference in the diagnostic criteria proposed by prof. Raoult and the Dutch Q Fever Consensus Group is the diagnostic value attributed to *C. burnetii* PCR positivity of blood samples, which is not considered proof of chronic infection by prof. Raoult. We showed that with prof. Raoult's criteria, approximately 30% of proven chronic Q fever cases would be missed, including at least four patients that eventually died due to chronic Q fever-related causes, and almost all probable and possible cases. Although specificity of the Dutch guideline might be lower, sensitivity is importantly higher.

In ***chapter 7*** we have studied the height of phase I IgG antibody titers at the time of positive PCR in blood, at diagnosis, and at peak levels in patients with proven, probable or possible chronic Q fever. Positive *C. burnetii* PCR on blood was seen in 65% of proven chronic Q fever cases, demonstrating the relative low sensitivity of PCR for the diagnosis of chronic Q fever. In contrast to previous reports, positive PCR was associated with high phase I IgG titers. In patients with proven chronic Q fever, phase I IgG titers were significantly higher compared to patients with probable and possible chronic Q fever. The positive predictive values (PPV) of phase I IgG

titers for proven chronic Q fever, when compared to possible chronic Q fever, at titers 1:1024, 1:2048, 1:4096, =1:8192, were 62%, 67%, 77%, >86%, respectively. On the other hand, sensitivity was 97.8%, 94.6%, 80.6% and <60%, respectively. These figures indicate that raising the cut-off titer for the diagnosis of chronic Q fever, would lead to an unacceptable number of missed chronic Q fever cases. Whereas, with the current cut-off of 1:1024, the number of chronic Q fever cases is overestimated.

Part 3. Chronic Q fever: Risk groups, morbidity and mortality

To reduce morbidity and mortality of chronic Q fever it is of importance to identify high-risk groups for the development of this manifestation. Knowledge on risk factors could be used in early case-finding, before complications occur, by screening of high-risk populations. In ***chapter 8*** a case-control study is presented, which aimed to identify and quantify risk factors for development of chronic Q fever after *C. burnetii* infection. Co-morbidity, cardiovascular risk factors, medication and demographic characteristics from patients with proven, probable or possible chronic Q fever (cases), were compared with patients who had acute Q fever in 2009, but did not develop chronic Q fever (controls). Previous valvular surgery, vascular prosthesis, aneurysms, renal insufficiency, and increasing age were identified as major risk factors for the development of chronic Q fever in this study. Remarkably, an association between (mild) non-surgical heart valve pathology and chronic Q fever was not found.

A previously defined high-risk group for chronic Q fever, namely patients with a history of cardiac valve surgery, was evaluated in ***chapter 9***. Patients with a history of cardiac valve surgery in the Q fever epidemic area were selected for microbiological screening, by means of analysis of phase I and II IgG against *C. burnetii*. If titers were above a defined cut-off level, *C. burnetii* PCR was performed. Chronic Q fever was diagnosed according to the consensus guideline of the Dutch Q Fever Consensus Group. We found a seroprevalence of *C. burnetii* antibodies of 20.4% in this population. Proven or probable chronic Q fever was identified in 7.8% of seropositive patients, which was remarkably lower than incidence figures in previous reports of this high-risk group. The risk for chronic Q fever seemed to be independent of type of valvular surgery, although numbers of patients with chronic Q fever were small.

In response to the rising number of chronic Q fever cases after 2009, and the many questions around this disease, a Dutch National Chronic Q Fever Database was implemented, to address these questions. All Dutch hospitals that treat chronic Q fever patients were actively approached to include patients in this database. In ***chapter 10*** we describe the characteristics and outcome of chronic Q fever patients included until five years after the start of the Q fever epidemic. We specifically focused on mortality of chronic Q fever. In total, 284 chronic Q fever patients were identified, of whom 151 (53.7%) had proven, 64 (22.5%) probable and 69 (24.3%) possible chronic Q fever. The majority of proven and probable chronic Q fever patients had a vascular focus of infection (56.7%), while endocarditis (34.9%) was less prevalent. Only 27.0% recalled an acute Q fever episode. In our population, mortality related to chronic Q fever was 13.0%: 9.3% among endocarditis patients and 18.0% among vascular chronic Q fever patients. Older age and presentation with vascular complications necessitating acute surgical intervention were the major risk factors for chronic Q fever mortality.

Part 4. Important examples of clinical manifestations of Q fever endocarditis

Endocarditis is one of the major manifestations of chronic Q fever. Nevertheless, it is not always recognized. Early treatment can prevent substantial morbidity and mortality. In this part we de-

scribe two manifestations of Q fever endocarditis, which may facilitate (early) case finding by clinicians.

Endocarditis due to a concomitant infection with *C. burnetii* and another causative agent has thus far been incidentally described in seven cases only. In ***chapter 11*** three other patients with endocarditis by *C. burnetii* concomitant with another bacterial pathogen are described.

Q fever endocarditis can present very subtle, with no or few symptoms. Cardiac valve infection with *C. burnetii* is difficult to differentiate from degenerative changes of cardiac valves on echocardiogram. In ***chapter 12*** we presented three cases of chronic Q fever, in which diagnosis was delayed until after cardiac valve surgery, warranting better surveillance for *C. burnetii* infection in case of cardiac valve surgery in Q fever endemic areas.

