Prognosis and treatment of chronic Q fever

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Prognose en behandeling van chronische Q-koorts
(met een samenvatting in het Nederlands)

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Chapter 1

General introduction and outline of thesis

Introduction

The Dutch Q fever outbreak

Q fever is caused by *Coxiella burnetii*, a Gram-negative, intracellular bacterium. Disease in humans and animals is reported worldwide with exception of New Zealand.[1] *C. burnetii* may cause disease in ongoing endemic settings and in the setting of large outbreaks.[1] In the Netherlands, a large Q fever outbreak was documented between 2007 and 2010.[2] Before 2007, there were signs of an increase in abortion rates among goat caused by *C. burnetii* in the Netherlands, but no signs of an increase in the number of human infections.[3,4] Between 2007 and 2010, over 4,000 human acute Q fever cases have been reported.[5] It is estimated that up to 40,000 – 50,000 people have been infected.[6,7] The peak of the Dutch Q fever outbreak was in 2009, in which year over 60% of infected individuals were notified (see figure 1).[5] After several control measures taken by the Dutch government, the epidemic resolved. Currently, approximately 10 - 30 cases of acute Q fever are reported each year.[4,5]

Figure 1. Number of notified acute Q fever cases during the Dutch Q fever outbreak[7]

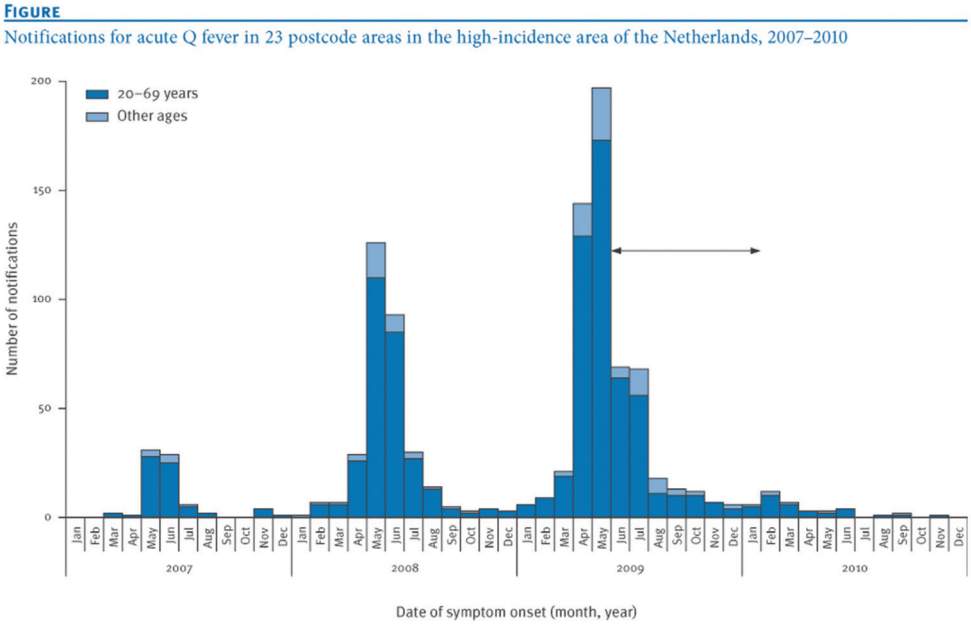

Image derived from van der Hoek W, Hogema BM, Dijkstra F et al. Relation between Q fever notifications and Coxiella burnetii during the 2009 outbreak in the Netherlands. Eurosurveillance, special edition Q fever; 2012; 11-5.

Coxiella burnetii: transmission to humans

C. burnetii can spread via the air, with a radius of thirty kilometers around the source.[8] During the Dutch Q fever outbreak, transmission of *C. burnetii* mainly occurred through infected goat shedding the bacterium in large amounts in birth products and milk, resulting in large numbers of human acute Q fever cases in springtime after inhalation of *C. burnetii*.[2] Besides inhalation of *C. burnetii* spreaded through the air, individuals can be infected by drinking contaminated milk, incidentally from human to human during sexual intercourse, and hypothetically through transplantation.[1,9] Transmission of *C. burnetii* through tissue transplantation has not been described in literature. However, single cases of likely transmission through blood transfusion and possible transmission through bone marrow transplantation to an immunocompromised recipient have been reported.[10,11] Furthermore, transmission through transplantation (liver, thymus and lymph nodes) in animals has been observed.[12] Therefore, it has been suggested that a potential risk for transmission of *C. burnetii* lies with transplantation of some tissues, such as heart valves, musculoskeletal tissues and skin.[9] After the Dutch Q fever outbreak, the risk of transmission through transplantation led to concerns. Therefore, the Dutch Health Council advised screening of all Dutch tissue- and cell donors. Screening of donors started in 2010 and is continued up to this date. However, the outcome of screening, in terms of seroprevalence of *C. burnetii* antibodies and the proportion of yet undetected chronic Q fever patients among Dutch tissue and cell donors, has not been evaluated. Moreover, it is unknown what patient characteristics predict seropositivity or presence of undiagnosed chronic Q fever. Evaluation of the program may aid in quantifying the risk of transmission, evaluating the efficacy of screening and identifying high risk donors.

Chronic Q fever

After inhalation by humans, the bacterium targets antigen-presenting cells, such as (alveolar) monocytes and macrophages.[13] Primary infection with *C. burnetii* is relatively mild. Most patients remain asymptomatic (60%); the remaining proportion of patients develops flu-like illness, pneumonia or hepatitis which is self-limiting in most cases. A small proportion of patients (1-5%) develops chronic Q fever after primary infection, most often presenting with infected arterial aneurysms, infected vascular prostheses or endocarditis as focus of infection.[14-16] In figure 2, an example of an infected vascular prosthesis is shown.[17] Most cases of chronic Q fever occur within one year after primary infection, but longer intervals have been described.[14,15] Establishing the diagnosis of chronic Q fever may be difficult: there is no single test to confirm or exclude the diagnosis. Therefore, sets of diagnostic criteria have been formulated, consisting of microbiological, radiographic and clinical characteristics by both Dutch and French research groups.[1,18]

Figure 2. Example of an infected arterial aneurysm, caused by *Coxiella burnetii*[17]

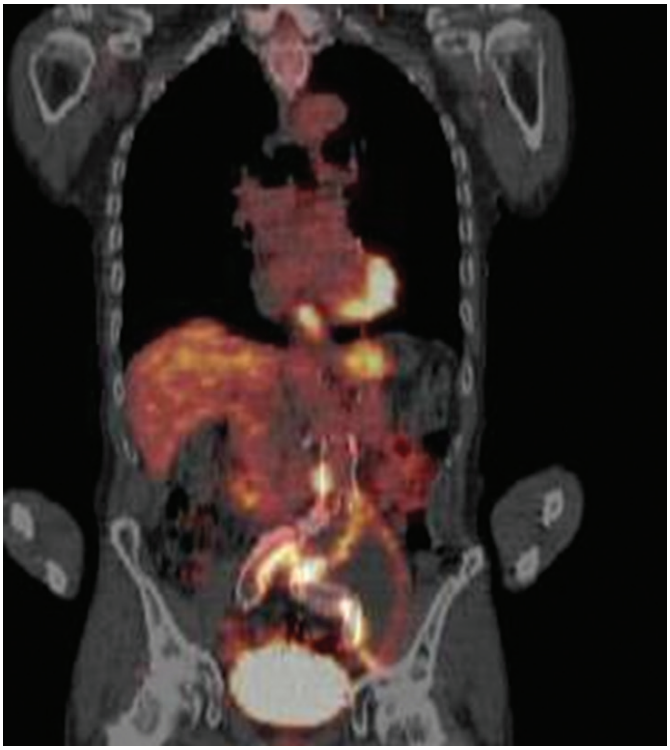

Image derived from Barten DG, Delsing CE, Keijmel SP. Localizing chronic Q fever: a challenging query. BMC infectious diseases, 2013, DOI: 10.1186/1471-2334-13-413.

The Dutch chronic Q fever consensus group criteria classify chronic Q fever patients as proven, probable or possible chronic Q fever patients. A requirement for any of these three diagnoses is having serological evidence for the disease, defined as a phase I IgG titer ≥ 1:1,024 in an immunocompetent host. Patients with proven chronic Q fever are those with an established endocarditis (according to the Duke criteria for infective endocarditis), those with an established vascular infection (confirmed by PET-CT or other imaging studies) or those with a positive polymerase chain reaction (PCR) for *C. burnetii* on serum or tissue. Probable chronic Q fever is defined as having a risk factor for the disease, signs and symptoms of chronic infection or a less typical focus of infection. Patients with possible chronic Q fever are those not fulfilling the definitions of proven or probable chronic Q fever: in other words, these patients have serological evidence for the disease without any focus, risk factor or signs and symptoms.[18] See table 1 for the criteria formulated by the Dutch chronic Q fever consensus group.[18] Eldin *et al*. formulated criteria based on focus of infection, with an additional factor of certainty (definite or possible).[1] Both criteria cover the same patients in essence: the diagnoses of proven or probable chronic Q fever according to the Dutch chronic Q fever consensus group criteria are similar to the definitions of definite or possible persistent focalized infection according to the criteria formulated by Eldin et al.[1]

Table 1. Diagnostic criteria for chronic Q fever, defined by the Dutch chronic Q fever consensus group[18]

Proven chronic Q fever	Probable chronic Q fever	Possible chronic Q fever
1. Positive *Coxiella burnetii* PCR in tissue and/or blood*	1. IFA phase I IgG titer ≥ 1:1,024	1. IFA phase I IgG titer ≥ 1:1,024 without fulfilling the definition of proven or probable chronic Q fever
OR	**AND**	
2. IFA phase I IgG titer ≥ 1:1,024	Valvulopathy without fulfilling the definition of definite endocarditis according to the 'modified Duke criteria' or	
AND	Known aneurysm, vascular prosthesis or valve prosthesis without signs of infection (e.g. on echo, PET-CT) or	
Definite endocarditis according to the 'modified Duke criteria' or		
Proven arterial or vascular prosthesis infection, radiographically confirmed (e.g. PET-CT)	Suspected atypical focus of infection such as osteomyelitis, pericarditis or hepatitis or	
	Pregnancy or	
	Signs and symptoms of chronic infection (e.g. fever, weight loss, glomerulo-nephritis, night sweats) or	
	Proven granulomatous infiltrate in tissue, confirmed by pathological evaluation or	
	Immunocompromised state	

**in absence of an acute infection. Abbreviations in table 1: PCR = polymerase chain reaction. IFA = indirect fluorescent-antibody assay.*

Prognosis and course of disease for the different diagnostic categories, as defined in both sets of criteria, have not been described in detail before. However, it has been reported that chronic Q fever can be accompanied by serious complications and mortality, in contrast to acute Q fever. Previously reported overall mortality was up to 25% for patients with proven vascular chronic Q fever and varying between 7%-26% for patients with proven chronic Q fever endocarditis after a follow-up duration of three years.[16,19-23] Symptomatic aneurysms, ruptured aneurysms, formation of fistula, abscesses and spondylodiscitis have been reported as complications of chronic Q fever.[21-23,26,27] The incidence of complications, timing of occurrence of complications and exact nature of complications are unknown. Moreover, it has never been studied which factors contribute to occurrence of complications. Besides disease-related outcomes, chronic Q fever may impact patients' quality of life. There is one

study that evaluated quality of life in chronic Q fever patients shortly after start of treatment. The effect of the disease and antibiotic treatment on long-term quality of life (QOL) however, has never been evaluated before. Patient-centered outcomes are of great importance when managing patients and awareness of the consequences of the disease and its treatment on long-term QOL among clinicians is crucial. Impaired QOL may have a bigger impact on patients' lives than clinical outcomes, and acknowledgement of disease burden and disadvantages of treatment is important for patients.[28] The importance of acknowledgement of the consequences of the Q fever outbreak is painfully visible in the Netherlands, where patients have sued the Dutch government and goat farmers after the Q fever outbreak of 2007 – 2010. They were accused of negligence and mismanagement, patients felt misinformed and unacknowledged with regard to the impact of the disease on their lives.[29]

Altogether, there is a lack of data on the prognosis of chronic Q fever and factors that predict adverse outcomes during the disease. Due to the rarity of chronic Q fever, large cohorts of patients are difficult to describe. Knowledge on the course of disease, complications and risk factors for adverse outcomes may aid clinicians to recognize the disease and identify individuals at risk for adverse outcomes. Moreover, better understanding of the prognosis of chronic Q fever, in terms of complications, mortality and QOL is important in managing patients and informing them on their disease. Therefore, there is need for a systematic and structured assessment of the prognosis of chronic Q fever in terms of adverse outcomes, and to study what factors are associated with adverse outcomes. Moreover, to fully comprehend the consequences of chronic Q fever, long-term QOL in chronic Q fever patients should be assessed.

Treatment of chronic Q fever

Similar to the diagnosis of chronic Q fever, treatment of chronic Q fever is also complicated. After antigen-presenting cells are invaded, *C. burnetii* forms an acidified phagosomal vacuole in which it replicates (see figure 3).[30] *C. burnetii* is intrinsically resistant to penicillin, which was soon noted after discovery of the bacterium.[31] In the nineteen-fifties, tetracyclines and chloramphenicol were found to be beneficial for treatment of chronic Q fever. For a long time, it was presumed that there was no definitive treatment and long-term suppression was the main goal of treatment.[32] In the nineteen-nineties, treatment of chronic infection with tetracyclines plus quinolones was found to be more effective than treatment with tetracyclines alone and curation appeared to be possible.[33] Later, it was hypothesized that tetracyclines would be more effective combined with an alkalizing agent such as hydroxychloroquine, since *C. burnetii* replicates within macrophages and monocytes where the acidified phagosomal compartment decreases the bactericidal efficacy of antibiotics.[30] Tetracyclines plus hydroxochloroquine was indeed found to be superior to tetracyclines plus quinolones in terms of relapse and treatment duration in a retrospective study of 35 patients.[34] However, evidence on superiority of tetracyclines plus hydroxychloroquine compared to other regimens is limited and inconsistent: in another study of patients with vascular chronic Q fever, treatment with tetracyclines

plus hydroxychloroquine was not more effective compared to tetracyclines plus quinolones or tetracyclines alone in reducing mortality.[19] It is currently advised to treat chronic Q fever patients with tetracyclines plus hydroxychloroquine, with tetracyclines plus quinolones considered as a potential alternative, for at least 18 months.[34] All three studies performed were small, observational studies. At most, 35 patients have been studied.[19,30,34] Treatment and follow-up procedures were unstandardized, and, therefore, all findings are subject to the risk of confounding by indication: physicians will treat patients with more severe disease differently than those with less severe disease, which leads to bias that can hardly be corrected for. Finally, all available data on treatment of chronic Q fever so far are published by one group. Validation of their findings in other settings has never been performed. Therefore, the quality of available evidence is limited. Apart from the limited evidence on treatment of chronic Q fever with tetracyclines plus hydroxychloroquine or tetracyclines plus quinolones, little is known on potential alternative treatment regimens. Altogether, there is an urgent need for additional studies on efficacy of diverse treatment strategies for chronic Q fever, in a large cohort of chronic Q fever patients.

Figure 3. Formation of large replicate vacuoles in monocyte-derived macrophages infected by *Coxiella burnetii*[35]

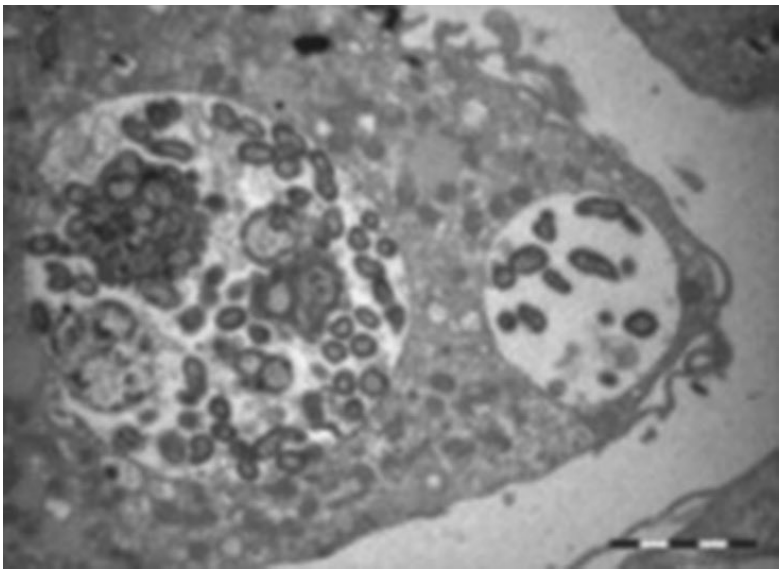

Image derived from Desnues B, Imbert G, Raoult D et al. Role of specific antibodies in Coxiella burnetii infection of macrophages. Clin Microbiol and Infect 2009;15(2):161-62.

During treatment, treatment efficacy can be verified in different ways. Naturally, the clinical course of disease (thus presence of complications, signs and symptoms of infection) is the most important measure in evaluating treatment efficacy. Moreover, there are data suggesting that a four-fold titer decrease after one year

predicts better outcomes.[20] However, both parameters are slow: waiting until a titer decrease or resolution of complications may take months. Culturing of *C. burnetii* is not routinely performed in clinical practice, since it is difficult and only allowed in laboratories with a biohazard safety level 3 facility.[36-38] Therefore, time to negativity of cultures is rarely used in clinical practice as a parameter for efficacy of treatment. A potential tool to gain insight in the adequacy of treatment, is measurement of serum doxycycline concentrations (SDC). Measuring SDC may be used to evaluate doxycycline dosage and compliance to therapy. In two small clinical studies, SDC >5µg/mL were associated with favorable serological response, and higher SDC to minimum inhibitory concentration (MIC) ratios were associated with a rapid decline in phase I IgG antibodies to *C. burnetii*.[39-41] This can be explained by doxycycline being more effective in higher concentrations, or (intermediate) resistance leading to decreased effectiveness in patients with lower doxycycline serum concentrations.[42-44] In two reports, resistance has been reported in 6-23% of isolates.[39,44] Despite the literature on favorable serological response, no evidence is available on the effect of measuring SDC on clinical outcomes, such as occurrence of complications, disease-related mortality and PCR-positivity. Since the relation between serological response and these clinical outcomes in itself has not been established yet, and monitoring of treatment efficacy by other means is complicated and not unambiguous, it is highly relevant to assess the relation between measurement of SDC and clinical outcomes.

Non-Hodgkin lymphoma as a long-term sequela of Q fever?

Serious concerns were raised after a report on a potentially increased risk of non-Hodgkin lymphoma (NHL) for Q fever patients was published in October 2015. Researchers found a 25-fold increased incidence of B-cell NHL in a cohort of Q fever patients from a tertiary referral center in France, compared to the incidence of NHL extracted from the French national registry. The association was based on seven cases. Moreover, the bacterium was found intracellular in NHL tissues in four patients. The researchers postulated the hypothesis that the bacterium itself induces the development of NHL.[45] The potential pathophysiological mechanisms could be that *C. burnetii* stimulates production of interleukin-10 (IL-10), an anti-inflammatory cytokine that prevents expression of pro-inflammatory cytokines and receptors, inhibits T-cell activation and promotes survival, proliferation, and differentiation of B-cells.[46-49] The promoting of B-cells could lead to development of lymphoma, B-cell NHL specifically. Besides IL-10, other cytokines such as IL-6 may explain lymphomagenesis after infection with *C. burnetii*. During primary and chronic infection with *C. burnetii*, production of IL-6 is increased, promoting both healthy and malignant T-cell and B-cell proliferation.[47,50,51] However, there were some questions with regard to the validity of the excess risk and the explanatory hypothesis. The population in the index study was highly selected (from a tertiary referral center), and extensive diagnostic work-up was performed in these patients.[45] This could both lead to selection bias and detection bias. Moreover, the association could also rely on common risk factors, such as immunocompromised state. Therefore, the existence of the association is questioned.

With regard to the finding of bacteria in NHL tissue samples, conclusions cannot be drawn yet either: *C. burnetii* may be latently present in tissues for years after primary infection.[52] Perhaps *C. burnetii* does not target tumor-infiltrating antigen-presenting cells specifically: the bacterium may target monocytes or macrophages at random, which could make the presence in tumor-infiltrating antigen-presenting cells a coincidence. Altogether, there is no convincing evidence on the existence of an association yet. Therefore, it is premature to draw conclusions, and the implications for clinical practice are unknown. Clarification of the association and the causal relationship between *C. burnetii* and NHL is highly relevant after the large Dutch Q fever outbreak, and in general for Q fever patients worldwide. Studies on the risk of NHL both after acute and chronic Q fever are needed. Moreover, further exploration of the implications of finding *C. burnetii* in tissues is important. The setting of the Dutch Q fever outbreak provides the unique opportunity to assess the association and its causation in depth.

Aims and outline of this thesis

Part I. Prognosis of chronic Q fever

In **chapter 2**, the prognosis of chronic Q fever patients is described in detail. The focus lies on complications and disease-related mortality, and factors associated with these outcomes. **Chapter 3** describes the cumulative incidence of arterial fistulae in patients with proven chronic Q fever, for those with a vascular focus of infection specifically. The outcomes of these patients and types of surgical intervention are described. In **chapter 4**, long-term QOL of chronic Q fever patients is reported. Moreover, factors associated with impaired long-term QOL are assessed.

