The Dutch human Q fever outbreak: exploratory clinical studies

Gijsbertus Joseph Marie Limonard

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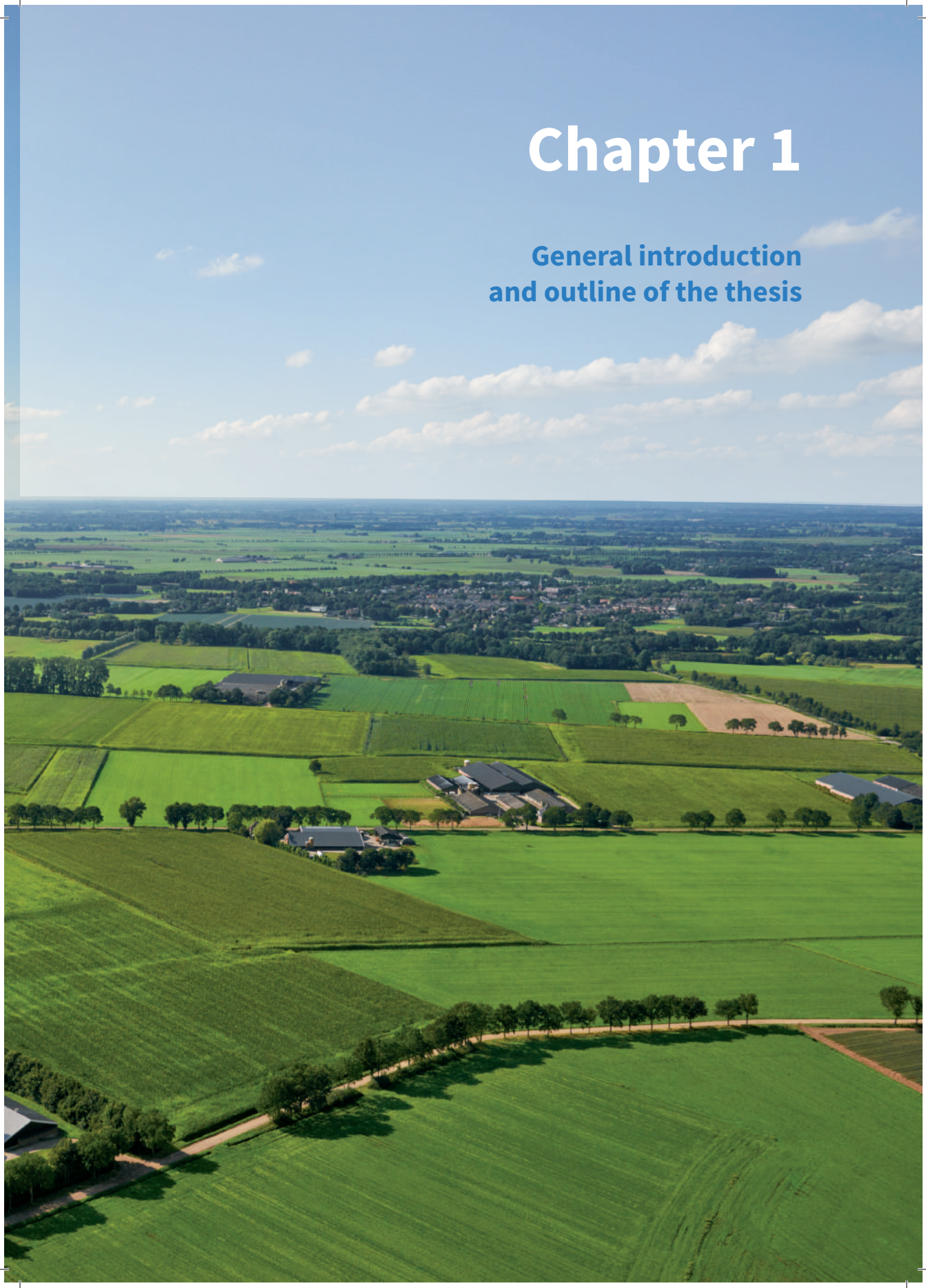
Where are the songs of Spring? Aye, where are they?
Think not of them. Thou hast thy music too –

John Keats – To Autumn

Contents

Chapter 1

General introduction and outline of the thesis



General introduction

Coxiella burnetii emerged as a human pathogen in the Netherlands in May 2007 [1]. The small, Gram-negative intracellular bacterium has its natural reservoir in small ruminants (goats, sheep, cattle) and can cause acute Q fever in humans; a mostly self limiting febrile illness, presenting as a flulike syndrome, pneumonia or hepatitis [2,3]. Long-term sequelae of the acute disease are progression to chronic infection (mostly as endocarditis or infection of a vascular aneurysm), and the post Q fever fatigue syndrome (QFS); a state of prolonged symptomatology (especially fatigue) that can last for years, associated with a marked sustained decreased in health status [3].

The first Dutch human Q fever outbreak in 2007 (168 cases) heralded a recurring and expanding annual seasonal outbreak pattern (figure 1) for 2008 (1000 cases) and 2009 (2354 cases), which were retrospectively linked to Q fever abortion waves on dairy goats and sheep farms farms starting in 2005 [4].

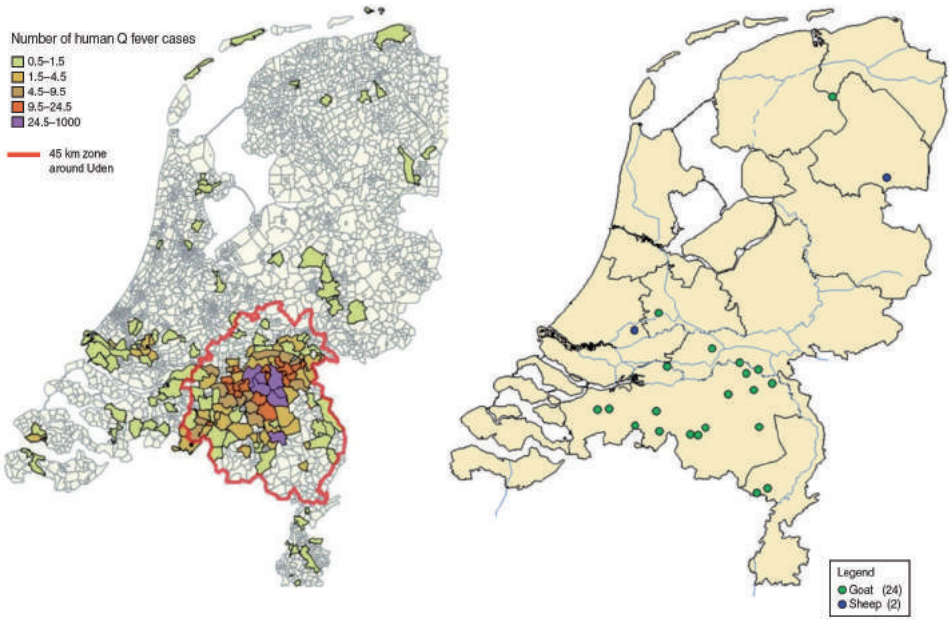


Figure 1. Map of the Netherlands. Left: Number of human cases in 2007 and 2008. The red line shows the dairy goat and dairy sheep voluntary vaccination area in 2008. Right: Dairy goat farms (green) [24] and dairy sheep farms (blue) [2] with Q fever abortion history between 2005 and 2008 (adapted from [4]).

At the outset of the epidemic, there were no validated protocols regarding diagnosis and follow-up of acute Q fever in the Netherlands [5]. Importantly, there was a lack of data on dynamics of the *C. burnetii* specific antibody response following acute Q fever and in case of chronic disease, as measured by comercially available kits. This posed a major challenge for clinicians and other health care workers in managing acute disease and preventing long-

term Q fever sequelae in the early stages of the epidemic [5,6].
After institution of drastic control measures including the culling of pregnant goats, the epidemic abated (figure 2) (504 cases in 2010, 81 cases in 2011) [5]. As of 2011, the Q fever disease burden amounted to more than 4000 notified human acute Q fever cases and an additional 44000 unnotified cases, as inferred from sero-epidemiological surveys [7,8].

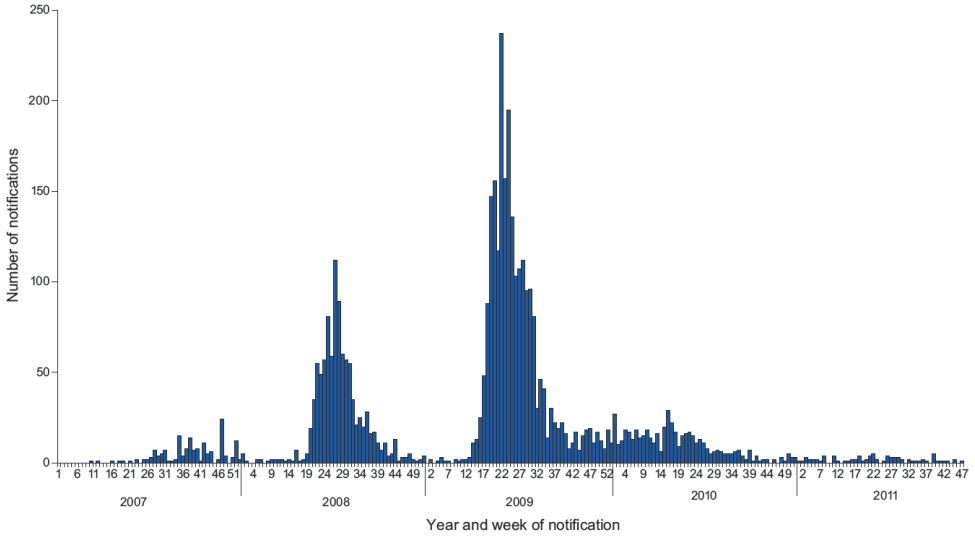


Figure 2. Acute Q fever notifications, The Netherlands, 1 January (week 1) 2007 – 30 november (week 48) 2011. (source: www.rivm.nl)

By the time the acute Q fever epidemic declined, the long-term effects of the disease became increasingly apparent. Research focus shifted from detection and management of acute disease to diagnosis and management of chronic Q fever and QFS [9].
This introduction provides background information on *C. burnetii* as the causative agent of Q fever, Q fever disease manifestations including its long-term sequelae, the impact and scope of the Dutch Q fever outbreak 2007-2011 and a detailed description of the aims and outline of this thesis.

History of Q fever

Edward Holbrook Derrick described the febrile illness he encountered in abattoir workers in Queensland, Australia in 1937 as Q [for 'query'] fever, "until fuller knowledge should allow a better name" [10]. Despite elucidation of the causative agent, the animal reservoir and route of transmission shortly thereafter, the name Q fever has stuck. Suggestions of 'abbatoir fever' and 'Queensland rickettsial fever' were not favored, probably to avoid negative associations with either the meat cattle industry or the Australian state of Queensland [11]. In addition, researchers possibly just like to quote the catchy name of the disease in their article titles [3].

Shortly after Derrick's description of the disease, Frank Macfarlane Burnet (Australia) isolated the causative bacterium in humans [12]. Almost simultaneously, Herald Rea Cox (USA) isolated the same microorganism in ticks [13]. They showed that the etiological agent of Q fever had both viral and rickettsial properties. Therefore the initial name of the Q fever agent was *Rickettsia burnetii.* Later, a new genus named *Coxiella* was put forward, and the Q fever agent was renamed *Coxiella burnetii,* eponominously honouring both scientists who isolated the organism, but in the process leaving the disease's discoverer's name unmentioned [14].

The pathogen Coxiella burnetii

Coxiella burnetti is a small, pleomorphic, Gram-stain negative, intracellular coccobacillus ref raoult, parker. Sequencing of the *C. burnetii* 16S rRNA and genome has established a high degree of homology with *Legionella pneumophila*; both bacteria belong to the gamma division of proteobacteria [3].

Morphologically, *C. burnetii* has a unique lifecycle in which 2 distinct forms can be identified, both with different densities and antigenic properties (figure 3). The 'large cell variant' (LCV) is metabolically active and is the intracellularly living form of *C. burnetii.* The extracellular 'small cell variant' (SCV) is metabolically inactive and can withstand intense environmental physical conditions and exposure to chemical agents, allowing prolonged survival in the environment of *C. burnetii* in this particular form [2,3].

In addition to these distinct morphological forms, *C. burnetii* can undergo phase variation into two distinct antigenic forms known as phase I and phase II. Antigenic phase II is less virulent and has marked differences in lipopolysaccharide (LPS) compared to the highly infectious wild-type form; antigenic phase I [2,3]. Phase I *C. burnetii* are isolated from specimens from infected humans. Phase II organisms can only be isolated in the laboratory following serial passage in fertilised eggs or tissue cultures. Morphologically, these two forms are indistinguishable when observed under a microscope. The antigenic phase shift is reflected in the host's antibody response to infection with *C. burnetii* and as such is used as a diagnostic tool [2,3]. Contrary to expectation, however, the antibody response in acute disease is mainly directed against phase II organisms whereas in chronic disease antibody titres against phase I organisms predominate [3] .

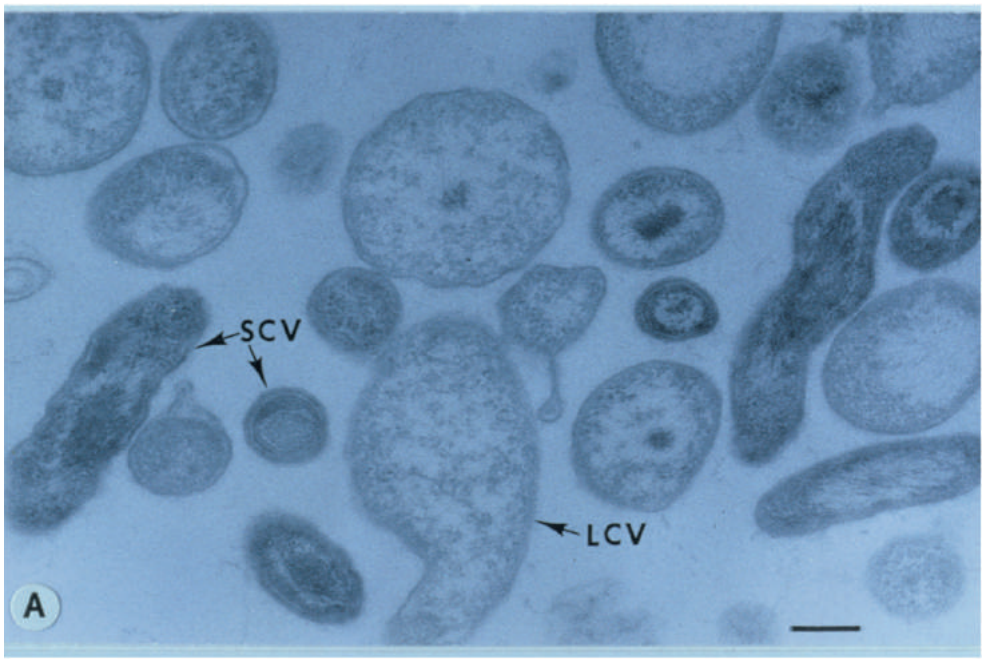


Figure 3. electron microscope image of C. burnetii showing the LCV and SCV forms (presented at the Seminar on Q fever in Cambridge, 26th of July 2008, courtesy of prof. BP Marmion).

Human infection

Q fever is a zoonosis with a widespread global distribution, notable exceptions being New Zealand and the continent of Antarctica [3]. The main animal reservoir of *C. burnetii* is small ruminants; goats, sheep and cattle, but also outbreaks related to pets and even wildlife have been described [2, 15]. Infected mammals shed large quantities of *C. burnetii* organisms in milk, urine, faeces and the products of parturition [2,3]. In the environment, the SCV form of *C. burnetii* can survive outside of the host for prolonged periods of time. *C. burnetii* contaminated dust particles particles can be taken up in aerosols, become airborn and carried by the wind, spread out over extensive geographical areas spanning kilometers [16]. Human infection mainly occurs by way of inhalation of contaminated dust or aerosols, rarely by direct contact with animal birth products or ingestion of infected animal dairy products such as milk or cheese [2,3]. Human-to-human transmission is extremely rare, but Q fever has been reported to follow breast milk feeding, sexual intercourse, bone marrow transplant and blood transfusion [2,17,18]. There are 2 earlier reports on respiratory spread after autopsies, and 1 case of infection of an obstetrician attending an infected pregnant woman, whose prematurely aborted fetus was also infected by vertical transmission of *C. burnetii* [19].

The host's immune response to C. burnetii

As is the case for other intracellular pathogens, both cell-mediated immunity and humoral immune mechanisms play an important role in the host's defence against *C. burnetii* infection.

T-cells and their associated cytokines, interferon-gamma being the most potent, have a pivotal role in countering primary *C. burnetii* infection and subsequent clearance or control of the intracellular pathogen [20]. The host's humoral immune response is characterised by antibody formation against both phase II and phase I antigens due to the antigenic phase variation of *C. burnetii* earlier described in this chapter.

Once the host has inhaled the contaminated aerosols, *C. burnetii* targets resting alveolar macrophages and other mononuclear phagocytes. After being engulfed and phagocytised by these cells, *C. burnetii* survives in a slightly acidic phagosome (pH 4,5) [21,22].

In primary infection, these *C. burnetii* laden phagosomes fuse with lysosomes to form phagolysosomes. Subsequently infected host cells undergo apoptosis, thereby controlling the infection. This process of phagolysosome fusion and apoptosis is very likely to be controlled by a T cell-mediated immune response, requiring interferon-gamma (IFN-gamma) [22,23].

In chronic Q fever patients, this IFN-gamma-mediated microbicidal capacity of mono- and macrophages is inhibited by over-production of inhibitory cytokines such as IL-10 [23].

In patients with post Q fever fatigue syndrome, there is some evidence to suggest an enhanced cell mediated immune response contributing to or causing the protracted symptoms. It has been put forward that this dysfunctional immune reponse is triggered by a persistence of *C. burnetii* specific antigens in the host after primary infection [24,25]. However, the putative role of the host's immune response in the pathophysiology of QFS remains to be elucidated and confirmed by other research groups.

Clinical manifestations of Q fever

Q fever is an infectious disease with a wide array of clinical manifestations, either acute or chronic. The clinical course (figure 4) can range from asymptomatic serocoversion to acute disease to to development of long-term complications such as the post Q fever fatigue syndrome and chronic Q fever in patients at risk.

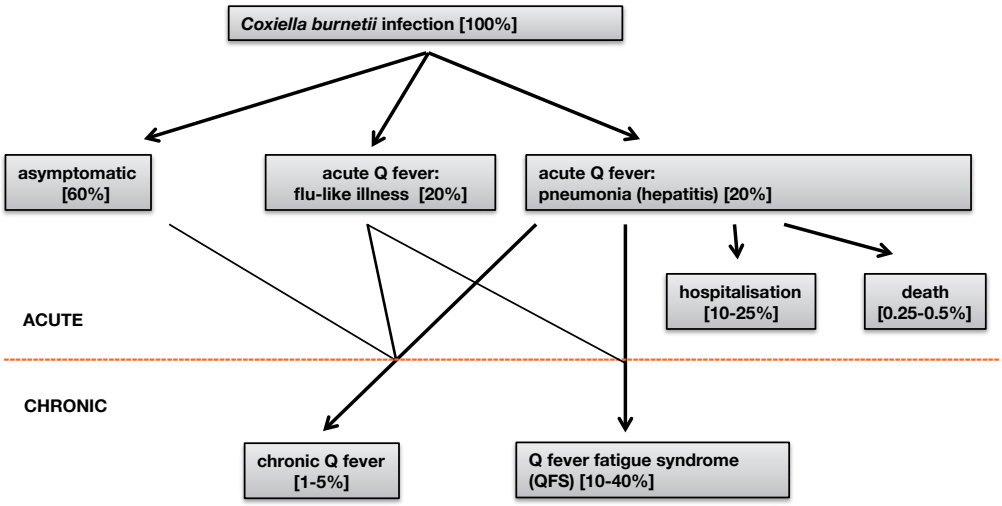


Figure 4. Schematic representation of clinical outcomes of *Coxiella burnetii* infection, both acute and chronic.

Primary C. burnetii infection; asymptomatic seroconversion or acute Q fever

A reported 50-60% of primary *C. burnetii* infections remain subclinical [2,3]. These patients exhibit no symptoms but can be at risk for later development of chronic Q fever. In the Netherlands the reported proportion of asymptomatic cases is much higher, with an estimated 12,6 unnotified incident cases for every notified Q fever case [8].

When symptomatic, primary infection with *C. burnetii* is called acute Q fever. After an incubation period of two to three weeks, most patients experience a rather sudden onset of a flu-like illness consisting of fever, headache, myalgia, fatigue and respiratory symptoms. Atypical pneumonia as defined by respiratory symptoms including chest x-ray abnormalities can be a more clinically severe presentation of acute Q fever. Severe acute hepatitis has been described by some authors, but has not been encountered in the Netherlands. Of note, however, slightly elevated liver function (transaminase) are often found in acute Q fever, but without clinical signs of hepatitis such as jaundice or abdominal pain [2,3]. Symptoms can last up to 3 months and generally resolve spontaneously. Antibiotic therapy shortens the symptomatic period and can speed up recovery of pneumonia. Doxycycline is the first line treatment for acute Q fever (200mg/day for 14 days), alternative antibiotics being co-trimoxazol or a quinolone. Hospitalisation rates for acute Q fever are reportedly low at 2%, depending on outbreak characteristics [2]. In the Netherlands hospitalisation rates in 2007 were much higher at 46%, dropping to 21% in 2009 [26]. The mortality rate of hospitalisation with acute Q fever in the Netherlands is an estimated 1%, tallying with earlier reports from France and the United Kingdom [27].

Q fever: long-term clinical outcome

Post-Q fever fatigue syndrome

Following the acute Q fever episode a large proportion of patients experience protracted symptomatology and a sustained decrease in health status generally referred to as the post-Q fever fatigue syndrome (QFS [28,29]. In contrast with chronic Q fever, which is a persistent infection with *C. burnetii,* in QFS no viable *C. burnetii* bacteria have ever been isolated. In addition the antibody titres in QFS patients are similar to those who fully recovered from primary *C. burnetii* infection.

Research groups in England, Australia and Canada have reported on a decreased health status in Q fever patients, using various questionnaires and study methods [28-30]. QFS's hallmark feature is persistent, excessive and undue fatigue, other symptoms being fatigue night sweats, headache, myalgia, arthralgia, dyspnea and blurred vision [28]. The incidence of QFS ranges from 20 to 42% and can last up to ten years after primary infection [28-30].

Despite these reports, the acceptance of QFS as a distinct disease entity, however, is not universal [31]. Indeed, post-infectious protracted fatigue syndromes have been reported for several other bacterial and viral pathogens such as *Borrelia burgdorferi, Legionella pneumophila,* Epstein Barr virus and West Nile Virus [32,33].

In addition, a lack of clear definition of QFS, small patient numbers in studies and different

study protocols do not allow for readily comparison of data and validation of obtained results.
Furthermore, the pathophysiology of QFS remains to be elucidated, but severity of the acute Q fever illness, genetic polymorphisms and abnormal and dysfunctional host immune response to *C. burnetii* infection have all been implicated [34-38]. Recently a new QFS paradigm has been put forward postulating a heightened dysfunctional immune respons [24].
In clinical practice, QFS patients remain indistinguishable from patients with a complete recovery after primary infection with *C. burnetii.* QFS patients do not meet the criteria for chronic Q fever and *C. burnetii* specific antibody titres are comparable [28-30]. QFS remains a purely clinical diagnosis.
In the Netherlands, QFS causes prolonged morbidity in a large section of Q fever patients and accounts for the largest portion of the total disease burden in socioeconomic terms due to increased healthcare consumption and absence from work [39].

Chronic Q fever

Primary *C. burnetii* infection, both symptomatic and asymptomatic, will develop into chronic Q fever in 1-5% of patients with an incubation period varying from months to years [2,3]. Until recently, endocarditis was reported to account for 75% of all chronic Q fever cases. In the Netherlands, however, infection of aortic aneurysms and vascular prosthesis is more prevalent, closely followed by endocarditis [40,41]. Less frequent clinical presentations of chronic infection with *C. burnetii* are pericarditis, hepatitis, osteomyelitis and placentitis in case of chronic Q fever in pregnancy [2,3].
Risk factors for development of chronic Q fever are increasing age, cardiac valve defects, vascular grafts and aneurysms, immunosuppression, and pregnancy [2,3]. Recently, Kampschreur et al. identified renal insufficiency as an independent risk factor [42]. The risk of developing chronic Q fever in case of known or previously unknown pre-existing cardiac valvulopathy has been estimated by one research group to be as high as 39%. This has led to the recommendation to perform an echocardiogram in every Q fever patient and start long-term antibiotic prophylaxis in case of any, be it trivial or clinically significant, cardiac valve defect [43-45].
Chronic Q fever has a very high mortality when left untreated. Timely diagnosis and start of antibiotic treatment has dramatically decreased mortality rates over the last years. Treatment of chronic Q fever consists of long-term administration of antibiotics for at least 18 to 24 months, preferably a combination of doxycycline and hydroxychloroquine [2,3]. Chloroquine heightens the pH in the phagoloysosome, thereby enhancing the efficacy of doxycycline when used in combination. The toxicity of this regimen is considerable and is rarely mentioned in articles on chronic Q fever and the prevention of disease in patients at risk. Doxycycline associated photosensitivity is reported in up to 100% of patients on long-term treatment [46,47]. Other frequently reported adverse effects are nausea, dizziness

and headache. In addition patients need to undergo regular ophtalmologic examinations as hydroxychloroquine can cause irreversible maculopathy. Intensive counselling and monitoring of therapeutic drug levels is required to secure optimal treatment regimen adherence [47].

Diagnosis of Q fever

A diagnosis of *C. burnetii* infection can be made by demonstrating in the host: 1) *C. burnetii* specific phase I and/or phase II antibodies, 2) the bacterium or (components of) its DNA in blood or tissue, 3) a *C. burnetii* specific cellular immune respons. Establishing a diagnosis of acute Q fever differs from that of chronic Q fever, the latter being a complex diagnosis based on a combination of symptomatology, detection of *C. burnetii* DNA, serology, host characteristics and diagnostic imaging techniques [40].

Cultivation of *C. burnetii* is not routinely undertaken because it is laboursome, time-consuming, has a low sensitivity for detecting the organism and requires a biosafety level 3 laboratory. Measuring the host's cellular immune reponse to *C. burnetii* in humans has been performed in experimental settings, including Q fever vaccination studies [48]. However, to date, there is no validated test measuring *C. burnetii* specific cellular immunity in humans, in order to diagnose acute Q fever or its long-term sequelae; chronic disease and QFS.

In routine daily clinical practice, diagnosis of acute Q fever relies almost exclusively on serology [2,3]. The most frequently used serological test is the immunofluorescence assay (IFA), but enzyme-linked immunosorbent assay (ELISA) or complement binding reaction (CBR) are widely used as well. The antibody response, however, can take up to 15 days after onset of symptoms. As a consequence of the antigenic phase variation of C. burnetii, the host's humoral immune response is characterised by antibody formation against both phase II antigens, which paradoxically appear first (as the virulent form of *C. burnetii* is phase I), and subsequently against phase I antigens (figure 5).

In recent years, PCR on serum has proven to be a valuable additional diagnostic tool in the early stages of primary infection. In the Netherlands, Schneeberger et al. showed a sensitivity of *C. burnetii* PCR on serum of >90% in the early stages of acute Q fever. The latest time point at which PCR was positive being 17 days after onset of symptoms. PCR invariably became negative as the serological response fully developed [49].

In chronic Q fever, viable *C. burnetii* bacteria persist in the host after primary infection. The onset of chronic Q fever can be insidious and it can take several months up to many years before the disease becomes clinically apparent. When symptoms do occur, they are often non-specific [2,3].

Establishing a chronic Q fever diagnosis can be difficult, as it based on a combination of symptomatology, detection of *C. burnetii* DNA, serology, host risk factors and diagnostic imaging techniques [40]. A positive PCR in blood or tissue in the absence of acute Q fever is proof of chronic Q fever, but sensitivity is low [51]. A high titre of phase I IgG might be indicative of chronic disease but has low positive predictive value on its own and should

therefore always be interpreted in combination with clinical findings and results of imaging studies. In medical literature, the widely cited cut-off value for phase I IgG of >1:800 was established using an in-house IFA developed in France [2,52]. As this test is not routinely available, a commercially available IFA (Focus diagnostics) is used in the Netherlands, and a cut-off value of =/>1:1024 is considered to be indicative of chronic disease [40].

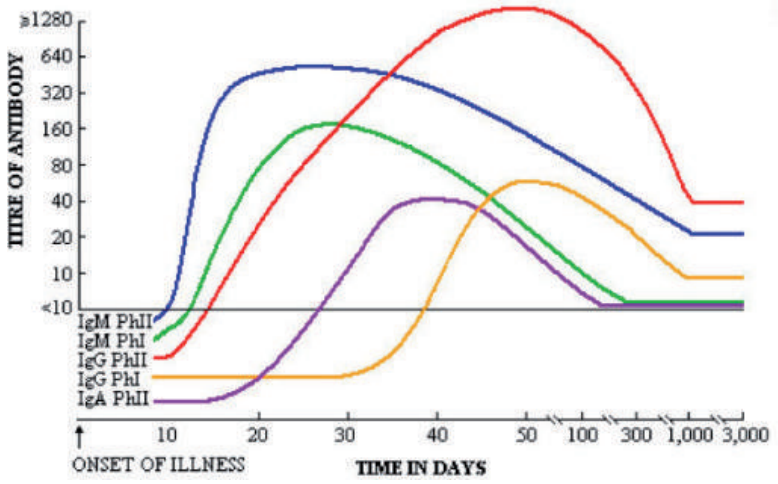


Figure 5. Idealised antibody response in acute Q fever as measured by IFA (from Munster et al. [50]; original from B.P. Marmion: Q fever; your questions answered. St Leonards, N.S.W. Medimedia Communications, 1999)

Q fever in the Netherlands: Coxiella burnetii as emerging pathogen

In the Netherlands, Q fever has been a mandatory notifiable diseasee since 1975. Before 2007, between 1 and 37 cases were reported annually, with an average of 17 cases per year [4].