GENERAL DISCUSSION

Incidence of acute and chronic Q fever

During the Q fever epidemic in the Netherlands, over 4000 cases of acute Q fever were registered.[1] This is an underestimation of the total number of *C. burnetii* infections, due to a significant amount of asymptomatic infections. The percentage of asymptomatic human *C. burnetii* infections is much higher in our studies than the previously reported 50-60%.[2;3] In chapter 9, only 4.9% of seropositive patients with a history of heart valve surgery had an actual history of acute Q fever. Seroprevalence studies, described in chapter 4, and among blood donors4, demonstrate a tenfold higher number of *C. burnetii* infected individuals, than the 4000 cases reported by the Dutch governmental health institutes. Follow-up of a former screening in the general population in which the incidence of Q fever antibodies was 2.4% in 2006,5 in combination with analysis of notified acute Q fever cases in this population, could give more insight in the percentage of asymptomatic Q fever infections and the number of people potentially at risk of chronic Q fever.

In the literature it is stated that 1-5% of patients develop chronic Q fever after acute Q fever,6;7 although definitions of chronic Q fever were based on serologic results only. We have found a similar incidence in our studies for all chronic Q fever cases, including possible chronic Q fever. Proven and probable chronic Q fever cases had experienced an episode of acute Q fever less often, whereas most patients with possible chronic Q fever were identified in follow-up programmes after acute Q fever. In chapter 10 we demonstrated that the risk for development of chronic Q fever after a known acute Q fever episode is approximately 2.5% if all chronic Q fever cases are taken into account, and 1.5% if limited to proven and probable chronic Q fever cases. These incidence numbers were confirmed by van der Hoek et al, who demonstrated 1.6% progression to chronic Q fever in a group of 686 unselected patients diagnosed with acute Q fever in 2007.[8] Nevertheless, we have also demonstrated that the majority of patients with chronic Q fever did not recall an episode of acute Q fever reflective of the fact that the percentage of asymptomatic infections is much larger than previously reported. Therefore, the percentage of people who will develop chronic Q fever after primary *C. burnetii* infection, whether symptomatic or asymptomatic, is presumably much lower than 1%.

Risk factors for chronic Q fever development and follow-up regimes

In the light of a high percentage of asymptomatic individuals with a primary *C. burnetii* infection, there is a large population at risk for chronic Q fever, who do not benefit from current follow-up programs for patients with acute Q fever. Follow-up programs in the Netherlands consist of sero-

logical examinations for at least one year after the acute Q fever episode. There is controversy in the international medical literature, whether all patients with acute Q fever should be screened for heart valve defects by echocardiogram.[6;9;10] A Dutch study demonstrated no benefit of a screening echocardiogram for all acute Q fever patients.[9] Moreover, in chapter 8 risk factors for chronic Q fever were analysed, in which (mild) cardiac valvulopathy, in contrast to previous cardiac valvular surgery, was not identified as risk factor. Incidence of chronic Q fever in case of *C. burnetii* infection and cardiac valvulopathy was previously estimated at 40%, and even higher in case of heart valve prosthesis. However, our screening study among patients with a history of heart valve surgery, demonstrated a much lower chronic Q fever incidence of 7.9%. Another recent Dutch screening study in a Q fever epidemic area of high risk groups for chronic Q fever, including both patients with heart valve prosthesis, aortic aneurysms and vascular prosthesis showed that 19% of *C. burnetii* seropositive patients had chronic Q fever.[11] Importantly, both studies detected patients with chronic Q fever before occurrence of complications, with no or minor symptoms, and, in case of suspected endocarditis, no abnormalities on echocardiogram. Previously proposed high risk factors consist, besides pre-existing cardiac valvulopathy, of vascular grafts and aneurysms, immunosuppression and pregnancy.[7;12-16] Identified risk factors for chronic Q fever in this thesis (chapter 8) were previous cardiac valvular surgery, vascular prosthesis, aneurysms, renal insufficiency, and increasing age. An association between immunosuppression and chronic Q fever could not be confirmed in this study, although total numbers of patients with immunosuppression were very small. However, in the international medical literature concerning chronic Q fever, clear definition of immunosuppression and statistical empowerment proving an association with chronic Q fever is lacking.[13] Numbers were too small to reliably evaluate pregnancy as a risk factor for chronic Q fever. However, during the Dutch Q fever epidemic only one case of pregnancy-related chronic Q fever has been identified (see also chapter 10).[17] A novel finding was the association between mild renal insufficiency and chronic Q fever, which may be explained by the fact that renal insufficiency is associated with vascular disease. Increasing age also predisposed for the development of chronic Q fever, which was also illustrated in another Dutch study by van der Hoek et al.[8] Explanation probably lies in the increased prevalence of cardiovascular diseases and the decrease of cellular immunity during aging.[18;19] Age >60 appeared the best age cut-off above which the risk for chronic Q fever is increased significantly. Confirmation of the findings of this study and elucidation of the risk factors immunosuppression, pregnancy and even non-surgical cardiac valvulopathy is needed from future research.