Part II. Treatment of chronic Q fever

In **chapter 5**, treatment of all proven and probable chronic Q fever patients from the Dutch national chronic Q fever database is described. The relation between treatment and disease-related outcomes is studied to assess clinical efficacy of these treatment regimens. Moreover, reasons for stopping and switching treatment and toxicity of different treatment regimens are described. **Chapter 6** focusses on the effect of measuring SDC on clinical outcomes during treatment of chronic Q fever with doxycycline and hydroxychloroquine.

Part III. *Coxiella burnetii* and the risk of non-Hodgkin lymphoma

In **chapter 7**, a case-report of a patient presenting with NHL and underlying vascular chronic Q fever is described. In this patient, *C. burnetii* was demonstrated intracellular in NHL tissue, which triggered further exploration of the potential association between *C. burnetii* and NHL. **Chapter 8** describes a population-based analysis with the aim to assess the association between *C. burnetii* exposure and NHL incidence, by assessing the incidence of NHL in the Netherlands for different Q fever incidence areas, before, during and after the Dutch Q fever outbreak. Moreover, the incidence of NHL in chronic Q fever patients is compared to the incidence of NHL in the general Dutch population. **Chapter 9** shows the results of an exploratory laboratory study, in which the presence of *C. burnetii* in both NHL and control tissues was assessed, to verify if the presence of *C. burnetii* in tissue samples is specific for NHL patients or not specific at all.

Part IV. Transmission of *Coxiella burnetii* through transplantation

Chapter 10 of this thesis describes the incidence of chronic Q fever among tissue and cell donors in the Netherlands. Moreover, seroprevalence of *C. burnetii* antibodies and factors associated with presence of these antibodies are described. Based on the incidence of chronic Q fever among tissue and cell donors, the discussion on the necessity of screening all tissue and cell donors is raised.

References

1. Eldin C, Melenotte C, Mediannikov O et al. From Q fever to *Coxiella burnetii* infection: a paradigm change. Clin Microbiol Rev 2017;30(1):115-90.
2. 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; 64(1): 3-12.
3. Roest HIJ, Tilburg JJHC, van der Hoek W et al. The Q fever epidemic in the Netherlands: history, onset, response and reflection. Epidemiol Infect 2011; 139: 1-12.
4. Notermans DW, Schimmer B, Harms MG et al. Sero-epidemiologie van Q-koorts in Nederland in 2006-2007. Infectieziekten Bulletin 2010; 21(9):314-6.
5. National Institute for Public Health and the Environment, RIVM, Accessed Augustus 2017 at http://www.rivm.nl/Onderwerpen/Q/Q_koorts.
6. Kampschreur LM, Hagenaars JC, Wielders CC et al. Screening for *Coxiella burnetii* seroprevalence in chronic Q fever high-risk groups reveals the magnitude of the Dutch Q fever outbreak. Epidemiol Infect 2013;141(4):847-51.
7. Van der Hoek W, Hogema BM, Dijkstra F et al. Relation between Q fever notifications and *Coxiella burnetii* during the 2009 outbreak in the Netherlands. Eurosurveillance, special edition Q fever; 2012; 11-5.
8. Tissot-Dupont H, Amadei M-A, Nezri M et al. 2004. Wind in November, Q fever in December. Emerg Infect Dis 2004; 10(7): 1264 –9.
9. Van Wijk MJ, Hogema BM, Maas DW et al. A Q fever outbreak in the Netherlands: consequences for tissue banking. Transfus Med Hemother 2011, 38:357–364.
10. Raoult D, Marrie T: Q fever. Clin Infect Dis 1995;20:489-95.
11. Kanfer E, Farrag N, Price C et al. Q fever following bone marrow transplantation. Bone Marrow Transplant 1988;3:165-6.
12. Criley JM, Carty AJ, Besch-Williford CL et al. *Coxiella burnetii* infection in C.B-17 scid-bg mice xenotransplanted with fetal bovine tissue. Comp Med 2001;51:357-60.
13. Amara AB, Bechah Y, Mege JL. Immune response and *Coxiella burnetii* invastion. Adv Exp Med Biol 2012; 984:287-98.
14. Maurin M, Raoult D. Q fever. Clin Microbiol Rev 1999; 12(4): 518-53.
15. Parker NR, Barralet JH, Bell AM. Q fever. Lancet 2006 Feb 25; 367(9511): 679-88.
16. 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; 52(5): 1637-43.
17. Barten DG, Delsing CE, Keijmel SP. Localizing chronic Q fever: a challenging query. BMC infectious diseases, 2013, DOI: 10.1186/1471-2334-13-413.
18. Wegdam-Blans MC, Kampschreur LM, Delsing CE et al. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Infect 2012;64(3):247-59.
19. Botelho-Nevers E, Fournier PE, Richet H et al. *Coxiella burnetii* infection of aortic aneurysms or vascular grafts: report of 30 new cases and evaluation of outcome. Eur J Clin Microbiol Infect Dis 2007;26(9):635-40.
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(8):527-35.
21. Eldin C, Mailhe M, Lions C et al. Treatment and prophylactic strategy for *Coxiella burnetii* infection of aneurysms and vascular grafts: a retrospective cohort study. Medicine 2016;95(12):e2810.
22. Broos PP, Hagenaars JC, Kampschreur LM et al. Vascular complications and surgical interventions after world's largest Q fever outbreak. J Vasc Surg 2015;62(5):1273-80.
23. Wedgam-Blans MC, Vainas T, van Sambeek MR et al. Vascular complications of Q-fever infections. Eur J Vasc Endovasc Surg 2011;42(3):384-92.
24. Siegman-Igra Y, Kaufman O, Keysary A et al. Q fever endocarditis in Israel and a worldwide review. Scand J Infect Dis 1997;29(1):41-9.
25. Mogollón MV, Anguita MP, Aguado JM et al. Q fever endocarditis in Spain. Clinical characteristics and outcome. Enferm Infecc Microbiol Clin 2011; 29(2):109-16.
26. Hagenaars JCP, Wever PC, van Petersen AS et al. Estimated prevalence of chronic Q fever among *Coxiella burnetii* seropositive patients with an abdominal aortic/ iliac aneurysm or aorto-iliac reconstruction after a large Dutch Q fever outbreak. J Infect 2014;69(2):154-60.
27. Fort S, Fraser AG, Fox KA. Extensive aortic valve ring abscess formation: a rare complication of Q fever endocarditis. Postgrad Med J 1989;65(764):384-6.
28. Higginson IJ, Carr AJ. Measuring Quality of life: using quality of life measures in the clinical setting. BMJ. 2001; 322:1297-300.
29. Website on the Q fever claim, http://www.qkoortsclaim.nl/, consulted on July 7th 2017.
30. Maurin M, Benoliel AM, Brongrand P et al. Phagolysosomal alkalinization and the bactericidal effect of antibiotics: the *Coxiella burnetii* paradigm. J Infect Dis, 1992; 166(5): 1097-102.
31. Clark WH, Lennette EH. Antibiotics in Q fever. Ann N Y Acad Sci, 1952; 55(6): 1004-18.
32. Kristinsson A, Bentall HH. Medical and surgical treatment of Q-fever endocarditis. The Lancet, 1967; 2(7518): 693-7.
33. Levy PY, Drancourt M, Etienne J et al. Comparison of different antibiotic regimens for therapy of 32 cases of Q fever endocarditis. Antimicrob Agents Chemother, 1991; 35(3): 533-7.
34. Raoult D, Houpikian P, Tissot Dupont H et al. Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydroxychloroquine. Arch Int Med, 1999; 159(2): 167-73.
35. Desnues B, Imbert G, Raoult D et al. Role of specific antibodies in *Coxiella burnetii* infection of macrophages. Clin Microbiol and Infect 2009;15(2):161-2.
36. Samuel JE, Hendrix LR. Laboratory maintenance of *Coxiella burnetii*. Curr Protoc Microbiol 2009;Chapter6C.
37. Vincent GA, Graves SR, Robson JM et al. Isolation of *Coxiella burnetii* from serum of patients with acute Q fever. J Microbiol Methods, 2015;119:74-8.
38. Omsland A, Hackstadt T, Heinzen RA. Bringing culture to the uncultured: *Coxiella burnetii* and lessons for obligate intracellular bacterial pathogens. PLoS Pathog 2013;9(9):e1003540.
39. Rolain JM, Mallet MN, Raoult D. Correlation between serum doxycycline concentrations and serologic evolution in patients with *Coxiella burnetii*

endocarditis. J Infect Dis 2003;188(9):1322-5.
40. Lecaillet A, Mallet MN, Raoult D et al. Therapeutic impact of the correlation of doxycycline serum concentrations and the decline of phase I antibodies in Q fever endocarditis. J Antimicrob Chemother 2009;63(4):771-4.
41. Rolain JM, Boulos A, Mallet MN et al. Correlation between ratio of serum doxycycline concentration to MIC and rapid decline of antibody levels during treatment of Q fever endocarditis. Antimicrob Agents Chemother 2005;49(7):2673-6.
42. Rouli L, Rolain JM, El Filali A et al. Genome sequence of *Coxiella burnetii* 109, a doxycycline-resistant clinical isolate. J Bacteriol 2012;194(24):6939.
43. Vranakis I, De Bock PJ, Papadioti A et al. Quantitative proteome profiling of *C. burnetii* under tetracycline stress conditions. PLoS One 2012;7(3):e33599.
44. Rolain JM, Lambert F, Raoult D. Activity of telithromycin against thirteen new isolates of *C. burnetii* including three resistant to doxycycline. Ann N Y Acad Sci 2005;1063:252-6.
45. Melenotte C, Million M, Audoly G et al. B-cell non Hodgkin-lymphoma linked to *Coxiella burnetii*. Blood 2016;127(1):113-21.
46. Capo C, Zaffran Y, Zugun F et al. Production of interleukin-10 and transforming growth factor beta by peripheral blood mononuclear cells in Q fever endocarditis. Infect Immun 1996;64(10):4143-7.
47. Honstettre A, Imbert G, Ghigo E et al. Dysregulation of cytokines in acute Q fever: role of interleukin-10 and tumor necrosis factor in chronic evolution of Q fever. J Infect Dis. 2003;187(6):956-62.
48. Ghigo E, Capo C, Raoult D et al. Interleukin-10 stimulates *Coxiella burnetii* replication in human monocytes through tumor necrosis factor down-modulation: role in microbicidal defect of Q fever. Infect Immun. 2001;69(4):2345-52.
49. Meghari S, Bechah Y, Capo C et al. Persistent *Coxiella burnetii* infection in mice overexpressing IL-10: an efficient model for chronic Q fever pathogenesis. PLoS Pathog. 2008;4(2):e23.
50. Akdis M, Aab A, Altunbulakli C et al. Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: receptors, functions, and roles in diseases. J Allergy Clin Immunol 2016; Published online 2016 August 28.
51. 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; 38(4): 1065-70.
52. Harris RJ, Storm PA, Lloyd A et al. Long-term persistence of *Coxiella burnetii* in the host after primary Q fever. Epidemiol Infect 2000;124:543-9.

Part I

Prognosis of chronic Q fever

Chapter 2

Chronic Q fever-related complications and mortality: data from a nationwide cohort

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Abstract

A large cohort of chronic Q fever patients was assessed to describe complications, factors associated with complications and related mortality. Complications occurred in 166/439 (38%) chronic Q fever patients: in 61% of proven (153/249), 15% of probable (11/74) and 2% of possible chronic Q fever patients (2/116). Most frequently observed complications were acute aneurysms (n=63; 14%), heart failure (n=55; 13%) and non-cardiac abscesses (n=45; 10%). PCR-positivity at any time during disease (OR2.25; 95%CI 1.36-3.72), presence of prosthetic material (OR1.79; 95%CI 1.07-2.99) and older age (OR1.04; 95%CI 1.02-1.06) were associated with complications. Overall mortality was 34% (n=110/323) for proven or probable chronic Q fever patients. Mortality was related to chronic Q fever in 63 proven (25%) and 3 probable (4%) chronic Q fever patients. Complications were associated with chronic Q fever-related mortality (OR8.20; 95%CI 3.65-18.45). We conclude that complications occur frequently and contribute to mortality in chronic Q fever patients.

Introduction

Q fever is a zoonosis caused by the intracellular Gram-negative coccobacillus *Coxiella burnetii*. Following primary infection, 1-5% of all patients develop chronic Q fever. Endocarditis, infected aneurysms or infected vascular prostheses are most frequently observed as the focus of infection during chronic Q fever. The duration between primary infection and manifestation of chronic infection may be several years.[1,2] Several risk factors for the development of chronic infection have been identified and include valvulopathy or prior valve surgery, aneurysm, vascular prostheses, renal insufficiency, older age, immunocompromised state and malignancy.[3,4] Diagnosing chronic Q fever is difficult as patients often present with nonspecific symptoms, such as fever, night sweats, weight loss, fatigue and malaise. A final diagnosis relies on a combination of clinical signs, serology, PCR on blood or tissue and radiological findings.[5,6]

Despite treatment, mortality in chronic Q fever patients is high. An overall mortality of 16% has been reported after a median follow-up of 14 months. Mortality rates are higher for specific subgroups of patients, such as patients with proven chronic Q fever (23% after a median follow-up of 14 months).[2] Limited evidence suggests that complications are associated with mortality in chronic Q fever. Nevertheless, prognosis of chronic Q fever patients with complications, nature of complications and risk factors associated with complications are largely unknown.[2,7-9]

Between 2007 and 2010, there was a large Q fever outbreak in the Netherlands. It is estimated that over 40.000 humans were infected with major impact on physical and psychological health.[10] Following this outbreak, all known chronic Q fever patients were included in an ongoing nationwide registration in which patients were classified as proven, probable or possible chronic Q fever patients according to the Dutch chronic Q fever consensus group guidelines.[5] Mortality and factors associated with mortality have been described before, but were updated. Complications, risk factors for complications and the risk for death following complications have not been described previously.[2] This cohort provides the unique opportunity to assess occurrence of complications, factors associated with complications and mortality in patients with complications in the largest cohort of chronic Q fever patients ever described.

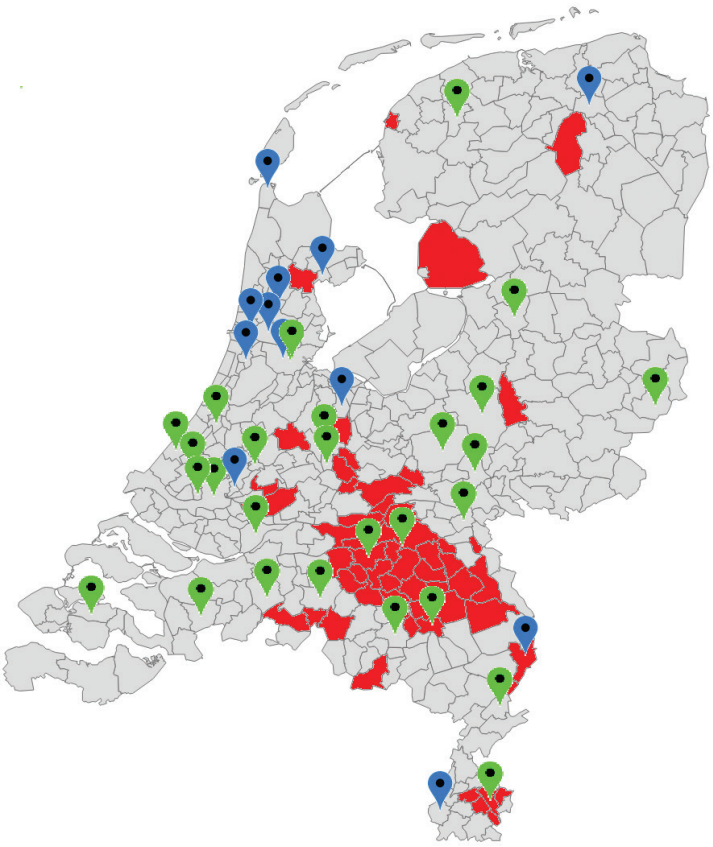
Methods

We assessed chronic Q fever-related complications and mortality and associated risk factors in a nationwide cohort of chronic Q fever patients. All known patients with chronic Q fever since the start of the Dutch Q fever outbreak (01-01-2007) were included.

Data collection and storage

Data were retrieved from the Dutch national chronic Q fever database. In this database, clinical, microbiological and radiological data are stored of all known proven, probable and possible chronic Q fever patients in the Netherlands.[5] Data were retrieved from electronically stored patient records or paper records if applicable and stored anonymously in a Microsoft Access 2010 database. Registration started in February 2011 and the last update ended in May 2016. Design of this database was approved by the Medical Ethical Committee of the University Medical Centre in Utrecht. All Dutch hospitals with microbiological laboratories were approached and asked to participate: all hospitals in the Q fever epidemic area participated with exception of one hospital. Patients were included from 28 hospitals in total, see figure 1 and appendix 1. Data were exported via R (version i384, 3.1.1) to SPSS for analysis (version 21.0).

Figure 1. Overview of participating and non-participating hospitals in the Netherlands



Green pin: participating hospitals. Blue pin: non-participating hospital. Red area: Q fever endemic area during the outbreak of 2007 – 2010. Only hospitals with own microbiological laboratory facilities are included. In case of multiple locations headquarter of regional laboratory or hospital is listed. Locations willing to participate but with not a single case of chronic Q fever are not shown on this map.

Patient inclusion

All patients 18 years or older at time of data collection with proven, probable or possible chronic Q fever infection according to the definitions formulated by the Dutch chronic Q fever consensus group were included in the database, see table 1.[5] Clinicians identified patients based on a positive PCR on serum or tissue and/or a *C. burnetii* phase I IgG antibody titer of ≥1:1024. Patients with a serological profile and clinical condition matching acute Q fever were excluded.

Table 1. Diagnostic criteria for chronic Q fever as defined by the 'Dutch Q fever consensus group [5]

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

** In absence of acute infection. Abbreviations in table 1: PCR = polymerase chain-reaction. IFA = indirect fluorescent-antibody assay. TEE = transesophageal echocardiography. TTE = transthoracal echocardiography. ESR = erythrocyte sedimentation rate. CRP = C-reactive protein.*

Definitions of outcome

Definitions for complications and mortality were formulated by four investigators (SR, PW, CB, JO). Complications considered potentially related to chronic Q fever are: rupture of aneurysm or dissection of aneurysm; arterial fistula (arterio-bronchial, arterio-cutaneous, arterio-caval or arterio-digestive fistula); endoleak of vascular prostheses; spondyl(odisc)itis; osteomyelitis (other than spondylitis); abscess (psoas region, intra-abdominal, retroperitoneal, intrathoracic, other); acute symptomatic aneurysm (without simultaneous intra-abdominal or retroperitoneal abscess /spondylodiscitis / rupture of aneurysm /dissection of aneurysm /fistula); cerebrovascular accident(hemorrhagic or ischemic)/transient ischemic attack; cardiac arrest; heart failure (with or without necessity of heart transplantation / Left Ventricular Assist Device); cardiac or valvular abscess or tamponade during pericarditis.

Cause of death was evaluated by two investigators (SR and CB). Disagreements were settled by consensus discussion. Definitions for relationship between death and chronic Q fever were as following:

- Definitely or probably related in case of active disease (defined as *C. burnetii* phase I IgG ≥1:1024 or positive PCR on serum or tissue) AND cause of death related to chronic Q fever: sepsis/fever episode with no other cause (with exception of dual pathogen infections), brain infarction or hemorrhage during endocarditis episode or due to cerebral aneurysm, arterial fistula, rupture or dissection of aneurysm, heart failure, fatal arrhythmia during endocarditis episode, cardiac arrest during endocarditis episode, due to surgical complications or side effects of antibiotic therapy or in case of clinical deterioration during active disease with no other cause;
- Definitely or probably related in case of proven chronic Q fever-related cause of death by autopsy;
- Definitely or probably related in case of presence of complications and active disease AND cause of death unknown;
- Definitely or probably related in case of active disease without adequate treatment AND cause of death unknown;
- Possibly related in case of inactive disease AND cause of death potentially related to consequences of chronic Q fever: after brain infarction or hemorrhage during endocarditis episode or due to cerebral aneurysm, arterial fistula, rupture or dissection of aneurysm, heart failure or fatal arrhythmia/cardiac arrest after endocarditis episode;
- Possibly related in case of active disease without known complications and with adequate treatment AND cause of death unknown;
- Unrelated if active disease AND cause unrelated to chronic Q fever: trauma, cancer, other infectious disease, cerebral hemorrhage or infarction without evidence of endocarditis, myocardial infarction in absence of organ involvement OR in case of inactive disease AND unknown cause of death. Since there is a potential association between B-cell non-Hodgkin lymphoma and Q fever, deaths caused by B-NHL were reported separately.[11]

Microbiological analysis

Microbiological testing consisted of an indirect fluorescent-antibody assay (IFA) for phase I and II IgG against *C. burnetii* on plasma or serum (Focus Diagnostics, Inc., Cypress, CA or Fuller Diagnostics, LLC., Anchorage, AK). Titration of antibody levels was carried out at different hospital sites with dilutions on a binary scale, with a cut-off of 1:32. Furthermore, results of polymerase chain-reaction (PCR) for *C. burnetii* DNA on serum or plasma were collected and if applicable on tissue samples (NucliSENS easyMAG; bioMérieux, Marcy l'Etoile, France).