In May 2007, an oubreak of atypical pneumonia cases was reported by a general practitioner in Herpen, a small rural village in the province of Noord Brabant, in the southern part of the Netherlands. In a 10 day period, 9 patients presented with flu-like symptoms and dyspnea and 6 of them were admited to hospital with a clinical suspicion of pneumonia. A few days prior, a medical microbiologist in the same region had reported to the public health service two cases of severe pneumonia unresponsive to antibiotics. Retrospective serological investigation on serum samples of 48 patients showed presence of *Mycoplasma pneumonia* IgM antibodies in 7 of the first 19 sera tested, so initially a local *M. Pneumonia* outbreak was suspected. Meanwhile, sera from 3 hospitalised patients from Herpen tested positive in the IFA test for *C. Burnetii*. Targeted testing for *C. burnetii* antibodies of the earlier collected sera using the complement binding reaction (CBR) was positive in 13 of the 48 sera. The putative causative agent of the outbreak was changed from *M. Pneumoniae* to *C. burnetii* [1,4].

This was the beginning of the first Dutch human Q fever outbreak that would entail 168 notified cases in 2007. Successive annual seasonal outbreaks in 2008 (1000 cases) and 2009

(2354 cases) spread out over a larger geographical area. In 2008 a definitive link to dairy goat farming was established and several measures controlling the dairy goat farming industry followed, eventually culminating in the culling of pregnant goats on infected farms starting in december 2009. In 2010 the Q fever epidemic abated and by 2011 the number of Q fever cases fell below the number of patients in 2007. By 2011 more than 4000 Q fever patients had been diagnosed with Q fever [4].
The majority of patients described in the studies of this thesis (Chapter 2 to 6) were diagnosed with Q fever in the initial stages of the outbreak and were followed up at the GP practice in Herpen.

Outline and aims of the thesis

The aim of this thesis is to describe observations on epidemiology, clinical aspects and long term health outcomes of Q fever patients diagnosed in the initial stages of the first Dutch human Q fever outbreak. In addition, this thesis aims to describe the potential clinical use of a newly developed diagnostic Q fever interferon gamma release assay, the *Coxiella* ELISPOT, which measures the host's cellular immune reponse to *Coxiella burnetii*.
Q fever is considered a new and emerging zoonosis in the Netherlands. Retrospective serological studies perfomed on available samples from the period leading up to the first Q fever outbreak in the Netherlands show a low seroprevalence of antibodies againt *C. burnetii* [53]. However, the highly epidemic municipalities in the epidemic's epicentre in the province of Noord-Brabant were excluded from analysis [54]. A description of the aetiology of clinically treated pneumonia cases in the epicentre of the first Q fever outbreak in 2007 is given in **Chapter 2**. The clinical presentation, diagnosis including antibody response and clinical outcome of Q fever in the Netherlands were unknown at the onset of the first epidemic wave in 2007. Available literature on the disease in its acute and chronic form, its diagnosis and management, was mostly retrospective and mainly derived from a single reference centre of expertise in France. **Chapter 3.** describes the follow-up in terms of clinical presentation, serology and echocardiographic findings of Dutch Q fever patients from the epicentre of the first outbreak. **Chapter 4.** describes the echocardiographic findings and outcomes of a larger group of Q fever patients from both the 2007 and 2008 cohort. In **Chapter 5.** the health status of Q fever patients from the first outbreak cohort is studied in detail, using a case-control design. In **Chapter 6.** the health status of the same cohort of patients is studied 4 years after acute Q fever, allowing for a direct comparison of detailed health status assessment on a group- and individual patient's level . **Chapter 7.** deals with the development and validation of the *Coxiella* ELISPOT; a *Coxiella burnetti* specific interferon gamma release assay (IGRA) measuring C. burnetii specific T-cell responses to both *C. burnetii* phase I and phase II antigens. In **Chapter 8.** the *Coxiella* ELISPOT is compared to a different whole blood *C. burnetii* specific IGRA in terms of diagnostic potential. **Chapter 9.** summarises the findings of the present thesis and formulates implications and opportunities for further research.

References

1. Van Steenbergen JE, Morroy G, Groot CA et al [An outbreak of Q fever in The Netherlands--possible link to goats]. Ned Tijdschr Geneeskd 2007 Sep 8;151(36):1998-2003.
2. Maurin M, Raoult D. Q fever. Clin Microbiol Rev 1999 Oct;12(4):518-53.
3. Parker NR, Barralet JH, Bell AM. Q fever. Lancet 2006 Feb 25;367(9511):679-88.
4. Roest HI, Tilburg JJ, Van der Hoek W et al. The Q fever epidemic in the Netherlands: history, onset, response and reflection. Epidemiology and Infection 2011 Jan;139(1):1-12.
5. Schneeberger PM, Wintenberger C, Van der Hoek W et al. Q fever in the Netherlands – 2007-2010: what we learned from the largest outbreak ever. Med Mal Infect. 2014 Aug;44(8):339-53.
6. Wielders CC, Morroy G, Wever PC et al. Strategies for early detection of chronic Q-fever: a systematic review. Eur J Clin Invest. 2013 Jun;43(6):616-39.
7. Kampschreur LM, Hagenaars JC, Wielders CC et al. Screening for Coxiella burnetii seroprevalence in chronic Q fever high-risk groups reveals magnitude of Dutch Q fever outbreak. Epidemiol Infect. 2012;13:1-5.
8. Van der Hoek W, Hogema BM, Dijkstra F et al. Relation between Q fever notifications and Coxiella burnetii infections during the 2009 outbreak in The Netherlands. Euro Surveill. 2012 Jan 19;17(3):20058.
9. Van der Hoek W, Schneeberger P, Oomen T et al. Shifting priorities in the aftermath of a Q fever epidemic in 2007 to 2009 in the Netherlands: from acute to chronic infection. Euro Surveill. 2012; 17(3):20059.
10. HoekDerrick E. Q fever, a new fever entity: clinical features, diagnosis and laboratory investigation. Med J Aust 1937; 2: 281–99.
11. Joseph E. McDade (1990). “Historical Aspects of Q Fever”. In Thomas J. Marrie. Q Fever, Volume I: The Disease. CRC Press. p. 8. ISBN 0-8493-5984-8.
12. Burnet FM, Freeman M. Experimental studies on the virus of “Q” fever. Med J Aust. 1937;2:299-305.
13. Davis GE, Cox HR. A filter-passing infectious agent isolated from ticks. I. Isolation from *Dermacentor andersoni*, reactions in animals, and filtration experiments. Public Health Rep. 1938;53:2259-76.
14. Philip CB. Observations on experimental Q fever. J Parasitol. 1948;34(6):457-64.
15. Comer JA, Paddock CD, Childs JE. Urban zoonoses caused by Bartonella, Coxiella, Ehrlichia, and Rickettsia species. Vector Borne Zoonotic Dis. 2001;1(2):91–118.
16. Van der Hoek W, Van de Kassteele J, Bom B et al. Smooth incidence maps give valuable insight into Q fever outbreaks in the Netherlands. Geospat. Health. 2012 Nov;7(1): 127-34.
17. Kruszewska D, Lembowicz K, Tylewska-Wierzbanowska S. Possible sexual transmission of Q fever among humans. Clin Infect Dis. 1996 Jun;22(6):1087-8.
18. Milazzo A, Hall R, Storm PA, Harris RJ, Winslow W, Marmion BP. Sexually transmitted Q fever. Clin Infect Dis. 2001 Aug 1;33(3):399-402. Epub 2001 Jul 5.
19. Racult D, Stein A. Q fever during pregnancy--a risk for women, fetuses, and obstetricians. N Engl J Med. 1994 Feb 3;330(5):371.

20. Andoh M, Zhang G, Russell-Lodrigue KE et al. T cells are essential for bacterial clearance, and gamma interferon, tumor necrosis factor alpha, and B cells are crucial for disease development in Coxiella burnetii infection in mice. Infect Immun. 2007 Jul;75(7):3245-55.
21. Shannon JG, Heinzen RA. Infection of human monocyte-derived macrophages with Coxiella burnetii. Methods Mol Biol. 2008;431:189-200.
22. Benoit M, Barbarat B, Bernard A et al. Coxiella burnetii, the agent of Q fever, stimulates an atypical M2 activation program in human macrophages. Eur J Immunol. 2008 Apr;38(4):1065-70.
23. Benoit M, Ghigo E, Capo C et al. The uptake of apoptotic cells drives Coxiella burnetii replication and macrophage polarization: a model for Q fever endocarditis. PLoS Pathog. 2008 May 16;4(5):e1000066.
24. Marmion BP et al. Q fever: persistence of antigenic non-viable cell residues of Coxiella burnetii in the host-implications for post Q fever infection fatigue syndrome and other chronic sequelae. QJM 2009; 102: 673-684.
25. Sukocheva OA et al. Long-term persistence after acute Q fever of non-infective Coxiella burnetii cell components, including antigens. QJM 2010; 103: 847-863.
26. Dijkstra F, van der Hoek W, Wijers N et al. The 2007–2010 Q fever epidemic in The Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. FEMS Immunol Med Microbiol. 2012 Feb;64(1):3-12.
27. Kampschreur LM, Wegdam-Blans MC, Thijsen SF et al. Acute Q fever related in-hospital mortality in the Netherlands. Neth J Med. 2010 Dec;68(12):408-13.
28. Marmion BP, Shannon M, Maddocks I et al. Protracted debility and fatigue after acute Q fever. Lancet 1996; 347:977.
29. Ayres JG, Smith EG, Flint N. Protracted fatigue and debility after acute Q fever. Lancet 1996; 347: 978.
30. Wildman MJ, Smith EG, Groves J et al. Chronic fatigue following infection by Coxiella burnetii (Q fever): Ten-year follow-up of the 1989 UK outbreak cohort. Q J Med 2002;95: 527–38.
31. Raoult D. Q fever: still a mysterious disease. QJM 2002; 95: 491-492.
32. Hickie I et al. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. British Medical Journal 2006; 16; 333.
33. Garcia MN et al. Evaluation of prolonged fatigue post-West Nile virus infection and association of fatigue with elevated antiviral and proinflammatory cytokines. Viral Immunology 2014; 27: 327-333.
34. Kerr JR et al. Gene expression subtypes in patients with chronic fatigue syndrome/myalgic encephalomyelitis. Journal of Infectious Diseases 2008; 15; 197: 1171-1184.
35. Helbig K et al. Immune response genes in the post-Q-fever fatigue syndrome, Q fever endocarditis and uncomplicated acute primary Q fever. QJM: An International Journal of Medicine 2005; 98: 565-574.
36. Piraino B, Vollmer-Conna U, Lloyd AR. Genetic associations of fatigue and other symptom domains of the acute sickness response to infection. Brain, Behaviour and Immunity 2012; 26: 552-558.
37. Penttila IA et al. Cytokine dysregulation in the post-Q-fever fatigue syndrome. QJM: An International Journal of Medicine 1998; 91: 549-560.

38. Vollmer-Conna U et al. Cytokine polymorphisms have a synergistic effect on severity of the acute sickness response to infection. Clinical Infectious Diseases 2008; 47: 1418-1425.
39. Morroy G, Bor HH, Polder J et al. Self-reported sick leave and long-term health symptoms of Q-fever patients. Eur J Public Health. 2012 Dec;22(6):814-9.
40. Wegdam-Blans MC, Kampschreur LM, Delsing CE et al.; Dutch Q fever Consensus Group. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Infect. 2012 Mar;64(3):247-59.
41. Kampschreur LM, Delsing CE, Groenwold RH et al. Chronic Q fever in the Netherlands 5 years after the start of the Q fever epidemic: results from the Dutch chronic Q fever database. J Clin Microbiol. 2014 May;52(5):1637-43.
42. Kampschreur LM, Dekker S, Hagenaars JC et al. Identification of risk factors for chronic Q fever, the Netherlands. Emerg Infect Dis. 2012 Apr;18(4):563-70.
43. Fenollar F, Fournier PE, Carrieri MP et al. Risks factors and prevention of Q fever endocarditis. Clin Infect Dis 2001 Aug 1;33(3):312-6.
44. Landais C, Fenollar F, Thuny F et al. From acute Q fever to endocarditis: serological follow-up strategy. Clin Infect Dis 2007 May 15;44(10):1337-40.
45. Fenollar F, Thuny F, Xeridat B et al. Endocarditis after acute Q fever in patients with previously undiagnosed valvulopathies. Clin Infect Dis 2006 Mar 15;42(6):818-21.
46. Raoult D, Houpikian P, Tissot DH et al. Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydroxychloroquine. Arch Intern Med. 1999;159(2):167-73.
47. Delsing CE, Kullberg BJ, Bleeker-Rovers CP. Q fever in the Netherlands from 2007 to 2010. Neth J Med. 2010 Dec;68(12):382-7.
48. Izzo AA, Marmion BP. Variation in interferon-gamma responses to Coxiella burnetii antigens with lymphocytes from vaccinated or naturally infected subjects. Clin Exp Immunol. 1993 Dec;94(3):507-15.
49. Schneeberger PM, Hermans MH, van Hannen EJ et al. Real-time PCR with serum samples is indispensable for early diagnosis of acute Q fever. Clin Vaccine Immunol. 2010 Feb;17(2):286-90.
50. Munster JM, Leenders AC, van der Hoek W 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 Nov 1;10:32.
51. Fenollar F, Fournier PE, Raoult D. Molecular detection of Coxiella burnetii in the sera of patients with Q fever endocarditis or vascular infection. J Clin Microbiol. 2004 Nov;42(11):4919-24.
52. Fournier PE, Casalta JP, Habib G et al. Modification of the diagnostic criteria proposed by the Duke Endocarditis Service to permit improved diagnosis of Q fever endocarditis. Am J Med 1996 Jun;100(6):629-33.
53. Schimmer B, et al. Low seroprevalence of Q fever in The Netherlands prior to a series of large outbreaks. Epidemiology and Infection 2011; 139: 1–9.
54. Van den Wijngaard CC, et al. In search of hidden Q-fever outbreaks: linking syndromic hospital clusters to infected goat farms. Epidemiology and Infection 2011; 139:19–26.

Chapter 2

Coxiella burnetii: a genuinely novel causative agent of pneumonia in the Netherlands since May 2007

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Abstract

Despite a reported low seroprevalence in the Netherlands in the period leading up to the outbreak, the question whether *C. burnetii* emerged as a cause of pneumonia in the geographical epicentre of the first Q fever outbreak in 2007 remained unanswered.

The contribution of *C. burnetii* as a causative agent of pneumonia in patients admitted to Bernhoven Hospital in the period January-July 2007 was assessed by retrospectively testing available patients' sera for the presence of *C. burnetii* antibodies. Of the total of 95 clinical pneumonia cases, *C. burnetii* was the causative agent in 21 (22%). The number of Q fever pneumonia cases in May (n=13) was significantly higher than in any other month of the study period (range 0-5). To examine these findings in a historical perspective, data on number of pneumonia cases treated at Bernhoven Hospital were retrieved from the local diagnosis registration system. In the 2 years preceding the outbreak, the number of pneumonia cases was stable with expected seasonal variation in 2005 (206 cases) and 2006 (170 cases), but with no sharp increase as was observed in 2007 (n=272).

The findings in this study support the hypothesis that *C. burnetii* emerged as a novel etiological agent of pneumonia in the epidemic area in the Netherlands in May 2007.

Coxiella burnetii: a genuinely novel causative agent of pneumonia in the Netherlands since May 2007

Q fever is considered a new and emerging zoonosis in the Netherlands since the first reported outbreak in late spring 2007 [1]. In support of this hypothesis, Schimmer et al. recently reported a low seroprevalence of 2,4% in the Netherlands in the period leading up to the outbreak (February 2006 – June 2007) retrospectively testing sera from the Dutch National Immunization Programme available from randomly selected municipalities all over the country [2]. However, the highly epidemic municipalities of 2007-2010 were excluded from the study sample, leaving unanswered the question whether *Coxiella burnetii* emerged as a genuinely novel infectious agent in those areas in May 2007.

We present here data from a retrospective study performed in the geographical epicentre of the first Q fever outbreak, investigating the etiology of clinically treated pneumonia cases, in the period leading up to the first reported outbreak. The outbreak's epicentre was located in the rural village of Herpen, in the province of Noord-Brabant. Herpen lies within the catchment area (catchment population 190000) of the nearby located community based Bernhoven hospital in Oss.

Aim of this study was to determine the contribution of *Coxiella burnetii* as a causative agent of pneumonia in patients admitted for pneumonia to the Bernhoven hospital, in the period January to July 2007. Pneumonia cases were identified retrospectively by retrieving all entries for 'pneumonia' from the hospital's electronic diagnosis registration system. A pneumonia case was defined by the presence of one or more clinical compatible symptoms (fever, cough, dyspnea, headache) and a (new) infiltrate or consolidation on chest imaging (chest x-ray or CT scan). If no causative agent had already been identified using standard cultures and serology, available sera were tested retrospectively for *Coxiella burnetii*, using both complement fixation test (CFT) and PCR.

Of the total of 95 clinical pneumonia cases identified, *C. burnetii* was the causative agent in 21 patients (22%). These patients showed a fourfold increase in CFT in paired samples. One Q fever patient had a positive serum PCR with negative CFT at presentation, but seroconverted in the follow-up CFT sample taken 3 months later.

In 7 cases (7%), a pathogen other than *C. burnetii* was found, in 23 cases (24%) no cause was found after serological testing. In the remaining 44 cases (47%) serology could not be obtained (Figure 1.). Mean number of pneumonia cases was 13 per month (range 5-29). The number of Q fever pneumonia cases in May (13) was significantly higher than any other month in the study period (range 0-5) ($p<0,05$). For all other groups (other pathogen, unknown etiology, no serology available), the number of cases did not differ significantly between months in the study period ($p>0,05$ for all groups). The contribution of *Coxiella burnetii* as a causative agent of clinically treated pneumonia sharply increased in May 2007 compared to January to April 2007. There was no evidence of underreporting of Q fever pneumonia in the period leading up to the outbreak.

In order to examine these data further in a historical perspective, we retrieved data on number of pneumonia cases from the local diagnosis registration system from the Bernhoven hospital for 2005 and 2006. In the 2 years preceding the 2007 outbreak, the number of pneumonia cases was stable with expected seasonal variations in 2005 (206 cases) and 2006 (170 cases), but with no sudden sharp increase as was observed in 2007 (272 cases). This significant increase in number of pneumonia cases, especially in late spring and summer, further supports the notion of the introduction of *Coxiella burnetii* as a new etiological agent of pneumonia in this region.

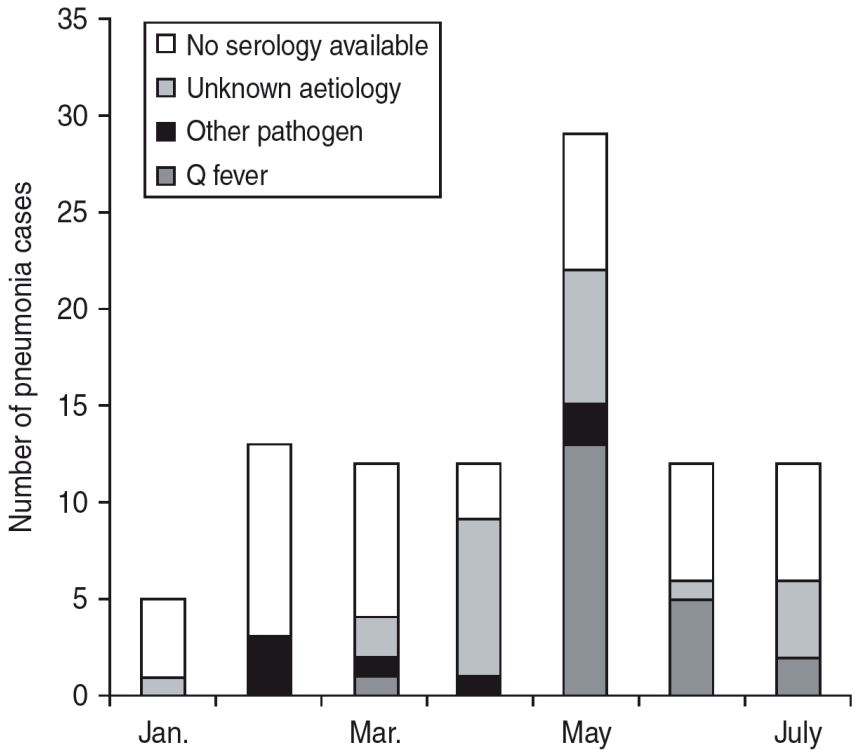

Figure 1. Etiology of pneumonia cases in the Bernhoven Hospital in the period leading up to the first reported Dutch Q fever outbreak.

A Q fever diagnosis can easily be overlooked as symptoms are non specific and the clinical course is generally mild. In addition, sheer unfamiliarity with *Coxiella burnetii* as an infectious agent could have led to an underreporting of Q fever cases by attending physicians. Interestingly in this regard, Van den Wijngaard et al. found retrospective statistical evidence of clustering of hospitalisations of possible and plausible Q fever pneumonia cases starting as early as 2005 [3]. However, lack of serological testing precluded definite confirmation of these outbreak clusters as being caused by Q fever.

Our data support the hypothesis that *Coxiella burnetii* emerged as a novel etiological agent of pneumonia in the epidemic area in the Netherlands in May 2007.

References

1. Q fever outbreak in the Netherlands: a preliminary report. Karagiannis I, Morroy G, Rietveld A, Horrevorts AM, Hamans M, Francken P, Schimmer B. Euro Surveill. 2007 Aug 9;12:E070809.2.
2. Low seroprevalence of Q fever in The Netherlands prior to a series of large outbreaks. Schimmer B, Notermans DW, Harms MG, Reimerink JH, Bakker J, Schneeberger P, Mollema L, Teunis P, VAN Pelt W, VAN Duynhoven Y. Epidemiol Infect. 2011 Feb 16:1-9.
3. In search of hidden Q-fever outbreaks: linking syndromic hospital clusters to infected goat farms. Van den Wijngaard CC, Dijkstra F, van Pelt W, van Asten L, Kretzschmar M, Schimmer B, Nagelkerke NJ, Vellema P, Donker GA, Koopmans MP. Epidemiol Infect. 2011 Jan;139:19-26. Epub 2010 May 18.

Chapter 3

One year follow-up of patients of the ongoing Dutch Q fever outbreak; clinical, serological and echocardiographic findings

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Abstract

Purpose

In 2007, a large goatfarming associated Q fever outbreak occurred in the Netherlands. Data on clinical outcome of Dutch Q fever patients are lacking. The current advocated follow-up strategy includes serological follow-up to detect evolution to chronic disease and cardiac screening at baseline to identify and prophylactically treat Q fever patients in case of valvulopathy. However, serological follow-up using commercially available tests is complicated by lack of validated cut-off values. Furthermore, cardiac screening in the setting of a large oubreak has not been implemented before. Therefore, we report here the clinical outcome, serological follow-up and cardiac screening data of the Q fever patients of the current ongoing outbreak.

Methods

Implementation of a protocol including clinical and serological follow-up at baseline 3, 6 and 12 months after acute Q fever and screening echocardiography at baseline.

Results

Eighty-five patients with acute Q fever were identified (male 62%, female 38%). An aspecific, flu-like illness was the most common clinical presentation. Persistent symptoms after acute Q fever were reported by 59% of patients at 6 months and 30% at 12 months follow-up. We observed a typical serological response to *Coxiella burnetii* infection in both anti-phase I and anti-phase II IgG antibodies, with an increase in antibody titers up to 3 months and a subsequent decrease in the following 9 months. Screening echocardiography was available for 66 (78%) out of 85 Q-fever patients. Cardiac valvulopathy was present in 39 (59%) patients. None of the 85 patients developed chronic Q fever.

Conclusions

Clinical, serological and echocardiographic data of the current ongoing Dutch Q fever outbreak cohort are presented. Screening echocardiography is no longer part of the standard work-up of Q fever patients in the Netherlands.

Introduction

In late spring 2007, a large Q fever outbreak in The Netherlands occurred in the province of Noord Brabant with a distinct epidemic centre around the town of Herpen [1]. Although no definitive source for the outbreak was identified, the preceding increased abortion rate amongst goats in the region suggested these ruminants as being the most likely reservoir [2,3].

Q fever is an ubiquitous zoonosis caused by the obligate intracellular bacterium *Coxiella burnetii.* The clinical manifestations of this acute infection are usually self-limiting and range from a mild flu-like febrile illness to atypical pneumonia and hepatitis [4]. Serological and clinical follow-up after primary infection is recommended because approximately 1-5% of patients will develop chronic Q fever, endocarditis being the clinical manifestation in 60-70% of cases [5]. Cardiac valve abnormalities, vascular prosthesis, compromised immunity and pregnancy constitute predisposing host factors for chronic Q fever [6]. Recently screening echocardiography has been added to the standard of care for Q fever patients [7,8]. In daily clinical practice however, adequate follow-up of patients after acute Q fever has practical difficulties. First, interpretation of serology obtained by commercially available tests is hampered by incomplete knowledge of the natural course of the antibody reponse to *Coxiella burnetii* and lack of validated cut-off values for chronic disease. Second, minor cardiac valvulopathies are frequently encountered in the general population, raising the question whether indeed all patients with cardiac valve abnormalities should receive prolonged prophylactic antibiotic treatment.

Faced with the forementioned Q fever outbreak in the Netherlands, a follow-up protocol was implemented, including clinical and serological follow-up for a 1-year period and Q fever patients were offered a screening echocardiography at baseline. The aim of this paper was to report the clinical characteristics and outcome, serological data and echocardiographic findings of the current ongoing Q fever outbreak in the Netherlands.

Methods

Q fever case definition

A case of acute Q fever was defined as any inhabitant of the outbreak cluster area who presented with one or more compatible clinical symptoms (fever, fatigue, chills, headache, myalgia, sweats, cough [4]) and demonstration of infection with *Coxiella burnetii* as evidenced by 1) a seroconversion or fourfold increase of antibody titer using a *C. burnetii* complement fixation test (CFT) in samples taken at least 14 days apart, 2) presence of both anti-phase II IgM and anti-phase II IgG antibodies in the C. burnetii Immunofluorescence Assay (IFA) with a 1:64 or greater dilution [1], or a positive serum PCR. For patients admitted to hospital and presenting with pneumonia, the severity of disease was assessed using the Pneumonia Severity Index (PSI) [9].

A case of chronic Q fever is defined as any inhabitant of the outbreak cluster area with a clinical entity compatible with chronic *Coxiella burnetii* infection as described in the literature

by Raoult (endocarditis, vascular infection, osteoarticular infection, chronic hepatitis, pregnancy), in the presence of an anti-Phase-I IgG titer ≥ 800, for ≥ 6 months after the initial day of illness [4,10].

Follow-up protocol

The follow-up protocol consisted of a complete history and physical examination at 6 and 12 months after the initial day of illness, serological testing at baseline followed by testing after 3, 6 and 12 months after a referral to a cardiologist for a single screening transthoracic echocardiogram. Data on symptoms were obtained by asking the patient an open question on the presence of any complaints. No structured questionnaire was used. As the Q fever outbreak was identified retrospectively, data on presenting symptoms at baseline were collected through review of all available medical records at the GP practice. Since this concerned an observational study, all interventions had been part of standard care. Therefore, patients were asked to cooperate and no specific ethical approval for this study was sought.

Serology and PCR

Sera were tested for *Coxiella burnetii* antibodies using a Complement Fixation Test (CFT, Institute Virion/Serion, GmbH, Würzburg, Germany), testing only anti-phase II antibodies, and an Immunofluorescence Assay (IFA, Focus Diagnostics, Cypress, California 90630, U.S.A.), assessing IgM and IgG antibodies to both phase I and II antibodies. Sera taken at baseline (T=0) were also tested by Polymerase Chain Reaction (PCR) as described in the literature [11]. The respective time-points for final analysis were defined as follows: baseline (T=0) is the date of the first available serological results within 6 weeks after the first day of illness. Serological results at 3 (T=3), 6 (T=6) and 12 months (T=12) after first day of illness were included if blood samples were drawn at these time points, with a margin of plus or minus 1 month.

Screening echocardiography

Structural cardiac abnormalities and valvular defects were classified according to the ASE guidelines [12-15]. The ASE guidelines are a consensus statement of the American College of Cardiology, the American Heart Association and the European Society of Cardiology. These guidelines provide a framework for standardized assessment of severity of valvular regurgitation and –stenosis, using well defined structural-, Doppler- and quantitative echocardiographic parameters. Major (or clinically significant) valvulopathies are defined as moderate and severe regurgitation or stenosis of the mitral and/or aortic valve. Minor valvulopathies are defined as trace or mild regurgitation or stenosis of the mitral and/or aortic valve, a bicuspid aortic valve and mitral valve prolaps without significant accompanying stenosis or regurgitation.

Data to calculate percentages were analysed using Microsoft Excell 7.0 and SPSS 17.0. GraphPad Prism 4.0 was used to make Figure 1.

Results

The Herpen Q fever outbreak cohort

A total of 85 patients with acute Q fever were identified in the outbreak cluster. Patient characteristics are given in Table 1.

The male-to-female ratio was 1,7. None of the female patients was pregnant. Co-morbidity was present in 26 patients (31%). Six patients had a known risk factor for developing chronic Q fever: 4 patients with previously documented significant cardiac valvulopathies, 1 patient using long-term high dose corticosteroids for idiopathic trombocytopenic purpura and 1 patient with an aortic vascular prosthesis. Complete baseline and follow-up data on symptoms and physical examination were available for all patients and are given in Table 2.

An aspecific, flu-like illness was the most common clinical presentation. All patients who were admitted to hospital (8 female, 16 male) presented with (atypical) pneumonia and 83% of these patients had a Pneumonia Severity Index (PSI) of class I or class II, representing a low disease severity [9]. At 6 months follow-up, more than half of all Q fever patients had persistent symptoms, fatigue being the most prevalent complaint. At one year, reports of persistent symptoms had roughly halved. Twenty-six percent of patients still reporting fatigue which they attributed to their acute Q fever episode. None of the 85 patients had developed chronic Q fever at the one-year follow-up point. One male patient died of a myocardial infarction at 8 months follow-up, unrelated to Q fever.