It could be debated if the current follow-up regime for detection of chronic Q fever needs adjustment. The benefit of screening all patients after acute Q fever is relatively low (1-2% of patients develop chronic Q fever), while mainly patients with possible chronic Q fever are detected. The total costs of the current follow-up regimes during and after the Dutch Q fever epidemic are unknown, but need evaluation. More importantly, a large amount of patients is missed with follow-up regimes, as most chronic Q fever patients, especially proven and probable cases, did not recall an episode of acute Q fever. Results of the studies described in this thesis indicate that early case-finding of chronic Q fever patients in case of a Q fever epidemic may benefit more from screening programs for risk populations. The highest risks for chronic Q fever were observed in patients with a history of valvular surgery, aortic aneurysms and vascular prosthesis. Therefore, we would recommend serological screening for chronic Q fever in epidemic areas in these patient groups. Older patients (age >60) and patients with mild renal disease seem also at risk, probably because of previous unidentified cardiovascular disease. The risks of chronic Q fever for immunocompromised patients and pregnant women are until now unclear. Cardiac non-surgical valvulopathy

was previously indicated as an important risk factor for chronic Q fever (risk of up to 40%), but could not be identified as such in our study. For patient groups with age >60 years, mild renal disease, non-surgical cardiac valvulopathy, immunosuppression, or pregnancy, we would recommend thorough follow-up after acute Q fever. In light of the identified risk factors in this thesis, it seems to be more beneficial in case of acute Q fever, to perform a thorough medical examination and history taking for determination of the follow-up plan, instead of follow-up of all acute Q fever cases. Screening for risk factors with abdominal ultrasound, especially when vascular risk factors are present, may be more profitable than performing an echocardiogram to detect (minor) valvulopathy.

Manifestations of chronic Q fever

The most remarkable difference compared to reports from the international literature concerning chronic Q fever is the large number of vascular infections we have identified in the Netherlands. This manifestation is numerically at least of equal importance as endocarditis. In chapter 10 we found significantly more patients with a vascular focus of infection (56.7%) than patients with endocarditis (35.3%). This disparity with international observations might be caused by strain-specific differences and regional differences in hosts genetic background. Nevertheless, chronic Q fever vascular infections are increasingly described in literature from other countries in the last years.[13;20-23] Therefore, another explanation might be the increased awareness for this previously relatively unacknowledged manifestation among clinicians and microbiologists, especially in the Netherlands. Of concern is the fact that prognosis of vascular chronic Q fever is far worse than of Q fever endocarditis as was previously described in the international literature but also observed in our studies.[6;12] In chapter 10 we described that the majority of patients who probably or definitely died of chronic Q fever-related complications, had a vascular focus of infection (79%). As vascular chronic Q fever often presents with severe life threatening complications like acute aneurysms or prosthetic dysfunction, it is possible that patients with this manifestation were missed, because they succumbed before diagnosis of chronic Q fever could be made.

Diagnosis of chronic Q fever

The diagnostic criteria of chronic Q fever have recently led to debate in the international literature. A positive *C. burnetii* PCR, in the absence of signs of acute Q fever, proves chronic Q fever, but this method has low sensitivity in blood, which was also demonstrated in this thesis (chapter 7). In contrast to previous reports, we found a positive association between the height of phase I IgG titers and positivity of PCR on blood samples. Sensitivity of *C. burnetii* PCR on infected tissue is probably much higher, but definite proof is currently lacking mainly because the focus of infection of chronic Q fever is usually situated in difficult accessible locations like heart valves, aneurysms and vascular prosthesis. Until now, diagnosis of chronic Q fever relied mainly on serological examination with IFA as most commonly used tool. An elevated phase I IgG titre of >1:800, based on a French in-house IFA, or >1:1024, based on a commercially available IFA (Focus Diagnostics), has been used for the serological diagnosis of chronic Q fever.[8;24] In this thesis, we have demonstrated that high phase I IgG titers have a high positive predictive value (PPV) to differentiate proven chronic Q fever from possible chronic Q fever, especially if titers exceed 1:4096. Nevertheless, sensitivity of high phase I IgG titers is low: 60% in case of phase I IgG titres >1:4096. In contrast, phase I IgG titers between 1:1024 and 1:4096 have high sensitivity (98% to 81%), but a low PPV (62.2% to 76.5%). These results of this study emphasizes that serology is not a diagnostic tool on its own, but should be interpreted in relation with the clinical background. Uniformity

in diagnosis is important for uniform treatment and follow-up advice and adequate comparison of results of future research on chronic Q fever. The diagnostic guideline of the Dutch Q fever Consensus Group combines PCR, serology, clinical data and radiological data. In this guideline, suspected chronic Q fever cases are divided in three groups in decreasing likelihood of chronic Q fever: proven, probable and possible. In comparison with the recent guideline proposed by prof. Raoult, we have demonstrated that the guideline of the Dutch Q fever Consensus Group has better sensitivity and also takes more rare manifestations of chronic Q fever into account. Although part of the patients with probable chronic Q fever and most patients with possible chronic Q fever will eventually not have actual chronic Q fever, we do think that these patients should all be analysed for a chronic Q fever focus and should remain under close follow-up. We do not advocate treatment for all these patients.