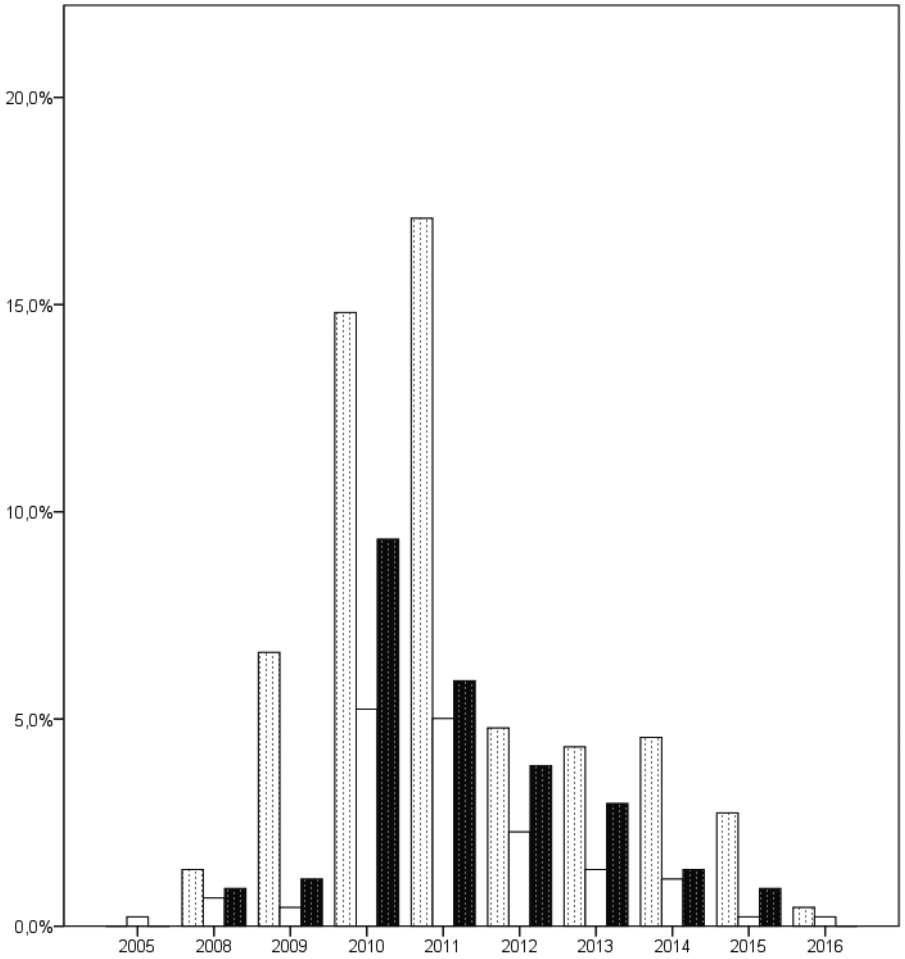
Statistical methods

Categorical data were compared by use of the Fischer exact test or Chi-square as appropriate. Mean values were compared by use of the independent samples t-test or one-way ANOVA as appropriate. Median values were compared by means of a Mann-Whitney test. Multiple logistic regression was performed, with stepwise modelling by backward Wald method. The threshold for excluding variables in the model was set at a p-value of 0.10. The significance level was set at a p-value <0.05. Survival between subgroups was analysed by comparing non-parametric survival curves (Kaplan-Meier), using a Tarone-Ware test.

Results

In total, 439 chronic Q fever patients were identified: 249 (57%) had proven, 74 (17%) probable and 116 (26%) possible chronic Q fever. Baseline characteristics are presented in table 2. Most patients were diagnosed in 2010 and 2011 (n=252; 57%; figure 2). Median follow-up was 4.3 years (interquartile range 2.0 – 5.4 years) for all patients, with significant longer follow-up for patients with probable and possible chronic Q fever (4.8 year, interquartile range 3.0 – 5.7 years) when compared to patients with proven chronic Q fever (3.6 years, interquartile range 1.4 – 5.2 years, p<0.001).

Figure 2. Percentage of diagnosed chronic Q fever patients in time



White bin black dotted: proven chronic Q fever patients. White: probable chronic Q fever patients. Black bin white dotted: probable chronic Q fever patient. X-axis: year of diagnosis of chronic Q fever. Y-axis: percentage of diagnosed patients. 2016 updated until May.

Table 2. Baseline characteristics of patients with chronic Q fever

	All patients	Proven chronic Q fever	Probable chronic Q fever	Possible chronic Q fever
n (%)	439 (100)	249 (57)	74 (17)	116 (26)
male gender (%)	322 (73)	192 (77)	57 (77)	73 (63)
mean age at diagnosis (range)	65 (17 - 92)	69 (20 - 92)	64 (22 - 86)	56 (17 - 84)
diagnosed episode acute Q fever (%)	166 (38)	57 (23)	31 (42)	78 (67)
adequate treatment acute Q fever (%)	136 (82)	44 (77)	23 (74)	69 (88)
median time between acute and chronic infection in weeks (interquartile range)	55 (35 - 92)	53 (35 - 93)	64 (34 - 96)	55 (34 - 88)
median maximum titer phase I IgG	1:4096	1:16384	1:4096	1:2048
Focus of chronic Q fever				
endocarditis (%)	84 (19)	68 (27)	16 (22)	-
vascular (prosthesis) infection (%)	153 (35)	125 (50)	28 (38)	-
endocarditis and vascular infection (%)	43 (10)	40 (16)	3 (4)	-
other focus (%)*	11 (3)	8 (3)	3 (4)	-
no focus identified (%)†	148 (34)	8 (3)**	24 (32)	116 (100)
Comorbidity				
peripheral vascular disease (%)	56 (13)	43 (17)	11 (15)	2 (2)
ischemic cardiac disease (%)	114 (26)	88 (35)	17 (23)	9 (8)
cerebrovascular disease (%)	56 (13)	40 (16)	10 (14)	6 (5)
diabetes (%)	63 (14)	33 (13)	14 (19)	16 (14)
Risk factors				
immunocompromised state (%)‡	48 (11)	33 (13)	15 (20)	-
vascular prosthesis (%)	97 (22)	81 (33)	16 (22)	-
arterial aneurysm (%)	49 (11)	41 (17)	8 (11)	-
valvulopathy (%)	84 (19)	66 (27)	18 (24)	-

**other foci: 4 placenta, 2 pericarditis, 2 pulmonary, 1 pleuritis, 1 combined endocarditis and pericarditis, 1 spondylodiscitis with no other focus. †patients with a positive PCR on blood, but no other focus of infection, are considered to have 'no focus of infection'. ‡immunocompromised state: 39 patients using immunosuppressives (usage of >5 mg prednisone daily > 30 days or cumulative dosage exceeding 750 mg, azathioprine, methotrexate, TNF-α blockers, mycophenolic acid, cyclosporine, sulfasalazine or a combination of these),1 patient with chemotherapy for non-hematological malignancy, 5 patients with hematological malignancies with cytopenia or requiring treatment (hairy cell leukemia, myelodysplastic syndrome, chronic lymphatic leukemia and non-Hodgkin lymphoma), 4 patients post-splenectomy and 2 patients with IgG deficiency/common variable immunodeficiency. Three patients fulfill two categories: all three with hematological malignancies (2 with immunosuppressive medication for other diseases and 1 post-splenectomy).*

Complications

In 166 patients (38%), 254 complications occurred. Most complications occurred prior to initiation of therapy (n=101; 61%; table 3). Acute aneurysms (non-fistula) (n=63; 14%), heart failure (n=55; 13%) and abscesses (non-cardiac) (n=45; 10%) were most frequently recorded complications. Patients with proven chronic Q fever had the highest risk of complications (n=153; 61%), with also the highest mean number of complications per patient. Complications occurred in 11 patients with probable chronic Q fever (15%) and 2 patients with possible chronic Q fever (2%). Table 4 shows an overview of mortality, complications and type of complications per focus of infection.

In multivariable analysis, factors independently associated with occurrence of complications in proven and probable chronic Q fever patients were positive serum PCR at any time during disease (OR 2.25; 95% CI 1.36-3.72), presence of prosthetic material prior to diagnosis of chronic Q fever (OR 1.79; 95%CI 1.07-2.99) and older age (OR 1.04; 95% CI 1.02-1.06 per year increase). Patients proven and probable chronic Q fever without evident focus of infection had a decreased risk of complications (OR 0.04, 95%CI 0.01-0.34), see table 5.

Table 3. Complications and mortality in all chronic Q fever patients

	All patients	Proven chronic Q fever	Probable chronic Q fever	Possible chronic Q fever
N (%)	439 (100)	249 (57)	74 (17)	116 (26)
Follow-up duration (median)	4.3 years	3.6 years	4.7 years	5.0 years
Complications of Q fever				
complications per patient	1.5	1.6	1.1	1.0
patients with complications (%)	166 (38)	153 (61)	11 (15)	2 (2)
time to first complication*(median)	0.0 years	0.0 years	0.0 years	4.2 years
before initiation of treatment*†(%)	101 (61)	98 (64)	3 (27)	-
< 12 weeks after start of treatment*†(%)	109 (66)	105 (69)	4 (36)	-
< 24 weeks after start of treatment*†(%)	113 (68)	108 (71)	5 (45)	-
Type of complication				
acute aneurysm (non-fistula)‡(%)	63 (14)	59 (24)	4 (5)	-
fistula (%)	24 (6)	24 (10)	-	-
abscess (non-cardiac) (%)	45 (10)	45 (18)	-	-
spondylodiscitis/osteomyelitis (%)	20 (5)	19 (8)	1 (1)	-
heart failure (%)	55 (13)	50 (20)	5 (7)	-
arterial embolic complications (%)	21 (5)	19 (8)	1 (1)	1 (1)
Mortality				
deceased (%)	118 (27)	94 (38)	16 (22)	8 (7)
definitely/probably chronic Q fever-related (%)	66 (15)	63 (25)	3 (4)	0
possibly Q fever-related (%)	13 (3)	8 (3)	5 (7)	0
time diagnosis to death §(median)	0.7 years	0.6 years	2.6 years	-
< 12 weeks after start of therapy†§ (%)	19 (29)	19 (30)	-	-
< 24 weeks after start of therapy†§ (%)	23 (35)	23 (37)	-	-
Cause of death				
complications of aneurysm (%)	24 (20)	23 (24)	1 (6)	-
cardiac causes¶ (%)	17 (14)	15 (16)	2 (13)	-
surgical complications (%)	10 (8)	10 (11)	-	-
arterial embolic complications (%)	6 (5)	6 (6)	-	-
other (%)	61 (52)	40 (43)	13 (81)	8 (100)

**data missing in one patient with proven and one patient with probable chronic Q fever. †not applicable in 24 patients: no treatment. ‡ definition of acute aneurysm: rupture of aneurysm, dissection of aneurysm, endoleak or symptomatic aneurysm (symptomatic aneurysm only counted in absence of abscess, fistula, spondylodiscitis, rupture or dissection). §for causes that are definitely or probably chronic Q fever-related. ¶heart failure, fatal arrhythmia, cardiac arrest.*

Table 4. Outcome of patients with proven and probable chronic Q fever per focus of infection

	Vascular	Endocarditis	Endocarditis & Vascular
No. patients	153	84	43
Complications	92 (60)	39 (46)	27 (63)
number of complications per patient	1.6	1.2	1.7
acute aneurysm (non-fistula)* (%)	52 (34)	1 (1)	10 (23)
fistula(%)	21 (14)	0	3 (7)
abscess (non-cardiac) (%)	34 (22)	2 (2)	9 (21)
spondylodiscitis/osteomyelitis(%)	15 (10)	1 (1)	3 (7)
heart failure(%)	7 (5)	30 (36)	15 (35)
arterial embolic complications(%)	9 (6)	8 (10)	2 (5)
Deceased	54 (35)	27 (32)	20 (47)
definitely/probably chronic Q fever related(%)	38 (25)	10 (12)	14 (33)
(possibly) related to Q fever(%)	2 (1)	7 (8)	3 (7)

**definition of acute aneurysm: rupture of aneurysm, dissection of aneurysm, endoleak or symptomatic aneurysm (symptomatic aneurysm only counted in absence of abscess, fistula, spondylodiscitis, rupture or dissection).*

Table 5. Factors associated with occurrence of complications in proven and probable chronic Q fever patients

Factor	Complications	No complications	Multivariable analysis (OR, 95%CI)
No. patients	164	159	
Patient related factors			
age (mean, OR per year increase)	72 years	64 years	1.04 (1.02 - 1.06)
presence of prosthetic material			
(vascular and/or valvular) (%)	105 (64)	61 (38)	1.79 (1.07 - 2.99)
use of statin (%)	88 (54)	67 (42)	-
use of platelet aggregation inhibitor (%)	83 (51)	61 (38)	-
Focus / classification			
vascular focus (%)	93 (57)	60 (38)	-
no focus identified (%)	1 (1)	31 (19)	0.04 (0.01 - 0.34)
Serological course / PCR			
positive serum PCR (%)*	89 (54)	52 (33)	2.25 (1.36 - 3.72)
decrease of titer to <1:1024 (%)	53 (32)	78 (49)	-
four-fold titer decrease (%)	52 (32)	68 (43)	-

** at any moment in course of disease. Univariable analysis comparison by Fisher exact test, Chi-square test or independent samples t-test as appropriate. Multivariable analysis performed by multiple logistic regression by backward Wald method. Factors assessed in univariable analysis, significant: age, presence of prosthetic material, use of statin, use of platelet aggregation inhibitor, vascular focus, no focus, positive serum PCR, serological cure, four-fold titer decrease. Factors assessed in univariable analysis, non-significant: male sexe, immunocompromised state, combined focus, valvular focus (endocarditis), prolonged positivity of PCR during treatment (>six months). In stepwise logistic regression use of statin, use of platelet aggregation inhibitor, vascular focus, decrease of titer to <1:1024 and four-fold titer decrease excluded as predictors in the model.*

Mortality

The overall mortality rate was 34% (n = 110/ 323) for proven or probable chronic Q fever patients. Overall five-year survival for proven and probable chronic Q fever patients was 64%. Mortality was considered to be definitely or probably related to chronic Q fever in 66 patients (15%) and possibly related to chronic Q fever in 13 patients (3%). There were significant differences in overall mortality between the different foci of infection ($p<0.001$), see figure 3.

Definitely or probably chronic Q fever-related mortality was highest in proven chronic Q fever patients (n=63; 25%), followed by probable chronic Q fever patients (n=3; 4%). Chronic Q fever-related mortality did not occur among possible chronic Q fever patients (table 3). Chronic Q fever-related mortality was highest in patients with a combined endocarditis and vascular focus (n=14; 33%), followed by patients with a vascular infection only (n=38; 25%) and endocarditis patients (n=10; 12%), see table 4. Median time from diagnosis to death was 0.7 years: 0.6 years for proven chronic Q fever patients and 2.6 years for probable chronic Q fever patients. Most frequent causes of death were complications of aneurysms (n=24; 20%) and cardiac causes (n=17; 14%), see table 3.

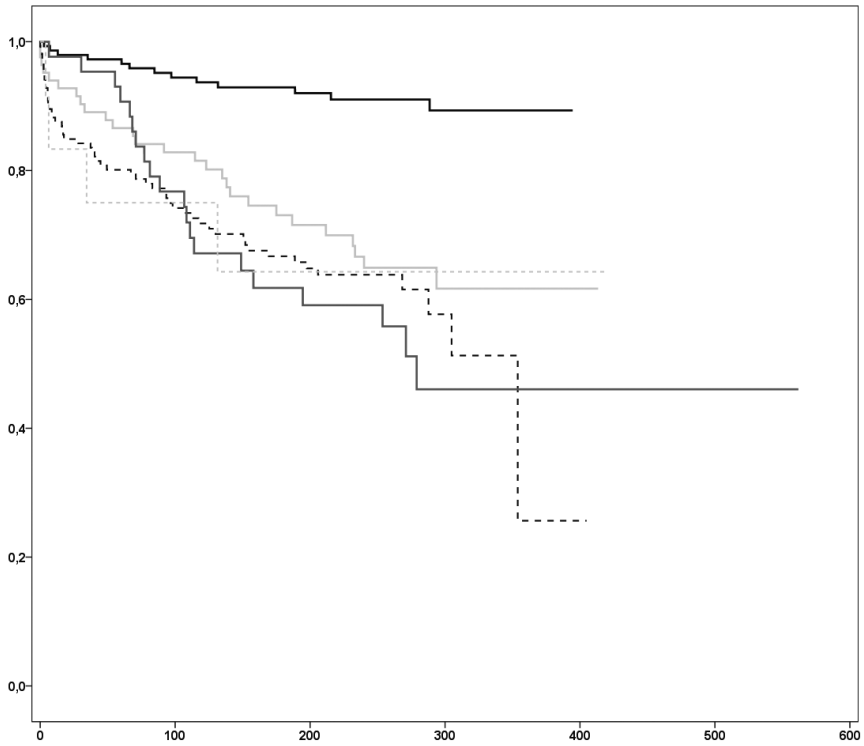
In multivariable analysis, complications (OR 8.20; 95% CI 3.65-18.45) and older age (OR 1.03; 95%CI 1.00-1.06) were independently associated with chronic Q fever-related mortality in multivariable analysis in proven and probable chronic Q fever patients. Four-fold titer decrease was associated with a decreased risk of chronic Q fever-related mortality (OR 0.27; 95%CI 0.12-0.58), see table 6.

Table 6. Factors associated with definitely and probably chronic Q fever-related mortality in proven and probable chronic Q fever patients

Factor	Deceased: Q fever-related*	Not deceased or death not Q fever-related	Multivariable analysis (OR, 95%CI)
No. patients	66	257	
Patient characteristics			
age (mean, OR per year increase)	74 years	67 years	1.03 (1.00 - 1.06)
presence of prosthetic material (vascular and/or valvular) (%)	43 (65)	123 (48)	-
use of platelet aggregation inhibitor (%)	39 (59)	105 (41)	-
Focus / classification			
no focus (%)	1 (2)	31 (12)	-
endocarditis (%)	10 (15)	74 (29)	-
endocarditis and vascular focus (%)	14 (21)	29 (11)	-
Serological course			
four-fold titer decrease (%)	10 (15)	110 (43)	0.27 (0.12 - 0.58)
decrease of titer to < 1:1024 (%)	12 (19)	119 (46)	-
PCR positivity† (%)	39 (59)	102 (40)	-
occurrence of complications	58 (88)	106 (41)	8.20 (3.65 - 18.45)

**Definitely and probably related. †at any moment in course of disease. Univariable analysis comparison by Fisher exact test, Chi-square test or independent samples t-test as appropriate. Multivariable analysis performed by multiple logistic regression by backward Wald method. Factors assessed in univariable analysis, significant: age, presence of prosthetic material, use of platelet aggregation inhibitor, valvular focus (endocarditis), combined focus, no focus, four-fold titer decrease, serological cure, positive serum PCR, occurrence of complications. Factors assessed in univariable analysis, non-significant: male sex, use of statin, immunocompromised state, vascular focus, prolonged positivity of PCR during treatment (>six months). In stepwise logistic regression presence of prosthetic material, use of platelet aggregation inhibitor, no focus, endocarditis, endocarditis and vascular focus, decrease of titer to <1:1024 and PCR positivity excluded as predictors in the model.*

Figure 3. Kaplan-Meier survival curve for patients with complications, without complications and all patients



Black line: patients with no focus of infection. Dark grey line: patients with endocarditis and a vascular focus of infection. Light grey line: patients with endocarditis. Dashed black line: patients with a vascular focus of infection. Dashed grey line: patients with other focus of infection. X-axis: Follow-up duration in weeks. Y-axis: Cumulative survival

Discussion

Complication and mortality rates in chronic Q fever patients are high: over 60% of patients with proven chronic Q fever develop complications and over 60% of complications occur before initiation of treatment. Overall mortality rate in proven chronic Q fever patients with a median follow-up duration of 3.6 years is 38%, of which 67% can be attributed to chronic Q fever. Complications are independently associated with chronic Q fever-related mortality, which underlines the importance of complete diagnostic work-up in order to identify the focus of disease, recognize complications and start adequate treatment. Our cohort showed a higher complication rate when compared to earlier studies, which showed complications such as symptomatic aneurysms, ruptured aneurysms, formation of fistula, abscesses and spondylodiscitis in 9-30% of vascular chronic Q fever patients and cardiac abscesses in up to 7% in patients with Q fever endocarditis.[6,8,9,12-14] The high complication rate observed in our cohort may theoretically be explained by the fact that most earlier studies only reported acute or complications visible on PET-CT scanning at time of diagnosis.[6,9,12,13] In our cohort, 92% of patients with a vascular focus of infection underwent PET-CT scanning or CT-abdomen at time of diagnosis. Furthermore, 48% of patients with a vascular focus of infection underwent PET-scanning > 3 months after diagnosis of chronic Q fever, enabling detection of new complications during course of the disease.