Table 1. Characteristics of the Q fever outbreak cohort (n=85)

male / female (n, (%))	53 (62) / 32 (38)
mean age (years, (range))	49 (18 - 80)
co-morbidity (n, (%))	26 (31)
cardiovascular	6 (7)
pulmonary	3 (4)
neurological	1 (1)
rheumatological	4 (5)
hematological	1 (1)
depression	3 (4)
diabetes	5 (6)
other	3 (4)
immunocompromised (n, (%))	1 (1)
vascular graft (n (%))	1 (1)
antibiotic treatment (n, (%))	
doxycyclin	5 (6)
moxifloxacin	35 (41)
beta-lactam antibiotic	32 (38)
azithromycin	1 (1)
none	12 (14)
mortality due to Q fever	0 (0)
overall one-year mortality	1 (1)
hospitalisation (n, (%))	24 (28)
Pneumonia Severity Index (PSI) of hospitalised patients	
PSI class I	12 (50)
PSI class II	8 (33)
PSI class III	4 (17)
PSI class IV and class V	0 (0)
intensive care treatment	0 (0)
duration of hospital admission (days, median)	4 (5)

Serology

Serological data were available at baseline (68%), at 3 month follow-up (49%), at 6 month follow-up (81%) and at 12 month follow-up (75%). Results are shown in figure 1. CFT and IFA showed similar profiles, but at a different titer level.

The numbers of patients with an anti-pase I IgG titer of equal or more than 800 (suggesting chronic disease) at the respective timepoints were 7 (T=0), 21 (T=3), 13 (T=6) and 2 (T=12). PCR was performed on 60 out of 85 first available sera. Seven of these 60 patients (11,6%) had a positive PCR and negative serology. All PCR positive patients subsequently seroconverted after 3 months.

Table 2. Clinical presentation and follow-up

	T=0 (n=85)	**T=6** (n=85)	**T=12** (n=84)*
Symptoms (%)			
any symptoms	100	59	30
fever	93	0	0
fatigue	69	52	26
headache	40	1	1
cough	39	0	0
myalgia	34	1	2
sweats	32	6	6
dyspnea	24	19	5
anorexia	17	0	0
nausea	14	0	0
arthralgia	11	1	2
abdominal pain	11	0	0
cognitive disturbance	0	4	1
Physical examination (%)			
cardiac murmur	5	8	7
pulmonary crackles	12	0	0
hepatosplenomegaly	2	0	0

* One patient died of a myocardial infarction, not related to Q fever, at 8 months follow-up.

Echocardiography

Screening transthoracic echocardiography was available for 66 (78%) out of 85 Q-fever patients. The remaining nineteen patients repeatedly failed to show up at their appointment for a transthoracic echocardiogram. Results are shown in Table 3. Cardiac valvulopathy was present in 39 (59%) patients, five of whom had a major or clinically significant (moderate or severe) valvulopathy. Four of these five patients were previously known to have a cardiac valvulopathy. One patient had a bicuspid aortic valve associated with a moderate aortic insufficiency, This anomaly was classified as a clinically significant valvulopathy. The remaining 34 patients had one or more minor cardiac valvular abnomalities.

Table 3. Cardiac Valvulopathies in Q fever patients (n=66)

	severity of valvulopathy (No. of patients)			
	trace	mild	moderate	severe
mitral valve regurgitation	23	9	2	1
mitral valve stenosis	0	0	0	0
aortic valve regurgitation	5	2	2	0
aortic valve stenosis	0	1	0	0

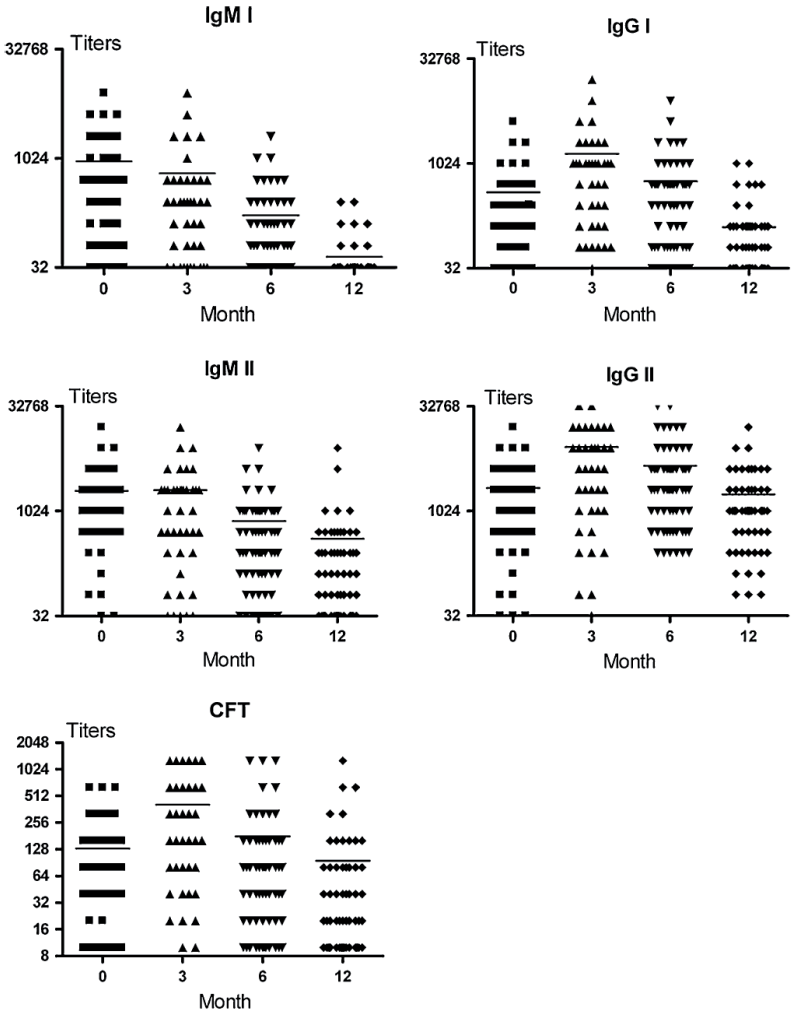

Figure 1. One-year follow-up of patients with acute Q fever by complement fixation test (CFT; antibodies to phase II) and immunofluorescence assay (IFA; IgG- and IgM-antibodies to phase I and phase II). The *horizontal bars* represent the mean.

Discussion

As expected, an aspecific, flu-like syndrome with or without signs of respiratory tract infection was the main clinical Q fever manifestation in this remarkably compliant cohort of patients. However, chest radiography was only performed in patients admitted to hospital, which could underestimate the prevalence of pneumonia in patients treated by their GP. Males were more likely to suffer from symptomatic Q fever than females, which is a well known phenomenon and appears to be the result of the gender related expression of sex hormones [16].

Fatigue was noted by Q fever patients in 52% at 6 months and 26% at 1 year following primary infection. Other research groups, using various questionnaires, have reported a comparably high proportion of fatigued patients [17-21]. However, fatigue levels in this outbreak cohort are self reported and a control group is lacking. Therefore these findings must be interpreted with extreme caution. Further studies are needed to evaluate long term symptoms and quality of life in Q fever patients in the Netherlands.

Using a commercially available IFA we observed a typical serological response to Coxiella burnetii infection with strikingly high levels of antibodies to both phase I and phase II antibodies. This serological response was characterized by an increase in antibody titers up to 3 months and a subsequent decrease in the following 9 months. The anti-phase I IgG kinetic, with a titer peak at 3 months, is different from available literature on IFA serological patterns in the follow-up of acute Q fever, that shows a rather slow and gradual increase of anti-phase I IgG antibodies [22,23]. The high proportion of Q fever patients in this cohort that received a beta-lactam antibiotic with no efficacy against *Coxiella burnetii* (38%) or no treatment at all (14%) could possibly account for this discrepancy. The CFT and the IFA test showed an identical serological pattern over time. Therefore, both tests might be useful for the follow-up of patients. Additional IFA should be performed when the CFT-titer is rising, to see the balance between anti-phase I and anti-phase II IgG antibodies.

Although at the various timepoints there were patients with an anti-phase I IgG antibody titer of 1024 or more, suggesting chronic disease, none of these patients developed a clinical picture compatible with chronic Q fever. Furthermore, at follow-up, all these patients showed a spontaneous subsequent decline in anti-phase I IgG titers, which in the case of a possible chronic Q fever is highly unlikely, given the natural detrimental course of chronic disease if left untreated.

For aiding in the diagnosis chronic Q fever, we used the well-established anti-phase I IgG antibody titer of $\geq$ 800 [10]. Unfortunately, this cut-off value is based on a single center experience using a home made IFA test. Additional studies are urgently needed to compare both tests and to give cutt-off levels that can be used with commercially available IFA tests.

Q fever endocarditis may develop in up to 39% of patient with known pre-existing valvulopathy [24]. Even minor cardiac valve abnormalities might be a risk factor for Q fever endocarditis [7]. An active search for cardiac valvular abnormalities and serological surveillance following acute Q fever have been advocated to identify chronic Q fever in an early stage and to trigger prolonged prophylactic antibiotic treatment in case of even minor cardiac valvulopathy [7,8,25].

No cases of chronic Q fever were observed during follow-up, despite the high incidence of minor valvulopathies found by screening echocardiography. Similar incidences of minor cardiac valvular abnormalities in the general population have been reported in large series [26,27]. For example, the prevalence of 'trace' mitral valve regurgitation is 40% and can therefore be considered to be a physiological phenomenon without clinical importance [12]. The prevalence of mitral valve prolaps and bicuspid aortic valve is estimated at 2-3% and circa 1% respectively [28,29]. The absolute risk of developing chronic Q fever for these minor valvulopathies remains unknown. The limited number of patients in this study does not allow to determine this risk, but it's clear that the absolute risk, at least in the case of a minor mitral valve insufficiency, is likely to be small enough to withhold prolonged prophylactical antibiotic treatment and closely monitor serology during the follow-up.

In the setting of the ongoing Q fever epidemic in the Netherlands that currently encompasses more than 3000 new Q fever patients, performing screening echocardiograms in all patients is costly [30]. Moreover, it is not likely that such a screening will detect cardiac valve abnormalities that in turn would influence clinical management. Therefore, we currently perform only serological and clinical follow-up after acute Q fever, omitting routine screening echocardiography. In other words, in the context of the large and continuous outbreak in the Netherlands, a pragmatic approach has been adopted for patients without known risk factors for chronic disease, consisting of closely serological monitoring for a period of one year (at 3, 6 and 12 months). Only when serological and/or clinical signs of chronic disease appear, is further investigation using PCR and echocardiography undertaken. In patients with known, pre-existing risk factors for chronic disease, including cardiac valvulopathy, decisions regarding follow-up and prophylactical antibiotic treatment are made in each individual case by a multidisciplinary team including a medical microbiologist, infectiologist and cardiologist. We realize that such a strategy can only be applied in countries with a low background prevalence of cardiac valvulopathies in the general population. Indeed in India, due to the high prevalence of rheumatic heart disease, *Coxiella burnetii* is responsible for 14% of culture negative endocarditis cases [31].

In conclusion, in the Dutch Q fever outbreak, an aspecific febrile illness with or without respiratory tract symptoms was the most common clinical presentation. Fatigue was present in 52% of patients at 6 months and dropped to 26% at one year follow-up. Using a commercially available IFA we observed a typical serological response to both phase I and phase II *Coxiella burnetii* antigens, characterized by an increase in antibody titers up to 3 months and a subsequent decrease in the following 9 months. Screening echocardiography at baseline revealed cardiac valve abnormalities in 59% of patients investigated, with only 7,6% having a clinically significant valvulopathy. None of the patients progressed to chronic disease. Baseline screening echocardiography is no longer part of the standard work-up of Q fever patients the Netherlands.

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References

1. Karagiannis I, Morroy G, Rietveld A, et al. Q fever outbreak in the Netherlands: a preliminary report. Euro Surveill 2007 Aug;12(8):E070809 2.
2. Van Steenbergen JE, Morroy G, Groot CA, Ruikes FG, Marcelis JH, Speelman P. [An outbreak of Q fever in The Netherlands--possible link to goats]. Ned Tijdschr Geneeskd 2007 Sep 8;151(36):1998-2003.
3. Karagiannis I, Schimmer B, Van Lier A, et al. Investigation of a Q fever outbreak in a rural area of The Netherlands. Epidemiol Infect 2009 Sep;137(9):1283-94.
4. Maurin M, Raoult D. Q fever. Clin Microbiol Rev 1999 Oct;12(4):518-53.
5. Parker NR, Barralet JH, Bell AM. Q fever. Lancet 2006 Feb 25;367(9511):679-88.
6. Raoult D, Marrie T, Mege J. Natural history and pathophysiology of Q fever. Lancet Infect Dis 2005 Apr;5(4):219-26.
7. Fenollar F, Thuny F, Xeridat B, Lepidi H, Raoult D. Endocarditis after acute Q fever in patients with previously undiagnosed valvulopathies. Clin Infect Dis 2006 Mar 15;42(6):818-21.
8. Landais C, Fenollar F, Thuny F, Raoult D. From acute Q fever to endocarditis: serological follow-up strategy. Clin Infect Dis 2007 May 15;44(10):1337-40.
9. Fine MJ, Auble TE, Yealy DM, et al. A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med 1997 Jan 23;336(4):243-50.
10. Fournier PE, Casalta JP, Habib G, Messana T, Raoult D. Modification of the diagnostic criteria proposed by the Duke Endocarditis Service to permit improved diagnosis of Q fever endocarditis. Am J Med 1996 Jun;100(6):629-33.
11. Fournier PE, Raoult D. Comparison of PCR and serology assays for early diagnosis of acute Q fever. J Clin Microbiol 2003 Nov;41(11):5094-8.
12. Zoghbi WA, Enriquez-Sarano M, Foster E, et al. Recommendations for evaluation of the severity of native valvular regurgitation with two-dimensional and Doppler echocardiography. J Am Soc Echocardiogr 2003 Jul;16(7):777-802.
13. Quinones MA, Otto CM, Stoddard M, Waggoner A, Zoghbi WA. Recommendations for quantification of Doppler echocardiography: a report from the Doppler Quantification Task Force of the Nomenclature and Standards Committee of the American Society of Echocardiography. J Am Soc Echocardiogr 2002 Feb;15(2):167-84.
14. Lang RM, Bierig M, Devereux RB, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005 Dec;18(12):1440-63.
15. Lang RM, Bierig M, Devereux RB, et al. Recommendations for chamber quantification. Eur J Echocardiogr 2006 Mar;7(2):79-108.
16. Leone M, Honstettre A, Lepidi H, et al. Effect of sex on Coxiella burnetii infection: protective role of 17beta-estradiol. J Infect Dis 2004 Jan 15;189(2):339-45.

17. Marmion BP, Shannon M, Maddocks I, Storm P, Penttila I. Protracted debility and fatigue after acute Q fever. Lancet 1996 Apr 6;347(9006):977-8.
18. Ayres JG, Smith EG, Flint N. Protracted fatigue and debility after acute Q fever. Lancet 1996 Apr 6;347(9006):978-9.
19. Wildman MJ, Smith EG, Groves J, Beattie JM, Caul EO, Ayres JG. Chronic fatigue following infection by Coxiella burnetii (Q fever): ten-year follow-up of the 1989 UK outbreak cohort. QJM 2002 Aug;95(8):527-38.
20. Hatchette TF, Hayes M, Merry H, Schlech WF, Marrie TJ. The effect of C. burnetii infection on the quality of life of patients following an outbreak of Q fever. Epidemiol Infect 2003 Jun;130(3):491-5.
21. Hickie I, Davenport T, Wakefield D, et al. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. BMJ 2006 Sep 16;333(7568):575.
22. Dupuis G, Peter O, Peacock M, Burgdorfer W, Haller E. Immunoglobulin responses in acute Q fever. J Clin Microbiol 1985 Oct;22(4):484-7.
23. Dupont HT, Thirion X, Raoult D. Q fever serology: cutoff determination for microimmunofluorescence. Clin Diagn Lab Immunol 1994 Mar;1(2):189-96.
24. Fenollar F, Fournier PE, Carrieri MP, Habib G, Messana T, Raoult D. Risks factors and prevention of Q fever endocarditis. Clin Infect Dis 2001 Aug 1;33(3):312-6.
25. Million M, Lepidi H, Raoult D. [Q fever: current diagnosis and treatment options]. Med Mal Infect 2009 Feb;39(2):82-94.
26. Singh JP, Evans JC, Levy D, et al. Prevalence and clinical determinants of mitral, tricuspid, and aortic regurgitation (the Framingham Heart Study). Am J Cardiol 1999 Mar 15;83(6):897-902.
27. Jones EC, Devereux RB, Roman MJ, et al. Prevalence and correlates of mitral regurgitation in a population-based sample (the Strong Heart Study). Am J Cardiol 2001 Feb 1;87(3):298-304.
28. Lewin MB, Otto CM. The bicuspid aortic valve: adverse outcomes from infancy to old age. Circulation 2005 Feb 22;111(7):832-4.
29. Hayek E, Gring CN, Griffin BP. Mitral valve prolapse. Lancet 2005 Feb 5-11;365(9458):507-18.
30. Schimmer B, Dijkstra F, Vellema P, et al. Sustained intensive transmission of Q fever in the south of the Netherlands, 2009. Euro Surveill 2009;14(19).
31. Balakrishnan N, Menon T, Fournier PE, Raoult D. Bartonella quintana and Coxiella burnetii as causes of endocarditis, India. Emerg Infect Dis 2008 Jul;14(7):1168-9.

Chapter 4

Prevention of Q fever endocarditis

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Abstract

The value of echocardiographic screening for cardiac valvular abnormalities of every acute Q fever patient and administration of longterm prophylactic antibiotic treatment in case of any, be it trivial or clinically significant, valvular defect has not been prospectively validated in an unselected population of Q fever patients.

A high prevalence of clinically insignificant valvular defects was found in al large group of Q fever patients from both the 2007 and 2008 cohort (n=134), but no patients developed chronic Q fever.

By contrast with existing publications, these data indicate a low risk of progression to Q fever endocarditis. This contrasting result may be explained by selection bias and lack of specification of valvular defect severity in published retrospective research.

In the setting of a massive Q fever outbreak, this raises the question whether the harm of low-treshold administration of long-term antibiotic prophylaxis for mild valvular defects outweighs the benefit. In addition, the associated health-care costs of such a follow-up strategy on a large scale are substantial.

Prevention of Q fever endocarditis

Confronted with more than 4000 human Q fever cases in the Netherlands since 2007, we appreciated the milestone article of Matthieu Million and colleagues' paper about Q fever endocarditis [1].

Since cardiac valvular disease is regarded as a major risk factor for this rare but potentially fatal complication, the investigators emphasise their follow-up strategy: systematic screening for cardiac valvular abnormalities of every acute Q fever patient and administration of longterm prophylactic antibiotic treatment in case of any, be it trivial or clinically significant, valvular defect [2].

However, this follow-up strategy has never been prospectively validated in an unselected population of patients with Q fever. We present here the outcome of 134 Dutch patients with Q fever from the 2007 and 2008 outbreaks, who had screening echocardiography and prospective serological follow-up for 1 year (tables 1 and 2). None of the patients received antibiotic prophylaxis and none developed clinical or serological signs of Q fever endocarditis. Additionally, none had developed clinical signs suggestive of chronic disease during the past 2 years.

Table 1. Characteristics of patients and echocardiographic findings

General	Number of patients (%)
Male sex	77 (57%)
Age (mean, range)	52,7 (19-87)
Cardiac valve surgery	0
Prosthetic valve	0
Antibiotic prophylaxis after acute Q fever	0
Origin of valvular disease*	
Rheumatic fever	1 (0,7%)
Bicuspid aortic valve	2 (1,5%)
Mitral valve prolaps	2 (1,5%)
History of infective endocarditis	0
Congenital (except bicuspid aortic valve)	0
Degenerative valvular disease**	29 (22%)
Unspecified valvulopathy	0

* Multiple predisposing valvular diseases are possible for a patient

** Trace regurgitation was not considered a degenerative valvulopathy

By contrast with existing publications, our data indicate a very low risk of progression to Q fever endocarditis. This contrasting result may be explained by selection bias and lack of specification of valvular defect severity in published retrospective research [3,4].

Table 2. Valvulopathy at screening echocardiography***

	trace	mild	moderate	severe
Mitral regurgitation	50	15	7	2
Aortic regurgitation	9	10	4	0
Aortic stenosis	0	2	0	0
Mitral stenosis	0	0	1	0

Severity of valvular defects were classified according to the American Society of Echocardiography guidelines

*** multiple valvulopathies are possible for a patient

In these studies, the risk of endocarditis in patients with acute Q fever and pre-existing cardiac valve defects was estimated to be 39%, but many of the included patients had prosthetic valves [3]. Million and colleagues [1] describe a highly selective cohort with valve defects mostly caused by rheumatic fever (30%, about half of all specified valvular disease), a disorder that is rare in most developed countries. Furthermore, 30% of their patients had infected prosthetic valves [1]. Degenerative valvular disease was present in only 5% [1] whereas this type of valvular defect is highly prevalent in the general population (22% in our study). Minor, clinically insignificant valvular disease (trace and mild defects) has a high prevalence in any unselected population [5]. In our study, these defects were not associated with development of chronic disease.
In our setting, the harm of low-threshold administration of long-term antibiotic prophylaxis might therefore outweigh the benefit. Additionally, the associated healthcare costs of this follow-up strategy on a large scale are substantial. Screening echocardiography is therefore no longer undertaken in patients with acute Q fever in the Netherlands.

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References

1. Million M, Thuny F, Richet H et al. Long-term outcome of Q fever endocarditis: a 26-year personal survey. Lancet Infect Dis 2010; 10: 527–35.
2. Landais C, Fenollar F, Thuny F et al. From acute Q fever to endocarditis: serological follow-up strategy. Clin Infect Dis 2007 May 15;44(10):1337-40.
3. Fenollar F, Fournier PE, Carrieri MP et al. Risks factors and prevention of Q fever endocarditis. Clin Infect Dis 2001 Aug 1;33(3):312-6.
4. Tissot-Dupont H, Vaillant V, Rey S et al. Role of sex, age, previous valve lesion, and pregnancy in the clinical expression and outcome of Q fever after a large outbreak. Clin Infect Dis 2007;44: 232–37.
5. Singh JP, Evans JC, Levy D, et al. Prevalence and clinical determinants of mitral, tricuspid, and aortic regurgitation (the Framingham Heart Study). Am J Cardiol 1999;83: 897–902.

Chapter 5

Detailed analysis of health status of Q fever patients 1 year after the first Dutch outbreak: a case–control study

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Abstract

Background

Q fever is a zoonosis caused by the obligate intracellular bacterium *Coxiella burnetii*. The two long-term complications, after primary infection, are chronic Q fever in 1% of patients, and a chronic fatigue syndrome in 10–20%. However, the existence of a protracted decreased health status after Q fever remains controversial.

Aim

To determine the health status of the patients of the Q fever outbreak in The Netherlands in 2007, 1 year after primary infection.

Design

Cross-sectional case–control study.

Methods

Health status of the patients from the 2007 Dutch Q fever outbreak was compared to age-, seks and geographically matched and Q fever seronegative controls. Health status of both patients and controls was assessed with the Nijmegen Clinical Screening Instrument (NCSI).

Results

Fifty-four Q fever patients provided 34 years of age- and sex-matched controls from the same neighbourhood. Eleven controls had positive Q fever serology and were excluded. Q fever patients had significantly more problems on the subdomains of symptoms and functional impairment. Overall quality of life was decreased in both patients and controls, 59% vs. 39%, respectively, ns). Severe fatigue levels were present in 52% of patients vs. 26% in controls ($P < 0.05$).

Conclusion

These data support a sustained decrease in many aspects of health status in Q fever patients in The Netherlands, 1 year after primary infection.

Introduction

Q fever is a zoonosis caused by the obligate intracellular bacterium *Coxiella burnetii* [1]. In its acute form, Q fever generally presents as a mild flu-like syndrome, atypical pneumonia or hepatitis [1,2]. After primary infection, approximately 1% of patients develop chronic Q fever, mainly as endocarditis in patients with pre-existing cardiac valvulopathies [1,3]. In recent years, research groups have drawn attention to another, less known, chronic sequel to primary Q fever, which takes the form of a debilitating chronic fatigue syndrome lasting more than 6 months in up to circa 20% of patients [4-9]. However, despite these reports on post Q fever fatigue, the existence of a 'post Q fever fatigue syndrome' or QFS as a distinct clinicopathological entity remains controversial, especially in France and the US [1,10]. In 2007, a goat-farming related Q fever outbreak of 73 cases was identified in the rural town of Herpen, the Netherlands [11]. Since then, an ongoing Q fever endemic has produced the Dutch province of North-Brabant as the currently most hyperendemic region in the world with more than 3000 acute Q fever cases in 2008 and 2009 [12,13]. No data exist on the impact on the long term impact on health status after acute Q fever in the Netherlands. The aim of the present study was to determine the health status of the patients of the Q fever outbreak in the Netherlands in 2007, one year after primary Q fever infection.

Methods

Patients

All patients from the Q fever outbreak cluster in Herpen (n=73) were asked to participate. A case of acute Q fever was defined as any inhabitant of the outbreak cluster area who presented with compatible clinical symptoms and a positive serology defined by immunofluorescence assay (IFA) (Focus diagnostics). Positive serology was defined as both anti-phase II IgM and anti-phase II IgG antibodies with a 1:64 or greater dilution or a seroconversion consisting of a fourfold increase of anti-Phase II IgG titer during follow-up. All Q fever patients were followed up serologically for a period of one year for antibodies against both Phase I and Phase II antigens, to exclude progression to chronic infection. As controls, Q fever patients were asked to bring along an age and sex matched control subject from their neighbourhood, without a history of Q fever. Control subjects had to be age (plus or minus 10 years) and sex matched to the patient. Control subjects were serologically tested for *Coxiella burnetii* antibodies using IFA. Positive serological findings of Q fever excluded controls from the primary analysis. Documentation on actual significant comorbidity was available for all participants. All patients provided written informed-consent. The study was approved by the local Ethical Board for Human Research. (Commissie Mensgebonden Onderzoek file-nr.: 2008/192, ABR nr.: NL24404.091.08)

Study design

The health status of the patients from the 2007 Q fever outbreak was compared to age-, sex- and geographically matched controls. Health status of both patients and controls was assessed with the Nijmegen Clinical Screening Instrument (NCSI) 1 year after the initial Q fever infection.

The Nijmegen Clinical Screening Instrument (NCSI)

In the literature, health status is defined as covering physiological functioning, symptoms, functional impairment in daily life, and quality of life as main domains [14,15]. These domains were shown empirically to be subdivided into many independent sub-domains [16]. The NCSI is an empirically composed battery of well validated instruments that enable a detailed measurement of these sub-domains of health status [17]. See table 1 for the tests and instruments by which the sub-domains of Health Status were measured. In the present study, the NCSI covers 8 sub-domains of the main domains 'symptoms', 'functional impairment' and 'quality of life'. The clinical meaning of these main domains is given hereafter.

Main domain Subjective Symptoms

The sub-domain Subjective Symptoms represents the patient's overall burden of dyspnea and experienced dyspnea during activities. The sub-domain Dyspnea Emotions embodies the level of frustration and anxiety a person experiences when dyspnoeic.

Main domain Functional Impairment

The sub-domain Behavioural Impairment represents the extent to which a person cannot perform specific and concrete activities, with respect to ambulation and activities at home, as a result of having the disease. The sub-domain Subjective Impairment represents the experienced degree of impairment.

Main domain Quality Of Life

The sub-domain General QoL covers mood and satisfaction with life as a whole. The sub-domain HRQoL represents satisfaction with physical functioning and confidence in the future. The sub-domain Satisfaction Relations represents the satisfaction with (or absence of) the relationships with spouse and others.

The NCSI provides normative data for each sub-domain; increasing scores indicating normal functioning, mild problems or severe problems.

Statistical analysis

All quantitative data are presented as mean ± se if normally distributed, otherwise median values (with range) are reported. Testing for differences between patients and controls was performed by Pearson's Chi^2 or Mann-Whitney test when appropriate. Statistical significance is set at a $p< 0.05$. Data were analyzed with SPSS 14.

Table 1. Main domains and subdomains of the NCSI, their corresponding instruments and scales [18]

Main domain	
Sub-domain	Instrument Subscale
Symptoms	
Subjective Symptoms	PARS-D Global Dyspnea Activity
	PARS-D Global Dyspnea Burden
Dyspnea Emotions	DEQ-Frustration
	DEQ-Anxiety
Fatigue	Checklist Individual Strength
Functional Impairment	
Behavioural Impairment	SIP Home Management
	SIP Ambulation
Subjective Impairment	QoL-RIQ General Activities
Quality of Life	
General QoL	BDI Primary Care
	SWLS-Total
HRQol	Satisfaction Physical
	Satisfaction Future
Satisfaction Relations	Satisfaction Spouse
	Satisfaction Social

Results

A total of 54 of the 73 (74%) Q fever patients from the 2007 Herpen outbreak agreed to participate. Thirty-four of these patients provided an age and sex-matched control from the same neighbourhood. Eleven of these controls had positive Q fever serology and were excluded, leaving 23 seronegative controls for comparison. Characteristics of the study and seronegative control subjects are given in table 2. Patients and controls proved to be well matched for age, sex, pre-existing comorbidity and smoking status.

Table 2. Patient characteristics expressed in number (%) unless stated otherwise of the patient group, and control group

	patient	control	
N	54	23	
Male	33 (61.1%)	10 (42.3%)	p>0.05*
Age (mean (sd))	53.1 (14.2)	53.6 (9.7)	p>0.05 $
range	20-81	38-73	
Comorbidity	22 (40.7%)	9 (39.1%)	p>0.05*
Smoking status			p>0.05*
current	24 (44.4%)	6 (26.1%)	
former	19 (35.2%)	8 (34.8%)	
never	11 (20.4%)	9 (39.1%)	

*Pearson Chi-Square, $mann-whitney test

Results on the sub-domains of the NCSI on a group level are provided in table 3. Q fever patients had significantly higher scores on all sub-domains of 'symptoms' (subjective pulmonary symptoms, dyspnea emotions, fatigue), 'functional impairment' (subjective impairment, behavioural impairment) and 'satisfaction with relations'. With respect to the main domain 'quality of life', there was a non-significant trend towards more problematic (i.e. higher) scores on the sub-domains 'general quality of life' (p = 0,09) and health related quality of life' (p = 0,073). In figure 1 results are presented on an individual level by the percentages of patients and controls scoring in the range of normal, mild, or severe problems. Fatigue scores of Q fever patients were abnormal (score: mild or severe) in 74% vs 48% in controls. Severe fatigue levels were present in 52% of patients vs. 26% in controls. Overall quality of life was decreased in a substantial number of patients and controls, but not significantly different between the two groups (Q fever patients 59% vs. controls 39%, ns). In figure 2 the percentage of patients and controls (y-axis) is given as a function of the number of sub-domains in which these patients and controls experience severe problems (x-axis) . In addition to the primary data analysis, we compared NCSI scores of the excluded seropositive controls (n=11) with the scores of seronegative control subjects (n=23). The NCSI scores of seropositive- and seronegative controls were not statistically different in all 8 measured sub-domains of health status (p > 0,05 for all sub-domains).