The best way to solve the dispute about chronic Q fever diagnostic criteria is a more reliable strategy to establish this manifestation of Q fever. With the current diagnostic arsenal, consisting of PCR and serology, differentiation between past and chronic Q fever is insufficient. A gold standard for the diagnosis of chronic Q fever is lacking. Research into better chronic Q fever diagnostic criteria and methods are in progress, but more time is needed to evaluate these new developments.[25-29]

PERSPECTIVES AND FUTURE RESEARCH

The recent outbreak of Q fever in the Netherlands has brought about new perspectives on this complex disease. We found some important dissimilarities between findings from the international medical literature on the topic of Q fever, and findings obtained from the Dutch Q fever epidemic. There are three main reasons for the observed dissimilarities between international studies and studies presented in this thesis. First, until now, most research was based on work from a single reference laboratory and clinic for *C. burnetii* infection (Marseille, France). While this centre has performed important and solid work on the topic of Q fever, using data from a reference centre can cause selection bias. Second, strain specific differences, and differences in host genetic background, could lead to distinct clinical presentations and risk profiles.[30] Third, former studies often analyzed cases from both endemic and epidemic origin, which were identified over a considerable time span, while the Dutch studies analyzed cases from a single four-year-lasting epidemic.

To determine better follow-up strategies after a Q fever outbreak, there is a need for clarification of the risk for chronic Q fever after *C. burnetii* infection. Although it is evident that patients with a history of valvular surgery, aortic aneurysms and vascular prosthesis possess a high-risk, this needs to be elucidated for other potential risk-factors like non-surgical cardiac valvulopathy, immunosuppression, (mild) renal insufficiency and pregnancy. Besides, it is important to determine the number of patients with asymptomatic primary infection, as we have proven that they are also at risk for chronic Q fever. Data from the Dutch epidemic indicate that the number of asymptomatic primary infections is far beyond the formerly stated 60%. Screening in the general population and comparing incidence of Q fever antibodies before and after the Dutch Q fever epidemic in combination with analysis of notified acute Q fever cases in this population, could give more insight in the percentage of asymptomatic Q fever infections. Moreover, with these data a new case-control study, as described in chapter 8 could be performed, now also including asymptomatic primary *C. burnetii* infections.

We have demonstrated that chronic Q fever still has a high mortality, especially in case of vascular infection. Early case-finding, by targeted screening in risk-groups, could improve prognosis. On the other hand, best treatment strategies are not fully elucidated yet. Although not subject of this thesis, in daily practice the antibiotic regime for chronic Q fever, consisting of doxycycline and hydroxychloroquine, has many side-effects and efficacy is unclear. Also, the role of surgical intervention in case of chronic Q fever needs clarification. The Dutch Chronic Q Fever Database could provide more answers about this topic in the future. Nevertheless, as most research on Q fever is descriptive and retrospective from nature, there is also a need for randomized trails, especially for treatment regimes.

CONCLUSION

This thesis illustrates that Q fever, and in particular chronic Q fever, is a complex disease with diverse manifestations and still many queries. We have specifically focused on long-term sequels of *C. burnetii* infection. The most important manifestations of chronic Q fever in the Netherlands are infections of aortic aneurysms, vascular prosthesis, and, to a slightly lesser extent, cardiac valves. Major risk factors for development of chronic Q fever are, correspondingly, aortic aneurysms, vascular prosthesis and history of valve surgery. Other risk factors, including increasing age, mild renal disease, non-surgical cardiac valvulopathy, immunosuppression, and pregnancy, should also be taken into account, but need further evaluation. Early case-finding in epidemic areas could diminish morbidity and mortality of chronic Q fever, for which we would like to recommend targeted screening for risk groups. Diagnosis of chronic Q fever is debated, but until further research offers better diagnostic tools, we feel that the diagnostic guideline of the Dutch Q fever Consensus Group is most accurate.

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Dutch summary

Samenvatting

SAMENVATTING

In ***hoofdstuk 1*** worden de doelen en achtergronden van dit proefschrift beschreven. Het doel van dit promotieonderzoek is om de gevolgen van de Q-koorts uitbraak in Nederland in kaart te brengen. Hierbij is vooral gefocust op chronische Q-koorts.

Q-koorts, welke wordt veroorzaakt door de intracellulaire bacterie *Cociella burnetii*, is een zoönose, wat wil zeggen dat de ziekte van dier op mens overdraagbaar is. *C. burnetii* heeft zijn belangrijkste reservoir in geiten en schapen. Humane Q-koorts kent zowel acute als chronische manifestaties. Acute Q-koorts presenteert zich doorgaans als een zelflimiterend, griepachtig beeld, met daarbij vaak aanwijzingnen voor een longontsteking. Soms is er ook sprake van een hepatitis. Ongeveer 50-60% van de mensen die besmet raken met *C. burnetii* heeft geen initiële klachten na besmetting. Chronische Q-koorts treedt op bij 1-5% van alle besmettingen met *C. burnetii* en kan zich jaren na de eerste besmetting presenteren. De belangrijkste verschijningsvormen zijn hartklepontsteking (endocarditis), infecties van aneurysmata of vaatprothesen en zwangerschap gerelateerde infecties (placentitis). Osteomyelitis, hepatitis en meningitis zijn zeldzamere verschijningsvormen. In geval van chronische Q-koorts is er een indicatie voor langdurige antibiotica behandeling (18-24 maanden). Indien onbehandeld, zijn de mortaliteit en morbiditeit van chronische Q-koorts hoog.