Mortality rates in our cohort are also higher in comparison to earlier studies and in comparison to evaluation of this cohort in May 2012.[2] It is remarkable, that more than nine years after start of the epidemic of 2007 – 2010, new patients are still identified and mortality rates (disease-related) continue to increase.

In our cohort, five-year survival for proven and probable chronic Q fever is 64%, which is comparable to the five-year survival of colorectal cancers in the USA.[16] Previously reported overall mortality was up to 25% for patients with proven vascular chronic Q fever and varying between 7%-26% for patients with proven chronic Q fever endocarditis after a follow-up duration of three years.[2,6-9,12,17,18] The cohort of endocarditis patients described by Million *et al*, which demonstrated 7% mortality after 3 years, showed a mortality of 27% after 10 years of follow-up.[8] A potential explanation for the high complication and mortality in our cohort may be the lack of a structural screening program in the Netherlands for chronic Q fever: all patients have been diagnosed in usual care. Since Q fever was very rare prior to the outbreak, clinicians had limited experience in diagnosing chronic Q fever. This may have led to a diagnostic delay. The costs and benefits of a nationwide serological screening program for chronic Q fever are currently evaluated by the Dutch government. The differences in fatality rates between our cohort and earlier studies may be caused by the fact that the patients in this study were included from 28 hospitals. Mortality rates as reported from single referral centers may not be comparable.[8] Patients that die early in the onset of disease, will never be referred. This may lead to a relative favorable prognosis for those who are sent to a referral center.

The large Q fever outbreak in the Netherlands led to a large number of chronic Q fever patients in a relatively short period. It has been suggested, although this has not been validated prospectively, that screening for valvulopathies is effective in prevention of chronic Q fever endocarditis.[4,19] This was not routinely performed in the Netherlands. If we assume that prophylaxis is indeed effective for prevention of Q fever endocarditis in practice, cases may have been prevented with systematic screening. However, of patients with proven or probable chronic Q fever, only 27% had a notified acute Q fever infection of which 25% had an endocarditis (either alone or combined with a vascular focus of infection). Therefore, the potential number of possible preventable cases in this cohort was limited.

Presence of prosthetic material prior to chronic Q fever diagnosis was an independent risk factor for occurrence of complications, but was not associated with mortality. The absence of an association between the presence of prosthetic material, adjusted for occurrence of complications, and mortality is in line with earlier findings.[2,9] It seems contradictory that presence of prosthetic material is an independent risk factor for occurrence of complications (which is associated with mortality), but not for mortality. A potential explanation is that patients with prosthetic material are regularly evaluated and monitored with radiographical imaging by their vascular surgeon, which leads to detection of complications at an earlier stage of chronic Q fever and earlier diagnosis of chronic Q fever, resulting in lower mortality rates.

Complications and Q fever-related mortality in probable chronic Q fever patients occur much less frequent than in proven chronic Q fever patients. Of all probable chronic Q fever patients, 15% experienced complications and 11% of patients had a cause of death definitely or probably related to chronic Q fever. The difference in occurrence of complications and chronic Q fever-related mortality between these two groups may be due to the fact that the group of probable chronic Q fever patients is heterogeneous with a broad variety of disease burden.

Only two patients with possible chronic Q fever were registered to have a (possible) complication: one patient underwent heart transplantation, but was known with a congenital cardiomyopathy. One patient had a cerebrovascular accident, without evidence of a mycotic aneurysm or endocarditis. It is unlikely that these events are related to chronic Q fever. Overall prognosis for patients with possible chronic Q fever, who only have a high phase I IgG titer and no signs of infection, is therefore highly favorable in terms of complications and mortality. Their serological profile might, therefore, reflect an (over)adequate immune response instead of an ongoing or chronic infection with *C. burnetii*.

No cases of osteomyelitis or lymphadenitis have been observed in this cohort. In another large cohort of chronic Q fever patients, osteo-articular infections accounted for 3% and isolated lymphadenitis for 1% of all cases.[11] The lack of patients with osteomyelitis or isolated lymphadenitis in this cohort may be explained by the fact that our clinicians do not recognize these entities (although they are not excluded by the definitions)[5] or that the strain causing the Dutch outbreak causes cardiovascular infections predominantly. Since most patients underwent total body PET-CT scanning

(including the lower extremities) other foci of osteomyelitis would probably have been diagnosed.

This study has several strengths. First, complication and mortality rates were extracted from a large cohort of chronic Q fever patients. With all (but one) hospitals from the epidemic area participating, we presume that almost all chronic Q fever patients in the Netherlands are included. Mortality has been evaluated before in this cohort.[2] However, to our knowledge, evaluation of occurrence of complications, evaluation of mortality following complications and identification of factors associated with chronic Q fever-related complications were not previously performed on such scale. This study provides better understanding in the course of disease of chronic Q fever and helps to identify patients at risk for complications and mortality.

Secondly, all patients are categorized based on the classification of the Dutch chronic Q fever consensus group. Classification of mortality and complications was pre-defined by four experienced clinicians and researchers, leading to a standardized classification and description of our outcomes.

A limitation of our study is the retrospective design: information was gathered from (electronically stored) patient records, potentially leading to information bias. However, since surgical, radiographical imaging and laboratory reports were reviewed, the risk for information bias was minimized. Secondly, causality cannot be assured based on these observational results. Complications and relation between mortality and chronic Q fever was used by predefined criteria and was not confirmed by pathological evaluation in all cases. By using strict criteria and assessing cause of death by two investigators, we minimized subjectivity in assessing complications and mortality.Furthermore, it is possible that we may have missed patients with undetected chronic Q fever. Due to our retrospective design, our inclusion depended on confirmed cases of chronic Q fever. For example, patients with undetected chronic Q fever that died of acute complications of chronic Q fever have been missed. Finally, we used the criteria as formulated by the Dutch chronic Q fever consensus group to classify likelihood of chronic Q fever infection in the patients in this cohort.[5] However, these criteria have been criticized.[20-22] They require complete work-up, otherwise patients could be misclassified as possible chronic Q fever patients. Furthermore, since clinical characteristics are embedded in these criteria, complications and mortality occur more often in patients with the highest likelihood of infection (i.e. proven chronic Q fever patients).[22] Using other criteria such as those formulated by the French study group [22], in which patients are classified based on focalized persistent infection and likelihood of infection is defined as definite or possible, would encounter the same classification difficulties. Additionally, focus of persistent infection is not always known at presentation.[22] The ongoing controversy underlines the difficulty of diagnosing chronic Q fever, and future collaborative research will have to ensure consensus on chronic Q fever diagnostic criteria.

In conclusion, incidence of complications and chronic Q fever-related mortality is high, especially in proven chronic Q fever patients. Furthermore, complications are strongly associated with mortality. Presence of prosthetic material is associated with

complications. Prognosis in patients with possible chronic Q fever is highly favorable in terms of complications and mortality, indicating that these patients may not have an actual chronic infection but merely a serological remnant of cleared infection with *C. burnetii*.

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References

1. Maurin M, Raoult D. Q fever. Clin Microbiol Rev 1999;12(4); 518-53.
2. Kampschreur LM, Delsing CE, Groenwold RH, Wegdam-Blans MC, Bleeker-Rovers CP, de Jager-Leclercq MG *et al*. Chronic Q fever in the Netherlands 5 year after the start of the Q fever epidemic: results from the Dutch chronic Q fever database. J Clin Microbiol 2014;52(5):1637-43.
3. Raoult D. Host factors in the severity of Q fever. Ann N Y Acad Sci 1990;590:33-8.
4. Fenollar F, Fournier PE, Carrieri MP, Habib G, Messana T, Raoult D. Risk factors and prevention of Q fever endocarditis. Clin Infect Dis 2001;33(3):312-6.
5. Wegdam-Blans MC, Kampschreur LM, Delsing CE, Bleeker-Rover CP, Sprong T, van Kasteren ME *et al*. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Infect 2012;64(3):247-59.
6. Broos PP, Hagenaars JC, Kampschreur LM, Wever PC, Bleeker-Rovers CP, Koning OH et al. Vascular complications and surgical interventions after world's largest Q fever outbreak. J Vasc Surg 2015;62(5):1273-80.
7. Botelho-Nevers E, Fournier PE, Richet H, Fenollar F, Lepidi H, Foucault C *et al*. *Coxiella burnetii* infection of aortic aneurysms or vascular grafts: report of 30 new cases and evaluation of outcome. Eur J Clin Microbiol Infect Dis 2007;26(9):635-40.
8. Million M, Thuny F, Richet H and Raoult D. Long-term outcome of Q fever endocarditis: a 26-year personal survey. Lancet Infect Dis, 2010;10(8):527-35.
9. Eldin C, Mailhe M, Lions C, Carrieri P, Safi H, Brouqui P *et al*. Treatment and prophylactic strategy for *Coxiella burnetii* infection of aneurysms and vascular grafts: a retrospective cohort study. Medicine 2016;95(12):e2810.
10. Kampschreur LM, Hagenaars JC, Wielders CC, Elsman P, Lestrade PJ, Koning OH et al. Screening for *Coxiella burnetii* seroprevalence in chronic Q fever high-risk groups reveals the magnitude of the Dutch Q fever outbreak. Epidemiol Infect 2013;141(4):847-51.
11. Melenotte C, Million M, Audoly G et al. B-cell non Hodgkin-lymphoma linked to *Coxiella burnetii*. Blood 2016;127(1):113-21.
12. Wedgam-Blans MC, Vainas T, van Sambeek MR, Cuypers PW, Tjhie HT, van Straten AH et al. Vascular complications of Q-fever infections. Eur J Vasc Endovasc Surg 2011;42(3):384-92.
13. Hagenaars JCP, Wever PC, van Petersen AS, Lestrade PJ, de Jager-Leclercq MG, Hermans MH et al. Estimated prevalence of chronic Q fever among *Coxiella burnetii* seropositive patients with an abdominal aortic/iliac aneurysm or aorto-iliac reconstruction after a large Dutch Q fever outbreak. J Infect 2014;69(2):154-60.
14. Fort S, Fraser AG, Fox KA. Extensive aortic valve ring abscess formation: a rare complication of Q fever endocarditis. Postgrad Med J 1989;65(764):384-6.
15. Website on the Q fever claim, http://www.qkoortsclaim.nl/, accessed on July 7th 2017.
16. Henley SJ, Singh SD, King J, Wilson R, O'Neil ME, Ryerson AB, Centers For Disease Control and Prevention (CDC). Invasive cancer incidence and survival –United States, 2011. MMWR Morb Mortal Wkly Rep 2015;64(9):237-42.
17. Siegman-Igra Y, Kaufman O, Keysary A, Rzotkiewicz S, Shalit I. Q fever endocarditis

in Israel and a worldwide review. Scand J Infect Dis 1997;29(1):41-9.
18. Mogollón MV, Anguita MP, Aguado JM, Tornos P, Miró JM, Gálvez-Acebal J *et al*. Q fever endocarditis in Spain. Clinical characteristics and outcome. Enferm Infecc Microbiol Clin 2011; 29(2):109-16.
19. 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;42(6):818-21.
20. Raoult D. Chronic Q fever: expert opinion versus literature analysis and consensus. J Infect 2012;65(2);102-8.
21. Kampschreur LM, Wegdam-Blans MC, Wever PW, Renders NH, Delsing CE, Sprong T et al. Chronic Q fever diagnosis – consensus guideline versus expert opinion. Emerg Infect Dis 2015;21(7):1183-88.
22. Eldin C, Melenotte C, Mediannikov O, Ghigo E, Million M, Edouard S *et al*. From Q fever to *Coxiella burnetii* infection: a paradigm change. Clin Microbiol Rev 2017;30(1):115-90.

Appendix 1 – List of participating hospitals

Participating hospitals: Sint Elisabeth Hospital in Tilburg, Rijnstate Hospital in Arnhem, Hospital Gelderse Vallei in Ede, St. Antonius Hospital in Nieuwegein, Diakonessenhuis in Utrecht, Radboudumc in Nijmegen, Jeroen Bosch Hospital in 's –Hertogenbosch, Elkerliek Hospital in Helmond, Leids University Medical Center in Leiden, Erasmus Medical Center in Rotterdam, Atrium Medical Center in Heerlen, Bernhoven Hospital in Uden, Canisius-Wilhelmina Hospital in Nijmegen, Catharina Hospital in Eindhoven, Maxima Medisch Centrum in Eindhoven, Izore Laboratory in Leeuwarden, Isala Clinic in Zwolle, Medisch Spectrum Twente in Enschede, Gelre Hospital in Apeldoorn, Meander Medical Center in Amersfoort, Onze Lieve Vrouwe Gasthuis in Amsterdam, Reinier de Graaf Hospital in Delft, Groene Hart Hospital in Gouda, Maasstad Hospital in Rotterdam, Amphia Hospital in Breda, Bravis Hospital in Roosendaal, Laurentius Hospital te Roermond and University Medical Center Utrecht in Utrecht.

Hospitals providing cooperation but without chronic Q fever patients: St. Jansdal in Harderwijk, Albert Schweitzer Hospital in Dordrecht, Bronovo Hospital in The Hague, St. Fransiscus gasthuis in Rotterdam, Vlietland Hospital in Schiedam, Admiraal de Ruyter Hospital in Goes.

Chapter 3

Primary and secondary arterial fistulae during chronic Q fever

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Abstract

Objective

After primary infection with *Coxiella burnetii* patients may develop acute Q fever, which is a relatively mild disease. A small proportion of patients (1-5%) develops chronic Q fever, which is accompanied by high mortality and can manifest as infected arterial or aortic aneurysms or infected vascular prostheses. The disease can be complicated by arterial fistulae, which are often fatal if left untreated. We aimed to assess the cumulative incidence of arterial fistulae and mortality in patients with proven chronic Q fever.

Methods

In a retrospective, observational study the cumulative incidence of arterial fistulae (aortoenteric, aortobronchial, aortovenous or arteriocutaneous) in patients with proven chronic Q fever (according to the Dutch chronic Q fever consensus group criteria) was assessed. Proven chronic Q fever with a vascular focus of infection was defined as a confirmed mycotic aneurysm or infected prosthesis on imaging studies or positive serum PCR for *Coxiella burnetii* in the presence of an arterial aneurysm or vascular prosthesis.

Results

Of 253 patients with proven chronic Q fever, 169 patients (67%) were diagnosed with a vascular focus of infection (of which 42/169 had a combined vascular focus and endocarditis). In total, 26 arterial fistulae were diagnosed in 25 patients (15% of patients with a vascular focus): aortoenteric (15), aortobronchial (2), aortocaval (4) and arteriocutaneous (5) fistulae (1 patient presented with both an aortocaval and arteriocutaneous fistula). Chronic Q fever-related mortality was 60% for patients with and 21% for patients without arterial fistula ($P < .0001$). Primary fistulae accounted for 42% and secondary fistulae for 58%. Of patients that underwent surgical intervention for chronic Q fever-related fistula (n = 17), 9 died of chronic Q fever-related causes (53%). Of patients that did not undergo any surgical intervention (n = 8), 6 died of chronic Q fever-related causes (75%).

Conclusions

The proportion of patients with proven chronic Q fever developing primary or secondary arterial fistulae is high: 15% of patients with a vascular focus of infection develops an arterial fistula. This observation suggests that *C. burnetii*, the causative agent of Q fever, plays a role in the development of fistulae in these patients. Chronic Q fever-related mortality in patients with arterial fistula is very high, in both patients that undergo and do not undergo surgical intervention.

Introduction

Arterial fistulae can develop to enteric, bronchial, venous or cutaneous structures, with a significant risk for severe bleeding leading to death if left untreated.[1,2] They can be divided in primary and secondary fistulae. The cause of primary aortic fistulae is most often an underlying aneurysm of the aorta.[2,3] Secondary fistulae occur following surgical aorta repair (both open and endovascular), abdominal trauma or radiation and are more common than primary aortic fistulae, although both are very rare.[2,4-7] Previously reported incidences vary from <0.1% for primary aortoenteric fistulae, and <1% for secondary aortoenteric fistulae.[5,7] Incidence of other types of aortic fistulae is even lower.[6] Clinical presentation of patients with fistulae is diverse and depends on the location of the involved artery and organ to which it connects. Patients with aortoenteric fistulae for example, present with gastro-intestinal bleeding.[2] However, typical symptom patterns may be absent and the condition is often not acknowledged until surgery, autopsy or not at all.[2]

The Netherlands faced the largest Q fever outbreak ever documented worldwide thus far between 2007 – 2010, with most cases reported between May 2007 and June 2010, and an estimated 40,000 infected individuals.[8,9] *Coxiella burnetii*, the causative agent of Q fever, causes zoonotic disease worldwide (with exception of New Zealand), both in ongoing endemic setting and in the setting of outbreaks.[10] The bacterium can spread through the air, with a radius of thirty kilometers around the source.[11] Therefore, patients may not have noticed exposure explicitly. During the Dutch Q fever outbreak, transmission of Q fever mainly occurred through infected goats, shedding *C. burnetii* in large amounts in birth products and milk, resulting in large numbers of human acute Q fever cases in springtime.[12] After primary infection patients may develop flu-like illness, pneumonia or hepatitis. Approximately 60% of patients remain asymptomatic. Mortality during acute Q fever is low (1%).[13] Approximately 1-5% of patients develops chronic Q fever after primary infection, which mainly manifests as endocarditis, infected aneurysms or vascular prostheses. Most cases of chronic Q fever occur within one year after primary infection, but longer intervals up to at least 7 years occur. Patients with a vascular prosthesis or arterial aneurysm have a 25-fold increased risk for proven chronic Q fever.[14] In contrast to acute Q fever, chronic Q fever is accompanied by complications and mortality.[10] The presence of prosthetic material specifically, is associated with occurrence of complications.[15] Chronic Q fever-related mortality rates of 25% after 3.5 years of follow-up have been described in patients with proven chronic Q fever.[15] The actual prevalence of chronic Q fever is unknown, but it is considered a rare disease: the largest cohorts of chronic Q fever patients, with various foci of infection, consist of 440 patients diagnosed over multiple years.[15,16] Arterial fistulae, both primary and secondary, have been described as a complication of chronic Q fever with a vascular focus of infection (vascular chronic Q fever).[17-20] However, little is known of the risk and clinical course of arterial fistulae in chronic Q fever patients. Therefore, we aimed to assess the cumulative incidence of arterial fistulae and subsequent mortality in a Dutch cohort of chronic Q fever patients, specifically those with a vascular focus of infection.

Methods

Study population and design

Patients above 18 years of age with proven chronic Q fever according to the Dutch national chronic Q fever consensus group criteria were included in this study, to ensure inclusion of patients with an established infection.

Data collection and storage

Data were retrieved from the Dutch national chronic Q fever database. In this database, clinical, microbiological and radiological data are stored of all known proven, probable and possible chronic Q fever patients in the Netherlands.[21] Data were retrieved from electronically stored patient records, or paper records if applicable, and stored anonymously in a Microsoft Access 2010® database. Registration started in February 2011 and the last update ended in May 2016. All patients diagnosed after January 2007 were included in the database. Design of this database was approved by the Medical Ethical Committee of the University Medical Centre in Utrecht. Data for this database are collected without informed consent: the informed consent procedure was waived since all data were provided anonymously. All Dutch hospitals with microbiological laboratories were approached and asked to participate, see appendix I for an overview of participating hospitals. Data were exported via R (version i384, 3.1.1) to SPSS for analysis (version 21.0).

Microbiological analysis

Serological tests were performed using an indirect fluorescent-antibody assay (IFA) for phase I and II IgG against *C. burnetii* on plasma or serum (Focus Diagnostics, Inc., Cypress, CA, US or Fuller Diagnostics, LLC., Anchorage, AK, US). Titration of antibody levels was carried out at different hospital sites with dilutions on a binary scale with a cut-off of 1:32. Furthermore, results of polymerase chain reaction (PCR) for *C. burnetii* DNA on serum, plasma and tissue if applicable were collected (NucliSENS easyMAG; bioMérieux, Marcy l'Etoile, France).

Statistical methods

Since all data were retrospectively collected, descriptive measures were used to describe the cohort concerning mortality and incidence of complications. The cumulative incidence of arterial fistula was the main outcome of interest. Survival between subgroups was analysed by comparing non-parametric survival curves (Kaplan-Meier) using a Log-rank or Tarone-Ware test as appropriate. Categorical data were compared by use of a Chi-square or Fisher exact test as appropriate. The significance level was set at a p-value <0.05.