Table 3. NCSI scores on all subdomains (the higher the score, the more problematic) (Mann-Whitney test)

Main			Patient		Control		
Domain	Sub-domain	min-max	mean	(sd)	mean	(sd)	Sign.
Symptoms							
	Subjective symptoms	2-20	7.26	(4.85)	4.57	(4.92)	0.002
	Dyspnea emotions	6-24	9.85	(4.36)	7.39	(3.16)	0.005
	Fatigue	8-56	34.35	(13.87)	23.87	(14.08)	0.004
Functional Impairment							
	Behavioral impairment	0-135.5	8.21	(11.65)	3.13	(6.37)	0.050
	Subjective impairment	4-28	9.70	(5.55)	6.00	(3.49)	<0.001
Quality of Life							
	General QoL	1-101.6	19.52	(17.84)	11.96	(9.98)	n.s.
	HRQoL	2-10	4.26	(2.04)	3.35	(1.40)	n.s.
	Satisfaction relations	2-10	3.72	(2.08)	2.70	(1.29)	0.015

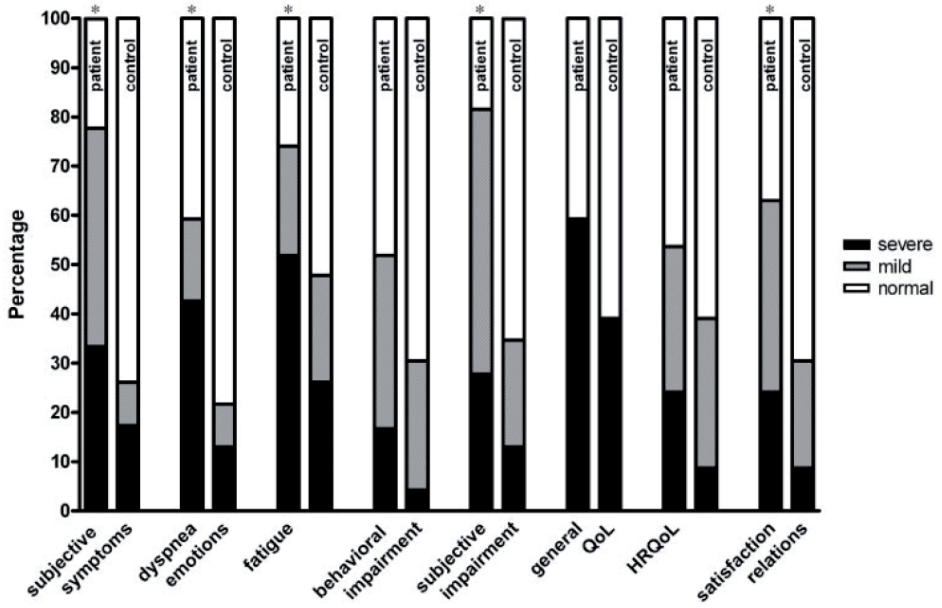

Figure 1. Percentages of mild and severe problems for each subdomain of the NCSI for the patient and control group (*p<0,05).

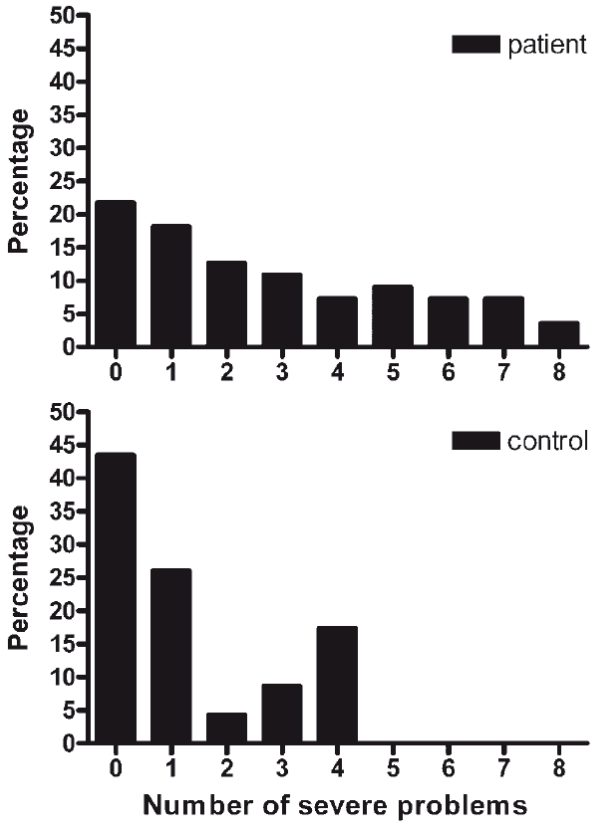

Figure 2. Frequency distribution of numbers of subdomains with severe problems in patients and controls.

Discussion

One year after primary infection, Q fever patients from the 2007 Herpen outbreak had a significantly lower health status in many sub-domains of the main domains 'symptoms' and 'functional impairment', when compared to age-, sex- and geographically-matched controls. Overall quality of life and health-related quality of life were significantly decreased in both patients and controls. Furthermore, on an individual level, patients had severe problems in more sub-domains than controls. Our findings lend support to the notion of a protracted reconvalescence phase after Q fever associated with decreased health status in many aspects. We found remarkably high clinically relevant (=severe) fatigue levels in roughly half (52%) of the Q fever patients one year after infection. In two separate case control studies published as letters the editor in the Lancet in 1996, Marmion et al. and Ayres et al. reported a syndrome of protracted fatigue and debility in Q fever patients for more than 5 years after primary infection with similar fatigue levels (67% (n=39) and 66% (n=71) respectively) [4,5]. Five- and 10-year follow-up of the large Q fever outbreak in the West Midlands, UK, also showed similar levels of chronic fatigue [6,7]. Dubbed the post Q fever fatigue syndrome (QFS), this protracted

fatigue state shares common features with the chronic fatigue syndromes following other (viral) pathogens such as Epstein-Barr virus and Ross River virus [9].

Although there was a significantly higher fatigue level in Q fever patients, the abnormally high fatigue level and low overall quality of life and health-related quality of life of the control group is striking. We postulate two explanations for this. First, the level of co-morbidity in this study is around 40%, which could partly account for the overall high scores on the NCSI sub-domains. Second, the original normal values for NCSI sub-domain scores were derived from healthy control subjects with normal pulmonary function tests. As these test were not available in the present study and given the significant smoking history equally present in patients and controls, undocumented pre-existing pulmonary morbidity may also have increased NCSI sub-domain scores in both groups.

Remarkably, NCSI scores from controls without a clinical history of Q fever but with serological evidence of exposure to *Coxiella burnetii* (and thus excluded from the primary analysis), were not statistically different from seronegative controls, suggesting that clinical expression of acute Q fever infection is an essential factor in the subsequent sustained decrease in health status. Severity of initial illness previously indeed has been shown to be the best predictor of subsequent development of a post-infective fatigue syndrome in both viral and non-viral pathogens, including Q fever [9]. Moreover, the same genetic polymorphisms in cytokine genes with critical roles in the inflammatory reponse to infection, underpin both the severity of the acute sickness and the average time to recovery across varied infections, including Q fever [19].

There are obvious difficulties with the credibility of QFS as a distinct clinico-pathological entity, as confounding factors such as financial compensation or insurance benefits following the acute sickness can be held responsible for the symptomatology and associated reduced quality of life. However, both the West Midlands outbreak mentioned earlier and the currently described Dutch outbreak were non-occupational and no litigation for financial compensation was pursued. A QFS diagnosis relies solely on the patient's own account of symptoms. In clinical practice, QFS patients remain indistinguishable from patients with a complete recovery after primary infection with *Coxiella burnetii*, as they do not meet the criteria for chronic Q fever infection: anti-phase I IgG titers are < 800 and appropriate cultures of the patients blood or tissues show no viable bacteria. Recently, an elegant new paradigm of persistence of *Coxiella* antigenic non-viable cell residues after primary infection in interaction with immunogenetic polymorphisms in the host has been put forward to better explain the chronic sequelae of acute Q fever, including QFS [20]. The importance of genetic host factors in QFS is supported by research done by Kerr et al. in the United Kingdom. They found significant differences in expression of 88 human genes, notably with a high proportion of genes involved in the immune response and infection, between patients with idiopathic chronic fatigue syndrome and normal controls. Remarkably, QFS patients were found to have similar patterns of gene expression to patients with idiopathic chronic fatigue syndrome [21,22].

Although our data support a decrease in many aspects of health status in many Q fever patients, some considerations have to be taken into account. First of all, patient numbers are small. However, Q fever patients were optimally matched, including serological testing in the controls. Furthermore, despite the small numbers, a statistically significant difference was found in 6 of the 8 tested sub-domains of the NCSI, supporting the notion of a rather large difference in health status between patients and controls. Second, the NCSI has proved to be a useful tool in assessing health status for use in research and care, but has mostly been applied in COPD patients. We used the NCSI in the setting of post-infectious health status assessment for the first time. Nevertheless, the various (parts of) questionnaires used to compile the NCSI function in their original and unaltered form. These generic questionnaires are not specified to assess only pulmonary disease and assess the different sub-domains of health status in the exact same way these instruments were originally designed and validated for. Moreover, the NCSI can be used by the clinician as an excellent tool to identify and monitor health status in its various sub-domains and can even guide therapeutic (psychological) interventions.

In conclusion, these data support a sustained decrease in health status in Q fever patients in the Netherlands, one year after primary infection. With more than 3000 new Q fever patients in the last 2 years in the setting of the ongoing Dutch Q fever epidemic, these are the first clinical data indicating a major long-term burden of the disease in the years to come.

References

1. Raoult D, Marrie T, Mege J. Natural history and pathophysiology of Q fever. Lancet Infect Dis. 2005 Apr;5(4):219-26.
2. Maurin M, Raoult D. Q fever. Clin Microbiol Rev. 1999 Oct;12(4):518-53.
3. Fenollar F, Fournier PE, Carrieri MP, Habib G, Messana T, Raoult D. Risks factors and prevention of Q fever endocarditis. Clin Infect Dis. 2001 Aug 1;33(3):312-6.
4. Marmion BP, Shannon M, Maddocks I, Storm P, Penttila I. Protracted debility and fatigue after acute Q fever. Lancet. 1996 Apr 6;347(9006):977-8.
5. Ayres JG, Smith EG, Flint N. Protracted fatigue and debility after acute Q fever. Lancet. 1996 Apr 6;347(9006):978-9.
6. Ayres JG, Flint N, Smith EG, Tunnicliffe WS, Fletcher TJ, Hammond K, Ward D, Marmion BP. Post-infection fatigue syndrome following Q fever. QJM. 1998 Feb;91(2):105-23.
7. Wildman MJ, Smith EG, Groves J, Beattie JM, Caul EO, Ayres JG. Chronic fatigue following infection by Coxiella burnetii (Q fever): ten-year follow-up of the 1989 UK outbreak cohort. QJM. 2002 Aug;95(8):527-38.
8. Hatchette TF, Hayes M, Merry H, Schlech WF, Marrie TJ. The effect of C. burnetii infection on the quality of life of patients following an outbreak of Q fever. Epidemiol Infect. 2003 Jun;130(3):491-5.
9. Hickie I, Davenport T, Wakefield D, Vollmer-Conna U, Cameron B, Vernon SD, Reeves WC, Lloyd A. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. BMJ. 2006 Sep 16;333(7568):575.
10. Raoult D. Q fever: still a mysterious disease. QJM2002 Aug;95(8):491-2.
11. Van Steenbergen JE, Morroy G, Groot CA, Ruikes FG, Marcelis JH, Speelman P. [An outbreak of Q fever in The Netherlands--possible link to goats]. Ned Tijdschr Geneeskd. 2007 Sep 8;151(36):1998-2003.
12. Schimmer B, Morroy G, Dijkstra F, Schneeberger PM, Weers-Pothoff G, Timen A, Wijkmans C, van der Hoek W. Large ongoing Q fever outbreak in the south of The Netherlands, 2008. Euro Surveill. 2008 Jul 31;13(31).
13. Schimmer B, Dijkstra F, Vellema P, Schneeberger PM, Hackert V, ter Schegget R, Wijkmans C, van Duynhoven Y, van der Hoek W. Sustained intensive transmission of Q fever in the south of the Netherlands, 2009. Euro Surveill. 2009;14(19).
14. Wilson IB, Cleary PD. Linking clinical variables with health-related quality of life. A conceptual model of patient outcomes. JAMA. 1995;273:59-65.
15. Taillefer SS, Kirmayer LJ, Robbins JM, Lasry JC. Psychological correlates of functional status in chronic fatigue syndrome. J Psychosom Res. 2002 Dec;53(6):1097-106.
16. Vercoulen JH, Daudey L, Molema J, Vos PJ, Peters JB, Top M, Folgering H. An Integral assessment framework of health status in chronic obstructive pulmonary disease (COPD). Int J Behav Med. 2008;15(4):263-79.
17. Peters JB, Daudey L, Heijdra YF, Molema J, Dekhuijzen PN, Vercoulen JH. Development of a battery of instruments for detailed measurement of health status in patients with COPD in routine care: the Nijmegen Clinical Screening Instrument. Qual Life Res. 2009 Sep;18(7):901-12.

18. Vercoulen JHMM, Swanink CMA, Galama JMD et al. Dimensional assessment in chronic fatigue syndrome. J Psychosom Res. 1994; 38:383-392.
19. Vollmer-Conna U, Piraino BF, Cameron B, Davenport T, Hickie I, Wakefield D, Lloyd AR. Cytokine polymorphisms have a synergistic effect on severity of the acute sickness response to infection. Clin Infect Dis 2008 Dec 1;47(11):1418-25.
20. Marmion BP, Sukocheva O, Storm PA, Lockhart M, Turra M, Kok T, Ayres J, Routledge H, Graves S. Q fever: persistence of antigenic non-viable cell residues of Coxiella burnetii in the host--implications for post Q fever infection fatigue syndrome and other chronic sequelae. QJM2009 Oct;102(10):673-84.
21. Gene expression subtypes in patients with chronic fatigue syndrome/myalgic encephalomyelitis. Kerr JR, Petty R, Burke B, Gough J, Fear D, Sinclair LI, Mattey DL, Richards SC, Montgomery J, Baldwin DA, Kellam P, Harrison TJ, Griffin GE, Main J, Enlander D, Nutt DJ, Holgate ST. J Infect Dis. 2008 Apr 15;197(8):1171-84.
22. Microbial infections in eight genomic subtypes of chronic fatigue syndrome/myalgic encephalomyelitis. Zhang L, Gough J, Christmas D, Mattey DL, Richards SC, Main J, Enlander D, Honeybourne D, Ayres JG, Nutt DJ, Kerr JR. J Clin Pathol. 2010 Feb;63(2):156-64.

Chapter 6

Persistence of impaired health status of Q fever patients 4 years after the first Dutch outbreak

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Abstract

A significant proportion of Q fever patients from the first Dutch Q fever outbreak in 2007 showed impairment in health status up to 1 year after infection. Interested in whether this decrease in health status persisted, we set out to determine the health status in the same cohort of patients, 4 years after primary infection and to compare health status scores on an individual patient's level between 1 and 4 years follow-up. Health status was assessed with the Nijmegen Clinical Screening Instrument (NCSI). Patients were serologically tested to exclude patients with possible, probable or proven chronic Q fever. Results on the sub-domains of the NCSI on a group level [2008 (n=54) and 2011 (n=46)] showed a persistent significant percentage of patients exhibiting clinically relevant ('severe') scores for all sub-domains of the NCSI. After 4 years, undue fatigue was present in 46% and exactly half of all patients experienced a severely impaired general quality of life. Patients with NCSI scores available in both 2008 and 2011 (n=37) showed no difference in all sub-domain scores, except for a small decrease in dyspnea emotions in 2011. In this group, a significant proportion of patients either improved or worsened in one or more sub-domains of health status. We conclude that on a group level, health status of Q fever patients remained impaired 4 years after primary infection. On an individual patient's level, health status may change.

Introduction

Q fever, an ubiquitous zoonosis caused by the intracellular bacterium *Coxiella burnetii*, emerged as a human pathogen in The Netherlands in 2007 [1]. Successive annual outbreaks linked to intensified goat farming have amounted to more than 4000 notified and a further estimated 44000 unnotified human cases, the majority of which occurred in the province of Noord-Brabant in the south of the Netherlands [2]. Following extensive governmental measures including restrictions on manure trafficking, vaccination and culling of dairy goats and sheep starting in 2010, the rate of acute human Q fever cases fell markedly. However, since then chronic Q fever has remained a significant clinical problem [3]. Accordingly, research efforts have shifted in recent years from management of acute disease to the long term sequelae of Q fever; chronic Q fever (vascular infection, endocarditis) and the Q fever fatigue syndrome (QFS) [4].

Early reports after the first outbreaks in 2007 and 2008 showed persistent symptomatology, especially fatigue, and a decreased health status of Q fever patients for a follow-up period of 12-26 months [5,6]. Health status scores of patients at 12 months versus 17-26 months follow-up did not differ significantly, suggesting a long term persistence of impaired health status of equal severity level after one year. The original small studies from Australia and England suggest persistent symptomatology and decreased health status can persist for 5-10 years [7,8].

For the patients of the Dutch Q fever outbreaks, it remains unknown whether impaired health status and symptomatology persist or can change after 1-2 years. In addition to the evaluation of long term health status on a group level, changes in health status of individual patients have not been studied before.

The first aim of this study was to determine the health status of patients from the first Q fever outbreak in the Netherlands 4 years after primary infection. Second aim was to compare health status scores on an individual patient's level between 1 and 4 years follow-up, for those patients for whom data were available at both time points.

Methods

Study design and population

Health status of patients from the 2007 Q fever outbreak was assessed with the Nijmegen Clinical Screening Instrument (NCSI), 4 year after the initial Q fever infection. The same questionnaire (NCSI) was used in an earlier assessment of health status of patients from the Herpen outbreak cluster at one year follow-up [5]. This allows for direct comparisons of scores on an individual patient level between time points 1 and 4 years follow-up. All surviving patients from the 2007 outbreak followed up in the GP's practice in Herpen were asked to participate [9]. Patients who did not respond to the initial invitation were contacted by mail and a single telephone call.

Clinical evaluation and questionnaire

Evaluation of participants included a complete history and physical examination by their own physician, completion of the NCSI questionnaire and a single venous blood sampling for serological testing to exclude patients with possible, probable or proven chronic Q fever. Serological testing consisted of the determination of IFA phase I IgG (Focus diagnostics, Cypress, CA, USA) antibodies. Following the diagnostic criteria of the Dutch Q fever Consensus Group, an IgG phase I of 1024 or more excluded patients from analysis [10].

All patients provided written informed consent. This study was approved by the local Ethical Board for Human Research. (Commissie Mensgebonden Onderzoek file-nr.: 2008/192, ABR nr.: NL24404.091.08, amendment dd 3 September 2010)

Questionnaire: the Nijmegen Clinical Screening Instrument (NCSI)

The NCSI has been used in previous studies assessing health status after Q fever infection [5, 6, 11-13]. It is based on an empirical definition of health status, covering 'physiological functioning', 'symptoms', 'functional impairment in daily life', and 'quality of life' as main domains. These domains are in turn subdivided into many independent sub-domains. The NCSI is an empirically composed battery of well validated instruments that enable a detailed measurement of these sub-domains of health status [14]. The NCSI provides normative data for each individual sub-domain; increasing scores indicating normal functioning, mild problems or severe problems. We excluded the domain 'physiological function' from the NCSI questionnaire as patients did not perform pulmonary function tests. In this study we measured the same main domains as in 2008; 'symptoms', 'functional impairment' and 'quality of life'. The corresponding sub-domains are 'subjective symptoms', 'dyspnea emotions*', 'fatigue', 'behavioral impairment', 'subjective impairment', 'general quality of life', 'health related quality of life' and 'satisfaction with relations' [5]. (The subdomain subjective symptoms represent the patient's overall burden of dyspnea and experienced dyspnea during activities. The subdomain 'dyspnea emotions' embodies the level of frustration and anxiety a person experiences when dyspnoeic.)

Statistical analysis

All quantitative data are presented as mean plus or minus standard error if normally distributed, otherwise median values (with range) are reported. Testing for differences between patients in 2008 and 2011 was performed by Pearson's Chi^2 or Mann-Whitney test when appropriate. Statistical significance was set at a $p< 0.05$. Data were analyzed with SPSS v. 14 (SPSS Inc., USA).

Results

A total of 47 (57%) of 82 surviving Q fever patients from the 2007 outbreak who were followed up in the Herpen GP's practice, agreed to participate and were included. All patients underwent clinical evaluation, provided a completed NCSI questionnaire and a blood sample. One of

the patients had a phase I IgG of 1024, indicating possible, probable or proven chronic Q fever, and was referred to an infectious diseases specialist for evaluation, leaving 46 patients for analysis. For 37 patients the NCSI scores were available at both the 1 and 4 year follow-up, allowing for longitudinal comparison of health status at the individual patient's level.
In table 1, patient characteristics of this study population (n=46) are given for comparison with the data on the patient group assessed in 2008 (n=54).

Table 1. Patient characteristics expressed in number (%) unless stated otherwise
see reference [5]

	2008#	2011
N	54	46
Male	33 (61%)	28 (61%)
Age (mean (sd))	53 (14)	56 (14)
range	20-81	23-84
Comorbidity	22 (41%)	22 (48%)
Smoking status		
current	24 (44%)	21 (46%)
former	19 (35%)	15 (33%)
never	11 (20%)	10 (22%)

Table 2. Percentage of patients scoring normal, elevated or severe scores on the NCSI in 2008 (n=54) and 2011 (n=46).

Main domain		2008			2011		
	Sub-domain	normal	elevated	severe	normal	elevated	severe
Symptoms							
	Subjective symptoms	22 %	44 %	33 %	20 %	46 %	35 %
	Dyspnea emotions	41 %	17 %	43 %	41 %	22 %	37 %
	Fatigue	26 %	22 %	52 %	33 %	22 %	46 %
Functional Impairment							
	Behavioral impairment	48 %	35 %	17 %	46 %	33 %	22 %
	Subjective impairment	19 %	54 %	28 %	28 %	41 %	30 %
Quality of Life							
	General QoL	41 %	--	59 %	50 %	--	50 %
	HRQoL	46%	30 %	24 %	50 %	22 %	28 %
	Satisfaction relations	37 %	39 %	24 %	59 %	24 %	17 %

Table 3. Comparison of NCSI scores for all sub-domains* on an individual patient's level between 2008 and 2011 (N=37). * the higher the score, the more problematic, for reference values for all subdomains see reference [14]

Main		2008		2011		
Domain	Sub-domain	mean	(sd)	mean	(sd)	sign.
Symptoms						
	Subjective symptoms	6.9	(4.9)	6.9	(5.5)	0.97 n.s.
	Dyspnea emotions	10.1	(4.5)	8.7	(3.9)	0.01
	Fatigue	33.4	(15.1)	31.5	(13.1)	0.26 n.s.
Functional Impairment						
	Behavioral impairment	8.7	(12.6)	9.5	(13.9)	0.63 n.s.
	Subjective impairment	9.8	(5.7)	9.2	(5.2)	0.46 n.s.
Quality of Life						
	General QoL	20.1	(19.5)	18.3	(19.15)	0.54 n.s.
	HRQoL	4.0	(1.9)	4.0	(2.1)	1.00 n.s.
	Satisfaction relations	3.5	(1.8)	3.4	(2.2)	0.71 n.s.

Table 4. Percentages of patients whose NCSI scores improved, remained equal or worsened* on an individual patient's level, between follow-up time points 2008 and 2011 (n=37).
* Differences between scores in 2008 and 2011 were calculated as follows: (Score 2008 – score 2011) / sd 2008. A difference on a subdomain score of < -0.5 corresponds with significant improvement; a score of > -0.5 and < 0.5 means no significant change and a score of > 0.5 corresponds to worsening of health status in that subdomain.

Main Domain	Sub-domain	Improved	Equal	Worsened
Symptoms				
	Subjective symptoms	24 %	58 %	18 %
	Dyspnea emotions	29 %	66 %	5 %
	Fatigue	34 %	45 %	2 %
Functional Impairment				
	Behavioral impairment	5 %	82 %	13 %
	Subjective impairment	32 %	50 %	18 %
Quality of Life				
	General QoL	26 %	55 %	18 %
	HRQoL	37 %	32 %	32 %
	Satisfaction relations	21 %	68 %	11 %

Results on the sub-domains of the NCSI on a group level (table 2) for 2008 (n=54) and 2011 (n=46) show a persistent significant percentage of patients exhibiting clinically relevant ('severe') scores for all sub-domains of the NCSI. No significant differences were found between patients with or without co-morbidities on any of the sub-domains of the NCSI. Overall, severe subjective symptoms were present in 35% of patients. After 4 years, undue fatigue was present in 46% (52% after 1 year) and exactly half of all patients experienced a severely impaired general quality of life.
Comparison of sub-domains for patients with NCSI scores available in both 2008 and 2011 (n=37, table 3.) showed no difference in all sub-domain scores, except for a small but statistically significant decrease in dyspnoea emotions.
Table 4. shows the dynamics of NCSI scores on an individual patient's level, as percentages of patients whose scores either improved, remained equal or worsened between 2008 and 2011. A significant proportion of all individual Q fever patients either improved or worsened

in one or more sub-domains of health status. Fatigue scores improved in about one-third of patients, but worsened in one in five (21%). More than 30% of patients showed significant improvement in the subdomains of 'fatigue', 'subjective impairment' and 'health related quality of life', but these improvements were cancelled out on a group level by patients with worsening scores.

Discussion

Persistence of impairment in many subdomains of health status after 4 years in the majority of affected Q fever patients is marked, and suggests an absence of further dynamics in symptomatology after 1 year. These findings tally with earlier research in England, Australia and Canada, on very long term sequelae post Q fever infection, reporting increased symptomatology including similar levels of fatigue up to 10 years after primary infection [15-17]. A further finding is that on an individual patient level, although the majority remains equally impaired, patients can improve or worsen on an individual basis in one or more subdomains of health status between 1 and 4 years.

This study has several strengths and weaknesses that need to be addressed. This is the first report on longitudinal long term health status of Q fever patients in the Netherlands for more than 2 years follow-up. The availability of detailed health status data at follow-up points 1 year and 4 years for the same patients allowed for comparison on an individual patient's level, a feature that has not been studied before. Also, serology was performed to exclude chronic disease. As the background prevalence of serological evidence of past Q fever infection in endemic regions in the Netherlands reaches up to 15% and chronic Q fever can develop in 1-5% of Q fever patients, this is highly relevant [18].

The limited patient number in this study is a weakness, and precludes generalisation of the obtained results to the entire Dutch Q fever outbreak population. The small patient number is largely due to the fact that the study-cohort comprised the very first, local group of Q fever patients in the Netherlands from the rural town of Herpen, in the province of Noord-Brabant [9]. Furthermore, the absence of a proper Q fever negative control group at the 2011 time point does not allow for an estimation of how much impairment of health status is actually directly caused by the primary infection with *Coxiella burnetii.*

This study provides further evidence for persistent symptomatology and long term decreased health status of Q fever patients in the Netherlands. Research groups in England, Australia and Canada have similarly found consistent and clear decreased long term health status outcomes in Q fever patients, using various/different questionnaires and study methods [15-17]. The acknowledgement and acceptance of a protracted fatigued state following acute Q fever in about 20% of patients, sometimes designated the post Q fever fatigue syndrome (PQFS), however, is not universal [19,20]. The main reasons for this reluctance seem to be the incompletely understood pathophysiology of PQFS, the absence of a diagnostic test and possibly, fear of patient's financial compensation claims. Moreover, post-infectious protracted fatigue states have been reported for several other bacterial and viral pathogens

such as *Bartonella, Legionella*, Epstein Barr virus, Cytomegalovirus and West Nile Virus [21, 22]. A recent Dutch study, using the NCSI and the Short Form 36 (SF36), compared the health status of 309 Q fever patients and 190 patients with Legionnaires' disease and found severe impairment of various health status subdomains including fatigue for both diseases 1 year after onset of illness [11]. Another smaller study from the Netherlands compared 50 patients with a lower respiratory tract infection (LRTI) caused by Q fever with 32 non Q fever LRTI patients with a follow up period of 10 to 19 months. With the exception of the sub-domain 'subjective pulmonary symptoms', no difference was observed in health status for all other measured subdomains using the NCSI [12]. Interestingly, as in our study, significant proportions of Q fever patients experienced severe fatigue (40%) and severely impaired general quality of life (40%).

There are numerous studies probing the underlying pathophysiology of PQFS that show significant differences between Q fever patients with and without PQFS with regard to gene expression [23-25], serum cytokine levels [26] and PBMC cytokine release patterns after stimulation with *Coxiella burnetii* antigens [26,27]. However, there are several shortcomings that impede a thorough interpretation and unifying concept of PQFS pathophysioloy; a lack of a clear definition of PQFS, small patient numbers and different lab techniques and protocols that do not allow for ready comparison and validation of obtained results. Recently, a new unifying paradigm has been put forward for PQFS, postulating PQFS symptomatology to be underpinned by the interaction of persistent *Coxiella* specific non-viable cell residues with immunogenetic polymorphisms in the host [28,29].