Van 2007-2010 was er een unieke, grote uitbraak van Q-koorts in (Zuidoost) Nederland, waarbij bij ruim 4000 patiënten acute Q-koorts is gediagnosticeerd. Daarnaast is een nog steeds groeiend aantal patiënten met chronische Q-koorts.

Deel 1. Ziektelast en grootte van de Q-koorts uitbraak in Nederland

In ***hoofdstuk 2*** is een schatting gemaakt van de acute Q-koorts-geassocieerde mortaliteit in Nederlandse ziekenhuizen tijdens de recente Q-koorts uitbraak. Deze studie illustreert de lage sterfte aan acute Q-koorts. Met de beschikbare informatie van zes ziekenhuizen in het Q-koorts getroffen gebied lieten wij zien dat er 749 patiënten opgenomen waren met acute Q-koorts, waarvan er negen patiënten (1%) overleden. De patiënten die overleden door, of met, acute Q-koorts hadden merendeels een oudere leeftijd (leeftijd >65 jaar), waren van het mannelijke geslacht, en hadden ernstig onderliggend lijden, wat reeds de a-priori kans op overlijden vergrootte. Het acute Q-koorts sterftecijfer van 1% is vergelijkbaar met eerdere studies in andere landen. Aangezien zowel de registratie van gehospitaliseerde patiënten met acute Q-koorts als sterfgevallen aan acute Q-koorts niet meldingsplichtig zijn, zijn deze cijfers slechts een schatting. Bovendien waren in het begin van de Q-koorts epidemie veel artsen zich niet bewust van deze ziekte en was polymerase chain reaction (PCR) als diagnostisch hulpmiddel voor acute Q-koorts nog niet in gebruik. Dit kan ertoe geleid hebben dat sommige acute Q-koorts gevallen niet gediagnosticeerd zijn.

In ***hoofdstuk 3*** onderzochten we of, in geval van acute Q-koorts, een vroege diagnose en start met antibiotica invloed hebben op de ontwikkeling van IgG antilichamen in het jaar na de infectie. Hierbij hebben we gekeken naar symptomatische acute Q-koorts patiënten die gediagnosticeerd en behandeld werden, hetzij vóór, of tijdens, de ontwikkeling van een serologische respons op *C. burnetii*. De serologische profielen van 181 acute Q-koorts patiënten werden geanalyseerd, van wie er 91 vroeg werden gediagnosticeerd (ED; gedefinieerd als negatieve IgM fase II met enzymelinked immunosorbent assay (ELISA) en een positieve PCR) en 90 laat werden gediagnosticeerd (LD; gedefinieerd als positief / twijfelachtig IgM fase II met ELISA en positieve immuunfluorescentie assay (IFA)). De serologische profielen bestaande uit IgG fase I en II titers werden drie, zes

en 12 maanden na diagnose bepaald met IFA. Na 12 maanden, had 28,6% van de ED patiënten en 19,5% van de LD patiënten hoge IgG antilichaam titers (gedefinieerd als IgG fase II titers = 1:1024 en IgG fase I = 1:256). Dit verschil was niet statistisch significant. Daarnaast vonden we ook geen significante verschillen in de IgG fase I en fase II-IgG antistoftiters bij de follow-up voor zowel de adequaat en niet-adequaat behandelde patiënten, als de vroeg en laat behandelde patiënten. Deze studie geeft aan dat vroege diagnose en behandeling met antibiotica van acute Q-koorts niet de ontwikkeling van de IgG-antilichaam respons vermindert.

In ***hoofdstuk 4*** hebben we een schatting gemaakt van de omvang van de Q-koorts uitbraak in Nederland. Hierbij zijn de data geëxtrapoleerd van twee screeningsstudies in 2010 en 2011 naar chronische Q-koorts onder hoogrisicopatiënten: patiënten met een hartklepoperatie in de voorgeschiedenis en patiënten met een aneurysma van de aorta of vasculaire chirurgie van de aorta en zijn vertakkingen. In totaal werden 785 patiënten gescreend in het verzorgingsgebied van het Jeroen Bosch Ziekenhuis (JBZ) in 's-Hertogenbosch, dat zich in het epicentrum van de recente Q-koorts uitbraak bevond. Fase II IgG tegen *C. burnetii* was aanwezig bij 10,7% van de gescreende patiënten (95% betrouwbaarheidsinterval (BI) = 8,5-12,9%). De seroprevalentie werd niet beïnvloed door leeftijd, geslacht of woonomgeving. Door middel van extrapolatie van deze gegevens schatten we dat 40.600 personen (95% BI = 32,200-48,900) in het JBZ verzorgingsgebied zijn besmet met *C. burnetii*. Dit aantal is veel groter dan het aantal gemelde symptomatische acute Q-koorts patiënten in dit gebied en illustreert de omvang van de Nederlandse Q-koorts uitbraak.