Definitions

Chronic Q fever was defined according to the Dutch chronic Q fever consensus group criteria, which implies having at least a phase I IgG titer of ≥ 1:1024 in absence of an acute infection.[21] Acute infection can be differentiated from a chronic infection based on clinical signs and symptoms, high levels of IgM antibody titers during acute infection and predominantly increased phase II titers. Moreover, chronic Q fever can only be diagnosed at least six months after onset of acute Q fever, if the moment of primary infection is known.[21] Patients with proven chronic Q fever are those with an established endocarditis (according to the Duke criteria), proven vascular focus of infection (by imaging studies such as PET-CT) or positive serum or tissue PCR for *C. burnetii*.[18,21] A 'focus' of infection is defined as the nidus or manifestation of chronic infection. A patient with proven chronic Q fever with a vascular focus of infection is defined as a patient having a proven mycotic aneurysm or infected prosthesis on imaging studies (such as PET-CT) or a positive serum PCR in presence of a known aneurysm of vascular prosthesis.[18,21]

Death was categorized as definitely or probably related to chronic Q fever in case of:

Active disease (defined as *C. burnetii* phase I IgG ≥1:1024 or positive PCR on serum or tissue) AND cause of death related to chronic Q fever: sepsis/feverish episode with no other cause (with exception of dual pathogen infections), brain infarct or hemorrhage during endocarditis episode or due to cerebral aneurysm, arterial fistulae, rupture or dissection of aneurysm, heart failure, fatal arrhythmia during endocarditis episode, cardiac arrest during endocarditis episode, due to surgical complications or side effects of antibiotic therapy or in case of clinical deterioration during active disease with no other cause;

- Proven chronic Q fever-related cause of death by autopsy;
- Presence of complications and active disease AND cause of death unknown;
- Active disease without adequate treatment AND cause of death unknown.

Cause of death was evaluated by two investigators (SR and CB). Arterial fistulae were defined as fistulae between the aorta, or branches of the aorta, and adjacent structures. Primary fistulae were defined as fistulae that developed prior to insertion of or in absence of a vascular prosthesis (at the specific site of development of the fistula). Secondary fistulae were defined as fistulae that developed after prior insertion of a vascular prosthetic graft (at the specific site of development of the fistula).

Results

We identified 253 patients with proven chronic Q fever. Of these, 169 (67%) had a vascular focus of infection: 127 patients had a vascular focus only (50%) and 42 patients had a vascular focus combined with endocarditis (17%). The remaining patients had endocarditis

(n = 68; 27%), other foci (n = 8; 3%) or unknown foci (n = 8; 3%). A vascular prosthesis was present in 82 patients (32%) prior to diagnosis of chronic Q fever. Arterial fistulae (n = 26) were diagnosed in 25 patients (10% of all patients with proven chronic Q fever), of which all had a vascular focus of infection. The cumulative incidence of arterial fistulae in patients with a vascular focus of infection was 15% (25/169). In patients with arterial fistula, previous acute Q fever was significantly less often notified compared to patients without arterial fistula (P = .02). An overview of proven chronic Q fever patients with and without fistulae is shown in table I.

The 26 arterial fistula consisted of 15 aortoenteric fistulae (6%), 2 arteriobronchial fistulae (<1%), 4 aortocaval fistulae (2%) and 5 aortocutaneous fistulae (2%). One patient had both an aortocaval and arteriocutaneous fistula. Primary fistulae accounted for 42% (n = 11). A vascular prosthesis was present prior to development of fistula in 58% (n = 15); these were classified as secondary fistulae. All primary fistulae occurred in the presence of an arterial aneurysm. Of 26 fistulae, 25 (96%) were associated with infected aortic aneurysms or aortic prostheses. In 13 of 25 (52%) aortic infections, one or both iliac arteries were infected simultaneously: in 3 of those 13 (23%) the arterial fistula originated from an iliac artery. In 1 patient, the arterial fistula originated from a femoral artery. Time from insertion of vascular prosthesis to development of a fistula varied per subtype, with a median of 29 months (interquartile range (IQR) 22 – 59 months). Overall, most fistulae were diagnosed shortly before or after diagnosis of chronic Q fever (median 0 months, IQR 0 – 4 months) and shortly before or after initiation of antibiotics (median 1 month, IQR 0 – 8 months). All cases with fistulae had clinical signs and symptoms, except for two cases in which aortocaval fistulae were diagnosed coincidently per-operatively or by radiographical imaging. An overview on how fistulae were diagnosed is shown in table II.

Table I. Overview of patients with proven chronic Q fever

	Fistula	Non-fistula
N (%)	25 (10)	228 (90)
Median follow-up in months (interquartile range (IQR))	9 (1 - 23)	48 (21 - 63)
Male gender (%)	19 (76)	177 (78)
Mean age at diagnosis (sd)	73 (11)	69 (12)
Median maximum *Coxiella burnetii* phase I IgG titer	1:4096	1:16384
PCR positivity on serum for *Coxiella burnetii* DNA	10 (40)	133 (58)
Episode of acute Q fever notified (%)	1 (4)	56 (25)
Median time between acute and chronic Q fever (months, IQR)	12[a]	16 (8 - 30)
Focus of chronic Q fever		
vascular (prosthesis) infection (%)	22 (88)	105 (46)
endocarditis & vascular combined (%)	3 (12)	39 (17)
Comorbidity		
peripheral vascular disease (%)	7 (28)	36 (16)
ischemic cardiac disease (%)	9 (36)	80 (35)
cerebrovascular disease (%)	6 (24)	35 (15)
diabetes (%)	2 (8)	31 (14)
current or past smoking (%)	19 (76)	161 (71)
Risk factors chronic Q fever		
immunocompromised state (%)	2 (8)	32 (14)
cardiac valve disease (%)	-	67 (29)
vascular prosthesis (%)[b]	13 (52)	69 (30)
arterial aneurysm (%)	4 (16)	38 (17)

[a] Only one value available, no IQR presented. [b] present before diagnosis of chronic Q fever, not before development of fistula (two patients developed secondary fistula after surgery that was performed after diagnosis of chronic Q fever, thus the prosthesis was inserted after diagnosis of chronic Q fever).

Table II. Clinical characteristics of fistula and establishment of diagnosis of fistula

	Aorto-enteral fistula	Aorto-bronchial fistula	Aorto-caval fistula	Arterio-cutaneous fistula
N[a]	15	2	4[a]	5[a]
Age (mean (sd))	76 (10)	72 (0)	72 (3)	63 (16)
Male sexe (%)	12 (80)	-	4 (100)	4 (80)
Type of fistula				
primary fistula (%)	9 (60)	-	2 (50)	-
secondary fistula (%)	6 (40)	2 (100)	2 (50)	5 (100)
Establishment of diagnosis				
clinical characteristics (%)	15 (100)	2 (100)	2 (50)	5 (100)
radiographical characteristics (%)	6 (40)	2 (100)	3 (75)	4 (80)
surgical findings (%)	8 (53)	1 (50)	3 (75)	2 (40)
endoscopy (%)	1 (7)	-	-	-
findings at autopsy (%)	1 (7)	1 (50)	-	-
Mortality				
overall (%)	13 (87)	2 (100)	-	2 (40)
chronic Q fever-related (%)	12 (80)	2 (100)	-	1 (20)
death < 4 weeks after diagnosis of fistula (%)	7 (47)	2 (100)	-	-
surgical intervention for fistula (%)	9 (60)	1 (50)	4 (100)	3 (60)

[a] *one patient with two fistula.*

Overall mortality was 68% for patients with arterial fistula (n = 17) and 34% (n = 77) for patients without arterial fistula (P < .0001). Chronic Q fever-related mortality was 60% (n = 15) in patients with (primary or secondary) arterial fistula. In patients without arterial fistula, chronic Q fever-related mortality was 21% (n = 48). The proportion of patients with and without a vascular focus and with and without an arterial fistula, that died of chronic Q fever-related causes, is demonstrated in figure I. Patients with an arterial fistulae had a significantly higher risk of chronic Q fever-related mortality (P < .0001) compared to patients without an arterial fistula (see figure II). The median time to chronic Q fever-related death was 1 month for patients with fistula (IQR 1-16), whereas it was 9 months for patients without fistula (IQR 1-24). Of 25 patients with an arterial fistula, 17 underwent surgical intervention (68%). After surgical intervention for arterial fistula (n = 17), 53% died of chronic Q fever-related causes (n = 9) while in patients without surgical intervention for an arterial fistula (n = 8), 75% died of chronic Q fever-related causes (n = 6). There were no significant differences in survival (P = .45). Of patients without arterial fistula, 32% (n = 73) underwent surgical intervention for chronic Q fever. See table II for additional details on mortality and surgical interventions per type of fistula.

Figure I. Composite figure demonstrating the proportion of deaths due to chronic Q fever-related mortality among patients with and without a vascular focus of infection, and patients with and without an arterial fistula.

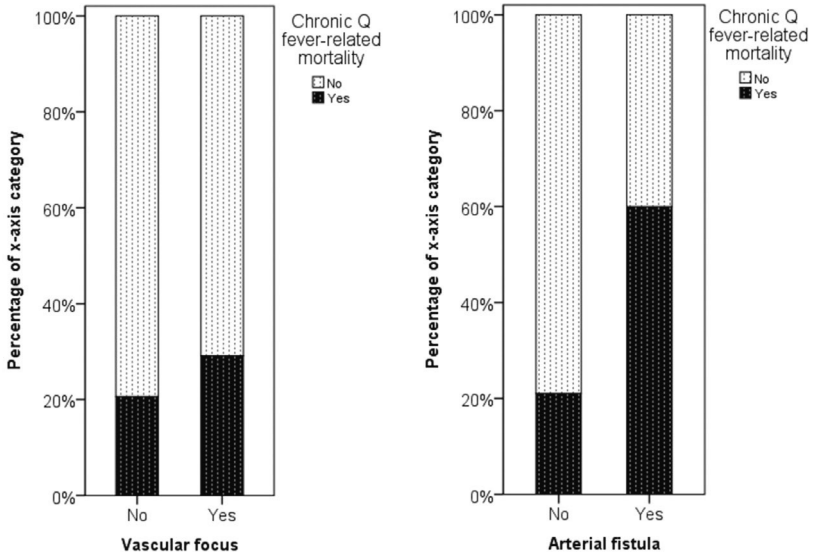
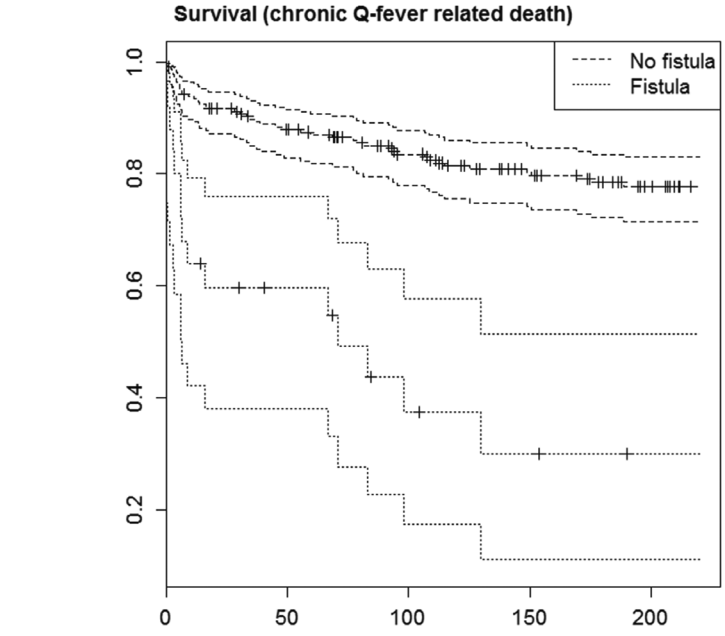


Figure II. Kaplan-Meier survival curves (surrounded by 95% confidence intervals) for patients with and without arterial fistula.



Number at risk

No fistula	228	189	162	137	118
Fistula	25	12	6	4	4

Types of surgical intervention were insertion of aortic (bifurcation) prosthesis by open procedure in 6 patients, endovascular aortic repair in 5 patients (abdominal in 2, thoracic in 1, fenestrated in 1, attempt not succeeded in 1), venous reconstruction in 4 patients, femoral-femoral cross-over bypass in 1 patient, and drainage of peri-aortic abscesses and an infected hematoma in 1 patient. In 1 patient treated with an aortic bifurcation prosthesis, an axillofemoral bypass procedure was performed later in the course of the disease after development of a second fistula. In 8 patients, no surgical intervention was performed. Reasons for withholding surgical treatment were premature death in 5 patients, on request of 2 patients (both with metastatic lung cancer), and unknown reasons in 1 patient.

Discussion

Arterial fistulae are commonly observed as a complication of chronic Q fever: 15% of all vascular proven chronic Q fever patients were diagnosed with arterial fistulae, with 58% accounted for by aortoenteric fistulae. Not only the high incidence of fistulae in patients with vascular chronic Q fever is remarkable, the proportion of primary fistulae is also higher than expected. Where previous reports noted a ratio of 1:10 between primary and secondary fistulae, we observed a ratio of 5:7.[5]

This high cumulative incidence suggests a highly increased risk of arterial fistulae in vascular chronic Q fever patients compared to patients with an aneurysm of the abdominal aorta not infected by *C. burnetii*. Incidences previously reported in literature vary from <0.1% for primary aortoenteric fistulae in patients with an aneurysm of the abdominal aorta and <1% for secondary fistula in patients with a vascular prosthesis (with exception of specific selected populations such as patients after emergency open aneurysm repair, were incidences are higher).[5,7,22] This finding implicates that infection with *C. burnetii* itself increases the risk for arterial fistulae, apart from the existence of an aneurysm itself.

Two other studies describing fistulae in vascular chronic Q fever patients both reported that 5% of patients developed arterial fistulae.[17,23] One study described patients from the Dutch national chronic Q fever database after a follow-up duration of 14 months: the considerably shorter follow-up duration may explain the lower incidence of fistulae.[17] A second study described patients from a French national referral center with a follow-up duration of three years.[23] Patients that are actively referred to a referral center may be a subgroup with more favorable prognosis (and thus less complications), since they are well enough for transport. Patients that die early in the course of disease or even at presentation will not be referred. In our study, we included patients from 29 hospitals, including small hospitals, all identified during or following one large outbreak including patients that died early in the course of disease or at presentation. Therefore, we think our cohort is more representative of an unselected population following a Q fever outbreak.

In the aftermath of the Dutch Q fever outbreak, up to 17% of patients with an abdominal aortic or iliac aneurysm or reconstruction living in the center of the epidemic area

were found to have antibodies against *C. burnetii*, of which 31% of patients showed serological evidence of chronic Q fever.[18] The high risk for chronic Q fever in these patients, and the high risk for lethal complications during vascular chronic Q fever, makes these patients vulnerable for adverse outcomes.

Besides aortoenteric, aortobronchial and aortovenous fistulae, we also observed arteriocutaneous fistulae. These types of fistulae are very rare and sporadically described.[24] The arteriocutaneous fistulae in our patients led to severe (intermittent) bleeding. All originated from vascular prostheses and were thus secondary fistulae. Since this type of fistula is very rare, the diagnosis chronic Q fever should strongly be considered in patients presenting with arteriocutaneous fistulae.

It is difficult to quantify the proportion of patients with underlying chronic Q fever in those presenting with arterial fistulae. Both diseases are rare and difficult to diagnose, thus the exact prevalence of chronic Q fever among patients presenting with arterial fistula is unknown to our knowledge. Diagnosing chronic Q fever is complicated by the fact that most patients present with nonspecific symptoms, such as night sweats, weight loss, fatigue and malaise. Fever is not always present. A definite diagnosis relies on a combination of clinical signs, serology, PCR on blood or tissue and radiological findings.[9,21] Nevertheless, the high incidence of arterial fistula among patients with chronic Q fever suggests that chronic Q fever significantly contributes to the incidence of arterial fistulae after the Dutch Q fever outbreak. Therefore, clinicians should be alert on signs and symptoms matching an arterial fistula in areas were Q fever has been epidemic, and consider the diagnosis chronic Q fever in patients presenting with an arterial fistula. It can be debated if patients should be screened for underlying chronic Q fever prior to insertion of a vascular prosthesis in regions were Q fever is endemic, and during and after Q fever outbreaks. However, patients may also present with primary arterial fistulae (in some patients underlying aortic disease was present but unknown). Moreover, patients with a vascular prosthesis are also at risk of development of arterial fistulae, as this study demonstrates. As mentioned before, patients with an abdominal aortic or iliac aneurysm or reconstruction are at highly increased risk for chronic Q fever.[18] Therefore, in the setting of an outbreak or ongoing endemic situation, all patients with aortic or iliac aneurysms or reconstructions could benefit from screening.

Disease-related mortality among patients presenting with arterial fistulae during chronic Q fever is 60% after a total median follow-up duration of 9 months. These findings are in line with earlier studies: mortality rates reported among patients with aortic fistulae in earlier studies ranged from 30-90%.[3,7] The risk for mortality is not significantly different between patients that underwent surgical intervention and those who did not. However, due to small number of cases and the retrospective observational nature of this study with the risk of confounding by indication, we cannot draw any conclusions on the effect of surgical intervention on prognosis. Nevertheless, it may be assumed that in case of life-threatening bleeding, a surgical intervention is the only potential therapeutic option. The poor prognosis of these patients underlines the importance of early recognition and vigilance amongst clinicians. The fact that significantly less patients with arterial fistula were previously

diagnosed with acute Q fever, suggests that early recognition of the disease may prevent complications: patients with acute Q fever often stay in follow-up, so chronic infection is detected at an earlier stage. Most patients without notified acute Q fever were diagnosed with chronic Q fever based on clinical signs and symptoms, and complication may have occurred already. Due to the low numbers of patients, we could not formally test differences in time between acute and chronic Q fever in this study for patients with and without arterial fistulae.

With this observational cohort study, we described a unique series of patients with vascular chronic Q fever and primary or secondary arterial fistulae. Although we evaluated a relatively large number of patients, identification of factors associated with development of fistulae and evaluation of the effect of surgical intervention was not possible, since numbers were still too small. Furthermore, it is possible that patients with arterial fistulae related to the Dutch outbreak have been missed, if cause of death was not established and especially if the diagnosis of chronic Q fever was unknown, and that we have missed patients with vascular chronic Q fever without arterial fistulae. This may lead to underestimation of the actual number of patients with chronic Q fever both with and without aortic fistulae and to distortion of the proportion of patients with fistulae among chronic Q fever patients.

Conclusion

In conclusion, the proportion of patients with proven chronic Q fever developing primary or secondary arterial fistulae is high: 15% of patients with vascular chronic Q fever develops an arterial fistula. This observation suggests that *C. burnetii* plays a role in the development of fistulae in these patients. Despite surgical intervention prognosis is highly unfavourable: chronic Q fever-related mortality is 60% in patients with an arterial fistula. Clinicians should take the diagnosis chronic Q fever into consideration in patients presenting with an arterial fistula, should be aware on the risk of fistulae in vascular chronic Q fever patients and should consider screening patients with aneurysms or vascular prosthesis for chronic Q fever in endemic or outbreak settings, to enable early initiation of treatment and prevent occurrence of complications.