Moreover, there is no validated evidence based treament for PQFS. To address this last issue, a large randomised placebo-controlled prospective clinical trial is underway in The Netherlands assessing the efficacy of longterm treatment with doxycycline and cognitive behavioral therapy in PQFS patients [30].

In conclusion, these data support an overall sustained impaired health status of Q fever patients in the Netherlands 4 years after primary infection. A minority of Q fever patients with impaired health status in 2008 showed improvement in various subdomains of health status by 2011, but on a group level this effect was canceled out by patients with worsening scores.

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Ethical Standards: The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

References

1. Van Steenbergen JE et al. [An outbreak of Q fever in The Netherlands--possible link to goats]. Nederlands Tijdschrift voor Geneeskunde 2007; 151: 1998-2003.
2. Van der Hoek W et al. Relation between Q fever notifications and Coxiella burnetii infections during the 2009 outbreak in The Netherlands. Eurosurveillance 2012; 19; 20058.
3. Schneeberger PM et al. Q fever in the Netherlands - 2007-2010: what we learned from the largest outbreak ever. Médecine et Maladies Infectieuses 2014; 44: 339-353.
4. Van der Hoek W et al. Shifting priorities in the aftermath of a Q fever epidemic in 2007 to 2009 in The Netherlands: from acute to chronic infection. Eurosurveillance 2012; 19: 20059.
5. Limonard GJ et al. Detailed analysis of health status of Q fever patients 1 year after the first Dutch outbreak: a case-control study. QJM: An International Journal of Medicine 2010; 103: 953-958.
6. Morroy G et al. The health status of Q-fever patients after long-term follow-up. BMC Infectious Diseases 2011; 18: 97.
7. Marmion BP et al. Protracted debility and fatigue after acute Q fever. Lancet 1996; 347: 977-978
8. Ayres JG, Smith EG, Flint N. Protracted fatigue and debility after acute Q fever. Lancet 1996; 347: 978-979.
9. Limonard GJ et al. One-year follow-up of patients of the ongoing Dutch Q fever outbreak: clinical, serological and echocardiographic findings. Infection 2010; 38: 471-477.
10. Wegdam-Blans MC et al. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. Journal of Infection 2012; 64: 247-259.
11. Van Loenhout JA et al. Serious long-term health consequences of Q-fever and Legionnaires' disease. Journal of Infection 2014; 68: 527-533.
12. Van Dam AS et al. A cross-sectional study to assess the long–term health status of patients with lower respiratory tract infections including Q fever. Epidemiology and Infection 2015; 143: 48-54.
13. Kremers MN et al. Correlations between peripheral blood Coxiella burnetii DNA load, interleukin-6 levels, and C-reactive protein levels in patients with acute Q fever. Clinical and Vaccine Immunology 2014; 21: 484-487.
14. Peters JB et al. Development of a battery of instruments for detailed measurement of health status in patients with COPD in routine care: the Nijmegen Clinical Screening Instrument. Quality of Life Research 2009; 18: 901-912.
15. Ayres JG et al. Post-infection fatigue syndrome following Q fever. QJM: An International Journal of Medicine 1998; 91: 105-123.
16. Wildman MJ et al. Chronic fatigue following infection by Coxiella burnetii (Q fever): ten-year follow-up of the 1989 UK outbreak cohort. QJM: An International Journal of Medicine 2002; 95: 527-538.
17. Hatchette TF et al. The effect of C. burnetii infection on the quality of life of patients following an outbreak of Q fever. Epidemiology and Infection 2003; 130: 491-495.
18. Van der Hoek W et al. Epidemic Q fever in humans in the Netherlands. Advances in Experimental Medicine and Biology 2012; 984: 329-364.

19. Raoult D. Q fever: still a mysterious disease. QJM: An International Journal of Medicine 2002; 95: 491-492.
20. Strauss B et al. Are fatigue symptoms and chronic fatigue syndrome following Q fever infection related to psychosocial variables? Journal of Psychosomatic Research 2012; 72: 300-304.
21. Hickie I et al. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. British Medical Journal 2006; 16; 333.
22. Garcia MN et al. Evaluation of prolonged fatigue post-West Nile virus infection and association of fatigue with elevated antiviral and proinflammatory cytokines. Viral Immunology 2014; 27: 327-333.
23. Kerr JR et al. Gene expression subtypes in patients with chronic fatigue syndrome/myalgic encephalomyelitis. Journal of Infectious Diseases 2008; 15; 197: 1171-1184.
24. Helbig K et al. Immune response genes in the post-Q-fever fatigue syndrome, Q fever endocarditis and uncomplicated acute primary Q fever. QJM: An International Journal of Medicine 2005; 98: 565-574.
25. Piraino B, Vollmer-Conna U, Lloyd AR. Genetic associations of fatigue and other symptom domains of the acute sickness response to infection. Brain, Behaviour and Immunity 2012; 26: 552-558.
26. Penttila IA et al. Cytokine dysregulation in the post-Q-fever fatigue syndrome. QJM: An International Journal of Medicine 1998; 91: 549-560.
27. Vollmer-Conna U et al. Cytokine polymorphisms have a synergistic effect on severity of the acute sickness response to infection. Clinical Infectious Diseases 2008; 47: 1418-1425.
28. Marmion BP et al. Q fever: persistence of antigenic non-viable cell residues of Coxiella burnetii in the host-implications for post Q fever infection fatigue syndrome and other chronic sequelae. QJM: An International Journal of Medicine 2009; 102: 673-684.
29. Sukocheva OA et al. Long-term persistence after acute Q fever of non-infective Coxiella burnetii cell components, including antigens. QJM: An International Journal of Medicine 2010; 103: 847-863.
30. Keijmel SP et al. The Qure study: Q fever fatigue syndrome--response to treatment; a randomized placebo-controlled trial. BMC Infectious Diseases 2013; 27; 157.

Chapter 7

Developing a new clinical tool for diagnosing chronic Q fever: the Coxiella ELISPOT

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Abstract

Definitively establishing a clinical diagnosis of chronic Q fever remains challenging, as the diagnostic performance of both conventional serological tests and PCR is limited. Given the importance of an early diagnosis of chronic Q fever, there is a need for a reliable diagnostic test.

We developed an enzymelinked immunospot assay to measure *Coxiella burnetii* (*C. burnetii*)-specific T-cell responses (*Coxiella* ELISPOT) to both phase I and phase II antigens and tested convalescent Q fever patients (without chronic disease, n = 9) and patients with an established diagnosis of chronic Q fever (n = 3). The *Coxiella* ELISPOT adequately identified convalescent Q fever patients from healthy controls by demonstrating *C. burnetii*-specific T-cell interferon-c production to both phase I and phase II antigens. Compared to convalescent Q fever patients, chronic Q fever patients showed a distinct *Coxiella* ELISPOT profile characterized by a much higher spot count for both phase I and phase II (18-fold for phase II, 8-fold higher for phase I) and a consistent shift towards more phase I reactivity.

The diagnostic potential of the *Coxiella* ELISPOT is promising and warrants further investigation.

Introduction

In the Netherlands, annual Q fever outbreaks associated with goat farming have amounted to more than 4000 new human Q fever cases since 2007 (Delsing et al., 2010). With an expected 2–5% of patients developing chronic Q fever, clinicians now increasingly face the challenges in accurately diagnosing this rare but potentially fatal complication of *Coxiella burnetii (C. burnetii)* infection (Delsing et al., 2010; Frankel et al., 2011). The main clinical manifestations of chronic disease following the Dutch outbreaks are infection of vascular aneurysms and prostheses, closely followed by endocarditis (Delsing et al., 2010). In routine clinical practice, definitively establishing a diagnosis of chronic Q fever diagnosis is challenging and relies heavily on the demonstration of a vigorous humoral immune response to the antigenic phase I *C. burnetii,* as evidenced by high levels of phase I IgG antibodies (Frankel et al., 2011). In conjunction with suggestive serology, a positive serum PCR for *Coxiella* DNA is considered diagnostic for chronic disease (Fenollar et al., 2004; Wegdam-Blans et al., 2011). This poses an important clinical problem, as the performance of serological tests and PCR is limited in both establishing and excluding a diagnosis of chronic disease. A possible explanation for the underperformance of current serological assays may be the fact that these tests only reflect the host's humoral immunity, whereas the most relevant immunological response to chronic infection with the obligate intracellular *C. burnetii* is first and foremost cellmediated (Andoh et al., 2007; Marmion et al., 2009).

Given the importance of an early diagnosis of chronic Q fever timely prompting long-term antibiotics and/or surgical intervention, testing a patient's cellular rather than humoral immunological response to *C. burnetii* might be a more reliable tool for diagnosing chronic Q fever in a clinical setting.

A recently developed paradigm indeed proposes clinical outcome after *C. burnetii* infection as a function of host immunity/pathogen interaction with a strong emphasis on dysfunctional cellular immunity (Marmion et al., 2009).

T-cells and their associated cytokines, interferon-γ (IFN-γ) being the most potent, have a pivotal role in dealing with primary infection and subsequent clearance or control of intracellular pathogens such as *Mycobacterium tuberculosis, Legionella pneumophila, Chlamydophila and C. burnetii* (Andoh et al., 2007). The long-term clinical outcome of Q fever might, therefore, be better characterized by measuring the host's C. burnetii-specific T-cell response. IFN-γ release assays (IGRA) such as ELISPOT can detect the IFN-γ production by antigen-specific T-cells ex vivo at an individual cell level. In tuberculosis, IFN-γ based tests are superior to tuberculin skin testing in detecting patients with latent infection and are better predictors of subsequent development of active disease (Santín Cerezales & Benítez, 2011). We hypothesize that different Q fever clinical outcomes (nonchronic past infection and chronic disease) are similarly associated with marked differences in a patient's *C. burnetii* antigenspecific T-cell IFN-γ production.

Aim

The first aim of this study was to develop an enzymelinked immunospot assay to measure *C. burnetii*-specific T-cell responses (*Coxiella* ELISPOT) in convalescent Q fever patients (without any evidence of chronic Q fever). The second aim was to test the Coxiella ELISPOT in patients with an established diagnosis of chronic Q fever.

Materials and methods

Coxiella ELISPOT

Mononuclear cells were isolated from whole blood specimens (lithium heparin) drawn at regular phlebotomy, using Leucosep tubes (Greiner Bio-One Ltd # 163288) in combination with T-cell Xtend (TTK.610; Oxford Imunnotec Ltd, Abingdon, UK). As stimulating antigens, we used the commercially available lyophilized phase I and phase II antigens (#1227 and #1123 respectively; Virion-Serion Immunodiagnostica GmbH, Wurzburg, Germany). Phytohaemagglutinin (PHA; Sigma L4144), anti-CD3 Mab (Mabtech, Sweden) and CEF-pool (CT370; U-Cy-Tech Biosciences, Utrecht, the Netherlands; a combination of CMV, EBV and influenza peptides) were used as stimulation controls. Negative control wells only contained mononuclear cells without stimulating antigens. Subsequently, 100 lL of mononuclear cells were seeded at a density of 250 000 cells per well in precoated wells of PVDF strip plates (ELISpotPRO; Mabtech) and incubated with 50 lL of antigens or controls during 16–20 h at 37 °C and 5% CO2. The resulting number of spots corresponds with the number of individual T-cells producing IFN-γ after stimulation with *C. burnetii* antigens. After detection spots were enumerated using an ELISpot reader (Auto Immun Diagnostika GmbH, Strassberg, Germany). This work was a proof of principle study for the TRIQ (T-cell Response In Q fever) study approved by the local Ethical Committee (VCMO NL35867.100.11). All study subjects gave informed consent.

Study population

Study subjects were classified into three groups. Healthy control subjects (n = 9): negative serology (immunofluorescence assay: IFA, Focus Diagnostics or ELISA, Serion Immundiagnostica) for *C. burnetii* and no pre-existing comorbidities. Convalescent Q fever patients (n = 9): a documented history of Q fever with complete clinical recovery; no residual complaints or clinical signs of chronic Q fever, serological evidence of a past *C. burnetii* infection and no evidence of chronic disease (PCR negative and IFA phase I IgG < 1024 at ≥6 months follow-up). Chronic Q fever patients (n = 3) fulfilled the diagnostic criteria of the Dutch consensus statement on chronic Q fever (Wegdam-Blans et al., 2011): a positive *C. burnetii* PCR in tissue and/or serum in the absence of acute Q fever infection (n = 2), or an IFA phase I IgG titre of ≥1024 and an endocarditis diagnosis using the Duke criteria or evidence of a vascular infection on PET/CT or ultrasound examination (n = 1).

Main study endpoint

The spot count in the *Coxiella* ELISPOT for the three groups were assessed: healthy controls, convalescent patients and chronic Q fever patients. Assuming a Gaussian distribution, differences in mean spot counts between groups were assessed by one-way ANOVA.

Results

Coxiella burnetii-specific T-cell responses (mean ± SE) in convalescent Q fever patients were 10 ± 5 spots to phase I antigen and 28 ± 13 spots to phase II antigen (Table 1). Using a cut-off value of five spots for phase II reactivity, ELISPOT sensitivity for a past Q fever infection was 89%. *Coxiella* burnetii-specific T-cell responses in chronic Q fever patients were more pronounced: 194 ± 46 spots to phase I antigen and 219 ± 36 spots to phase II antigen. T-cell responses in chronic Q fever patients were significantly higher than those in convalescent patients: 18-fold for phase I antigen (P < 0.0001) and 8-fold for phase II antigen (P 0.0002).

Table 1. Clinical characteristics and *Coxiella* ELISPOT results

	healthy controls n=9	convalescent Q fever n=9	chronic Q fever n=3	p value*
mean (±SD) age, range (years)	44,2 (10,8), 31-64	57,0 (15,5), 28-82	52,6 (15,3), 31-64	
female / male	3 / 6	3 / 6	0 / 3	
median months since diagnosis (range)	n/a	8 (6-22)	1 (0-1)	
phase I spots mean (±SD), range	0,8 (±1,0), 0-3	10 (±5), 1-42	194 (±46), 104-217	<0.01
phase II spots mean (±SD), range	0,2 (±0,4), 0-1	28 (±13), 1-120	219 (±36), 164-205	<0.01
PHA positive control spots mean (±SD), range	240 (±56), 58-350	272 (±25), 102-350	240 (±56), 172-350	0.87
CEF positive control spots mean (±SD), range	126 (±54), 16-211	101 (±62), 0-233	104 (±67), 18-236	0.95
negative control spots mean (±SD), range	0	0	0	

* differences between means of all three groups, significance set at p<0.05

The proportion of phase II to phase I response was expressed as a ratio or stimulation index (SI = phase II spots/phase I spots). The SI value in convalescent patients was 2.8 compared to 1.1 for chronic patients, indicating a tendency to shift to a phase I predominance in chronic disease. Stimulation controls in all patients and controls indicated adequate T-cell responses to the stimuli. Mean (± SE) T-cell responses in PHA-stimulated PBMCs (peripheral blood mononuclear cells) were adequate and not significantly different between all groups (P 0.87). Mean (± SE) T-cell responses in CEF-stimulated PBMCs did not significantly differ between all three groups (P 0.95). Responses in the healthy control group were negligible. We did not detect more than one spot after stimulation with either phase I or phase II antigens.

Discussion

In the Netherlands, a definitive chronic Q fever diagnosis currently is made on the basis of serology in combination with a positive serum PCR and in most cases, the presence of symptoms and a compatible clinical picture of endocarditis or vascular infection (Wegdam-

Blans et al., 2011). In many Q fever patients, however, high levels of IgG phase I antibodies are found without a positive PCR and/or clinical symptoms (Delsing et al., 2010). The positive predictive value (PPV) of the previously widely advocated IFA phase I IgG titre of ≥800 for diagnosing chronic Q fever is 37%. Recent adjustment of this cut-off titre to ≥1600 only increased the PPV to 59% (Frankel et al., 2011). The sensitivity of PCR on a fresh serum sample in chronic Q fever is 64% (specificity 100%) (Fenollar et al., 2004). Therefore, a negative PCR in the presence of highly elevated antibody levels does not exclude chronic disease. For clinicians, there clearly is a need for a more reliable diagnostic test for chronic Q fever.

In this exploratory study, the *Coxiella* ELISPOT adequately identified convalescent Q fever patients from healthy controls by demonstrating *C. burnetii*-specific T-cell IFN-γ production to both phase I and phase II antigens. Analogous to conventional serology, there was a clear phase II predominance. Specificity of the *Coxiella* ELISPOT for a past Q fever infection appears to be high, as T-cells from healthy controls did not react to both phase I and phase II *C. burnetii* antigens. False positive results because of cross-reactivity of the *Coxiella* ELISPOTs with other pathogens, however, remain a theoretical possibility.

Chronic Q fever patients showed a distinct *Coxiella* ELISPOT profile. First, spot count for both phase I and phase II was much higher (18-fold for phase II, 8-fold higher for phase I) indicating a more vigorous immune response in vitro to both antigenic stimuli. This is analogous to conventional serology, classically showing both highly elevated IFA phase I and phase II IgG antibody levels (Delsing et al., 2010; Frankel et al., 2011). Second, *Coxiella* ELISPOT results from chronic Q fever patients were consistently shifted towards more phase I reactivity as evidenced by a significantly lower SI (phase II/phase I spots). This balanced phase I and phase II reactivity is not a consistent feature in the conventionally assayed IFA antibody titres (Delsing et al., 2010).

There are some limitations to this pilot study. First, the limited number of patients does not allow for determination of a cut-off value for *Coxiella* ELISPOT results for diagnosing chronic Q fever. The consistent findings in phase I and phase II reactivity in chronic disease, however, are already statistically significant, but need confirmation in a larger cohort. Second, we used commercially available lyophilized *C. burnetii* antigens. These antigens are isolated from infected cells and can still contain tissue cell substrates, which could lead to false positive results. As we did not observe any reactivity for both antigens in healthy controls, such an aspecific reaction to these 'impure' antigens in the *Coxiella* ELISPOT is highly unlikely. Third, in contrast to the well-characterized conventional serological response to *C. burnetii* infection in time, the kinetic properties of the *Coxiella* ELISPOT assay are not yet known, precluding determination of cut-off values for diagnosing acute or chronic Q fever.

Anticipating further research, we hypothesize that measuringa patient's *C. burnetii*-specific T-cell responsiveness may better aid the clinician in accurately diagnosing a chronic from a non-chronic Q fever infection. In addition, such a test might prove a better tool for monitoring and guiding therapy than conventional serology. In the Netherlands, following the annual major Q fever outbreaks from 2007 to 2010, already more than 200 patients with a definite

or probable diagnosis of chronic Q fever have been identified (L.M. Kampschreur, pers. commun.). To validate the *Coxiella* ELISPOT for clinical use, a study comparing this assay to conventional serodiagnostics (serology and PCR) in a large cohort of Q fever patients is currently underway (the TRIQ study).

References

1. Andoh M, Zhang G, Russell-Lodrigue KE et al. (2007) T cells are essential for bacterial clearance, and gamma interferon, tumor necrosis factor alpha, and B cells are crucial for disease development in Coxiella burnetii infection in mice. Infect Immun. Jul;75(7):3245-55.
2. Delsing CE, Kullberg BJ, Bleeker-Rovers CP (2010). Q fever in the Netherlands from 2007 to 2010. Neth J Med. Dec;68(12):382-7.
3. Fenollar F, Fournier PE, Raoult D (2004) Molecular detection of Coxiella burnetii in the sera of patients with Q fever endocarditis or vascular infection. J Clin Microbiol. Nov;42(11):4919-24.
4. Frankel D, Richet H, Renvoisé A et al. (2011) Q fever in France, 1985-2009. Emerg Infect Dis. Mar;17(3):350-6.
5. Marmion BP, Sukocheva O, Storm PA et al. (2009) Q fever: persistence of antigenic non-viable cell residues of Coxiella burnetii in the host--implications for post Q fever infection fatigue syndrome and other chronic sequelae. QJM Oct;102(10):673-84.
6. Santín Cerezales M, Benítez JD (2011) Diagnosis of tuberculosis infection using interferon-γ-based assays. Enferm Infecc Microbiol Clin. Mar;29 Suppl 1:26-33.
7. Wegdam-Blans MCA, Kampschreur LM, Nabuurs-Franssen MH et al. (2011) Nederlandse consensus chronische Q koorts. Tijdschr Infect 6:71-3

Chapter 8

Diagnosis of Coxiella burnetii Infection: Comparison of a Whole Blood Interferon-Gamma Production Assay and a Coxiella ELISPOT

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Abstract

Diagnosis of ongoing or past infection with *Coxiella burnetii*, the causative agent of Q fever, relies heavily on serology: the measurement of *C. burnetii*-specific antibodies, reflecting the host's humoral immune response. However, cell-mediated immune responses play an important, probably even more relevant, role in infections caused by the intracellular *C. burnetii* bacterium. Recent studies have investigated interferon-gamma (IFN-γ) based assays, including a whole-blood IFN-γ production assay and a *Coxiella* enzyme-linked immunospot (*Coxiella* ELISPOT), as potential diagnostic tools for Q fever diagnosis. Both are in-house developed assays using stimulating antigens of different origin.

The main objective of this study was to compare the test performance of the IFN-γ production assay and the *Coxiella* ELISPOT for detecting a cellular immune response to *C. burnetii* in Q fever patients, and to assess the correlation between both assays. To that end, both tests were performed in a well-defined patient group of chronic Q fever patients (n = 16) and a group of healthy seronegative individuals (n = 17). Among patients, both the *Coxiella* ELISPOT and the IFN-γ production assay detected positive response in 14/16. Among controls, none were positive in the *Coxiella* ELISPOT, whereas the IFN-γ production assay detected positive results in 1/17 and 3/17, when using Henzerling and Nine Mile as stimulating antigens, respectively.

These results suggest the *Coxiella* ELISPOT has a somewhat higher specificity than the IFN-γ production assay when Nine Mile is used as antigen stimulus. The assays showed moderate correlation: the Spearman correlation coefficient r ranged between 0.37–0.60, depending on the antigens used. Further investigation of the diagnostic potential for *C. burnetii* infection of both assays is warranted.

Introduction

Q fever is a zoonotic disease that occurs worldwide and is caused by the gram-negative, intracellular bacterium *Coxiella burnetii.* The Netherlands experienced a major Q fever outbreak between 2007 and 2010, with over 4,000 reported human infections [1]. Acute Q fever mostly presents as a flu-like illness, pneumonia or hepatitis, but initial infections can be asymptomatic in more than 50% of cases [2]. Chronic Q fever is a rare but serious complication that occurs in approximately 5% of all patients following the acute infection. This persisting infection typically presents with endocarditis or vascular infection, and has significant mortality rates, especially in case of diagnostic and therapeutic delay [3].
Evaluating chronic *C. burnetii* infection is challenging. Measurement of serum antibodies against *C. burnetii* is currently the 'gold standard' for Q fever diagnosis [3]. Diagnosis of chronic Q fever relies heavily on detection of high IgG antibody titers against phase 1 C. burnetii. This serological criterion is combined with PCR for *C. burnetii* DNA on blood or tissue (if available) and clinical assessment of any nidus of chronic infection [4,5].
Both humoral and cell-mediated immune responses are involved in the host's immunity against the intracellular *C. burnetii* bacterium [6–8]. Therefore, it makes sense to explore the value of complementing conventional serology with an assessment of host specific cell-mediated immune responses to detect a chronic infection with *C. burnetii*. To this end, new immunological blood tests have been developed that are based on cellular immunity, measuring T-cell derived interferon-c (IFN-γ) production in response to stimulation with *C. burnetii*. The first, the wholeblood IFN-γ production assay, was extensively investigated during a Q fever vaccination campaign and for the diagnosis of chronic Q fever [9–11]. The second, the Coxiella enzyme-linked immunospot (*Coxiella* ELISPOT), was explored in a small series of patients with past or chronic Q fever [12]. The IFN-γ production assay measures the amount of *C. burnetii* specific IFN-γ production,while the *Coxiella* ELISPOT measures the number of *C. burnetii* specific IFN-γ producing cells. These IFN-γ based assays are not yet routinely used for Q fever diagnosis. Both are in-house assays, using different stimulating antigens. The IFN-γ production assay uses in-house cultured Nine Mile phase 1 and the Q-vax vaccine containing Henzerling phase 1, while the *Coxiella* ELISPOT uses commercially available Nine Mile phase 1 and phase 2 antigens.
In the aftermath of the Dutch Q fever outbreak, we had the opportunity to use both IFN-γ based tests in parallel in a group of well-defined chronic Q fever patients. Volunteers with no history of Q fever and with negative Q fever serology served as a control group.
The purpose of this study was to compare the test performance (sensitivity and specificity) of the IFN-γ production assay and the *Coxiella* ELISPOT for detecting a cellular immune response to *C. burnetii* in Q fever patients and to determine the correlation between the assays.

Materials and Methods

Patients and control subjects

Chronic Q fever patients (n = 16) were recruited from the outpatient clinics of the participating hospitals. All fulfilled the criteria for probable (n= 4) or proven (n= 12, of which 3 Q fevermendocarditis and 9 Q fever vascular infection) chronic Q fever according to the Dutch consensus statements on chronic Q fever [4]. Fourteen of the 16 patients were on long-term antibiotic treatment at inclusion. The median duration of antibiotic treatment at the time of blood collection was 21 months (range 0–59 months). The control individuals (n =17) were similar with respect gender, somewhat younger, but had no history of Q fever and had negative Q fever serology as measured by immunofluorescence assay (Focus Diagnostics, Cypress, CA, USA) (Table 1). Informed consent was obtained from all subjects before blood donation and the study was approved by the local ethics committee (CMO regio Arnhem-Nijmegen).

Table 1. Characteristics of chronic Q fever patients and control individuals.

	Patients (n = 16)	Controls (n = 17)
Age in yrs, median (range)	68 (31–80)	46 (25–64)
Males, number (%)	13 (81)	14 (82)
IgG anti-phase I titer[a], median (range)	2048 (128–32768)	<16 (n.a.)
IgG anti-phase II titer[a], median (range)	3072 (256–32768)	<16 (n.a.)
Duration of antibiotic treatment in months[a], median (range)	21 (0–59)	n.a.

n.a., not applicable.
[a]At the time of bloodsampling.

IFN-γ production assay

The IFN-γ production assay was performed as previously described [9]. In short, heparinized whole blood was aliquoted into four separate 1.5 mL tubes at 0.5 mL per tube. The tubes were inoculated with either 10 mg/mL PHA (Sigma, St. Louis, MO), 100 ng/ml Q vax vaccine (see below), 10‘7/mL heatinactivated *C. burnetii* Nine Mile phase I (see below), or nothing. The tubes were incubated in-vitro for 24 hours at 37uC and 5% CO2. The IFN-γ production was measured in the supernatant by ELISA (Pelikine compact, Sanquin, Amsterdam). Net IFN response was expressed as the concentration of IFN-γ in the stimulated sample minus that in the unstimulated sample. The Q-vax vaccine (CSL Biotherapies, Vic., Australia), contains formalin-inactivated whole cell phase 1 Henzerling strain. Heat-inactivated phase 1 *C. burnetii* Nine Mile (NMI, RSA493) was kindly provided by H.I. Roest (Central Veterinary Institute, Lelystad, the Netherlands). Details about culture and preparation of this stimulating antigen are described elsewhere [9].

Coxiella ELISPOT

The *Coxiella* ELISPOT was performed on peripheral blood mononuclear cells (PBMCs) isolated from heparinized blood and stimulated for 16–20 hours, as described before [12]. In precoated wells of PVDF strip plates (ELISpotpro; Mabtech) 100 mL of mononuclear cells were seeded at a density of 250,000 cells per well, and incubated with 50 mL of antigens, PHA (2.5 mg/mL) or nothing. As stimulating antigens, commercially available formalin-inactivated phase 1 and phase 2 Nine Mile (NMI and NMII, Virion-Serion Immunodiagnostica

GmbH, Wu¨rzburg, Germany) were used. After incubation, the resulting number of spots, representing the number of individual T-cells producing IFN-γ following stimulation with *C. burnetii* antigens, were detected and enumerated using an ELISpot reader (Auto Immun Diagnostika GmbH, Strassberg, Germany).

Statistics

Statistical analysis was performed using GraphPad Prism 5. Median (6 interquartile range) IFN-γ production and number of spots were compared between groups using Mann-Whitney Utests. Receiver Operating Characteristics (ROC) curves analysis were used to derive a cutoff for positivity of either assay. The cutoff was determined from the ROC curve by choosing the value that yielded empirical optimal sensitivity and specificity. Correlation was reported by calculating the Spearman's r with 95% Confidence Interval.

Results and Discussion

The amount of IFN-γ as measured in the IFN-γ production assay and the spot count obtained in the *Coxiella* ELISPOT were evaluated. The individual values are shown in Figure 1 for patients and control subjects separately. The median IFN-γ response to the Henzerling antigen in the patients was 440 pg/mL (IQR 62–612 pg/mL), whereas in control subjects, this was 0 pg/mL (IQR 0–0 pg/mL). The median IFN-c response to the NMI antigen in the patients was 3238 pg/mL (IQR 534–6564 pg/mL), whereas in control subjects, this was 126 pg/mL (IQR 14–250 pg/mL). The median ELISPOT spot count after NMI stimulation was 44 (IQR 15–94) in patients and 0 (IQR 0–0) in controls, and after NMII stimulation 71 (IQR 23–100) in patients and 0 (IQR 0–0) in controls. In both assays, the differences between patients and control subjects were significant for each stimulating antigen. All of the samples tested responded to PHA mitogen.