Deel 2: Diagnose en classificatie van chronische Q-koorts

Na 2009 werden steeds meer chronische Q-koorts patiënten gezien, met daarbij aanzienlijke morbiditeit en mortaliteit. De diagnose van chronische Q-koorts bleek ingewikkeld en uniformiteit in de diagnostiek van chronische Q-koorts ontbrak. In ***hoofdstuk 5*** wordt een review van de internationale literatuur over de diagnostiek van chronische Q-koorts besproken. Aan de hand van dit review en de ervaringen tijdens de recente Nederlandse Q-koorts uitbraak, werd een nieuwe diagnostische consensus richtlijn gepresenteerd, welke in samenspraak met de Nederlandse Consensusgroep Diagnostiek Q-koorts is opgesteld. Deze nieuwe richtlijn combineert klinische, radiologische en microbiologische factoren. Patiënten worden ingedeeld in bewezen (proven), waarschijnlijke (probable) en mogelijke (possible) chronische Q-koorts. De diagnose bewezen chronische Q-koorts wordt gesteld indien de *C. burnetii* PCR (of kweek) positief is in bloed of weefsel, in afwezigheid van een acute infectie, óf in geval van fase I IgG = 1:800 of fase I IgG = 1024 (afhankelijk van welke IFA techniek gebruikt wordt), in combinatie met één of meerdere major herziene Duke criteria voor endocarditis, of een evidente infectie van een aneurysma of vasculair transplantaat op computertomografie (CT), positron emissie tomografie (PET)-CT, duplex echografie of magnetic resonance imaging (MRI). Langdurige behandeling met antibiotica wordt geadviseerd in geval van bewezen chronische Q-koorts en daarbij frequente klinische en serologische controle, tenminste elke drie maanden. Eventueel chirurgische ingrijpen dient te worden overwogen in afwezigheid van een gunstige respons op de behandeling. De diagnose waarschijnlijke chronische Q-koorts wordt gesteld bij patiënten met fase I IgG = 1:1024, die risicofactoren hebben voor chronische Q-koorts, die echocardiografische afwijkingen hebben welke niet voldoen aan de major herziene Duke criteria, die zeldzame manifestaties van Q-koorts hebben (bijvoorbeeld hepatitis, osteomyelitis), of tekenen hebben van een systemische ontsteking. Het wel of niet starten van behandeling met antibiotica moet overlegd worden in een multidisciplinair team met ervaring op het gebied van Q-koorts. Drie-maandelijkse klinische en microbiologische follow-up, en eventueel radiografische beeldvorming (echocardiogram, PET / CT) wanneer kli-

nische verbetering stagneert, wordt geadviseerd. Mogelijke chronische Q-koorts wordt gediagnosticeerd bij patiënten met uitsluitend een fase I IgG = 1:1024, zonder één van de manifestaties in de categorieën bewezen en waarschijnlijke chronische Q-koorts, en weerspiegelt grotendeels patiënten zonder chronische Q-koorts, maar met alleen verhoogde antistoffen tegen *C. burnetii*. Behandeling met antibiotica wordt niet aangeraden. De follow-up is gelijk aan patiënten met een waarschijnlijke chronische Q-koorts.

Dit review leidde na publicatie tot discussie in de internationale literatuur. Professor Didier Raoult, expert op het gebied van Q-koorts, formuleerde vervolgens een eigen richtlijn voor de diagnostiek van chronische Q-koorts. In ***hoofdstuk 6*** wordt een vergelijking tussen de richtlijn van prof. Raoult en de richtlijn van de Nederlandse Consensusgroep Diagnostiek Q-koorts gemaakt. Een belangrijk verschil in de diagnostische criteria voorgesteld door prof. Raoult en de Nederlandse Consensusgroep Diagnostiek Q-koorts, is de diagnostische waarde die wordt toegekend aan een positieve *C. burnetii* PCR in bloedmonsters (in afwezigheid van tekenen van acute Q-koorts). Dit wordt door prof. Raoult, niet beschouwd als bewijs voor een chronische Q-koorts. We toonden aan dat met de criteria opgesteld door prof. Raoult ongeveer 30% van de bewezen chronische Q-koorts gevallen zou worden gemist. Daarbij waren vier patiënten die uiteindelijk stierven als gevolg van chronische Q-koorts-gerelateerde complicaties. Bovendien zouden vrijwel alle waarschijnlijk en mogelijk chronische Q-koorts patiënten ook niet gediagnosticeerd worden. Hoewel de specificiteit van de Nederlandse richtlijn wellicht lager is, is de sensitiviteit beduidend hoger.