References

1. Mosquera VX, Marini M, Pombo-Felipe F. Gómez-Martinez P, Velasco C, Herrera-Noreña JM et al. Predictors of outcome and different management of aortobronchial and aortoesophageal fistulas. J Thorac Cardiovasc Surg 2014;148(6):3020-6.
2. Sipe A, McWilliams SR, Saling L, Raptis C, Mellnick V, Bhalla, S. The red connection: a review of aortic and arterial fistulae with an emphasis on CT findings. Emerg Radiol 2017;24(1):73-80.
3. Saers SJ, Scheltinga MR. Primary aortoenteric fistulae. Br J Surg 2005;92(2):143-152.
4. Malik M, Ucbilek E, Sherwal A. Critical gastrointestinal bleed due to secondary aortoenteric fistulae. J Community Hosp Intern Med Perspect 2015;5(6):29677.
5. 5. Lozano SF, Muñoz-Bellvis L, San Norberto E, Garcia-Plaza A, Gonzalez-Porras JR. Primary aortoduodenal fistula: new case reports and a review of the literature. J Gastrointest Surg 2008;12(9):1561-5.
6. Czerny M, Reser D, Eggebrecht H, Janata K, Sodeck G, Etz C et al. Aorto-bronchial and aorto-pulmonary fistulation after thoracic endovascular aortic repair: an analysis from the European registry of Endovascular Aortic Repair Complications. Eur J Cardiothorac.Surg, 2015;48(2):252-7.
7. Bergqvist D, Björck M. Secondary arterioenteric fistulation -- a systematic literature analysis. Eur J Endovasc Surg 2009;37(1):31-42.
8. Kampschreur LM, Hagenaars JC, Wielders CC, Elsman P, Lestrade PJ, Koning OH et al. Screening for *Coxiella burnetii* seroprevalence in chronic Q fever high-risk groups reveals the magnitude of the Dutch Q fever outbreak. Epidemiol Infect 2013;141(4):847-51.
9. van der Hoek W, Hogema BM, Dijkstra F, Rietveld A, Wijkmans CJ, Schneeberger PM et al. Relation between Q fever notifications and *Coxiella burnetii* during the 2009 outbreak in the Netherlands. Eurosurveillance; 2012; 17(3):11-5.
10. Eldin C, Melenotte C, Mediannikov O, Ghigo E, Million M, Edouard S et al. From Q fever to *Coxiella burnetii* infection: a paradigm change. Clin Microbiol Rev 2017;30(1):115-90.
11. Tissot-Dupont H, Amadei M-A, Nezri M, Raoult D. 2004. Wind in November, Q fever in December. Emerg Infect Dis 2004; 10(7):1264 –9.
12. Dijkstra F, van der Hoek W, Wijers N, Schimmer B, Rietveld A, Wijkmans CJ et al. The 2007 – 2010 Q fever epidemic in the Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. FEMS Immunol Med Microbiol 2012; 64(1):3-12.
13. Kampschreur LM, Wegdam-Blans MCA, Thijsen SFT, Groot CA, Schneeberger PM, Hollander AA et al. Acute Q fever related in-hospital mortality in the Netherlands. Neth J Med 2010; 68(12):408-13.
14. Kampschreur LM, Dekker S, Hagenaars JCJP, Lestrade PJ, Renders NH, de Jager-Leclercq et al. Identification of risk factors for chronic Q fever, the Netherlands. EID 2012, 18(4):563-70.
15. van Roeden SE, Wever PC, Kampschreur LM, van der Hoek W, Hoepelman AIM, Bleeker-Rovers CP et al. Chronic Q fever-related complications and mortality: data from a nationwide cohort. Abstract oral presentation at ESCMID 2017, Vienna.
16. Melenotte C, Million M, Audoly G, Gorse A, Dutronc H, Roland G et al. B-cell non

Hodgkin-lymphoma linked to *Coxiella burnetii*. Blood 2016; 127(1):113-21.
17. Broos PP, Hagenaars, JC, Kampschreur LM, Wever PC, Bleeker-Rovers CP, Koning OH et al. Vascular complications and surgical interventions after world's largest Q fever outbreak. J Vasc Surg. 2015;62(5):1273-80.
18. Hagenaars JCP, Wever PC, van Petersen AS, Lestrade PJ, de Jager-Leclercq MG, Hermans MH et al. Estimated prevalence of chronic Q fever among *Coxiella burnetii* seropositive patients with an abdominal aortic/iliac aneurysm or aorto-iliac recontstruction after a large Dutch Q fever outbreak. J Infect 2014;69(2):154-60.
19. Eldin C, Mailhe M, Lions C, Carrieri P, Safi H, Brouqui P *et al*. Treatment and prophylactic strategy for *Coxiella burnetii* infection of aneurysms and vascular grafts: a retrospective cohort study. Medicine 2016;95(12):e2810.
20. Sigterman TA, Bendermacher BL, Welten RJ, Krasznai A, Bouwman LH. Primary aortoduodenal fistula and Q-fever. Vasc Med 2013;18(6):347-9.
21. Wegdam-Blans MC, Kampschreur LM, Delsing CE, Bleeker-Rovers CP, Sprong T, van Kasteren ME et al. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Infect 2012;64:247-59.
22. Tagowski M, Vieweg H, Wissgott C, Andresen R. Aortoenteric fistulae as a complication of open reconstruction and endovascular repair of abdominal aorta. Radiol Res Pract 2014;383159.
23. Botelho-Nevers E, Fournier PE, Richet H, Fenollar F, Lepidi H, Foucault C et al. *Coxiella burnetii* infection of aortic aneurysms or vascular grafts: report of 30 new cases and evaluation of outcome. Eur J Clin Microbiol Infect Dis 2007;26(9):635-40.
24. Torres-Blanco Á, Gómez-Palonés F, Edo-Fleta G. Arteriocutaneous fistula associated with bilateral femoral pseudoaneurysms caused by Bacillus Calmette-Guérin. Apropos of a case and review of literature. Ann Vasc Surg 2017;39:291.

Chapter 4

Chronic Q fever: patient and treatment-related factors influencing long-term quality of life and patients' perception of antibiotic treatment

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Abstract

Purpose

Chronic Q fever is accompanied by high mortality and morbidity, and requires prolonged antibiotic treatment. Little is known on long-term quality of life (LQOL) in chronic Q fever patients treated with antibiotics.

Methods

We performed a cross-sectional study to identify patient and treatment-related factors associated with impaired LQOL in chronic Q fever patients treated with antibiotics, and to assess patients' perception on treatment. LQOL was assessed with a validated questionnaire from the Nijmegen Clinical Screening Instrument. Patients' perception on treatment was measured with three newly developed questions.

Results

We included 64 patients: LQOL was impaired in 55% (n=35) after a median follow-up of 5 years. Median treatment duration was 27 months. In multivariable analysis, treatment duration was significantly associated with impaired LQOL (OR1.07; 95%CI 1.02-1.12, p<0.01 per month increase). Age, gender, number of antibiotic regimens, surgical intervention, complications, diagnostic classification, focus of infection or registration of side effects during treatment were not associated with impaired LQOL. After start of treatment, 17 patients (27%) perceived improvement of their condition. Disadvantages of treatment were experienced on a daily basis by 24 patients (69%) with impaired LQOL and 13 patients (46%) without impaired LQOL (p=0.04).

Conclusions

LQOL in chronic Q fever patients treated with antibiotics is impaired in more than half of patients 5 years after diagnosis. Antibiotic treatment duration was the only variable associated with impaired LQOL. The majority of patients experienced disadvantages on a daily basis, highlighting the high burden of disease and treatment.

Key words

Coxiella burnetii; chronic Q fever; antibiotic treatment; antibiotics; quality of life.

Introduction

Between 2007 and 2010, the Netherlands faced the largest Q fever outbreak ever recorded, with over 4,000 reported and 40,000 estimated cases of primary infected patients [1]. Q fever is caused by *Coxiella burnetii*, an intracellular bacterium that can be spread through air by cattle, mostly goats and sheep, after for example shedding in milk, excreta or birth products [2].

Besides symptomatic primary infection, long-term consequences consist of severe fatigue in 10-40% and chronic infection in approximately 1-5% of patients that were primarily infected [3,4]. Most common manifestations of chronic Q fever are endocarditis, infected aneurysms or infected vascular prostheses. It mandates long-term antibiotic treatment, for at least 18 months [1,2]. Treatment is initiated to reduce morbidity and mortality and to increase quality of life (QOL).

However, treatment itself is often accompanied by serious toxicity such as gastro-intestinal complaints, photosensitivity, retinopathy and hyperpigmentation [5,6]. One small study performed in 26 patients with vascular chronic Q fever, showed that physical health scores declined during treatment, while mental health scores improved [7].

Long-term QOL (LQOL) in chronic Q fever patients who were treated with antibiotics has not been studied before. Our aim was to identify patient and treatment-related factors associated with impaired LQOL in these patients and to assess patients' perception on treatment.

Methods

Study design and population

We performed a cross-sectional study to identify patient and treatment-related factors associated with impaired LQOL in chronic Q fever patients treated with antibiotics and to assess patients' perception on antibiotic treatment.

Patients with proven or probable chronic Q fever according to the Dutch chronic Q fever consensus guidelines ≥18 years of age were considered eligible [8]. Patients with possible chronic Q fever were excluded, since these patients do not have an established infection and do not have an indication for treatment with antibiotics.

This study was designed in cooperation with researchers from the Radboudumc of the ImpaQt study (DR and EJ), which is a study on long-term psychosocial functioning in chronic Q fever patients (Reukers et al, ImpaQt study, unpublished data). All patients in the ImpaQt study were recruited via their (former) treating physicians from the Radboudumc in Nijmegen, Jeroen Bosch Hospital in 's-Hertogenbosch, Sint Elisabeth Hospital in Tilburg, and Bernhoven Hospital in Uden (all hospitals located in the center of the epidemic area during the 2007 – 2010 outbreak). Additionally,

we approached patients via the newsletter of the patient organization ("Q-uestion") to recruit patients from other hospitals. The data collection started in February 2016 and ended in December 2016. Design of the ImpaQt study was approved by the Medical Ethics Committee of the Radboud university medical center, design of this study was approved by the Medical Ethics Committee of the University Medical Center Utrecht.

Data collection

Patients gave consent to answer questionnaires on LQOL for both the ImpaQt study and the current study, and for retrieval of their clinical data from the Dutch national chronic Q fever database to match clinical data with LQOL data. LQOL and clinical data were matched using an anonymous identifier. In the chronic Q fever database, clinical, microbiological, and radiological data are stored of all known proven, probable and possible chronic Q fever patients ≥18 years of age. For details on design of this database, we refer to a previous article [9]. Quality of life was measured with the Nijmegen Clinical Screening Instrument (NCSI), which combines the score from an instrument measuring depression (Beck's depression index) and an instrument measuring satisfaction with life [10,11]. The QOL score ranged from 0 to 100; a higher score corresponded with a lower QOL. A cut-off value for impaired QOL was 13.33: below this threshold QOL was considered normal; above the threshold QOL was considered impaired [12]. Perception of treatment was measured with three questions, targeting perception on their change in condition after initiation of treatment, overall judgment of the treatment effect (advantages versus disadvantages) and the frequency of experiencing disadvantages (see supplement 1 for these questions).

Statistical analysis

Data were stored in SPSS version 21.0. All analyses were performed in R3.2.2 (studio version). For univariable comparison of categorical variables, a Pearson's Chi-squared test or Fisher exact test as appropriate was used. Medians were compared by means of a Mann-Whitney U test. Univariable odds ratio's with 95% confidence intervals (profiling likelihood) were calculated. Binary logistic regression was performed, with stepwise modelling by backward Wald method. The threshold for excluding variables in the model was set at a p-value of 0.10. Since this is an exploratory study, all patient and treatment related variables potentially influencing QOL on theoretical grounds were entered in the model. The selected variables were age, gender, presence of comorbidities, diagnostic classification (proven or probable chronic Q fever), focus of infection, presence of complications, surgical treatment of chronic Q fever, number of antibiotic treatment regimens, duration of treatment in months and reporting of side effects during treatment. Comorbidity was defined as having a history of diabetes mellitus, ischemic cardiovascular disease, cerebrovascular disease, peripheral arterial disease, immunodeficiency, vascular prosthesis or arterial aneurysm, valvulopathy or valve prosthesis, chronic obstructive pulmonary disease (COPD), malignancy (both non-hematological and hematological), renal insufficiency or heart failure. Odds ratios

with 95% (profiling likelihood) confidence intervals were reported. The significance level was set at a p-value <0.05. Missing data were reported below tables.

Results

Inclusion of patients

In total, 249 proven chronic Q fever patients and 74 probable chronic Q fever patients were identified for inclusion in the chronic Q fever database. At start of this study, 212 were known to be alive, of which 145 patients (68%) were approached via the four hospitals involved in this study. Overall response rate of recruitment via hospitals and the newsletter was 33% (69 patients). Of these 69 patients, 5 were excluded from analysis (they did not receive antibiotic treatment for unknown reasons). Therefore, we had data of 64 patients available for analysis: 52 with proven chronic Q fever (81%) and 12 with probable chronic Q fever (19%), see figure 1.

Baseline characteristics

Median age for all patients was 72 years (interquartile range (IQR) 65 - 79) and 84% of patients (n=54) were male. Any comorbidity was present in 52 patients (81%), most frequently arterial aneurysms or vascular prostheses (n=32; 50%), ischemic cardiovascular diseases (n=19; 30%) and valvulopathy or valve prostheses (n=13; 20%). Focus of chronic Q fever was vascular in 40 patients (63%), endocarditis in 10 patients (16%), and a combination of both in 9 patients (14%). Median time since diagnosis of chronic Q fever was 5.0 years (IQR 2.7 – 5.9 years). Most patients were treated with tetracyclines plus hydroxychloroquine (n=61; 95%), little over half of patients had finished antibiotic treatment at time of inclusion in our study (n =34; 53 %) see table 1.

Figure. 1 Flowchart of inclusion.

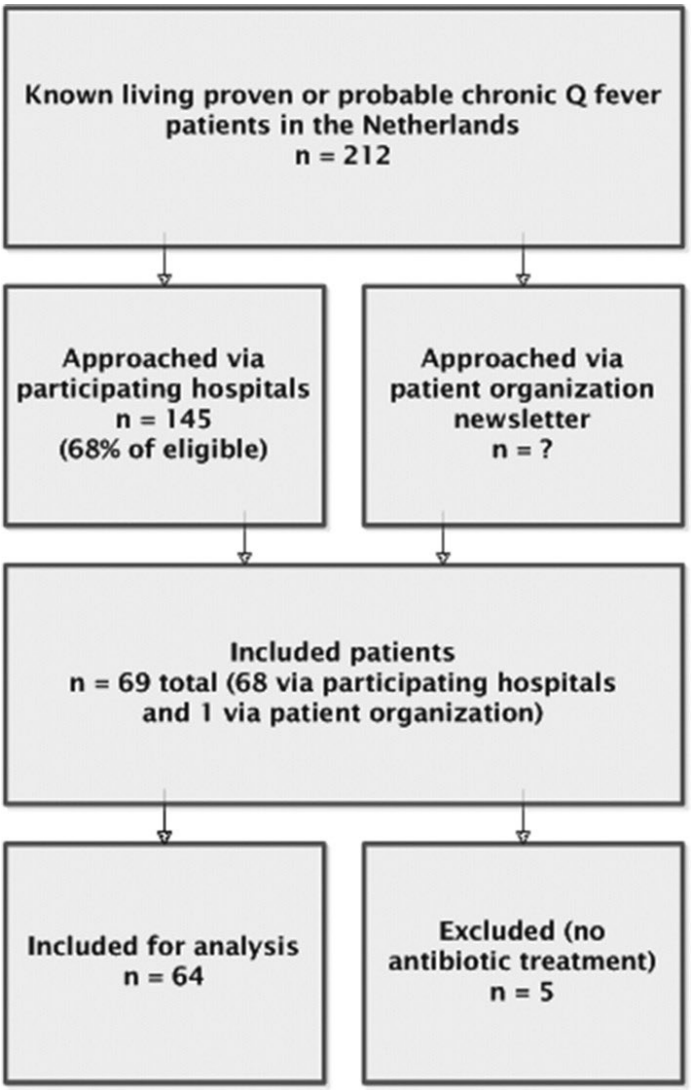

Table 1. Overview of clinical and treatment characteristics of chronic Q fever patients.

Characteristics	All patients	Impaired LQOL	Normal LQOL
Number of patients (%)	64	35 (55)	28 (44)
Median age (interquartile range (IQR))	72 (65 - 79)	70 (64 - 79)	73 (68 - 79)
Male sex (%)	54 (84)	28 (80)	25 (89)
Smoking (prior or current) (%)[a]	51 (80)	29 (83)	21 (75)
Comorbidity[a]			
Diabetes mellitus (%)	8 (13)	5 (14)	2 (7)
Ischemic cardiovascular disease (%)	19 (30)	12 (34)	6 (21)
Cerebrovascular disease (%)	10 (16)	7 (20)	3 (11)
Peripheral arterial disease (%)	7 (11)	4 (11)	3 (11)
Immunodeficiency (%)	6 (9)	3 (9)	3 (11)
Vascular prosthesis or arterial aneurysm (%)	32 (50)	19 (54)	12 (43)
Valvulopathy or valve prosthesis (%)	13 (20)	7 (20)	6 (21)
COPD (%)	7 (11)	6 (17)	1 (4)
Malignancy ((non-)hematological) (%)	8 (13)	6 (17)	2 (7)
Renal insufficiency (%)	5 (8)	3 (9)	2 (7)
Heart failure (%)	2 (3)	2 (6)	0
Chronic Q fever risk factor (%)[b]	41 (64)	23 (66)	17 (61)
Any comorbidity (all above excluding smoking)	52 (81)	30 (86)	21 (75)
Diagnostic classification			
Proven chronic Q fever (%)	52 (81)	27 (77)	25 (89)
Probable chronic Q fever (%)	12 (19)	8 (23)	3 (11)
Focus of infection			
Vascular infection (%)	40 (63)	23 (66)	16 (57)
Endocarditis (%)	10 (16)	4 (11)	6 (21)
Vascular infection & Endocarditis (%)	9 (14)	6 (17)	3 (11)
Other (%)	5 (8)	2[c] (6)	3 (11)[d]
Course of disease			
Median time since diagnosis in years (IQR)	5.0 (2.7 – 5.9)	5.0 (3.0 – 6.0)	4.9 (2.3 – 5.6)
Complications of chronic Q fever (%)	25 (39)	13 (37)	12 (43)
Surgical intervention for chronic Q fever (%)	29 (45)	17 (49)	12 (43)
Vascular surgical intervention (%)	23 (36)	14 (40)	9 (32)
Cardiac surgical intervention (%)	3 (5)	1 (3)	2 (7)
C. burnetii PCR positivity (%)	37 (58)	20 (57)	17 (61)
Four-fold titer decrease (%)	27 (42)	13 (37)	14 (50)
Decrease of titer to <1:1024 (%)	20 (31)	10 (27)	10 (36)

Treatment[e]			
Median number of antibiotic regimens (IQR)	2 (1 – 3)	3 (1 - 3)	2 (1-3)
Initiation of antibiotics < 12 weeks after diagnosis of chronic Q fever (%)	60 (94)	34 (97)	26 (93)
Finished treatment currently (%)	34 (53)	17 (49)	16 (57)
Median treatment duration in months (IQR)	27 (20 - 36)	30 (23 - 41)	24 (18 - 33)
Treatment with TET/HCQ (%)	61 (95)	34 (97)	26 (93)
Median duration TET/HCQ in months (IQR)	16 (6 - 23)	16 (6 - 25)	17 (5 - 23)
> 18 months TET/HCQ (%)	27 (42)	14 (40)	13 (46)
Treatment with TET/QNL (%)	16 (25)	9 (26)	7 (25)
Treatment with TET/QNL/HCQ (%)	9 (14)	6 (17)	3 (11)
Treatment with QNL (%)	27 (42)	18 (51)	8 (29)
Treatment with TET (%)	17 (27)	11 (31)	6 (21)
Treatment with other (%)	21 (33)	12 (34)	8 (29)
Side effects of antibiotic treatment			
Reporting of any side effects	54 (84)	31 (89)	22 (79)
Stop antibiotics due to side effects	34 (53)	21 (60)	12 (43)

LQOL = long-term quality of life. Missing NCSI score (LQOL) in one patient. [a]Missing data in two patients. [b]Chronic Q fever risk factors defined as valvulopathy or valve prosthesis, aneurysm or vascular prosthesis and immunocompromised state. [c]2 patients without focus of infection. [d]1 patient with pericarditis and endocarditis native valve, 2 patients without focus. [e]TET= tetracycline; HCQ= hydroxychloroquine; QNL = quinolone.

LQOL and factors predicting impaired LQOL

LQOL was impaired in 35 patients (55%). Median NCSI score for those with normal LQOL was 7 (IQR 4-10), and 29 (IQR 20-39) for those with impaired LQOL. In multivariable analysis, treatment duration was significantly associated with impaired LQOL (OR 1.07; 95%CI 1.02–1.12 per month increase, p<0.01) and probable chronic Q fever patients showed a trend towards a higher risk for impaired LQOL (OR4.08; 95%CI 0.94–22.91, p=0.08). None of the other factors were significantly associated with impaired LQOL (table 2).

Table 2. Multivariable logistic regression (backward stepwise approach) exploring what factors are associated with impaired long-term quality of life.

Variable	Univariable OR (95%CI) for impaired LQOL	Multivariable OR (95%CI) for impaired LQOL	Corresponding p-value multivariable OR
Age (per years increase)	0.98 (0.92 – 1.04)	-	-
Male gender	0.48 (0.10 – 1.93)	-	
Treatment duration (per month increase)	1.06 (1.02 – 1.11)	1.07 (1.02 – 1.12)	<0.01
Number of antibiotic regimens	1.48 (0.95 – 2.42)	-	-
Presence of any comorbidity	2.00 (0.56 – 7.58)	-	-
Surgical intervention for chronic Q fever	1.26 (0.46 – 3.46)	-	-
Presence of complications	0.79 (0.28 – 2.18)	-	-
Probable chronic Q fever	2.47 (0.63 – 12.24)	4.08 (0.94 – 22.91)	0.08
Focus of infection	[a]	-	-
Side effects during treatment	2.11 (0.54 – 9.11)	-	-

Variables without OR / p-value were excluded from the model in the backward stepwise modeling process. Missing data (NCSI score) in one patient. [a]endocarditis OR 0.67 (0.06 – 7.55), vascular focus OR 1.44 (0.16 – 13.02), combined [a]endocarditis and vascular focus 2.00 (0.17 – 25.23), other focus OR <0.01 (0 - ∞).

Patients' perception on antibiotic treatment

After start of treatment, 17 patients (27%) experienced improvement of their condition during treatment compared to prior to start of treatment, 23 patients (36%) did not notice any change, and 20 patients (32%) experienced deterioration of their condition during treatment compared to prior to start of treatment. Patients with impaired LQOL experienced more often a large deterioration in their condition after start of treatment (n=10; 29%) compared to those with normal LQOL (n=1; 4%), this difference showed a trend towards significance (p=0.05). In total, 22 patients (34%) judged that advantages of treatment did outweigh the disadvantages, without significant differences between those with and without impaired LQOL. Patients that had finished antibiotic treatment answered more often that disadvantages did not outweigh advantages (n=10/31; 32%) compared to those that were still on antibiotic treatment (n=4/30; 13%,), with a trend towards significance (p = 0.10). Median treatment duration was not significantly different between those that had finished and were still on antibiotic treatment (25 months vs. 29 months, p=0.45). Disadvantages of treatment were experienced on a daily basis by 38 patients (59%) while 9 patients (14%) never experienced any disadvantages. In those with impaired LQOL, a significant higher proportion experienced disadvantages on a daily basis compared to patients with normal LQOL (n=24; 69% versus n=13; 46%, p=0.04), while a relatively large proportion of patients with normal quality of life never perceived any disadvantages (table 3).