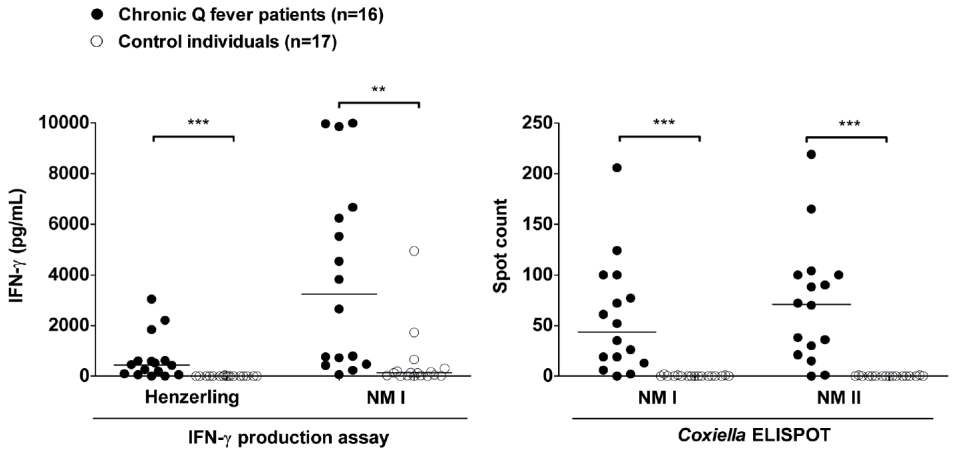

Figure 1. Results of the IFN-γ production assay and *Coxiella* ELISPOT in chronic Q fever patients and control subjects. In vitro IFN production by whole blood in response to Henzerling and Nine Mile phase 1 antigens was measured in the IFN-γ production assay. The number of IFN-γ positive cells in response to Nine Mile phase 1 and Nine Mile phase 2 antigens was measured in the *Coxiella* ELISPOT. Individual values of patients and controls are shown separately, and the lines indicate the medians. Patients and controls were compared using the Mann-Whitney U-test. ***P,0.001, **P,0.01. Abbreviations: NMI, Nine Mile phase 1; NMII, Nine Mile phase 2; IFN-c, interferon-gamma.

To establish a cutoff for a positive response in both assays for each of the antigens, ROC curves were constructed (Figure 2). Cutoffs were derived from ROC curve analysis to yield empirical optimal sensitivity and specificity. For the IFN-γ production assay this resulted in a cutoff of 45 pg/mL or 365 pg/mL when using the Henzerling or the NMI as stimulating antigen respectively. For the *Coxiella* ELISPOT, this resulted in a cutoff of 5 spots to both NMI and NMII. Given these cutoffs, the IFN-γ production assay detected positive response in 14/16 and the *Coxiella* ELISPOT was positive in 14/16 among the patients. Among the controls, the IFN-γ production assay detected positive response in 1/17 and 3/17, when using Henzerling and Nine Mile as stimulating antigens respectively, whereas none were positive in the *Coxiella* ELISPOT.

These results suggest that the assays have comparable sensitivity in this population, but the *Coxiella* ELISPOT has a higher specificity than the IFN-c production assay with Nine Mile as an antigen stimulus. This might be due to different antigens being exposed after heat-inactivation of the NMI strain as compared to the antigens present on the formalin-inactivated bacteria.

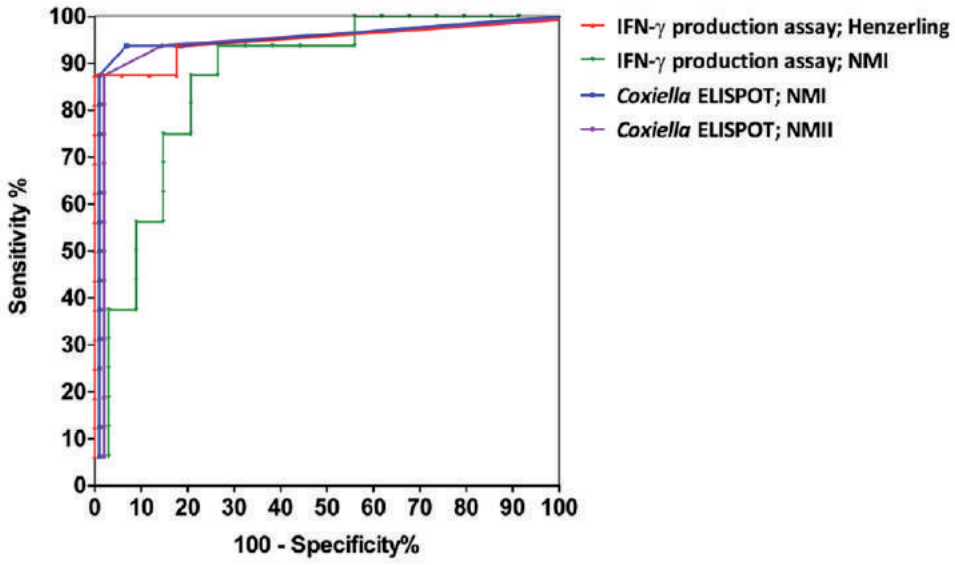

Figure 2. ROC curves of the IFN-γ production assay and the *Coxiella* ELISPOT. ROC curves are shown for each assay with each stimulating antigen separately. Abbreviations: NMI, Nine Mile phase 1; NMII, Nine Mile phase 2; IFN-γ, interferon-gamma.

Furthermore, it is very well possible that the IFN-γ production assay and the *Coxiella* ELISPOT assay do not measure the same features of the immune response. Although both tests measure *Coxiella* specific IFN-γ production, the *Coxiella* ELISPOT assay singles out and quantifies the T lymphocyte compartment, whereas whole blood stimulation also includes blood IFN-γ producing NK cells, reflecting also the innate part of the immune response. However, true positives in the control group cannot be excluded which in turn may

underestimate specificity. A seroprevalence study in the Q fever epidemic area among patients with a valvular risk factor for chronic Q fever found 20.4% of the people to be seropositive for *C. burnetii* [13]. As the control subjects lived close to or in the epidemic area, although not having a Q fever history or detectable anti-*C. burnetii* antibodies, it is not unthinkable that any of the control subject could have a past exposure to *C. burnetii* that was not picked up by serology. Of note, two of the three control subjects that produced substantial amounts of IFN-γ after NMI antigen stimulation, also showed minimal response below the cutoff in the *Coxiella* ELISPOT.
In the patient samples, we determined the correlation between the amount of IFN-γ produced in the IFN-γ production assay and the number of IFN-γ-producing cells in the *Coxiella* ELISPOT (Figure 3). The correlation between the values obtained in the two assays, each employing two stimulating antigens, was determined separately, resulting in a total of four comparisons.

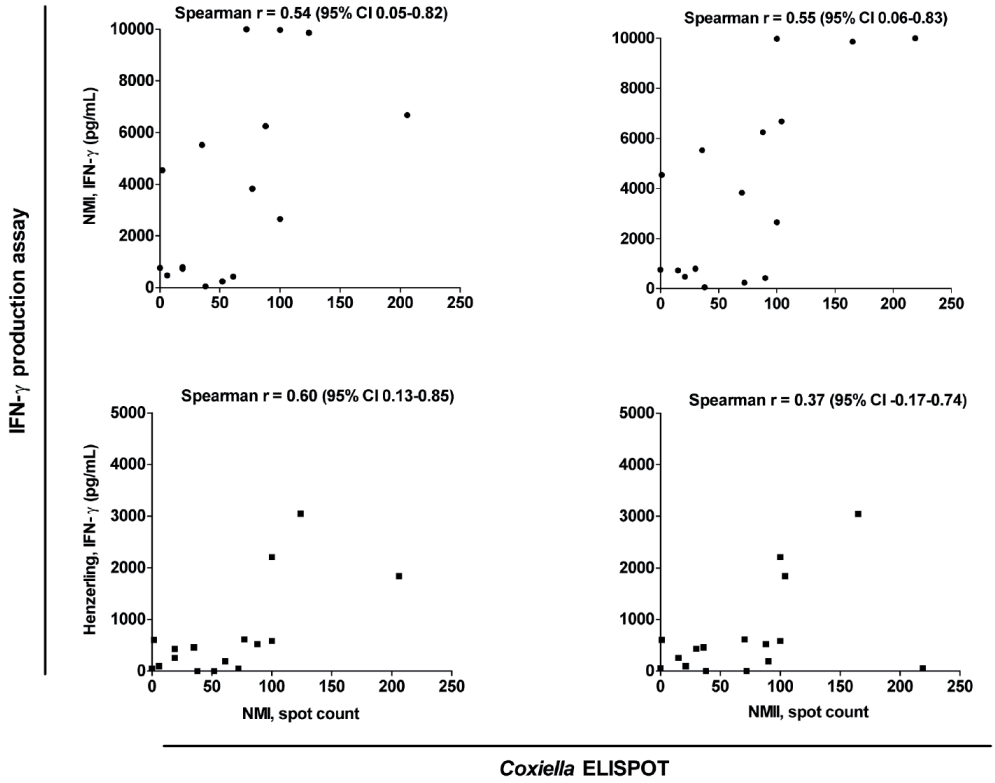

Figure 3. Correlation between the amount of *C. burnetii*-specific IFN-γ production and the number of IFN-γ positive cells. The individual values of chronic Q fever patients (n = 16) were used to determine the correlation between the IFN-γ production as measured with the IFN-γ production assay, and the number of IFN-γ positive cells as measured with the *Coxiella* ELISPOT. Each graph shows the correlation between the resulting values of the two assays, with either one of the stimulating antigens. On the Y-axes, the IFN-γ production is shown after stimulation with NMI (upper graphs) and Henzerling antigens (lower graphs). On the X-axes, the spot count is shown after stimulation with NMI (left graphs) and NMII (right graphs) antigens. The Spearman's correlation coefficient r (95% Confidence Interval) is given for each comparison. Abbreviations: NMI, Nine Mile phase 1; NMII, Nine Mile phase 2; IFN-γ, interferon-gamma.

The Spearman correlation coefficient r ranged between 0.37–0.60, indicating a moderate to strong correlation. The discrepancies between both types of assays can be explained by the different origins and concentrations of stimulating antigens, the different phase variation, or, as mentioned previously, the method of inactivation of the antigen stimuli. Moreover, it may well be true that the amount of IFN-γ released is not directly related to the number of IFN-γ positive cells. IFN-γ based assays are used for detection of immunity to Mycobacterium tuberculosis and commercial kits are available for the two types of assays, e.g. the QuantiFERON-TB and the T Spot TB [14]. These assays have been reported to be at least as accurate as the tuberculin skin test to detect exposure to M. tuberculosis. The correlation between the two types of IFN-γ based assays for tuberculosis as previously reported in the literature, are better (r= 0.69 and r =0.80) than what we observed evaluating these diagnostic platforms in *C. burnetii* infection [15,16].

In conclusion, the two IFN-γ based assays had a similarly high sensitivity for detecting *C. burnetii* infection and the correlation between both test was moderate. The IFN-γ production assay in whole blood has the practical advantage of relative technical simplicity over the more laboriously intensive *Coxiella* ELISPOT. The *Coxiella* ELISPOT, however, seemed to be more specific with Nine Mile as an antigen stimulus. These observations, and previous studies of these IFN-γ based assays in *C. burnetii* infection, show the potential of measuring cell-mediated immune response in Q fever and warrant further investigation of these assays in larger cohorts of Q fever patients.

Acknowledgments

We thank the patients and the volunteers for donating blood for this study. A patent application has been submitted for diagnosis of Q fever using the *Coxiella* burnetii specific IFN-γ production assay and is registered by the number PCT/NL 2011/050564.

Author Contributions

Conceived and designed the experiments: T. Schoffelen GJML JJMB MNF JWMvdM T. Sprong MvD. Performed the experiments: T. Schoffelen GJML JJMB. Analyzed the data: T. Schoffelen GJML T. Sprong MvD. Contributed to the writing of the manuscript: T. Schoffelen GJML CPBR JJMB MNF JWMvdM T. Sprong MvD. Contributed to the inclusion of patients: T. Schoffelen CPBR.

References

1. van der Hoek W, Schneeberger PM, Oomen T, Wegdam-Blans MC, Dijkstra F, et al. (2012) Shifting priorities in the aftermath of a Q fever epidemic in 2007 to 2009 in The Netherlands: from acute to chronic infection. Euro Surveill 17:20059.
2. Raoult D, Marrie T, Mege J (2005) Natural history and pathophysiology of Q fever. Lancet Infect Dis 5: 219–226.
3. Maurin M, Raoult D (1999) Q fever. Clin Microbiol Rev 12: 518–553.
4. Wegdam-Blans MC, Kampschreur LM, Delsing CE, Bleeker-Rovers CP, Sprong T, et al. (2012) Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Infect 64: 247–259.
5. Anderson A, Bijlmer H, Fournier PE, Graves S, Hartzell J, et al. (2013) Diagnosis and management of Q fever–United States, 2013: recommendations from CDC and the Q Fever Working Group. MMWR Recomm Rep 62: 1–30.
6. Ghigo E, Pretat L, Desnues B, Capo C, Raoult D, et al. (2009) Intracellular life of Coxiella burnetii in macrophages. Ann N Y Acad Sci 1166: 55–66.
7. Andoh M, Zhang G, Russell-Lodrigue KE, Shive HR, Weeks BR, et al. (2007) T cells are essential for bacterial clearance, and gamma interferon, tumor necrosis factor alpha, and B cells are crucial for disease development in Coxiella burnetii infection in mice. Infect Immun 75: 3245–3255.
8. Read AJ, Erickson S, Harmsen AG (2010) Role of CD4+ and CD8+ T cells in clearance of primary pulmonary infection with Coxiella burnetii. Infect Immun 78: 3019–3026.
9. Schoffelen T, Joosten LA, Herremans T, de Haan AF, Ammerdorffer A, et al. (2013) Specific interferon γ detection for the diagnosis of previous Q fever. Clin Infect Dis 56: 1742–1751.
10. Schoffelen T, Herremans T, Sprong T, Nabuurs-Franssen M, Wever PC, et al. (2013) Limited humoral and cellular responses to Q fever vaccination in older adults with risk factors for chronic Q fever. J Infect 67: 565–573.
11. Schoffelen T, Sprong T, Bleeker-Rovers CP, Wegdam-Blans MC, Ammerdorffer A, et al. (2013) A Combination of IFN-c and IL-2 Production by Coxiella burnetii Stimulated Circulating Cells Discriminates Between Chronic Q Fever and Past Q Fever. Clin Microbiol Infect. [Epub ahead of printing].
12. Limonard GJ, Thijsen SF, Bossink AW, Asscheman A, Bouwman JJ (2012) Developing a new clinical tool for diagnosing chronic Q fever: the Coxiella ELISPOT. FEMS Immunol Med Microbiol 64: 57–60.
13. Kampschreur LM, Oosterheert JJ, Hoepelman AI, Lestrade PJ, Renders NH, et al. (2012) Prevalence of chronic Q fever in patients with a history of cardiac valve surgery in an area where Coxiella burnetii is epidemic. Clin Vaccine Immunol 19: 1165–1169.
14. Pai M, Riley LW, Colford JM (2004) Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. Lancet Infect Dis 4:761–776.
15. Schölvinck E, Wilkinson KA, Whelan AO, Martineau AR, Levin M, et al. (2004) Gamma interferon-based immunodiagnosis of tuberculosis: comparison between whole-blood and enzyme-linked immunospot methods. J Clin Microbiol 42:829–831.
16. Goletti D, Vincenti D, Carrara S, Butera O, Bizzoni F, et al. (2005) Selected RD1 peptides for active tuberculosis diagnosis: comparison of a gamma interferon whole-blood enzyme-linked immunosorbent assay and an enzymelinked immunospot assay. Clin Diagn Lab Immunol 12: 1311–1316.

Chapter 9

General discussion and perspectives for future research

General discussion

The first Dutch human Q fever outbreak presented clinicians with many challenges regarding diagnosis and management of the disease. At the same time, it provided ample opportunity for researchers to observe, explore and investigate this complex disease from the vantage point of various scientific research disciplines, including the fields of epidemiology, microbiology, clinical medicine and immunology. This thesis contains several clinical studies performed mainly in patients from the early stages of the outbreak, exploring various aspects of Q fever and its longterm clinical outcomes.

Emergence of C. burnetii as a novel causative agent of pneumonia

In the epicenter of what would be the start of the largest documented Q fever outbreak ever reported, our data indeed support the hypothesis that *C. burnetii* emerged as a novel aetiological agent of pneumonia in May 2007. The aspecific nature of Q fever symptoms, its generally mild clinical course and perhaps sheer infamiliarity of attending physicians with the disease at the outset of the outbreak could have led to an underreporting of Q fever cases in 2007 and the preceding years. Interestingly in this regard, Van den Wijngaard et al. found retrospective statistical evidence of clustering of hospitalizations of possible and plausible Q fever pneumonia cases starting as early as 2005. Lack of possibility of serological testing, however, precluded definite confirmation of these outbreak clusters as being a Q fever outbreak [1]. Schimmer et al. Found a low sero-prevalence of 2,4% in the Netherlands in the period leading up to the outbreak [2]. In 2014 a sero-epidemiological study performed in Herpen included 70% of its total adult population and found a staggering 33,8% seroprevalence for *C. burnetii* antibodies, further underlining the massive impact and scope of Q fever in the epidemic region since its emergence in the spring of 2007 [3].

Clinical presentation, serological and echocardiographic findings of Q fever patients from the initial stages of the outbreak: implications for clinical practice

The finding of an aspecific, flu-like syndrome with or without atypical respiratory tract infection as the main clinical Q fever manifestation in the first cohort of patients tallies with earlier research [4,5]. There was a striking level of incapacitating and protracted fatigue in our Q fever patients in 52% at 6 months and 26% at 1 year following primary infection. Other research groups have reported similar findings in combination with a marked reduced quality of life [6-9]. The finding of prolonged symptomatology will be discussed in the following paragraph of this chapter.

Using a commercially available IFA we observed a typical serological response to Coxiella burnetii infection with strikingly high levels of antibodies to both phase I and phase II antibodies. This serological response was characterized by an increase in antibody titers up to 3 months and a subsequent decrease in the following 9 months. The phase I IgG antibody kinetic, with a titer peak at 3 months, is different from available literature on IFA serological

patterns in the follow-up of acute Q fever, that shows a rather slow and gradual increase of phase I IgG antibodies [10,11]. None of our patients developed a clinical picture compatible with chronic Q fever and all patients showed a spontaneous subsequent decline in antibody titres. This finding has considerable clinical importance as the appearance of high antibody levels, especially phase I IgG, can wrongly be taken as supposed proof of chronic disease. To aid in the diagnosis and management of chronic Q fever, a new guideline was proposed by the Dutch Q fever consensus group in 2012, stressing the importance of the fact that chronic *C. burnetii* infection is first and foremost a clinical diagnosis, its definite diagnosis requiring integration of data on *C. burnetii* antibody titres and PCR, imaging techniques and the presence of predisposing risk factors in the host [12].

An active search for cardiac valvular abnormalities and serological surveillance following acute Q fever have been advocated to identify chronic Q fever in an early stage and to trigger prolonged prophylactic antibiotic treatment in case of even minor cardiac valvulopathy [13-15]. Q fever endocarditis is reported to develop in up to 39% of patient with known pre-existing cardiac valvulopathy [16]. Interestingly, minor, clinically insignificant valvular disease (trace and mild defects) has a high prevalence in any unselected population [17]. For example, the prevalence of 'trace' mitral valve regurgitation is 40% and can therefore be considered to be a physiological phenomenon without clinical importance. The prevalence of mitral valve prolaps and bicuspid aortic valve is estimated at 2-3% and circa 1% respectively [18,19]. During follow-up of the outbreak cluster in Herpen, echocardiography was performed in most Q fever patients, confirming a high prevalence of minor cardiac valvulopathies, but none of the patients developed chronic disease. By contrast with existing publications, our data indicate a low risk of progression to Q fever endocarditis.

This contrasting result may be explained by several factors including selection bias, lack of specification of valvular defect severity in published retrospective research, strain differences of *C. burnetii* and differences in host characteristics. In addition, these studies were carried out in the setting of a large Q fever epidemic, as opposed to most studies on chronic Q fever incorporating and collecting Q fever patients from endemic regions over prolonged periods of time [13,14,16,20]. In these studies, the risk of endocarditis in patients with acute Q fever and pre-existing cardiac valve defects was estimated to be up to 39%, but many of the patients had prosthetic valves [16]. In their landmark study on Q fever endocarditis, Million and colleagues describe a highly selective cohort with valve defects mostly caused by rheumatic fever (30%, about half of all specified valvular disease), a disorder that is rare in most developed countries [20]. Furthermore, 30% of their patients had infected prosthetic valves. Degenerative valvular disease was present in only 5%,1 whereas this type of valvular defect is highly prevalent in the general population (22% in our study) [17].

The limited number of patients in our studies do not allow to determine the relative risk of developing chronic Q fever for patients with mild or even clinically significant degenerative valvulopathy, but it's clear that the absolute risk, at least in the case of a minor valve insufficiency, is likely to be small enough to withhold prolonged profylactical

antibiotic treatment and perform only serological follow-up. The a priori chance of finding a relevant but clinically silent cardiac valve abnormality by general cardiac screening is low. Furthermore, the previously estimated 39% chance of developing chronic Q fever is very likely not applicable for all valvulopathies and probably lower than has previously been estimated in case of minor valvulopathies. Interestingly in this regard, in 2012, Kampschreur et al. found a prevalence of 7,8% of probable and proven chronic Q fever cases in a group of patients in the Netherlands with a history of cardiac valve surgery and seropositivity for *C. burnetii* antibodies [21]. In addition, a recent study by Kampschreur et al. identified and quantified risk factors for development of chronic Q fever after *C. burnetii* infection. Using a case-control design, several major independant risk factors for development of chronic disease were identified: increasing age, previous valvular surgery, aneurysms and renal insufficiency. Of note and in contrast with previous studies on this subject, no association was found between (mild) non-surgical heart valve pathology and chronic Q fever [22].

In response to the findings of these and other studies in the Netherlands, the French research group of professor Raoult, from the worldwide centre of expertise on Q fever in Marseille, published data of the French database of Q fever patients stating a high relative risk of patients with chronic Q fever to have a bicuspid aortic valve, a mitral valve prolapse or a moderate mitral or aortic valve insufficiency [23]. In this letter to the editor, there was no mention of minor valvulopathies (trace and mild defects) being significantly associated with chronic Q fever. In addition, it remains unclear whether the described valvulpathies were clinically silent and whether the congenital valve abnormalities (bicuspid aortic valve or mitral valve prolapse) were uncomplicated or complicated by an accompanying valvular insufficiency and/or stenosis.

In 2014, the same research group reported the results of a follow-up prospective study of acute Q fever patients with significant valvulopathy (n=31) stating an invariable (13/13) progression rate to Q fever endocarditis in patients without or incomplete antibiotic prophylaxis [24]. Conversely, patients with significant cardiac valvulopathy who received antibiotic prophylaxis invariably (18/18) did not developed chronic disease. Again there are several concerns regarding the applicability of these data in clinical practice; data are from a highly selective cohort of patients analysed and treated in a worldwide reference centre, patients had a very high prevalence of significant cardiac valvulopathy (43%), and diagnosis of chronic disease was made mostly on marginally increased serological titres within an unusually short time frame after acute Q fever, suggesting the possibility of an inadvertent misdiagnosis of chronic Q fever in at least some of the cases [25].

In the years after the first outbreak, additional follow-up studies from the Netherlands have been published, including larger patient numbers, providing further insight into the longterm outcome of acute *C. burnetii* infection and risk factors associated with the development of chronic Q fever. Van der Hoek et al. identified 11 chronic Q fever cases in a group of 686 Q fever patients with a 12 month serological follow up period [26]. Complete clinical data were available from 9 of these 11 chronic Q fever patients, 3 of whom developed endocarditis. All

these 3 patients had known pre-existing cardiac valve disease and no cases of endocarditis due to clinically silent cardiac valvulopathy were encountered. In 2014, the entire adult population (n=2161) of the town of Herpen was offered serological screening for Q fever [27]. Of the 1517 participants (70,3%), 33,8% were seropositive and 3 patients had developed clinically diagnosed chronic Q fever, all of whom had known risk factors (1 patient with an aortic aneurysm and stent, 1 patient with a history of cardiac valve surgery and 1 patient with impaired renal function). No unexpected Q fever endocarditis cases were detected, 7 years after the first outbreak [27].

Identifying patients at risk of developing chronic Q fever is very important, as early detection and treatment of chronic disease is associated with better survival [15,24]. The association of chronic Q fever with (minor) non-cardiac surgery cardiac valve defects is likely to be smaller than has previously been estimated and calls into question the use of screening echocardiography for every Q fever patient. The harm of low-threshold administration of long-term antibiotic prophylaxis in case of minor valvulopathies might outweigh the possible benefit. Clearly, prophylactic antibiotic treatment after acute Q fever episode has the potential to prevent evolution to chronic disease of high-risk patients, but exactly which patients are most likely to benefit from such a strategy remains to be determined [25]. In the Netherlands, invariably performing a screening echocardiography for every Q fever patient was abandoned in favor of a pragmatic approach, employing serological follow-up for all Q fever patients and targeted screening of known risk groups [28,29].

Long term health status of Q fever patients

One year after primary infection, Q fever patients from the 2007 Herpen outbreak had a significantly lower health status in many subdomains of the main domains 'symptoms' and 'functional impairment', when compared to age-, sex- and geographically matched controls. Overall QoL and health-related QoL were significantly decreased in both patients and controls. This impairment in health status persisted in the same group of patients from the first outbreak at 4 years follow-up. This persistence of impairment in many sub-domains of health status after 4 years in the majority of affected Q fever patients is marked, and suggests an absence of dynamics in symptomatology after 1 year. Research groups in England, Australia and Canada have similarly found consistent and clearly decreased very long-term health status outcomes in Q fever patients, using various questionnaires and study designs [6-9].

Remarkably, health status scores of controls without a clinical history of Q fever but with serological evidence of exposure to C. burnetii were not statistically different from seronegative controls, suggesting that clinical expression of acute Q fever infection is an essential factor in the subsequent sustained decrease in health status. Severity of initial illness previously indeed has been shown to be the best predictor of subsequent development of a post-infective fatigue syndrome in both viral and non-viral pathogens, including Q fever [30]. Moreover, the same genetic polymorphisms in cytokine genes with critical roles in the

inflammatory reponse to infection, underpin both the severity of the acute sickness and the average time to recovery across varied infections, including Q fever [31-34].

Our findings lend support to the notion of an extremely protracted reconvalescence phase and decreased sustained health status after primary infection in a significant proportion of Q fever patients.

Additional studies assessing longterm health status in Q fever patients in the Netherlands found similar results. Morroy et al. found similar levels of fatigue and decreased health status in 515 Q fever patients with a follow-up period of 12-26 months [9]. Interestingly in this study, health status score did not differ significantly at 12 months vs. 17-26 months follow-up, suggesting an absence of dynamics in health status after 1 year. Van Loenhout et al. studied a group of 336 Q fever patients and found a reduced health status at 24 months in more than one out of three patients [35].

The acknowledgement and acceptance of a protracted fatigued state following acute Q fever in about 20% of patients, sometimes designated post-Q fever fatigue syndrome (QFS); however, is not universal [36,37]. A QFS diagnosis relies solely on the patient's own account of symptoms. In clinical practice, QFS patients remain indistinguishable from patients with a complete recovery after primary infection with *C. burnetii,* as they do not meet the criteria for chronic Q fever infection [38,39].

The main reasons for this reluctance seem to be the incompletely understood pathophysiology of QFS, the absence of a diagnostic test and possibly, fear of patients' financial compensation claims. Moreover, post-infectious protracted fatigue states following other bacterial and viral pathogens such as Bartonella, Legionella, Epstein–Barr virus, cytomegalovirus and West Nile virus, suggest a non pathogen-specific pathogenesis of postinfectious fatigue syndromes [30,40].

Interestingly in this regard, a recent Dutch study, using the NCSI and the Short Form 36 (SF-36), compared the health status of 309 Q fever patients and 190 patients with Legionnaires' disease and found severe impairment of various health status sub-domains including fatigue for both diseases 1 year after onset of illness [41]. Another smaller study from The Netherlands compared 50 patients with a lower respiratory tract infection (LRTI) caused by Q fever with 32 non-Q fever LRTI patients with a follow-up period of 10–19 months. With the exception of the sub-domain 'subjective pulmonary symptoms', no difference was observed in health status for all other measured subdomains using the NCSI [42]. Interestingly, as in our study, significant proportions of Q fever patients experienced severe fatigue (40%) and severely impaired general quality of life (40%).

Recently, an elegant new paradigm of persistence of *Coxiella* antigenic non-viable cell residues after primary infection in interaction with immunogenetic polymorphisms in the host has been put forward to better explain the chronic sequelae of acute Q fever, including QFS [38].

There are numerous studies probing the underlying pathophysiology of QFS that show significant differences between Q fever patients with and without QFS with regard to

gene expression [32,33,43], serum cytokine levels [34] and peripheral blood mononuclear cells cytokine release patterns after stimulation with *C. burnetii* antigens [31,34]. There are, however, several shortcomings that impede a thorough interpretation and unifying concept of QFS pathophysioloy; a lack of a clear definition of QFS, small patient numbers and different laboratory techniques and protocols that do not allow for comparison and validation of obtained results. Moreover, there is no validated evidence-based treatment for QFS. To address this issue, a large randomized placebo-controlled prospective clinical trial is underway in The Netherlands, assessing the efficacy of longterm treatment with doxycycline and cognitive behavioural therapy in patients with QFS [44].

Developing a new diagnostic tool for Q fever: measuring the host's C. burnetii specific interferon gamma production

In routine clinical practice, diagnosis of acute Q fever can readily be established with a high degree of sensitivity and specificity by demonstrating the patient's *C. burnetii* specific antibody response [4,5]. Contrastingly, serology has a rather poor diagnostic performance in identifying chronic Q fever and has no known diagnostic potential in diagnosing QFS [38,39]. Clearly, there is a need for a better test that can adequately identify or even predict patients who (will) develop an adverse clinical outcome after acute Q fever. We hypothesized measuring a patient's cellular immunity might better identify different clinical outcomes after *C. burnetii* infection. Analogous to IFN gamma release assays for tuberculosis, we devised a new platform using the ELISPOT technique, specifically modified to measure the patient's T-cell IFN gamma release to stimulation with *C. burnetii* specific antigens: the *Coxiella* ELISPOT (see Chapter 7).

The *Coxiella* ELISPOT adequately identifies convalescent Q fever patients from healthy controls by demonstrating *C. burnetii*-specific T-cell IFN-c production to both phase I and phase II antigens. Specificity of the *Coxiella* ELISPOT for a past Q fever infection appears to be high. We showed that measuring the host's T-cell *C. burnetii* specific interferon gamma production is feasible and different clinical outcomes (past non-chronic Q fever infection and chronic *C. burnetii* infection) after acute Q fever were associated with different *Coxiella* ELISPOT profiles. Comparison of our *Coxiella* ELISPOT with a IFN-gamma production assay devised by the research group from Nijmegen (Schoffelen et al.) showed a similarly high sensitivity for detecting *C. burnetii* infection with a moderate correlation between both tests (see Chapter 8). These observations, and previous studies of these IFN-gamma based assays in *C. burnetii* infection, show the potential of measuring cell-mediated immuneresponse in Q fever.