In ***hoofdstuk 7*** wordt gekeken naar verschil in antistoftiters, door middel van IFA, tussen bewezen, waarschijnlijke en mogelijke chronische Q-koorts patiënten én patiënten met en zonder positieve *C. burnetii* PCR. Er werd daarbij gekeken naar de titers bij stellen van de diagnose chronische Q-koorts, de hoogste gemeten titers en de titers ten tijde van een PCR-bepaling in bloed. Een positieve *C. burnetii* PCR op bloed werd gezien in 65% van de bewezen chronische Q-koorts patiënten. In tegenstelling tot eerdere publicaties was een positieve PCR geassocieerd met hoge fase I IgG titers. Bij patiënten met bewezen chronische Q-koorts, waren de fase I IgG titers significant hoger in vergelijking met patiënten met een waarschijnlijke en mogelijke chronische Q-koorts. De positief voorspellende waarden van fase I IgG titers voor bewezen chronische Q-koorts, in vergelijking met mogelijke chronische Q-koorts, bij titers 1:1024, 1:2048, 1:4096, = 1:8192, waren respectievelijk 62%, 67 %, 77%, >86%. Anderzijds, bedroeg de sensitiviteit respectievelijk 98%, 95%, 81% en <60%. Deze uitkomsten geven aan dat het verhogen van de afkap-titer voor de diagnose van chronische Q-koorts zou leiden tot een onaanvaardbaar aantal gemiste chronische Q-koorts patienten. Anderzijds, wordt met de huidige afkap-titer van 1:1024, het aantal chronische Q-koorts gevallen overschat.

Deel 3: Chronische Q-koorts; risicogroepen, morbiditeit en mortaliteit

Het is van belang om hoog-riscogroepen voor de ontwikkeling van chronische Q-koorts te identificeren. Deze informatie kan gebruikt worden voor vroege opsporing en behandeling van chronische Q-koorts patiënten om daarmee de morbiditeit en mortaliteit van deze ziekte te verminderen. In ***hoofdstuk 8*** wordt een case-control studie naar risicofactoren voor het ontwikkelen van chronische Q-koorts besproken. In dit hoofdstuk wordt een groep chronische Q-koorts patiënten (cases) vergeleken wordt met een groep patiënten die een acute Q-koorts heeft doorgemaakt zonder dat zich daarna chronische Q-koorts ontwikkelde (controls), met het doel risicofactoren voor de ontwikkeling van chronische Q-koorts te identificeren en te kwantificeren. Er werd gekeken naar comorbiditeit, cardiovasculaire risicofactoren, medicatie en demografische kenmerken van

patiënten. Een hartklepoperatie in de voorgeschiedenis, vaatprothesen, aneurysmata, nierinsufficiëntie, en toenemende leeftijd werden geïdentificeerd als de belangrijkste risicofactoren voor het ontwikkelen van chronische Q-koorts in deze studie. Opmerkelijk genoeg, is een verband tussen (milde) niet-chirurgische hartkleppathologie en chronische Q-koorts niet gevonden.

In ***hoofdstuk 9*** wordt een screeningsstudie beschreven naar de prevalentie van *C. burnetii* antistoffen en chronische Q-koorts in patiënten met een hartklepoperatie in de voorgeschiedenis. Deze patiënten worden in de literatuur aangeduid als een hoogrisico groep voor het ontwikkelen van chronische Q-koorts. Tevens wordt gekeken of het type hartklepoperatie en de locatie van de nieuwe hartklep van invloed is op het risico op chronische Q-koorts. Patiënten met een hartklepoperatie in de voorgeschiedenis in de regio van het JBZ werden gescreend door het bepalen van fase I en II IgG tegen *C. burnetii*. Indien geïndiceerd, werd er een *C. burnetii* PCR uitgevoerd. In deze populatie vonden wij een seroprevalentie van *C. burnetii* antilichamen van 20,4%. Bewezen of waarschijnlijke chronische Q-koorts werd vastgesteld in 7,8% van de seropositieve patiënten, wat opmerkelijk lager was dan beschreven in de internationale literatuur. Hoewel het gevonden aantal patiënten met chronische Q-koorts klein was, leek het risico op chronische Q-koorts niet afhankelijk van het type hartklepoperatie.

In samenwerking met ziekenhuizen uit de regio's waarin Q-koorts het meest voorkomt, is een database opgezet waarin alle chronische Q-koorts patiënten in Nederland opgenomen zullen worden. ***Hoofdstuk 10*** geeft een beschrijving van alle chronische Q-koorts patiënten die geïncludeerd zijn in de Nationale Chronische Q-koorts Database, vijf jaar na de start van de Q-koorts epidemie. Speciale focus ligt hierbij op de mortaliteit van chronische Q-koorts. In totaal werden 284 chronische Q-koorts patiënten geïdentificeerd, van wie er 151 (53,7%) bewezen chronische Q-koorts, 64 (22,5%) waarschijnlijk chronische Q-koorts en 69 (24,3%) mogelijk chronische Q-koorts hadden. De meerderheid van de bewezen en waarschijnlijke chronische Q-koorts patiënten had een vasculair infectiefocus (56,7%), terwijl endocarditis minder voorkwam (34,9%). Een acute Q-koorts episode werd herinnerd door 27,0% van de patiënten. In de bewezen en waarschijnlijke chronische Q-koorts groep, was de sterfte ten gevolge van chronische Q-koorts 13,0%: 9,3% bij endocarditis patiënten en 18,0% bij vasculaire chronische Q-koorts patiënten. Oudere leeftijd en presentatie met vasculaire complicaties waarvoor acuut chirurgisch ingrijpen noodzakelijk was, waren de belangrijkste risicofactoren voor sterfte door chronische Q-koorts.