Table 3. Subjective perception on treatment for all treated chronic Q fever patients.

Characteristics	All patients	Impaired	Normal	Pearson's
Number of treated patients (%)	64	35 (55)	28 (44)	
Overall condition after start treatment[a]				p = 0.05
Large improvement (%)	8 (13)	2 (6)	6 (21)	
Slight improvement (%)	9 (14)	4 (11)	5 (18)	
No change (%)	23 (36)	12 (34)	11 (39)	
Slight deterioration (%)	8 (13)	5 (14)	3 (11)	
Large deterioration (%)	12 (19)	10 (29)	1 (4)	*
Overall judgment on treatment effect[b]				p = 0.18
Advantages outweigh disadvantages (%)	22 (34)	10 (29)	12 (43)	
Neutral (%)	25 (39)	13 (37)	12 (43)	
Disadvantages outweigh advantages (%)	14 (22)	10 (29)	3 (11)	
Frequency of experiencing disadvantages[c]				p = 0.04
Daily (%)	38 (59)	24 (69)	13 (46)	*
Weekly (%)	6 (9)	4 (11)	2 (7)	
Monthly (%)	3 (4)	2 (6)	1 (4)	
<Once monthly (%)	4 (6)	1 (3)	3 (11)	
Never (%)	9 (14)	1 (3)	8 (29)	*

*LQOL = long-term quality of life. [a]missing NCSI score (LQOL) in 1 patient. [b]missing data in 4 patients. [c]missing data in 3 patients. *indicates difference between column proportions.*

Discussion

LQOL is impaired in more than half of proven and probable chronic Q fever patients that were treated with antibiotics. Treatment duration was the only variable that was associated significantly with impaired LQOL. Diagnostic classification as probable chronic Q fever was associated with a near-significant higher risk for impaired LQOL. Comorbidity, age, gender, surgical intervention, presence of complications, number of antibiotic regimens or reported side effects of antibiotic treatment were not associated with LQOL. The association between treatment duration and impaired LQOL could be due to the fact that treatment duration is a proxy parameter for disease severity, which may influence LQOL. Another potential explanation could be that treatment itself influences LQOL, apart from the disease, for example by causing side effects. Patients with probable chronic Q fever had a trend towards a higher risk of impaired LQOL. This is remarkable, since none of these patients had complications or underwent surgical intervention for their disease and comorbidities were not present more often in these patients. The higher risk for impaired LQOL in probable chronic Q fever patients, the absence of an association between surgical interventions or presence of complications supports the hypothesis that treatment itself may influence LQOL: these variables can all be considered to be proxy parameters for severity of disease. Although reporting of any side effects to the treating physician during treatment was not associated with LQOL, the proportion of patients reporting side effects on a daily basis in this study was higher among those with impaired LQOL, which supports the hypothesis of a negative influence on LQOL of treatment. The difference in patient-reported and 'doctor-reported' side effects may be explained by recall bias: patients with worse LQOL may have a better recall of disadvantages and report side effects more often or more intense than those with a normal quality of life (while there may have been no actual difference during treatment).

Probably, LQOL in chronic Q fever patients is influenced by many factors. It is very well possible that both disease severity and perception of treatment play an important role in LQOL of chronic Q fever patients. This study highlights the large impact of chronic Q fever on LQOL, besides the high morbidity and mortality (van Roeden et al, unpublished data). Because of the high risk for mortality and complications, treatment is inevitable. Nevertheless, awareness of the consequences of the disease and its treatment on LQOL among clinicians is crucial: impaired QOL may have a bigger impact on patients than clinical outcomes, and acknowledgement of disease burden and disadvantages of treatment is important for patients [13]. The importance of acknowledgement of the consequences of the Q fever outbreak is painfully visible in the Netherlands, where patients have sued the Dutch government and goat farmers after the Q fever outbreak of 2007 – 2010. They were accused of negligence and mismanagement, patients felt misinformed and unacknowledged with regard to the impact of the disease on their lives [14]. The current study was conducted shortly before start of the juridical trial. However, it is possible that our study results may be influenced by the ongoing public debate.

Our study has several strengths. Data on QOL in chronic Q fever patients are scarce and the patients' perception on treatment of chronic Q fever was not described previously. Furthermore, we had the opportunity to assess clinical characteristics and their relation to the outcome, thanks to the extensive Dutch national chronic Q fever database, which contains detailed medical data of all chronic Q fever patients.
In this study, we did not have a comparison group. Very few patients were not treated with antibiotics, and those who are left untreated often do not have comparable disease severity (e.g. patients with possible chronic Q fever or probable chronic Q fever based on a risk factor without any symptoms or signs of infection). These patients are incomparable in terms of prognosis and disease burden. Therefore, we could not assess the effect of treatment itself on LQOL. To our knowledge, LQOL has not been studied in patients with endocarditis or infected vascular prostheses by other pathogens. Therefore, it is difficult to compare the impact of the disease to other comparable conditions. Furthermore, we did not compare the influence of different antibiotic regimens on LQOL: most patients used multiple regimens consecutively, and numbers per subgroup were small. Finally, questions regarding patients' perception on treatment have not been validated in prior studies, thus interpretation of these questions should be done cautiously. However, the answers to these questions provide an interesting insight in patients' perception during treatment and demonstrate the burden of treatment experienced by these patients.
LQOL in proven and probable chronic Q fever patients receiving antibiotic treatment is impaired in more than half of patients five years after diagnosis. Antibiotic treatment duration was the only variable that was associated with impaired QOL. The majority of patients did not notice improvement of their condition after start of treatment and most patients experienced disadvantages on a daily basis, highlighting the high burden of disease and treatment in these patients.

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References

1. Kampschreur LM, Hagenaars JC, Wielders CC et al. Screening for *Coxiella burnetii* seroprevalence in chronic Q fever high-risk groups reveals the magnitude of the Dutch Q fever outbreak. Epidemiol Infect. 2013;141(4):847-51.
2. Eldin C, Melenotte C, Mediannikov O et al. From Q fever to *Coxiella burnetii*: a paradigm change. Clin Microbiol Rev. 2017;30(1):115-90.
3. 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;11:97.
4. Maurin M, Raoult D. Q fever. Clin Microbial Rev. 1999; 12(4):518-53.
5. Anderson A, Bijlmer H, Fournier PE et al. Diagnosis and Management of Q fever--United States 2013: Recommendations from CDC and the Q fever Working Group. MMWR Recomm Rep. 2013; 62(RR-03):1-30.
6. Keijmel SP, van Kasteren ME, Blokx WA et al. Cutaneous hyperpigmentation induced by doxycycline: a case series. Neth J Med. 2015;73(1):37-40.
7. Hagenaars JCJP, Wever PC, Shamelian SO et al. Vascular chronic Q fever: quality of life. Epidemiol Infect. 2015;143(13):2903-9.
8. Wegdam-Blans MC, Kampschreur LM, Delsing CE et al. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Infect. 2012;64(3):247-59.
9. Kampschreur LM, Delsing CE, Groenwold RH et al. Chronic Q fever in the Netherlands 5 year after the start of the Q fever epidemic: results from the Dutch chronic Q fever database. J Clin Microbiol. 2014;52(5):1637-43.
10. Beck AT, Guth D, Steer RA et al. Screening for major depression disorders in medical inpatients with the Beck Depression Inventory for Primary Care. Behav Res Ther. 1997;35(8):785-91.
11. Diener E, Emmons RA, Larsen RJ et al. The Satisfaction With Life Scale. J Pers Assess. 1985;49(1):71-5.
12. Peters JB, Daudey L, Heijdra YF 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. Qual Life Res. 2009;18(7):902-12.
13. Higginson IJ, Carr AJ. Measuring Quality of life: using quality of life measures in the clinical setting. BMJ. 2001; 322:1297-300.
14. Website on the Q fever claim, http://www.qkoortsclaim.nl/, consulted on July 7th 2017.

Supplement 1: Questions on perception of treatment

The questions were originally in Dutch, the text was translated for this article.

1. **How did you feel during treatment?**
   - ☐ Much better compared to prior to treatment
   - ☐ Somewhat better compared to prior to treatment
   - ☐ Equal compared to prior to treatment
   - ☐ Somewhat worse compared to prior to treatment
   - ☐ Much worse compared to prior to treatment

2. **Did the advantages of treatment outweigh disadvantages of treatment in your opinion?**
   - ☐ Advantages outweigh disadvantages
   - ☐ Neutral
   - ☐ Disadvantages do not outweigh advantages

3. **How often did you experience disadvantages of treatment?**
   - ☐ Daily
   - ☐ Weekly
   - ☐ Monthly
   - ☐ Less often than once monthly
   - ☐ Never

Part II

Treatment of chronic Q fever

Chapter 5

Treatment of chronic Q fever: clinical efficacy and toxicity of antibiotic regimens

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Abstract

Background

Evidence on effectivity of first line treatment for chronic Q fever, tetracyclines (TET) plus hydroxychloroquine (HCQ), and potential alternatives is scarce.

Methods

We performed a retrospective, observational cohort study to assess efficacy of treatment with TET plus quinolones (QNL), TET plus QNL plus HCQ, QNL monotherapy or TET monotherapy compared to TET plus HCQ in chronic Q fever patients. We used a time-dependent Cox proportional hazards model to assess our primary (all-cause mortality) and secondary outcomes (first disease-related event and therapy failure).

Results

We assessed 322 chronic Q fever patients: 276 (86%) received antibiotics. Compared to TET plus HCQ (n=254;92%), treatment with TET plus QNL (n=49;17%), TET plus QNL plus HCQ (n=29, 10%), QNL monotherapy (n = 93;34%) or TET monotherapy (n=54;20%) were not associated with primary or secondary outcomes. QNL and TET monotherapy were frequently discontinued due to insufficient clinical response. (n=27;29% and n=32;59%). TET plus HCQ, TET plus QNL, and TET plus QNL plus HCQ were most frequently discontinued due to side effects (n=110;43%, n=13;27% and n=12;41%).

Conclusions

Treatment of chronic Q fever with TET plus QNL appears to be a safe alternative for TET plus HCQ, for example if TET plus HCQ cannot be tolerated due to side effects. Treatment with TET plus QNL plus HCQ was not superior to treatment with TET plus HCQ, although this may be caused by confounding by indication. Treatment with TET or QNL monotherapy should be avoided, switches because of subjective insufficient clinical response were frequently observed.

Introduction

Chronic Q fever is a zoonosis caused by the intracellular coccobacillus *Coxiella burnetii*.[1,2] Shortly after discovery of *C. burnetii*, it was noticed that penicillin was not beneficial as treatment for Q fever, in contrast to tetracyclines.[3] In the fifties, Q fever endocarditis was first recognized.[4,5] It was presumed that there was no definitive treatment, but that suppression was achieved with tetracyclines and chloramphenicol.[5]

In the nineties, treatment of chronic infection with tetracyclines plus quinolones was found to be more effective than treatment with tetracyclines alone.[6] Later, it was hypothesized that tetracyclines would be more effective combined with an alkalizing agent such as hydroxychloroquine, since *C. burnetii* replicates within macrophages and monocytes where the acidified phagosomal compartment decreases the bactericidal efficacy of antibiotics.[7]

Tetracyclines plus hydroxochloroquine was indeed found to be superior to tetracyclines plus quinolones in terms of relapse and treatment duration in a retrospective study of 35 patients.[8] However, evidence on superiority of tetracyclines plus hydroxychloroquine compared to other regimens is limited and inconsistent: in another study of 30 patients with vascular chronic Q fever, tetracyclines plus hydroxychloroquine was not superior to tetracyclines plus quinolones or tetracyclines alone in reducing mortality.[9] It is currently advised to treat chronic Q fever patients with tetracyclines plus hydroxychloroquine, with tetracyclines plus quinolones considered as a potential alternative.[8]

All available evidence on optimal treatment is derived from small, observational studies.[7-9] Both tetracyclines and hydroxychloroquine may cause significant toxicity, such as photosensitivity, retinopathy and black hyperpigmentation.[10-11] Therefore, there is need for additional evidence on treatment options for chronic Q fever.

Since chronic Q fever is rare, it is difficult to identify the optimal antibiotic treatment regimen regarding mortality, morbidity, and side effects. After a large Q fever outbreak in the Netherlands (2007-2010)[12], we identified all known patients with chronic Q fever and evaluated effectivity of different treatment strategies.

Methods

Data collection

We used data from the Dutch national chronic Q fever database. In this database, clinical, microbiological and imaging data are stored of all known chronic Q fever patients in the Netherlands. Registration started February 2011 and the last update ended May 2016. Data collection within this cohort was approved by the Medical Ethical Committee of the University Medical Centre in Utrecht, The Netherlands. All Dutch hospitals with microbiological laboratories were approached and asked to participate: twenty-eight hospitals participated, and all but one hospital in the Q fever epidemic area participated.

Patient inclusion

Patients ≥18 years of age at time of data collection with proven or probable chronic Q fever according to the definitions formulated by the Dutch chronic Q fever consensus group were included.[13] In the Netherlands, all patients are categorized according to these criteria after publishing a national guideline on diagnosis of chronic Q fever.[14] The criteria overlap substantially with the criteria formulated by Eldin et al, except for the fact that classification depends on likelihood of infection instead of focus of infection.[13,15] Diagnostic classification was reviewed in all patients by four investigators (CB, PW, JJO and SR). Possible chronic Q fever patients were not evaluated, since infection is not established in these patients and there is no indication for treatment. Microbiologists identified patients based on a positive polymerase chain reaction (PCR) on serum or tissue and/or *C. burnetii* phase I IgG antibodies ≥1:1024 in serum. Patients with a serological profile and clinical condition matching acute Q fever were excluded. Antibody titers were analyzed through indirect fluorescent-antibody assay (IFA) for phase I and II IgG against *C. burnetii* on plasma or serum (Focus Diagnostics, Inc., Cypress, CA, USA or Fuller Diagnostics, LLC., Anchorage, AK, USA). Titration of antibody levels was carried out at different hospital sites with dilutions on a binary scale with a cut-off of 1:32. Furthermore, results of PCR for *C. burnetii* DNA on serum or plasma were collected and, if applicable, on tissue samples (NucliSENS easyMAG; bioMérieux, Marcy l'Etoile, France).

Treatment regimens

Clinicians treated their patients based on national guidelines, local guidelines, literature or own experience. The Dutch national guideline advises doxycycline (200 mg once daily) plus hydroxychloroquine (200 mg 3 times daily), and suggests potential alternatives (either quinolones, rifampicin or co-trimoxazole combined with doxycycline).[16] We categorized and analyzed tetracyclines plus hydroxychloroquine (reference category), tetracyclines plus quinolone, tetracyclines plus quinolones plus hydroxychloroquine, tetracycline monotherapy, and quinolone monotherapy. Despite the overlap between tetracyclines plus quinolones plus hydroxychloroquine, and

tetracyclines with only hydroxychloroquine or quinolones, we analyzed this group separately because this treatment regimen is significantly more intensive. Antibiotics not belonging to any of these categories were integrated in the model as 'other antibiotics' and reported. Single antibiotics used in addition to the above regimens were reported but not analyzed separately. End of the observation period was defined as May 1st 2016. Thus, for patients still treated at the end of data collection (May 1st 2016) treatment duration was calculated until May 1st 2016.

Outcome and complications

The primary outcome of this study was all-cause mortality. Secondary outcomes were first disease-related event during treatment (new complication or chronic Q fever related-mortality) and therapy failure (new complication during treatment or a new positive PCR having been negative for at least 3 months or a persistent positive PCR for more than 6 months or chronic Q fever related-mortality). All-cause mortality was chosen as primary outcome, since this is the only endpoint not subject to subjectivity. Conditions considered complications of chronic Q fever were: rupture or dissection of aneurysm; acute symptomatic aneurysm; arterial fistula; endoleak of vascular prosthesis; spondyl(odisc)itis or osteomyelitis; (cardiac) abscess; cerebrovascular accident(hemorrhagic or ischaemic)/transient ischaemic attack; cardiac arrest or tamponade during pericarditis. Definitions of complications were based on complications occurring during intravascular infections reported in literature.[17-21]
Causes of death were reviewed by two investigators (CB en SR) and classification of the relationship between death and chronic Q fever was performed by reaching consensus. Cause of death was defined as definitely or probably related to chronic Q fever in case of active disease AND cause of death potentially related to chronic Q fever. Active disease was defined as *C. burnetii* phase I IgG ≥1:1024 or positive PCR on serum or tissue. Causes of death potentially related to chronic Q fever were defined as sepsis/feverish episode with no other cause; brain infarct or hemorrhage during endocarditis or due to cerebral aneurysm; arterial fistula; ruptured/dissected aneurysm; heart failure; fatal arrhythmia or cardiac arrest during endocarditis; surgical complications; side effects of antibiotic therapy; clinical deterioration during active disease with no other cause; Q fever as cause of death proven by autopsy; unknown cause in the presence of Q fever-related complications or unknown cause without adequate Q fever treatment. PCR relapse was defined as a newly positive PCR after stopping treatment.

Statistical methods

Categorical data were compared by use of the Fischer exact test or Chi-square as appropriate. Continuous variables were compared by use of the independent samples t-test or Mann-Whitney U test as appropriate.

To compare the effect of different antibiotics for chronic Q fever on the occurrence of primary and secondary outcomes, we used a Cox proportional hazards model

with the determinant (antibiotics for chronic Q fever) incorporated as categorical time-varying covariate. A time-varying covariate allows the studying of multiple consecutive treatment regimens over time within patients. The currently advised first-line treatment, tetracyclines plus hydroxychloroquine, was set as reference category. Propensity scores were integrated in the model to adjust for heterogeneity in prognosis and potential confounders. Variables were selected based on prior studies exploring which factors predict adverse outcomes in patients with chronic Q fever (van Roeden SE, manuscript in preparation). Additionally, surgical interventions and comorbidities influencing prognosis were included. In the propensity score for overall mortality, age, gender, immunocompromised state, presence of prosthetic material prior to chronic Q fever diagnosis, focus of infection, PCR-positivity, use of statins or platelet aggregation inhibitors, surgical intervention, pre-existing heart failure or renal insufficiency, smoking, diagnostic classification and time to start antibiotics after diagnosis were included. In the propensity score for the first disease-related events, equal variables were included with exception of surgical intervention since this may be an intermediate variable. In the propensity score for therapy failure, equal variables were included with again exception of surgical intervention and with exception of PCR-positivity, since this is incorporated in the outcome.

For combined endpoints, we specifically studied the first event, since consecutive events per patient are not independent. We calculated cause-specific hazards for all-cause mortality and subdistribution hazards for combined endpoints (first disease-related event and therapy failure), in order to optimally account for competing risks. In chronic Q fever, there is a delay between start of antibiotics and clinical effect. Therefore, the models were fitted with different 'lagged times' for treatment (0, 2 and 4 weeks). The model with the lowest Aikake's Information Criterion (AIC) was selected for analysis. This model had a lagtime of 4 weeks. Longer lagtimes were considered to be unrealistic, based on advice of experienced clinicians. Sensitivity analyses for the most nearby lagtimes (2 and 6 weeks) were performed to ensure robustness of the data. Potential left-censoring was accounted for by stratification for the presence of complications before start of treatment.

To correct for clustering of patients within the same hospital and differences between hospitals, a random effect for hospital was included by fitting shared-frailty terms in the model (assuming a Gaussian distribution of the frailty parameter).

The Cox-regression models were fitted with the "cmprsk" and "survival" packages in R studio, version 3.2.2. [22] The proportional hazard assumption was verified and confirmed both with formal tests and graphically, using Schoenfeld residuals. Descriptive data were generated with SPSS, version 21.0.

Missing data

In one patient, all data regarding covariates, exposure or outcome were missing. This patient was excluded of analysis. In all other patients, data regarding exposure and outcome were complete. Missing data on covariates, baseline characteristics or side

effects were described beneath concerning tables. In the Cox regression analysis, missing data on covariates were included as separate categories in the analysis, since it is unlikely that these data are missing at random.

Results

We identified 439 chronic Q fever patients. One patient was excluded because detailed data on treatment of this patient were missing and 116 patients were excluded because they had possible chronic Q fever. Of 322 remaining patients, 248 (77%) patients had proven chronic Q fever and 74 (23%) probable chronic Q fever. Baseline characteristics are shown in table 1.

Overview of therapy

Overall, 276 (86%) patients were treated with antibiotics. Tetracyclines plus hydroxychloroquine were most frequently prescribed (n=254; 92%): 232 (84%) patients started with tetracyclines plus hydroxychloroquine. A complete overview of antibiotic treatment regimens is presented in table 2. Treatment was de-escalated from a combination of antibiotics to single antibiotics in 64 patients (23%), intensified (from monotherapy to combination therapy) in 12 patients (4%), both in 56 patients (20%) and neither in the remaining 144 patients (52%). Reasons for withholding treatment from patients with proven chronic Q fever were refusal (n=4), death before start treatment (n=7), chronic Q fever not recognized by the clinician (n=6) and unknown (n=4). Reasons for withholding treatment from patients with probable chronic Q fever were refusal (n=4), chronic Q fever not recognized by the clinician (n=4), clinician doubted the diagnosis (n=6) and unknown (n=11).