Since these first reports, the value of a *C. burnetii* IGRA (the IFN-gamma production assay) has been demonstrated in diagnosing past (non-chronic) Q fever [45], measuring *C. burnetii* vaccination response [46], and combined with measurement of IL-2 release, identifying QFS patients [47], chronic Q fever patients [48] and monitoring therapeutic succes in the treatment of chronic Q fever [49].

Perspectives for future research

Infection with the *C. burnetii* bacterium can take on many clinical guises, if any, and produces an adverse long term clinical outcome in a significant proportion of affected patients. Acute symptomatic infection, recognised in the appropriate epidemiological setting, is readily diagnosed using conventional serology and effectively treated with doxycycline. In stark contrast, diagnosis and treatment of the long term adverse outcomes of *C. burnetii* infection; chronic disease and QFS, are often very arduous and require a highly individualised patient-tailored approach.

In the case of chronic Q fever, there is a need for further clarification which risk factors, both those present in the host and the pathogen, contribute to the development of a chronic form of *C. burnetii* infection. For every clinically manifest case of Q fever during the successive Q fever outbreaks in the Netherlands, an estimated 12,6 cases occurred subclinically, making this patient group a large reservoir at risk for development of chronic disease [50]. Expanding research to identify risk factors for development of chronic Q fever in this population can aid in early detection, treatment and possibly even prevention of chronic disease.

In the case of QFS, as this long term adverse outcome by far constitutes the largest portion of the total disease burden, further elucidation of the pathofysiological mechanisms is urgently needed. In addition, a clear and internationally accepted uniform definition of this syndrome and follow-up parameters, will aid in comparising obtained research results between research groups and help devise new study protocols in search for an effective treatment strategem.

The current shortcomings in conventional diagnostic and monitoring tools in chronic Q fever and QFS, highlight the largely incomplete understanding of the pathophysiology of *C. burnetii* infection. The promising results of diagnostic tools that include measurement of the cellular immune respons emphasize the pivotal role of host-pathogen interaction in Q fever. Measuring the host's immune response to better identify and follow-up chronic disease and possibly even QFS is feasible and deserves to be explored.

Anticipating further research, we hypothesize that a test measuring a patient's *C. burnetii*-specific cellular responsiveness may better aid the clinician in accurately diagnosing *C. burnetii* infection in all its manifestations, especially in the setting of chronic Q fever and QFS. In addition, such a test might prove a better tool for monitoring and guiding therapeutic interventions.

Conclusions

This thesis contains several exploratory studies on Q fever in humans, with an emphasis on clinical observations, long-term quality of life following infection and the development of a new diagnostic Q fever assay.

The data in this thesis support the hypothesis that *Coxiella burnetii* emerged as a novel causative agent of pneumonia in the Netherlands in May 2007.

The first systematic observations on clinical presentation, serology and echocardiographic findings in Q fever patients from the initial stages of the outbreak in the Netherlands are

presented. Q fever mainly presented as a mild febrile flulike illness. Overall prevalence of mild cardiac valvulopathy was high, but the incidence of chronic Q fever in case of mild, clinically insignificant valvulopathy was lower than has previously been estimated. This calls into question the use of screening echocardiography and application of long-term profylactic antibiotic treatment for these mild cardiac valve defects.

A significant proportion of Q fever patients exhibit high levels of undue fatigue and a sustained decrease in many aspects of health status at 1 and 4 year follow-up.

Detecting *C. burnetii* specific interferon gamma production for diagnosing Q fever by the *Coxiella* ELISPOT is feasible and shows promise in terms of diagnostic performance. Comparison of the *Coxiella* ELISPOT with the Whole Blood Interferon Gamma production Assay showed moderate correlation. Further investigation of the diagnostic potential for *C. burnetii* infection of both assays is warranted.

References

1. Van den Wijngaard CC, Dijkstra F, van Pelt W et al. In search of hidden Q-fever outbreaks: linking syndromic hospital clusters to infected goat farms. Epidemiol Infect. 2011 Jan;139(1):19-26.
2. Schimmer B, Notermans DW, Harms MG et al. Low seroprevalence of Q fever in The Netherlands prior to a series of large outbreaks. Epidemiol Infect. 2012 Jan;140(1):27-35.
3. Morroy G, Van der Hoek W, Nanver ZD et al. The health status of a village population, 7 years after a major Q fever outbreak. Epidemiol Infect. 2016 Apr;144(6):1153-62.
4. Maurin M, Raoult D. Q fever. Clin Microbiol Rev 1999 Oct;12(4):518-53.
5. Parker NR, Barralet JH, Bell AM. Q fever. Lancet 2006 Feb 25;367(9511):679-88.
6. Marmion BP, Shannon M, Maddocks I et al. Protracted debility and fatigue after acute Q fever. Lancet 1996; 347:977.
7. Ayres JG, Smith EG, Flint N. Protracted fatigue and debility after acute Q fever. Lancet 1996; 347: 978.
8. Wildman MJ, Smith EG, Groves J et al. Chronic fatigue following infection by Coxiella burnetii (Q fever): Ten-year follow-up of the 1989 UK outbreak cohort. Q J Med 2002;95: 527–38.
9. Morroy G, Peters JB, van Nieuwenhof M et al. The health status of Q-fever patients after long-term follow-up. BMC Infect Dis. 2011 Apr 18;11:97.
10. Dupuis G, Peter O, Peacock M et al. Immunoglobulin responses in acute Q fever. J Clin Microbiol 1985 Oct;22(4):484-7.
11. Dupont HT, Thirion X, Raoult D. Q fever serology: cutoff determination for microimmunofluorescence. Clin Diagn Lab Immunol 1994 Mar;1(2):189-96.
12. Wegdam-Blans MC, Kampschreur LM, Delsing CE et al.; Dutch Q fever Consensus Group. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Infect. 2012 Mar;64(3):247-59.
13. Fenollar F, Thuny F, Xeridat B et al. Endocarditis after acute Q fever in patients with previously undiagnosed valvulopathies. Clin Infect Dis 2006 Mar 15;42(6):818-21.
14. Landais C, Fenollar F, Thuny F et al. From acute Q fever to endocarditis: serological follow-up strategy. Clin Infect Dis 2007 May 15;44(10):1337-40.
15. Million M, Lepidi H, Raoult D. [Q fever: current diagnosis and treatment options]. Med Mal Infect 2009 Feb;39(2):82-94.
16. Fenollar F, Fournier PE, Carrieri MP et al. Risks factors and prevention of Q fever endocarditis. Clin Infect Dis 2001 Aug 1;33(3):312-6.
17. Singh JP, Evans JC, Levy D, et al. Prevalence and clinical determinants of mitral, tricuspid, and aortic regurgitation (the Framingham Heart Study). Am J Cardiol 1999 Mar 15;83(6):897-902.
18. Jones EC, Devereux RB, Roman MJ, et al. Prevalence and correlates of mitral regurgitation in a population-based sample (the Strong Heart Study). Am J Cardiol 2001 Feb 1;87(3):298-304.
19. Lewin MB, Otto CM. The bicuspid aortic valve: adverse outcomes from infancy to old age. Circulation 2005 Feb 22;111(7):832-4.
20. Million M, Thuny F, Richet H et al. Long-term outcome of Q fever endocarditis: a 26-year personal survey. Lancet Infect Dis 2010; 10: 527–35.

21. Kampschreur LM, Oosterheert JJ, Hoepelman AI et al. Prevalence of chronic Q fever in patients with a history of cardiac valve surgery in an area where Coxiella burnetii is epidemic. Clin Vaccine Immunol. 2012 Aug;19(8):1165-9.
22. Kampschreur LM, Dekker S, Hagenaars JC et al. Identification of risk factors for chronic Q fever, the Netherlands. Emerg Infect Dis. 2012 Apr;18(4):563-70.
23. Raoult D, Million M, Thuny F et al. Chronic q Fever detection in the Netherlands. Clin Infect Dis. 2011 Dec;53(11):1170-1.
24. Million M, Walter G, Thuny F et al. Evolution from acute Q fever to endocarditis is associated with underlying valvulopathy and age and can be prevented by prolonged antibiotic treatment. Clin Infect Dis. 2013 Sep;57(6):836-44.
25. Kampschreur LM, Oosterheert JJ, Wever PC et al. Antibiotic prophylaxis for high-risk patients with acute Q Fever: no definitive answers yet. Clin Infect Dis. 2014 Feb;58(3):446-7.
26. Van der Hoek W, Versteeg B, Meekelenkamp JC et al. Follow-up of 686 patients with acute Q fever and detection of chronic infection. Clin Infect Dis. 2011 Jun 15;52(12):1431-6.
27. Morroy G, van der Hoek W, Albers J et al. Population Screening for Chronic Q-Fever Seven Years after a Major Outbreak. PLoS One. 2015 Jul 1;10(7):e0131777.
28. Wielders CC, van Loenhout JA, Morroy G et al. Long-Term Serological Follow-Up of Acute Q-Fever Patients after a Large Epidemic. PLoS One. 2015 Jul 10;10(7):e0131848.
29. Wielders CC, Morroy G, Wever PC et al. Strategies for early detection of chronic Q-fever: a systematic review. Eur J Clin Invest. 2013 Jun;43(6):616-39.
30. Hickie I, Davenport T, Wakefield D et al. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. BMJ. 2006 Sep 16;333(7568):575.
31. Vollmer-Conna U, Piraino BF, Cameron B et al. Cytokine polymorphisms have a synergistic effect on severity of the acute sickness response to infection. Clin Infect Dis2008 Dec 1;47(11):1418-25.
32. Kerr JR, Petty R, Burke B et al. Gene expression subtypes in patients with chronic fatigue syndrome/myalgic encephalomyelitis. J Infect Dis. 2008 Apr 15;197(8):1171-84.
33. Helbig K, Harris R, Ayres J et al. Immune response genes in the post-Q-fever fatigue syndrome, Q fever endocarditis and uncomplicated acute primary Q fever. QJM. 2005 Aug;98(8):565-74.
34. Penttila IA, Harris RJ, Storm P et al. Cytokine dysregulation in the post-Q-fever fatigue syndrome. QJM. 1998 Aug;91(8):549-60.
35. Van Loenhout JA, Hautvast JL, Vercoulen JH et al. Q-fever patients suffer from impaired health status long after the acute phase of the illness: results from a 24-month cohort study. J Infect. 2015 Mar;70(3):237-46.
36. Raoult D. Q fever: still a mysterious disease. QJM2002 Aug;95(8):491-2.
37. Strauss B, Löschau M, Seidel T et al. Are fatigue symptoms and chronic fatigue syndrome following Q fever infection related to psychosocial variables? J Psychosom Res. 2012 Apr;72(4):300-4.
38. Marmion BP, Sukocheva O, Storm PA et al. Q fever: persistence of antigenic non-viable cell residues of Coxiella burnetii in the host--implications for post Q fever infection fatigue syndrome and other chronic sequelae. QJM. 2009 Oct;102(10):673-84.

39. Sukocheva OA, Marmion BP, Storm PA et al. Long-term persistence after acute Q fever of non-infective Coxiella burnetii cell components, including antigens. QJM. 2010 Nov;103(11):847-63.
40. Garcia MN, Hause AM, Walker CM et al. Evaluation of prolonged fatigue post-West Nile virus infection and association of fatigue with elevated antiviral and proinflammatory cytokines. Viral Immunol. 2014 Sep;27(7):327-33.
41. Van Loenhout JA, van Tiel HH, van den Heuvel J et al. Serious long-term health consequences of Q-fever and Legionnaires' disease. J Infect. 2014 Jun;68(6):527-33.
42. Van Dam AS, van Loenhout JA, Peters JB et al. A cross-sectional study to assess the long –term health status of patients with lower respiratory tract infections including, Q fever. Epidemiol Infect. 2015 Jan;143(1):48-54.
43. Piraino B, Vollmer-Conna U, Lloyd AR. Genetic associations of fatigue and other symptom domains of the acute sickness response to infection. Brain Behav Immun. 2012 May;26(4):552-8.
44. Keijmel SP, Delsing CE, Sprong T et al. The Qure study: Q fever fatigue syndrome--response to treatment; a randomized placebo-controlled trial. BMC Infect Dis. 2013 Mar 27;13:157.
45. Schoffelen T, Joosten LA, Herremans T et al. Specific interferon γ detection for the diagnosis of previous Q fever. Clin Infect Dis. 2013 Jun;56(12):1742-51.
46. Schoffelen T, Herremans T, Sprong T et al. Limited humoral and cellular responses to Q fever vaccination in older adults with risk factors for chronic Q fever. J Infect. 2013 Dec;67(6):565-73.
47. Keijmel SP, Raijmakers RP, Bleeker-Rovers CP et al. Altered interferon-γ response in patients with Q-fever fatigue syndrome. J Infect. 2016 Apr;72(4):478-85.
48. Schoffelen T, Sprong T, Bleeker-Rovers CP et al. A combination of interferon-gamma and interleukin-2 production by Coxiella burnetii-stimulated circulating cells discriminates between chronic Q fever and past Q fever. Clin Microbiol Infect. 2014 Jul;20(7):642-50.
49. Schoffelen T, Wegdam-Blans MC, Ammerdorffer A et al. Specific in vitro interferon-gamma and IL-2 production as biomarkers during treatment of chronic Q fever. Front Microbiol. 2015 Feb 12;6:93.
50. Van der Hoek W, Hogema BM, Dijkstra F et al. Relation between Q fever notifications and Coxiella burnetii infections during the 2009 outbreak in The Netherlands. Euro Surveill. 2012 Jan 19;17(3):20058.

Summary

Summary

The first Dutch human Q fever cases in the spring of 2007 heralded a massive outbreak that would progressively unfold itself in annual seasonal incidence waves until 2011. The emergence of *C. burnetii* as a human pathogen on this unprecedented and unforeseen scale was linked to Q fever abortion waves on dairy goats and sheep farms in the epidemic region starting as early as 2005. As of 2011, the human Q fever disease burden amounted to more than 4000 notified acute Q fever cases and an additional estimated 44000 unnotified cases, as can be inferred from sero-epidemiological surveys.
At the outset of the epidemic, evidence-based validated protocols regarding diagnosis and follow-up of acute Q fever were lacking in the Netherlands. Available literature on diagnosis and follow-up of acute and chronic Q fever had almost exclusively been generated by a dedicated research group from a single reference centre of expertise from France. It remained unclear whether these research findings could be extrapolated and whether recommendations regarding clincial diagnosis and follow-up could be applied to the same effect in the setting of the unfolding massive Dutch Q fever outbreak. On the one hand, this posed a major challenge for clinicians and other health care workers in managing acute disease and preventing long-term Q fever sequelae in the early stages of the epidemic. On the other hand, the Q fever outbreak provided ample opportunity for researchers to observe, explore and investigate this complex disease from the vantage point of various scientific research disciplines, including the fields of veterinary medicine, epidemiology, microbiology, clinical medicine and immunology.

The aim of this thesis is to describe observations on epidemiology, clinical aspects and long term health outcomes of Q fever patients diagnosed in the initial stages of the first Dutch human Q fever outbreak. Additionally, this thesis aims to describe the performance and potential clinical use of a newly developed diagnostic Q fever interferon gamma release assay, the *Coxiella* ELISPOT, which measures the host's cellular immune reponse to *Coxiella burnetii*.

Chapter 1. provides a general introduction and contains background information on Q fever; its pathogenesis, clinical manifestations and long-term sequelae, the host's immune response to *C. burnetii,* and the impact and scope of the Dutch human Q fever outbreak.
Q fever is considered a new and emerging zoonosis in the Netherlands. Retrospective serological studies performed on available samples from the period leading up to the first Q fever outbreak in the Netherlands show a low seroprevalence of antibodies againt *C. burnetii.* However, the highly epidemic municipalities in the epidemic's epicentre in the province of Noord-Brabant were excluded from this analysis.
Chapter 2. reports on the aetiology of clinically treated pneumonia cases in the epicentre of the first Q fever outbreak in 2007, showing the emergence of *C. burnetii* as a novel causative

agent of pneumonia in this area. The contribution of *C. burnetii* as a causative agent of pneumonia in patients admitted to Bernhoven Hospital (Herpen lies within the catchment population of this community-based hospital) in the period January-July 2007 was assessed by retrospectively testing available patients' sera for the presence of *C. burnetii* antibodies. Of the total of 95 clinical pneumonia cases, *C. burnetii* was the causative agent in 21 (22%). The number of Q fever pneumonia cases in May (n=13) was significantly higher than in any other month of the study period (range 0-5). To examine these findings in a historical perspective, data on number of pneumonia cases treated at Bernhoven Hospital were retrieved from the local diagnosis registration system. In the 2 years preceding the outbreak, the number of pneumonia cases was stable with expected seasonal variation in 2005 (206 cases) and 2006 (170 cases), but with no sharp increase as was observed in 2007 (n=272). The findings in this study support the hypothesis that *C. burnetii* emerged as a novel etiological agent of pneumonia in the epidemic area in the Netherlands in May 2007.

The clinical presentation, diagnosis including antibody response and clinical outcome of Q fever in the Netherlands were unknown at the onset of the first epidemic wave in 2007. Available literature on the disease in its acute and chronic form, its diagnosis and management, was mostly retrospective and mainly derived from a single reference centre of expertise in France. In order to provide a uniform and systematic clinical follow-up for the Dutch Q fever patients from the first outbreak, Q fever patient care was centralized in and coordinated from the GP practice of Herpen, a rural town in the province of Noord-Brabant, and the epicentre of the first outbreak.

Chapter 3. describes clinical presentation, serology and echocardiographic findings of Q fever patients (n=85) from the first outbreak, who received follow-up at the GP practice in Herpen. An aspecific flu-like illness was the most common clinical presentation. Persistent fatigue after acute Q fever was present in 59% at 6 months and in 30% at 12 months follow-up. A typical serological response pattern was noted in both phase I and phase II antibodies, showing an increase in antibody titres up to 3 months, and a subsequent decline in the following 9 months of follow-up. None of the patients developed chronic Q fever. As could be expected on the basis of known background prevalence of minor cardiac valve defects in the general population, baseline echocardiography revealed a similarly high background prevalence of mild, clinically insignificant cardiac valve defects in this Q fever patient population. In the setting of a large outbreak, screening echocardiography is no longer part of the standard work-up of Q fever patients.

Chapter 4. describes the echocardiographic findings and outcomes of a larger group of Q fever patients from both the 2007 and 2008 cohort (n=134). Again a high proportion of clinically insignificant valvular defects were found and no patients developed chronic Q fever. By contrast with existing publications, these data indicate a low risk of progression to Q fever endocarditis. This contrasting result may be explained by selection bias and lack of specification of valvular defect severity in published retrospective research. In the setting of a massive Q fever outbreak, this raises the question whether the harm of low-treshold

administration of long-term antibiotic prophylaxis for mild valvular defects outweighs the benefit. In addition, the associated health-care costs of such a follow-up strategy on a large scale are substantial.

Following the observation of a striking level of undue long-term fatigue in many of the Q fever patients who were followed-up at the GP practice in Herpen (as described in Chapter 3.) two studies were undertaken to further characterize this symptomatology and its impact on health status.

In **Chapter 5.** the health status of Q fever patients from the first outbreak cohort is studied in detail. Health status was assessed with the Nijmegen Clinical Screening Instrument (NCSI). All patients were serologically tested for *C. burnetii* antibodies. Using a case-control design the study showed a sustained decrease in many aspects of health status 1 year after primary infection. Q fever patients had significantly more problems on the subdomains of symptoms and functional impairment. Severe fatigue levels were present in 52% of patients versus 26% in seronegative controls ($p<0,05$). NCSI score did not differ between seronegative (n=23) and seropositive (n=11) controls. These data support a sustained decrease in health status of Dutch Q fever patients after 1 year of follow-up.

For the patients of the Dutch Q fever outbreak, it remained unknown whether impaired health status and symptomatology persist (very) long-term or can change over time in the individual patient.

In **Chapter 6.** the health status of the same cohort of patients from the 2007 outbreak is studied 4 years after acute Q fever. A persistent significant percentage of patients exhibited clinically relevant ("severe") scores for all sub-domains of the NCSI. After 4 years, undue fatigue was present in 46% and exactly half of all patients experienced a severly impaired general quality of life. Patients with NCSI scores available in both 2008 and 2011 showed no difference in all sub-domain scores, except for a small decrease in dyspnoea emotions in 2011. In this group, a significant proportion of patients either improved or worsened in one or more sub-domains of health status. A minority of Q fever patients with impaired health status in 2008 showed improvement in various sub-domains of health status in 2011, but at the group level this effect was cancelled out by patients with worsening scores. These data support an overall persistence of impaired health status of Q fever patients in the Netherlands 4 years after primary infection.

Chapter 7. deals with the development and validation of the *Coxiella* ELISPOT; a *Coxiella burnetti* specific interferon gamma release assay (IGRA) measuring *C. burnetii* specific T-cell responses to both *C. burnetii* phase I and phase II antigens. Definitively establishing a diagnosis of chronic Q fever remains challenging, as the diagnostic performance of both conventional serological tests and PCR is limited. Given the importance of an early diagnosis of chronic Q fever, there is a need for a reliable diagnostic test.

The *Coxiella* ELISPOT adequately identified convalescent Q fever patients (n=9) from healthy controls (n=9). Compared to convalescent Q fever patients, chronic Q fever patients (n=3) showed a distinct *Coxiella* ELISPOT profile characterized by a much higher spot count for

both phase I and phase II (18-fold for phase II, 8-fold higher for phase I) andf a consistent shift towards more phase I reactivity. The diagnostic potential of the *Coxiella* ELISPOT is promising and warrants further investigation.

Following development and publication of another Q fever specific IGRA (the Whole Blood Interferon-Gamma Production Assay) by Schoffelen et al. in 2013, a comparison study was performed to assess the diagnostic performance of both tests in diagnosing Q fever.

In **Chapter 8.** the *Coxiella* ELISPOT is compared to a different whole blood *C. burnetii* specific IGRA in terms of diagnostic potential. Both tests were performed in a well-defined patient group of chronic Q fever patients (n=16) and a group of healthy seronegative individuals (n=17). Among patients, both tests detected a positive response in 14 out of 16 patients. Among controls, none were positive in the *Coxiella* ELISPOT whereas the Whole Blood Assay detected positive results in 1/17 and 3/17 individuals, when using Henzerling and Nine Mile as stimulating antigens, respectively. These data suggest that the *Coxiella* ELISPOT has a somewhat higher specificity than the Whole Blood Assay when Nine Mile is used as antigen stimulus. The assays showed moderate corrrelation: the Spearman correlation coefficient ranged between 0,37-0,60, depending on the antigen used. Further investigation of the diagnostic potential for *C. burnetii* infection of both assays is warranted.

Samenvatting

Samenvatting

De eerste Nederlandse menselijke Q koorts gevallen in de lente van 2007 waren het begin van een massale epidemie die zich in jaarlijkse seizoensgebonden uitbraken zou ontwikkelen tot 2011. De opkomst van *Coxiella burnetii* als een humaan pathogeen op deze onvoorziene schaal werd in verband gebracht met door Q koorts veroorzaakte abortus-golven op geiten- en schapenmelkbedrijven die al startten in 2005. In 2011, omvatte de Q koorts uitbraak meer dan 4000 gemelde Q koorts patiënten en een geschatte 44000 niet gemelde Q koorts gevallen. Aan het begin van de Nederlandse epidemie waren er geen evidence-based gevalideerde diagnose- en behandelprotocollen voorhanden. Beschikbare literatuur was tot dan toe hoofdzakelijk gegenereerd door een onderzoeksgroep van het WHO referentie laboratorium voor Q koorts in Marseille, Frankrijk. Het was onduidelijk of deze onderzoeksbevindingen toepasbaar waren in de setting van de massale uitbraak in Nederland. Enerzijds was dit een grote uitdaging voor clinici en andere gezondheidsprofessionals in het management van acute Q koorts en het voorkomen van lange-termijn gevolgen in de vroege fase van de epidemie. Anderzijds bood de Q koorts uitbraak een ongekende kans voor onderzoekers van verschillende disciplines om deze complexe infectieziekte te observeren, te verkennen en te onderzoeken.
Het doel van dit proefschrift is een beschrijving te geven van observaties over epidemiologische en klinische aspecten en lange-termijn gezondheidsuitkomsten van Q koorts patiënten van de initiële fase (2007-2008) van de eerste Nederlandse Q koorts uitbraak. In aanvulling hierop is het doel van dit proefschrift een beschrijving te geven van de diagnostische 'performance' en mogelijke klinische toepassingen van een nieuw ontwikkelde diagnostische interferon-gamma release test voor Q koorts: de *Coxiella* ELISPOT.

Hoofdstuk 1. geeft een algemene introductie en bevat achtergrondinformatie over Q koorts; de pathogenese, klinische manifestaties en lange termijns-gevolgen, de immuunrespons van de gastheer tegen *C. burnetii* en de impact en omvang van de Nederlandse humane Q koorts uitbraak.
In Nederland wordt Q koorts beschouwd als een nieuwe zoönose. In aanloop naar de eerste Q koorts uitbraak in Nederland in 2007 was de seroprevalentie van *C. burnetii* specifieke antistoffen laag in de algemene populatie. Echter, de gemeentes in het episch centrum van de epidemie waren niet vertegenwoordigd in deze seroprevalentie studie.
In **hoofdstuk 2.** wordt de etiologie van klinisch behandelde pneumonie gevallen in het episch centrum van de eerste Q koorts uitbraak in 2007 onderzocht en wordt de opkomst van *C. burnetii* als nieuw oorzakelijk agens van pneumonie aangetoond. De bijdrage van *C. burnetii* als oorzaak van pneumonie bij patiënten behandeld in het Bernhoven ziekenhuis (Herpen ligt in het adherentiegebied van dit algemeen ziekenhuis) in de periode januari-juli 2007 werd getest door retrospectief patiënten-sera te testen op de aanwezigheid van *C. burnetii* antistoffen. Van de 95 klinische pneumonie gevallen was *C. burnetii* het oorzakelijk pathogeen in 21 patiënten (22%). Het aantal Q koorts pneumonie gevallen in mei (n=13) was

aanmerkelijk hoger dan in elke andere maand in de studie periode (range 0-5). Om deze data in een historisch perspectief te onderzoeken werd retrospectief data over het aantal pneumonie gevallen in het Bernhoven ziekenhuis van de voorafgaande 2 jaar verzameld. In de 2 jaar voorafgaand aan de epidemie van 2007 was het aantal pneumonie gevallen stabiel met een verwachte seizoensgebonden variatie in 2005 (n=206) en 2006 (n=170), maar niet met een scherpe stijging in mei zoals waargenomen in 2007 (n=272). De bevindingen in deze studie ondersteunen de hypothese dat *C. burnetii* opgekomen is als een nieuwe oorzaak van pneumonie in het epidemisch gebied in mei 2007.

De klinische presentatie, diagnose inclusief antistof respons en klinische uitkomst van Q koorts in Nederland was onbekend bij aanvang van de eerste uitbraak in 2007. Om een uniforme en systematische follow-up te regelen voor de eerste Nederlandse Q koorts patiënten werd de patiëntenzorg gecentraliseerd en gecoördineerd in de huisartsenpraktijk te Herpen, een kleine gemeente in Noord-Brabant in het episch centrum van de eerste uitbraak.

Hoofdstuk 3. beschrijft de klinische presentatie, serologie en echocardiografische bevindingen van Q koorts patiënten (n=85) van de eerste uitbraak opgevolgd in de huisartsenpraktijk te Herpen. Een aspecifieke griepachtig beeld was de meest frequente klinische presentatie. Aanhoudende moeheid na acute Q koorts was aanwezig in 59% na 6 maanden en in 30% na 12 maanden follow-up. Er werd een karakteristiek serologische respons patroon waargenomen voor zowel fase I als fase II antistoffen, met een toename in antistoftiter tot 3 maanden en een daling in de daaropvolgende 9 maanden. Geen van de patiënten ontwikkelde chronische Q koorts. Zoals werd verwacht op basis van een bekende hoge prevalentie van milde hartklepgebreken in de algemene populatie, liet echocardiografie een hoge prevalentie zien van milde, klinisch onbelangrijke hartklepgebreken in dit Q koorts patiënt cohort. In de setting van een grote uitbraak is screening echocardiografie niet langer onderdeel van de standaard work-up van Q koorts patiënten.

Hoofdstuk 4. beschrijft de echocardiografische bevindingen en uitkomsten van een grotere groep Q koorts patiënten van zowel het 2007 en 2008 patiënten-cohort (n=134). Wederom werd een hoge prevalentie gevonden van milde, klinisch onbelangrijke hartklepgebreken gevonden en geen van de patiënten ontwikkelde chronische Q koorts. In tegenstelling tot beschikbare literatuur waarin een hoog risico tot ontwikkelen van Q koorts endocarditis bij klepgebreken wordt beschreven, wijzen deze data op een veel lager risico. In de setting van een massale Q koorts uitbraak roept dit de vraag op of de risico's van het laagdrempelig toedienen van langdurige antibiotische profylaxe (12 tot 18 maanden) opweegt tegen de mogelijke voordelen. Ook de kosten die gepaard gaan met een dergelijke follow-up strategie op deze schaal zijn aanzienlijk.

Een aanzienlijk deel van de Q koorts patiënten van de eerste Nederlandse Q koorts uitbraak hadden last van aanhoudende vermoeidheid (zoals beschreven in hoofdstuk 3). De hiernavolgende twee studies werden uitgevoerd om deze vermoeidheid en de gevolgen voor de gezondheidsstatus van Q koorts patiënten verder te onderzoeken.