Deel 4 : Belangrijke klinische manifestaties van Q-koorts endocarditis

Endocarditis is een van de belangrijkste manifestaties van chronische Q-koorts, maar wordt niet altijd onderkend. Vroege diagnose en behandeling kan de morbiditeit en mortaliteit reduceren. Om vroegtijdige herkenning door clinici te bevorderen, beschrijven we in dit deel enkele bijzondere manifestaties van Q-koorts endocarditis.

In ***hoofdstuk 11*** wordt een drietal casus beschreven met een "dual pathogen endodocarditis", waarbij de endocarditis werd veroorzaakt door een tweetal verwekkers waarvan *C. burnetii* er één was.

Hoofdstuk 12 geeft een drietal casus weer waarin de diagnose chronische Q-koorts endocarditis pas gesteld werd nadat er een operatieve correctie had plaatsgevonden van een hartklepafwijking, waarbij preoperatief de Q-koorts status niet was geëvalueerd.

In ***hoofdstuk 13*** worden de resultaten van de vorige hoofdstukken samengevat en worden daaruit conclusies getrokken en vervolgens aanbevelingen gedaan.

CONCLUSIE

Dit proefschrift toont aan dat Q-koorts, en in het bijzonder chronische Q-koorts, een complexe ziekte is met diverse manifestaties waarover nog vele vragen bestaan. De belangrijkste manifestaties van chronische Q-koorts in Nederland zijn infecties van aneurysmata en vaatprothesen, en in iets mindere mate, endocarditis. Belangrijke risicofactoren voor het ontwikkelen van chronische Q-koorts zijn dan ook aneurysmata, vaatprothesen en een voorgeschiedenis met hartklepchirurgie. Andere risicofactoren, zoals hogere leeftijd, milde nierziekte, hartklepaandoening (zonder hartklepchirurgie), immunosuppressie en zwangerschap, lijken ook geassocieerd te zijn met chronische Q-koorts, maar dat zou in toekomstig onderzoek nog verder geëvalueerd moeten worden. Omdat vroege opsporing van chronische Q-koorts in epidemische gebieden morbiditeit en mortaliteit van chronische Q-koorts zou kunnen verminderen, bevelen we gerichte screening aan voor risicogroepen. Hoewel er in de internationale literatuur nog geen overeenstemming is over de diagnostische strategie voor het vaststellen van chronische Q-koorts, denken we dat de diagnostische richtlijn van de Nederlandse Consensus Groep Diagnostiek Q-koorts het meest adequaat is. Toekomstig onderzoek op het gebied van chronische Q-koorts biedt hier hopelijk meer duidelijkheid over.

List of publications

LIST OF PUBLICATIONS

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Curriculum Vitae

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Linda Kampschreur was born in Beek (gemeente Bergh) on February 6th, 1980, and grew up in Beek and Groessen, The Netherlands. She graduated from secondary school (Liemers College, Zevenaar) in 1998. She performed her medical training at the Radboud University of Nijmegen, from which she graduated in 2005. During her study she performed a research project at the Universidad Mayor de San Simon (UMSS), Cochabamba, Bolivia entitled 'Water & Health: A need assessment in a low income area of Cochabamba Bolivia' to gain insight in infectious diseases in a poor area of Cochabamba with shortage of water supply. In December 2005, she her medical career at the Department of Internal Medicine of hospital 'de Gelderse Vallei' in Ede, and in September 2006 she started the specialization of Internal Medicine at the Radboud University Nijmegen Medical Center in Nijmegen. She was affiliated to the Jeroen Bosch Hospital, 's Hertogenbosch from September 2006 until September 2009. During that time Q fever became epidemic in the Southeast of the Netherlands, which was importantly concentrated in the area of the Jeroen Bosch Hospital. At this time her interest in this topic was raised. From September 2009 until January 2010, she continued her residency at the Radboud University Nijmegen Medical Center in Nijmegen. She switched her residency to the University Medical Center Utrecht in Utrecht at January 2010. From March 2010 on, she started to work, one day a week, at the Q fever outpatient clinic of the Jeroen Bosch Hospital. In September 2010 she interrupted her residency for a period of two years, to work as a research-resident at the Department of Internal Medicine and the Department of Medical Microbiology and Infection Control of the Jeroen Bosch Hospital, and the Department of Internal Medicine and Infectious Diseases of the University Medical Center Utrecht, under supervision of prof. dr. A.I.M. Hoepelman, dr. P.C. Wever and dr. J.J. Oosterheert. From December 2012 on, she will start her Infectious Diseases fellowship? under supervision of prof. dr. A.I.M. Hoepelman at the University Medical Center Utrecht.