Hoofdstuk 5. beschrijft gedetailleerd de gezondheidsstatus van Q koorts patiënten van het

eerste uitbraak cohort (n=54). De gezondheidsstatus werd gemeten met het Nijmegen Clinical Screening Instrument (NCSI). Patiënten en controles werden serologisch gecontroleerd. Van de 34 controle proefpersonen bleken 11 positieve *C. burnetii* serologie te hebben. Met behulp van een case-control design werd een vermindering van meerdere aspecten van de gezondheidsstatus gevonden. Q koorts patiënten hadden significant meer problemen in de sub-domeinen 'symptomen' en 'functionele beperking'. Ernstige vermoeidheid was aanwezig in 52% van de patiënten (n=54) tegen 26% in seronegatieve controle proefpersonen (n=23) ($p<0,05$). De NCSI scores verschilden niet tussen seropositieve (n=11) en seronegatieve (n=23) controle proefpersonen. Deze studie laat een duidelijke vermindering in gezondheidsstatus zien van Q koorts patiënten 1 jaar na acute Q koorts.

Het is onbekend of de vermindering in gezondheidsstatus van Q koorts patiënten aanhoudt op de zeer lange termijn (na 1 jaar) en of de gezondheidsstatus van een individuele patiënt kan variëren in de tijd.

Hoofdstuk 6. beschrijft de gezondheidsstatus van hetzelfde cohort Q koorts patiënten van de uitbraak in 2007 na 4 jaar follow-up. De gezondheidsstatus werd gemeten met het Nijmegen Clinical Screening Instrument (NCSI). NCSI scores in 2008 (n=54) en 2011 (46) werden vergeleken op het niveau van de groep en op individueel patiënt-niveau. Een groot deel van de patiënten vertoonden klinisch relevante ('ernstige') scores voor alle sub-domeinen van het NCSI. Na 4 jaar was overmatige vermoeidheid aanwezig in 46% en precies de helft van alle patiënten hadden een ernstig vermindering in algehele 'quality of life'. Voor patiënten met scores in 2008 en 2011 (n=37) was er geen verschil in alle sub-domein scores, met uitzondering van een vermindering van 'dyspneu emoties' in 2011. Een significant deel van patiënten in deze groep (n=37) verbeterden of verslechterden in een of meerdere sub-domeinen van de gezondheidsstatus. Een minderheid van Q koorts patiënten met verminderde gezondheidsstatus in 2008 vertoonde een verbetering in verschillende sub-domeinen van het NCSI in 2011, maar op groepsniveau werd dit effect tenietgedaan door patiënt met verslechterende scores. Deze data tonen een aanhouden van verminderde gezondheidsstatus van Nederlandse Q koorts patiënten 4 jaar na acute Q koorts.

Hoofdstuk 7. beschrijft de ontwikkeling en validatie van de *Coxiella* ELISPOT; een *Coxiella burnetii* specifiek interferon-gamma release assay (IGRA) die de *C. burnetii* specifieke T-cel respons op zowel *C. burnetii* fase I en fase II antigenen. Het stellen van een definitieve diagnose chronische Q koorts is uitdagend omdat de diagnostische waarde van serologie en PCR beperkt is. Het stellen van de diagnose chronische Q koorts is belangrijk voor een tijdige behandeling en het voorkomen van complicaties.

De *Coxiella* ELISPOT onderscheidde nauwkeurig convalescente Q koorts patiënten (patiënten met een doorgemaakte, niet chronische infectie) (n=9) van gezonde controle proefpersonen (n=9). Vergeleken met convalescente proefpersonen vertoonden patiënten met chronische Q koorts (n=3) een karakteristiek *Coxiella* ELISPOT profiel met een veel hogere spot-count voor zowel fase I (8-voudig verhoogd) als fase II (18-voudig verhoogd) antigenen, en een consistente verschuiving richting meer fase I reactiviteit. De diagnostische waarde van de

Coxiella ELISPOT is veelbelovend en verdient verder onderzoek.
Na de ontwikkeling en publicatie van een andere Q koorts specifiek IGRA (the Whole Blood Interferon-Gamma Production Assay) door Schoffelen et al. in 2013 werd een vergelijkende studie uitgevoerd om de diagnostische 'performance' van beide assays te meten in het diagnosticeren van een *C. burnetii* infectie.
In **hoofdstuk 8.** wordt de diagnostische 'performance' vergeleken van de *Coxiella* ELISPOT met de 'Whole Blood Interferon-Gamma Production Assay'. Beide assays werden uitgevoerd in een strikt gedefinieerde groep patiënten met chronische Q koorts (n=16) en een groep gezonde seronegatieve proefpersonen (n=17). In de patiëntengroep identificeerde beide assays een positieve respons in 14 van de 16 patiënten. In de controle groep was geen van de proefpersonen positief in de *Coxiella* ELISPOT assay. De Whole Blood Assay was positief in 1/17 en 3/17 controle proefpersonen wanneer respectievelijk de 'Henzerling' of 'Nine Mile' gebruikt werd als stimulerend antigen. Deze data suggereren dat de *Coxiella* ELISPOT een iets hogere specificiteit heeft dan de Whole Blood Assay als 'Nine Mile' als stimulerend antigen gebruikt wordt. De assays vertoonden een matige correlatie: de Spearman correlatie coëfficiënt varieerde tussen 0,37-0,60, afhankelijk van welk stimulerend antigen werd gebruikt.

Conclusies
Dit proefschrift bevat verkennende studies over Q koorts bij mensen, met een accent op klinische observaties, lange-termijn gezondheidsstatus na infectie en de ontwikkeling van een nieuwe diagnostische test voor Q koorts.
De bevindingen in dit proefschrift ondersteunen de hypothese dat *Coxiella burnetii* is opgekomen in Nederland als een nieuwe oorzaak van pneumonie in mei 2007. De eerste systematische observaties van klinische presentatie, serologie en echocardiografische bevindingen bij Q koorts patiënten van de initiële fase van de uitbraak in Nederlands worden gepresenteerd.
Q koorts manifesteerde zich voornamelijk als een mild griepachtig ziektebeeld. De prevalentie van milde hartklepgebreken bij Q koorts patiënten was hoog maar vergelijkbaar met de verwachte prevalentie in de algehele bevolking. De incidentie van chronische Q koorts in het geval van deze milde, klinisch niet belangrijke hartklepvitia was veel lager dan eerder werd aangenomen. Dit roept de vraag op of echocardiografische screening en het toedienen van langdurige antibiotische profylaxe in het geval van deze milde hartklepgebreken nuttig is.
En groot deel van de Q koorts patiënten heeft last van overmatige vermoeidheid en een aanhoudend verminderde gezondheidsstatus na 1 en 4 jaar follow-up.
Het meten van de *C. burnetii* specifieke interferon-gamma productie door menselijke T-cellen is haalbaar en heeft diagnostisch potentieel voor het diagnosticeren van een *C. burnetii* infectie. Een vergelijking van twee *C. burnetii* specifieke IGRA's; de *Coxiella* ELISPOT en de Whole Blood Interferon Gamma Production Assay, laat een hoge sensitiviteit zien voor het detecteren van een *C. burnetii* specifieke interferon-gamma respons bij chronische Q koorts patiënten. Nader onderzoek naar het diagnostisch potentieel van beide testen is aan te bevelen.

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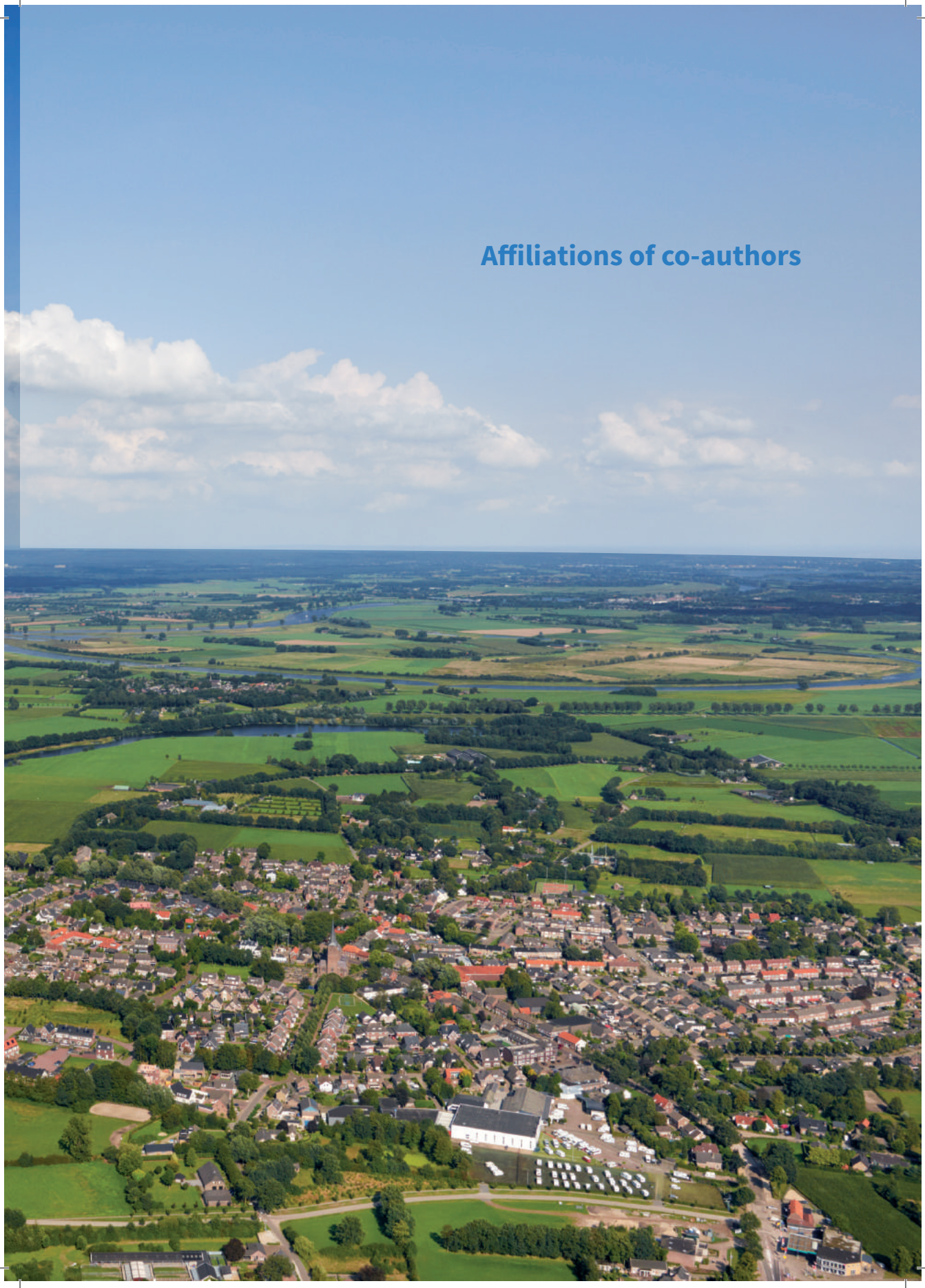

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Q koorts is een vreemde ziekte. Het doen van onderzoek naar een 'niche-aandoening' zoals infectie door *Coxiella burnetii* levert dan ook met name steeds betere onderzoeksvragen op.

Als het promotietraject al bij tijden een stormachtige beproeving leek, kwam steevast de opmerking van Trinculo uit Shakespeare's 'The Tempest' in mijn hoofd bovendrijven: "Misery acquaints a man with strange bedfellows". De kennismaking met zoveel eigenzinnige, prikkelende maar vooral inspirerende mensen was misschien wel het grootste plezier van het onderzoek en het schrijven van dit proefschrift. Velen hebben bewust en/of onbewust bijgedragen; ik dank allen hier en nu.

Het begon in Noord-Brabant, in Herpen. De Q koorts patiënten en inwoners van Herpen werden vanaf 2007 onverwacht proefpersonen in de inmiddels meest intens bestudeerde Q koorts epidemie ooit. Zonder jullie geen onderzoek, geen proefschrift. Mijn grootste dank gaat uit naar jullie en dit proefschrift is van en voor jullie. Ik hoop dat dit onderzoek bijdraagt aan een beter begrip van deze complexe ziekte en uiteindelijk zal resulteren in een betere diagnostiek en behandeling van Q koorts patiënten.

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Radboud universiteit medisch centrum, Nijmegen

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UK

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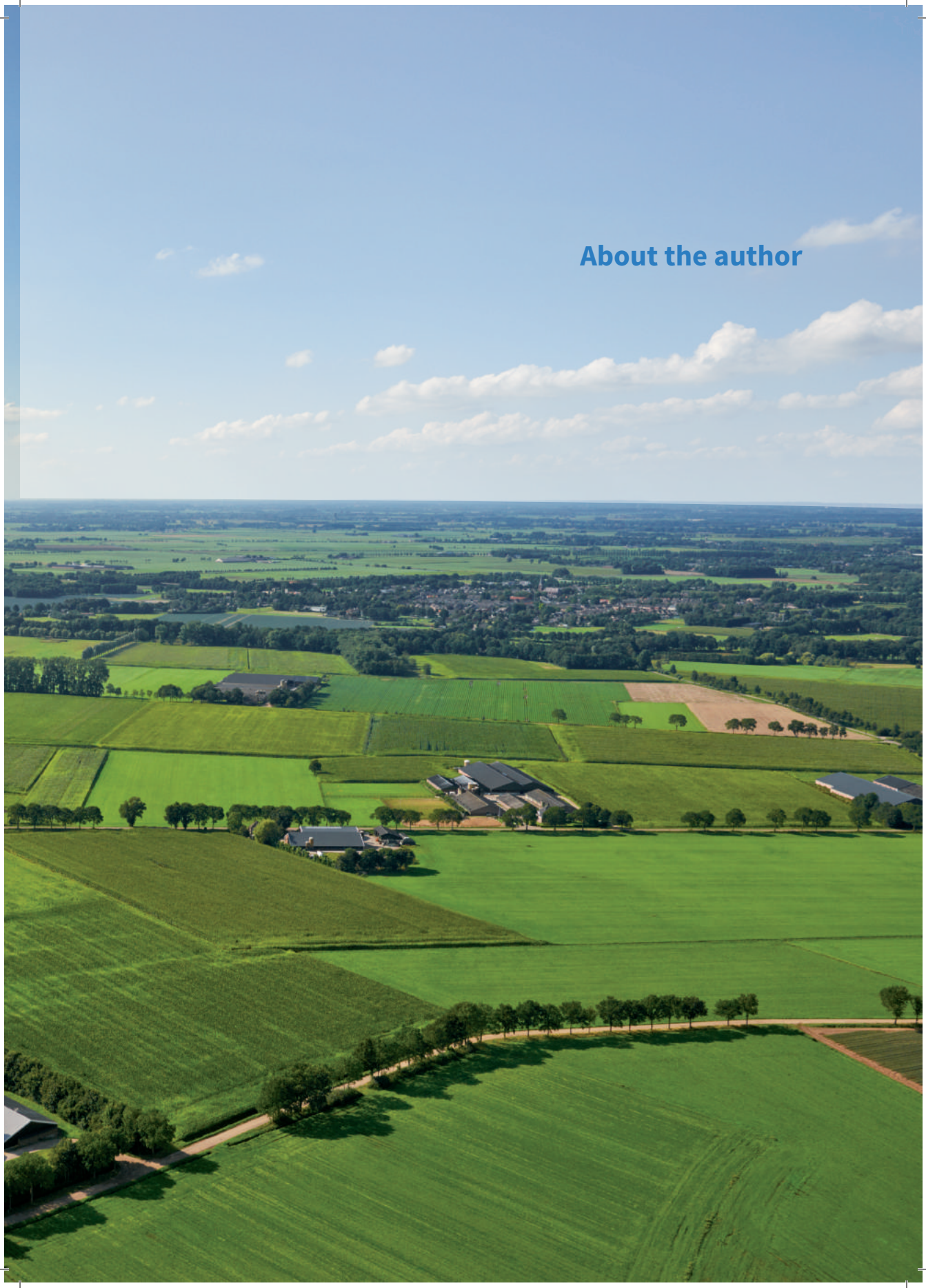
I'm gonna love you
Till the wheels come off
Oh, yeah

About the author



About the author

Gijs Limonard (Nijmegen, 1978) completed his secondary education (Gymnasium β) at the Jeanne d'Arc college in Maastricht in 1996.

Due to the numerus fixus at the time, he was deferred from starting his medical studies in the Netherlands and thereupon decided to pursue his academic medical education abroad, at the Catholic University of Leuven (KU Leuven), Belgium.

In 2003 he obtained his MD cum laude from the KU Leuven, after 5 years of formal medical training in Leuven and 2 years of internships in Belgium (Brasschaat, Bonheiden, Leuven), the Netherlands (Tilburg) and the United Kingdom (Ashington). During his studies he developed a keen interest in respiratory diseases and started to explore the opportunity of specializing in this clinical field as a pulmonologist.

From 2003 to 2009 he completed his training as a pulmonologist at the Department of Pulmonary medicine at the Canisius-Wilhelmina Hospital in Nijmegen, The Netherlands (clinical supervisor dr. J.P. Janssen). Part of this training was done at the department of Pulmonary Diseases at the Radboud University Nijmegen Medical Center from January to July 2009 (clinical supervisors prof. dr. P.N.R. Dekhuijzen and dr. Y. Heijdra). In spring 2007 the first ever Dutch human Q fever outbreak occurred, and in autumn of that year, research opportunity knocked when the first systematic clinical investigations were started in the region.

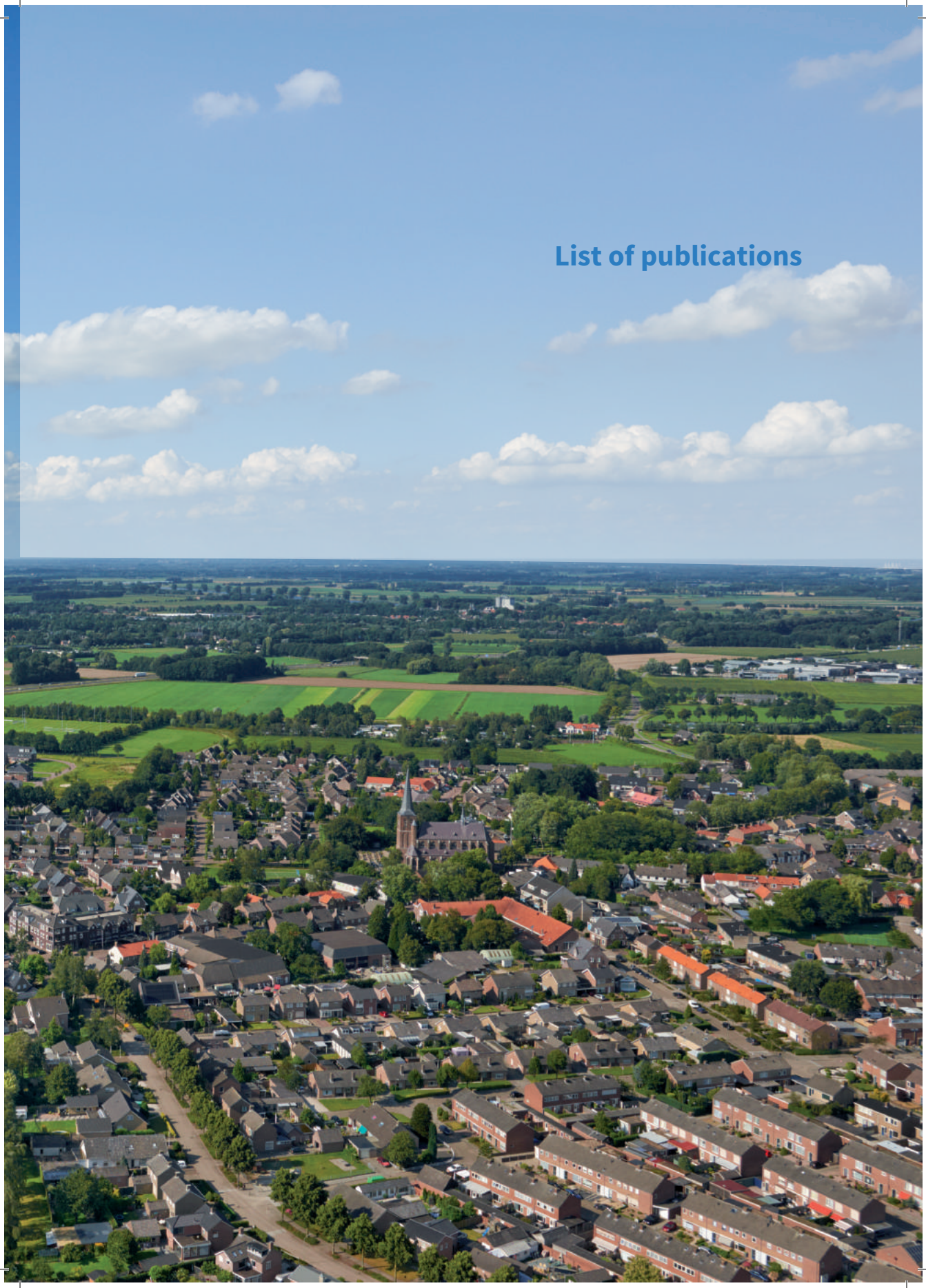
In 2009 he qualified as a pulmonologist registrar and started working as a full-time pulmonologist clinician at the Diakonessenhuis in Utrecht. Areas of clinical expertise include respiratory infections (including tuberculosis), interstitial lung diseases and sleep related breathing disorders. The research on Q fever was conducted in 2007 to 2011 in both Nijmegen and Utrecht, and completed and published during these last years in Utrecht.

In 2015 he qualified as clinical supervisor in pulmonology.

In his spare time, he enjoys; playing guitar (acoustic and electric), cycling, traveling, and reading and studying English literature and poetry; his favourite poets among many (aside from William Shakespeare, of course) being Christopher Marlowe, John Keats, Wilfred Owen and Philip Larkin.

He is married to Manon Leenders, they live in Utrecht.

List of publications



List of publications
Publications on Q fever

1. **Limonard GJ**, Peters JB, Besselink R, Groot CAR, Dekhuijzen PNR, Vercoulen JH, Nabuurs-Franssen MH. Persistence of impaired health status of Q fever patients 4 years after the first Dutch outbreak. Epidemiol Infect. 2016 Apr;144(6):1142-7.
2. Schoffelen T, **Limonard GJ**, Bleeker-Rovers CP, Bouwman JJ, van der Meer JW, Nabuurs-Franssen M, Sprong T, van Deuren M. Diagnosis of Coxiella burnetii infection: comparison of a whole blood interferon-gamma production assay and a Coxiella ELISPOT. Plos One. 2014 Aug 1;9(8):e103749.
3. Anderson A, Bijlmer H, Fournier PE, Graves S, Hartzell J, Kersh GJ, **Limonard G**, Marrie TJ, Massung RF, McQuiston JH, Nicholson WL, Paddock CD, Sexton DJ. Diagnosis and management of Q fever—United States, 2013: recommendations from CDC and the Q Fever Working Group. MMWR Recomm Rep. 2013 Mar 29;62(RR-03):1-30. Erratum in MMWR Recomm Rep. 2013 Sep 6;62(35):730.
4. **Limonard GJ**, Thijsen SF, Bossink AW, Asscheman A, Bouwman JJ. Developing a new clinical tool for diagnosing chronic Q fever: the Coxiella ELISPOT. FEMS Immunol Med Microbiol. 2012 Feb;64(1):57-60.
5. **Limonard GJ**, Groot CA, Dekhuijzen PN, Nabuurs-Franssen MH. Coxiella burnetii: a genuinely novel causative agent of pneumonia in The Netherlands since May 2007. Epidemiol Infect. 2012 May; 140(5):865-6.
6. **Limonard GJ**, Nabuurs-Franssen MH, Dekhuijzen PN, Groot CA. Prevention of Q fever endocarditis. Lancet Infect Dis. 2011 Feb;11(2):82-3.
7. **Limonard GJ**, Nabuurs-Franssen MH, Weers-Pothoff G, Wijkmans C, Besselink R, Horrevorts AM, Schneeberger PM, Groot CA. One-year follow-up of patients of the ongoing Dutch Q fever outbreak: clinical, serological and echocardiographic findings. Infection. 2010 Dec;38(6):471-7.
8. **Limonard GJ**, Peters JB, Nabuurs-Franssen MH, Weers-Pothoff G, Besselink R, Groot CA, Dekhuijzen PN, Vercoulen JH. Detailed analysis of health status of Q fever patients 1 year after the first Dutch outbreak: a case-control study. QJM. 2010 Dec;103(12):953-8.

Publications on other topics

1. Van Roeden SE, Thijsen SF, Sankatsing SUC, **Limonard GJ**. Clinical relevance of C. Pseudodiphteriticum in lower respiratory tract specimens. Infect Dis (Lond). 2015;47(12):862-8.
2. Ebisch IM, **Limonard GJ**, Vreuls W, Sporken JM. Herhaalde miskraam of paraneoplastisch syndroom? Een mogelijke valkuil. [Recurrent miscarriage turns out to be lung cancer]. Ned Tijdschr Geneeskd. 2013;157(44):A6487. Dutch.
3. Hermens FH, **Limonard GJ**, Hoevenaars BM, de Kievit I, Janssen JP. Diagnostic value of histology compared with cytology in transbronchial aspiration samples by histology needle. J Bronchology Interv Pulmonol. 2010 Jan;17(1):19-21.
4. Peek H, van der Bruggen W, **Limonard G**. Pleural FDG uptake more than a decade after talc pleurodesis. Case Rep Med. 2009;2009:650864.
5. **Limonard G**, Joosten J, Berk Y, De Kievit I, Zomer S, Keemers M. A 37-year-old woman with an incidentally found mediastinal nodule. Chest. 2008 Jun;133(6):1508-11.
6. Hermens FH, **Limonard GJ**, Termeer R, van den Berg W, Visser FJ, Hol BE, Janssen JP. Learning curve of conventional transbronchial needle aspiration in pulmonologists experienced in bronchoscopy. Respiration. 2008;75(2):189-92.
7. Jaspers R, Barendregt W, **Limonard G**, Visser F. Necessary resection of the left lower lobe due to systemic arterial supply. J Thorac Cardiovasc Surg. 2007 May;133(5):1384-5.

Book Chapter

Gijs Limonard en Julius Janssen. Hoofdstuk 'Unilateraal pleuravocht'. Probleemgeorienteerd denken in de longgeneeskunde. 1ste Druk/editie 2010. Uitgeverij de Tijdstroom. ISBN 10: 9058981762, ISBN 13: 9789058981769.

Abstracts presented

1. Persistence of impaired health status of Q fever patients 4 years after the first Dutch outbreak. Limonard GJM, Peters JB, Besselink R, Groot CAR, Dekhuijzen PNR, Vercoulen JH, Nabuurs-Franssen MH. International Q fever symposium 2012, Amsterdam.
2. Potential value of an ELISPOT interferon gamma release assay as a diagnostic tool in Q fever infection. G.J.M. LImonard, J.J.M. Bouwman, A. Asscheman, S.F.T. Thijsen, B. Vlaminckx, A. Bossink. Voorjaarsvergadering van de Nederlandse Vereniging voor Medische Microbiologie (NVMM) en de Nederlandse Vereniging voor Microbiologie (NVvM) 2011, Arnhem
3. Detailed assessment of health status of Q fever patients one year after the first Dutch outbreak: a case control study. G.J.M. Limonard, M.H. Nabuurs-Franssen, J.B. Peters, G. Weers-Pothoff, R. Besselink, J.H. Vercoulen, P.N.R. Dekhuijzen, C.A.R. Groot – Ned Tijdschr Med Microbiol 2010;18:Supplement. Voorjaarsvergadering van de Nederlandse Vereniging voor Medische Microbiologie (NVMM) en de Nederlandse Vereniging voor Microbiologie (NVvM) 2010, Arnhem
4. Potential value of an ELISPOT interferon gamma release assay as a diagnostic tool in Q fever infection. Limonard GJM, Bouwman JJM, Asscheman A, Thijsen SFT, Vlaminckx B, Bossink A. Congress of the European Respiratory Society 2011, Amsterdam
5. Coxiella burnetii: a genuinely novel causative agent of pneumonia in the Netherlands. G.J.M. Limonard, M.H. Nabuurs-Franssen, G. Weers-Potthof, C. Wijkmans, R. Besselink, A.M. Horrevorts, P.M. Schneeberger, P.N.R. Dekhuijzen, C.A.R. Groot. ESCMID/ASR 6th International Conference on Rickettsiae and Rickettsial Diseases, Heraklion 2011, Kreta
6. Developing a Coxiella burnetii ELISPOT assay measuring T-cell responses in Q fever. Bouwman JJM, Limonard, GJM (presenting), Asscheman A , Bossink AW, Hannen EJ, Thijsen SFT. ESCMID/ASR 6th International Conference on Rickettsiae and Rickettsial Diseases, Heraklion 2011, Kreta
7. Potential value of an ELISPOT interferon gamma release assay as a diagnostic tool in Q fever infection. Limonard GJM, Bouwman JJM, Asscheman A, Thijsen SFT, Vlaminckx B, Bossink A. ESCMID/ASR 6th International Conference on Rickettsiae and Rickettsial Diseases 2011, Heraklion, Kreta
8. Detailed analysis of health status of patients one year after the first Dutch Q fever outbreak: a case control study. Limonard G, Peters JB, Nabuurs-Franssen MH, Weers-Pothoff G, Besselink R, Groot CAR, Dekhuijzen PNR, Vercoulen J – Eur Respir J 2010;36: Suppl. 54. Congress of the European Respiratory Society 2010, Barcelona
9. Screening echocardiography in the follow-up strategy of a large Q fever pneumonia outbreak in the Netherlands. Limonard G, Nabuurs-Franssen M, Wijkmans C, Weers-Pothoff G, Besselink R, Horrevorts A, Groot CAR – Eur Respir J 2009;34: Suppl. 53. Congress of the European Respiratory Society 2009, Vienna
10. Learning curve of transbronchial needle aspiration (TBNA) for pulmonologists experienced in bronchoscopy G. Limonard, F. Hermens, J. Janssen - Eur Respir J 2007;30: Suppl. 51, 108s. Congress of the European Respiratory Society 2007, Stockholm
11. Collapse of a self expanding metallic stent in five patients with central airway obstruction. G. J. M. Limonard, F. M. N. H. Schramel, J. J. Mager, L. N. A. Willems, J. A. Burgers, O. C. J. Schuurbiers, J. P. Janssen - Eur Respir J 2007;30: Suppl. 51, 108s. Congress of the European Respiratory Society 2007, Stockholm