Table 1. Baseline characteristics of patients with proven or probable chronic Q fever.

	All patients with	Proven	Probable
n (%)	322	248 (77)	74 (23)
Male gender (%)	248 (77)	191 (77)	57 (77)
Median age at diagnosis (interquartile range (IQR))	7 (65-75)	71 (64-78)	65 (57-74)
Focus of chronic Q fever			
endocarditis (%)	84 (26)	68 (27)	16 (22)
vascular (prosthesis) infection (%)	153 (48)	125 (50)	28 (38)
endocarditis and vascular focus combined (%)	43 (13)	40 (16)	3 (4)
other focus (%)*	10 (3)	7 (3)	3 (4)
no focus (%)	32 (10)	8 (3)	24 (32)
Comorbidity			
peripheral vascular disease (%)	54 (17)	43 (17)	11 (15)
ischemic cardiac disease (%)	105 (33)	88 (35)	17 (23)
cerebrovascular disease (%)	50 (16)	40 (16)	10 (14)
diabetes mellitus (%)	46 (14)	32 (13)	14 (19)
presence of prosthetic material prior to complication (%)	166 (52)	142 (57)	24 (32)
immunocompromised state (%)†	48 (15)	33 (13)	15 (20)
Course of disease			
median follow-up duration in years (IQR)	3·9 (1·7 – 5·3)	3·6 (1·4 - 5·2)	4·6 (2·6 - 5·5)
Deceased (%)	109 (34)	93 (38)	16 (22)
related to Q fever (%)	65 (20)	62 (25)	3 (4)
median time to death from diagnosis in years (IQR)	1·4 (0·3 – 2·7)	1·3 (0·2 – 2·6)	2·4 (0·9 – 2·8)
Complications of Q fever			
no. patients with complications (%)	163 (51)	152 (61)	11 (15)
median time to 1st complication from diagnosis in years (IQR)	0 (0 - 0·3)	0 (0 - 0·3)	0 (0 - 0·7)
complication before initiation of treatment (%)‡	101 (62)	98 (64)	3 (27)
PCR-positivity			
PCR+ anytime during disease (%)	141 (44)	141 (57)	-
PCR+ relapse after stop of treatment (%)	5 (2)	5 (2)	-

**other foci: 4 placenta, 2 pericarditis, 2 pulmonary, 1 pleuritis, 1 combined endocarditis and pericarditis, 1 spondylodiscitis with no other focus. †immunocompromised state: 39 patients using immunosuppressives (usage of >5 mg prednisone daily > 30 days or cumulative dosage exceeding 750 mg, azathioprine, methotrexate, TNF-α blockers, mycophenolic acid, cyclosporine, sulfasalazine or a combination of these),1 patient with chemotherapy for non-hematological malignancy, 5 patients with hematological malignancies with cytopenia or requiring treatment (hairy cell leukemia, myelodysplastic syndrome, chronic lymphatic leukemia and non-Hodgkin lymphoma), 4 patients post-splenectomy and 2 patients with IgG deficiency/ common variable immunodeficiency. Three patients fulfill two categories: all three with hematological malignancies (2 with immunosuppressive medication for other diseases and 1 post-splenectomy). ‡Not applicable in 23 patients.*

Table 2. Overview of antibiotic treatment of chronic Q fever patients.

	All patients with	Proven	Probable
N	322	248 (77)	74 (23)
Patients receiving antibiotics (%)	276 (86)	227 (92)	49 (66)
Duration of treatment			
median number of antibiotic regimens (IQR)	2 (1-3)	2 (1-3)	1 (1-2)
median time to start antibiotics from diagnosis, in weeks (IQR)	1 (0 – 3)	1 (0 – 2)	1 (0 – 8)
median total treatment duration, in weeks (IQR)	95 (58 – 140)	96 (57 – 149)	83 (58 – 117)
antibiotics stopped, all reasons including death (%*)†	202 (73)	162 (71)	40 (82)
Antibiotics			
tetracyclines/hydroxochloroquine (%)*‡	254 (92)	210 (93)	44 (90)
median total duration, weeks (IQR)	63 (16 – 96)	64 (15 – 96)	61 (29 – 96)
tetracyclines/quinolones (%)*§	49 (17)	42 (19)	7 (14)
median total duration, weeks (IQR)	44 (8 - 70)	39 (9 - 72)	44 (2 - 54)
tetracyclines/quinolones/hydroxychloroquine (%)*‖	29 (10)	28 (12)	1 (2)
median total duration, weeks (IQR)	29 (7 - 57)	31 (9 - 58)	1
tetracyclines (%)*	54 (20)	43 (19)	11 (22)
median total duration, weeks (IQR)	7 (2 – 47)	7 (2 – 47)	21 (2 – 48)
quinolones (%)*¶	93 (34)	78 (34)	15 (31)
median total duration, weeks (IQR)	33 (10 – 83)	34 (9 – 83)	25 (10 – 88)
other(%)*††	56 (20)	49 (22)	7 (14)
median total duration, weeks (IQR)	37 (10 – 98)	37 (13 – 100)	4 (3 – 77)

*Tetracyclines prescribed: doxycycline for 267 patients, minocycline for 5 patients. Quinolones prescribed: moxifloxacine for 104 patients, ciprofloxacin for 58 patients, ofloxacine for 1 patient (multiple tetracyclines or quinolones within one individual possible). 100% of patients on doxycycline started on standard dosage (200 mg daily), dosage was adjusted later on in 29%. 95% of patients on hydroxychloroquine started on standard dosage (600 mg daily), dosage was adjusted later on in 22%. Ciprofloxacine was started in an alternative dosage in 43% of patients (adjusted on renal function), dosage was adjusted later on in 16%. Moxifloxacine was started on standard dosage in 99% of patients, dosage was adjusted later on in 2% of patients. *percentage of patients receiving treatment. †Of those finished, 185 were treated with tetracyclines plus hydroxychloroquine, 34 with tetracyclines plus quinolones, 13 with tetracyclines plus quinolones plus hydroxychloroquine (some patients used multiple combinations). ‡simultaneous use of other (combination of)antibiotics >1 month in 7 patients (4 co-trimoxazole, 1 clindamycine, 3 rifampicine, 1 clarithromycin). §simultaneous use of (combination of) other antibiotics >1 month in 2 patients (2 rifampicine). ‖simulataneous use of (combination of) other antibiotics >1 month in 2 patients (1 rifampicine, 1 clindamycine, 1 co-trimoxazole). ¶simultaneous use of (combination of)other antibiotics >1 month in 19 patients (10 rifampicine, 6 hydroxychloroquine, 2 co-trimoxazole, 1 clarithromycin). ††Other antibiotics: 19 claritromycin, 16 cotrimoxazole, 8 hydroxychloroquin, 1 clindamycin, 1 azitromycin/clindamycin, 1 erytromycin/co-tirmoxazole, 1 claritromycin/co-trimoxazole, 14 doxycyclin with other than quinolone or hydroxychloroquine (7 rifampicin, 2 chloroquine, 2 co-trimoxazole, 1 claritromycin, 1 rifampicin/co-trimoxazole, 1 rifampicin/clarithromycin).*

Clinical outcomes

Numbers of events occurring during different antibiotics regimens are shown in table 3. Treatment with tetracyclines plus quinolones was not associated with all-cause mortality (HR1·07; 95%CI 0·37-3·14, p=0·90), first disease-related event (HR1·51; 95%CI 0·66-3·47, p=0·33) or therapy failure (HR0·93; 95%CI 0·39-2·19, p=0·86) as compared to treatment with tetracyclines plus hydroxychloroquine. Treatment with tetracyclines plus quinolones plus hydroxychloroquine, tetracycline monotherapy or quinolone monotherapy was neither associated with primary or secondary outcomes (table 3). Sensitivity analyses for the most nearby lagtimes (2 and 6 weeks) did not change the hazard ratios significantly. None of the outcomes were associated with de-escalation or intensifying treatment in univariable analysis.

Antibiotic changes and discontinuation

Multiple consecutive antibiotic regimes were used by 161 (58%) patients (supplementary figure 1). Tetracyclines plus hydroxychloroquine was most frequently discontinued due to side effects (n=110; 43%), followed by tetracyclines plus quinolones plus hydroxychloroquine (n=12; 41%). Tetracycline monotherapy and quinolone monotherapy were most often discontinued due to insufficient clinical response (n=32; 59% and n=27; 29%), see table 4.

Side effects

Side effect were most frequently observed during treatment with tetracyclines plus quinolones plus hydroxychloroquine (n=24; 83%), tetracyclines plus quinolones (n=40; 82%) and tetracyclines plus hydroxychloroquine (n=190; 75%). Severity of side effects is described in table 4. In three patients treated with tetracyclines plus hydroxychloroquine, side effects were considered potentially fatal (n=3; 1%): all three cases were due to severe dehydration based on gastro-intestinal side effects. Most frequently reported side effects were gastro-intestinal complaints and photosensitivity.

Table 3. Number of events and (subdistribution) hazard ratio's for primary and secondary outcomes.

Endpoint / Treatment strategy	Number of events	HR	95%CI	p-value
All-cause mortality*		**HR**		
Reference: TET/HCQ	30	1·00	n/a	n/a
TET/QNL	5	1·07	0·37 - 3·14	0·90
TET/QNL/HCQ	3	2·19	0·73 - 6·56	0·16
TET	3	0·70	0·21 - 2·36	0·57
QNL	14	1·60	0·76 - 3·39	0·22
First disease-related event†		**SHR**		
Reference: TET/HCQ	44	1·00	n/a	n/a
TET/QNL	7	1·51	0·66 - 3·47	0·33
TET/QNL/HCQ	2	0·94	0·22 - 3·96	0·93
TET	7	1·27	0·53 - 3·06	0·59
QNL	12	1·22	0·57 - 2·59	0·61
Therapy failure‡		**SHR**		
Reference: TET/HCQ	60	1·00	n/a	n/a
TET/QNL	7	0·93	0·39 - 2·19	0·86
TET/QNL/HCQ	4	1·21	0·37 - 3·96	0·75
TET	9	1·43	0·67 - 3·04	0·35
QNL	15	1·26	0·68 - 2·35	0·47

*Time-varying Cox-regression analysis. HR= hazard ratio. SHR: subdistribution hazard ratio. TET=tetracycline. QNL=quinolone. HCQ = hydroxychloroquine.*significant covariate: therapy exposure category 'treatment finished' (HR 0·36, 0·18 – 0·75, p<0·01) and propensity score (HR 18.74 per point increase, 95% CI 7.54 – 46.55, p<0.01). Mean propensity score deceased: 0.48; mean propensity score non-deceased 0.26. †significant covariate: therapy exposure category 'treatment finished' (HR 0·40, 0·20 – 0·80, p<0·01) and propensity score (HR12.32 per point increase, 95%CI 5.01 – 30.30, p <0.01). Mean propensity score with event: 0.53; mean propensity score without event 0.30. ‡significant covariate: therapy exposure category 'treatment finished' (HR 0·32, 0·17 – 0·61, p<0·01) and propensity score (HR9.71 per point increase, 95%CI 4.04 – 23.33, p <0.01). Mean propensity score with therapy failure: 0.59; mean propensity score without therapy failure: 0.36.*

Table 4. Severity and nature of side effects and reasons for switch or stop.

Endpoint / Treatment strategy	TET/HCQ	TET/QNL	TET/QNL	TET	QNL
n (%)*	254 (92)	49 (18)	29 (11)	54 (20)	93 (34)
Severity of side effects					
any (%)	190 (75) †	40 (82)‡	24 (83)‡	31 (57)§	41 (44)‖
resulting in stop antibiotics (%)	110 (43)	13 (27)	12 (41)	7 (13)	25 (27)
leading to hospitalization (%)	17 (7)	1 (2)	-	6 (11)	2 (2)
leading to permanent damage (%)	16 (6)	1 (2)	-	-	1 (1)
life threatening (%)	5 (2)	**2 (4)**	3 (10)	-	3 (3)
fatal (%)	3 (1)¶	-	-	-	-
Specification of side effect					
gastro-intestinal	101 (40)	23 (47)	9 (31)	23 (43)	19 (20)
photosensitivity	81 (32)	18 (37)	11 (38)	10 (19)	-
hyperpigmentation	13 (5)	1 (2)	3 (10)	3 (6)	-
ocular toxicity	10 (4)	-	-	-	-
psychological side effects	13 (5)	5 (10)	3 (10)	3 (6)	9 (10)
decreased renal function	7 (3)	1 (1)	-	-	1 (1)
Other	35 (14)	9 (19)	9 (31)	2 (4)	11 (12)
Reason for stop/switch					
side effects	110 (43)	13 (27)	12 (41)	7 (13)	25 (27)
deceased	30 (12)	5 (10)	3 (10)	3 (6)	11 (12)
adequate treatment finished	63 (25)	11 (22)	4 (14)	8 (15)	15 (16)
insufficient clinical response	31 (12)	8 (16)	3 (10)	32 (59)	27 (29)
end of observationperiod (treatment unfinished)	29 (11)	5 (10)	8 (28)	4 (7)	19 (20)
other reason	38 (15)	10 (20)	1 (3)	8 (15)	11 (12)

*TET=tetracycline. QNL=quinolone. HCQ = hydroxychloroquine. *percentage of treated patients with proven or probable chronic Q fever, n = 276. †missing data in 9 patients. ‡missing data in 1 patient. §missing data in 3 patients. ‖missing data in 2 patients. ¶One case of severe dehydration due to diarrhea induced by antibiotics, deceased due to heart failure after rehydration. Two cases of severe dehydration due to diarrhea with renal failure and consequential death.*

Course of disease after stop of treatment

In total, 161 patients finished antibiotic treatment before the end of study (not because of death) or paused antibiotic treatment at some point. Thus, 161 patients were at risk for PCR-positive relapse or chronic Q fever-related mortality after stop of treatment (table 5). We observed 5 patients (3%) with PCR-positive relapse and 16 chronic Q fever-related deaths (10%) after stop of antibiotics. Patients with these events were significantly less often treated with 18 months of tetracyclines plus hydroxychloroquine or tetracyclines plus quinolones or tetracyclines plus quinolones plus hydroxychloroquine (n=3; 14%) compared to patients without these

events (n=75; 54%, p=0·001). Total treatment duration was significantly longer in patients without PCR-positive relapse or chronic Q fever-related mortality after stop of treatment (median 101 weeks; IQR 79-136) compared to patients with these events (median 71; IQR 19-99, p=0·02). In a supplementary table 1, detailed clinical characteristics of patients that died of chronic Q fever-related causes or had PCR-positive relapse after stop of antibiotics are shown.

Table 5. Chronic Q fever-related mortality or PCR-positive relapses after stop of treatment.

	TET/HCQ	TET/QNL	TET/QNL
N (patient treated with antibiotics) (%)	276 (86)	227 (92)	49 (66)
N (antibiotics stopped before May 1st 2016 or paused during treatment) (%)	161 (58)	124 (55)	37 (76)
chronic Q fever-related mortality after stop antibiotics (%)	16 (10)	14 (11)	2 (5)
median time to death after stop antibiotics in weeks (IQR)	8 (1 - 51)	3 (0 - 57)	34 – 54*
PCR-positive relapse after stop antibiotics (%)	5 (3)	**5 (4)**	-
median time to PCR-positive relapse after stop antibiotics in weeks (IQR)	6 (4 - 46)	6 (4 - 46)	-
chronic Q fever-related mortality or PCR-positive relapse after stop antibiotics (%)	21 (13)	19 (15)	2 (5)
median time to chronic Q fever-related mortality or PCR-positive relapse after stop antibiotics (IQR)	22 (2 - 54)	**11 (1 - 53)**	34 – 44*
total treatment duration (median, IQR)	71 (19 - 99)	58 (18 - 102)	81 - 82*
treatment duration with TET/HCQ (median, IQR)	19 (4 - 56)	15 (2 - 54)	44 - 51*
treatment duration with TET/HCQ, TET/QNL or TET/QNL/HCQ (median, IQR)	44 (7 - 62)	33 (5 - 65)	44 - 52*
Reason for stop†			
sufficient clinical response (%)	7 (33)	5 (26)	2 (100)
side effects (%)	8 (38)	8 (42)	-
refusal treatment by patient (%)	3 (14)	3 (16)	-
other reasons (%)	1 (5)	1 (5)	-
unknown (%)	3 (14)	3 (16)	-
Cause of death			
acute aneurysm (%)	5 (31)	4 (29)	1 (50)
sepsis/feverish episode e.c.i., no other pathogen (%)	1 (6)	1 (7)	-
cardiac complications (%)	2 (13)	2 (14)	-
surgical complications, Q fever-related (%)	3 (19)	3 (21)	-
severe side effects (%)	1 (6)	1 (7)	-
insufficient treatment and active disease, clinical deteriortation or cause unknown (%)	4 (25)	3 (19)	1 (50)

*TET= tetracycline. QNL=quinolone. HCQ=hydroxychloroquine. Half weeks truncated to whole weeks. †proportion of patients with chronic Q fever related mortality or PCR-positive relapse after stop of antibiotics. Multiple reasons for stop within 1 patient possible. *instead of median and IQR: minimum and maximum value since there are only 2 patients.*

Discussion

Treatment of chronic Q fever with tetracyclines plus quinolones was not inferior to treatment with tetracyclines plus hydroxychloroquine in terms of all-cause mortality, chronic Q fever-related events or therapy failure. Therefore, tetracyclines plus quinolones seems to be a viable alternative for treatment with tetracyclines plus hydroxychloroquine, for example if the initial therapy cannot be tolerated due to side effects. This is in line with other studies, showing that treatment with tetracyclines plus quinolones is equally effective in terms of mortality.[8,9] Treatment with tetracyclines plus quinolones plus hydroxychloroquine, tetracycline or quinolone monotherapy was neither associated with any of the outcomes.

Since this is a retrospective study, treatment decisions were unstandardized. As a consequence, there is a substantial risk of confounding by indication. Theoretically, patients with most severe disease could be switched more often to combination therapy, leading to underestimation of the effect of these treatment strategies. Patients with mild disease are more likely to be switched to monotherapy, resulting in favorable outcome regardless of the treatment strategy. Treatment with tetracycline or quinolone monotherapy was not inferior to the reference treatment. However, conclusions should be drawn with caution. The fact that patients treated with quinolone monotherapy (29%) or tetracycline monotherapy (59%) were frequently switched to other therapies due to insufficient clinical response, subjectively interpreted by the treating clinician, emphasizes the importance of vigilant interpretation.

After stopping treatment, chronic Q fever-related mortality or PCR-positive relapses were observed in 13% of patients. These patients were significantly less often treated with 18 months of appropriate antibiotics compared to patients without these events after stop of antibiotics, which underlines the importance of adequate treatment.

Beside treatment effectivity, we described adverse drug reactions and reasons for switching. Patients switched frequently between different treatment strategies. The proportion of patients experiencing side effects was high, especially for combination regimens. Correspondingly, combination regimens were most often discontinued due to side effects, which highlights the toxicity of these regimens.

The major strength of this study is the large number of patients studied. Chronic Q fever is rare and difficult to diagnose. Earlier, at most 35 patients have been studied.[6,8,9] Furthermore, effectivity of treatment in terms of complications has not been studied before, making this a unique study. Moreover, all chronic Q fever patients are diagnosed in a standardized way and clinical data were well documented. Finally, median follow-up duration is almost four years, providing adequate follow-up. Hence, this well-documented study in this comprehensive cohort provides the best available evidence on treatment of chronic Q fever patients at this moment. All patients diagnosed with chronic Q fever were included, since we selected our patients based on microbiological results. Naturally, undiagnosed chronic Q fever patients and patients in non-collaborating hospitals were missed. Since the participation of hospitals in the endemic area is near-complete, we probably identified most Dutch

chronic Q fever cases in the past ten years.
The most important drawback of this study is the observational nature. To overcome the issues of a non-experimental study we used advanced methodologically techniques, including different subsequent treatment strategies and a lagtime of the effect. Yet despite all these efforts, bias might still be present. Careful interpretation of the results is therefore essential. Moreover, we chose outcome based on hard clinical endpoints but did not use serological course as an outcome measure. There is limited evidence suggesting that the course of phase I IgG titers predicts outcomes. [23] Furthermore, we did not account for progression of complications. Serological course and progression of complications detected by imaging studies could explain the proportion of patients that stopped quinolone monotherapy due to insufficient clinical response as judged by the clinician, without newly diagnosed complications or mortality. Another limitation is that data on occurrence of side effects were retrieved from patient records, potentially leading to underestimation of mild side effects due to underreporting. Finally, we did not model antibiotic dosage or compliance. Due to the complexity of the model, this would lead to uninterpretable results.

In conclusion, treatment of chronic Q fever with tetracyclines plus quinolones appears to be a safe alternative, for example if tetracyclines plus hydroxychloroquine cannot be tolerated. Treatment with tetracyclines plus quinolones plus hydroxychloroquine was not superior to treatment with tetracyclines plus hydroxychloroquine, although this may be caused by confounding by indication. Treatment with tetracycline or quinolone monotherapy should be avoided, switches because of subjective insufficient clinical response were frequently observed.

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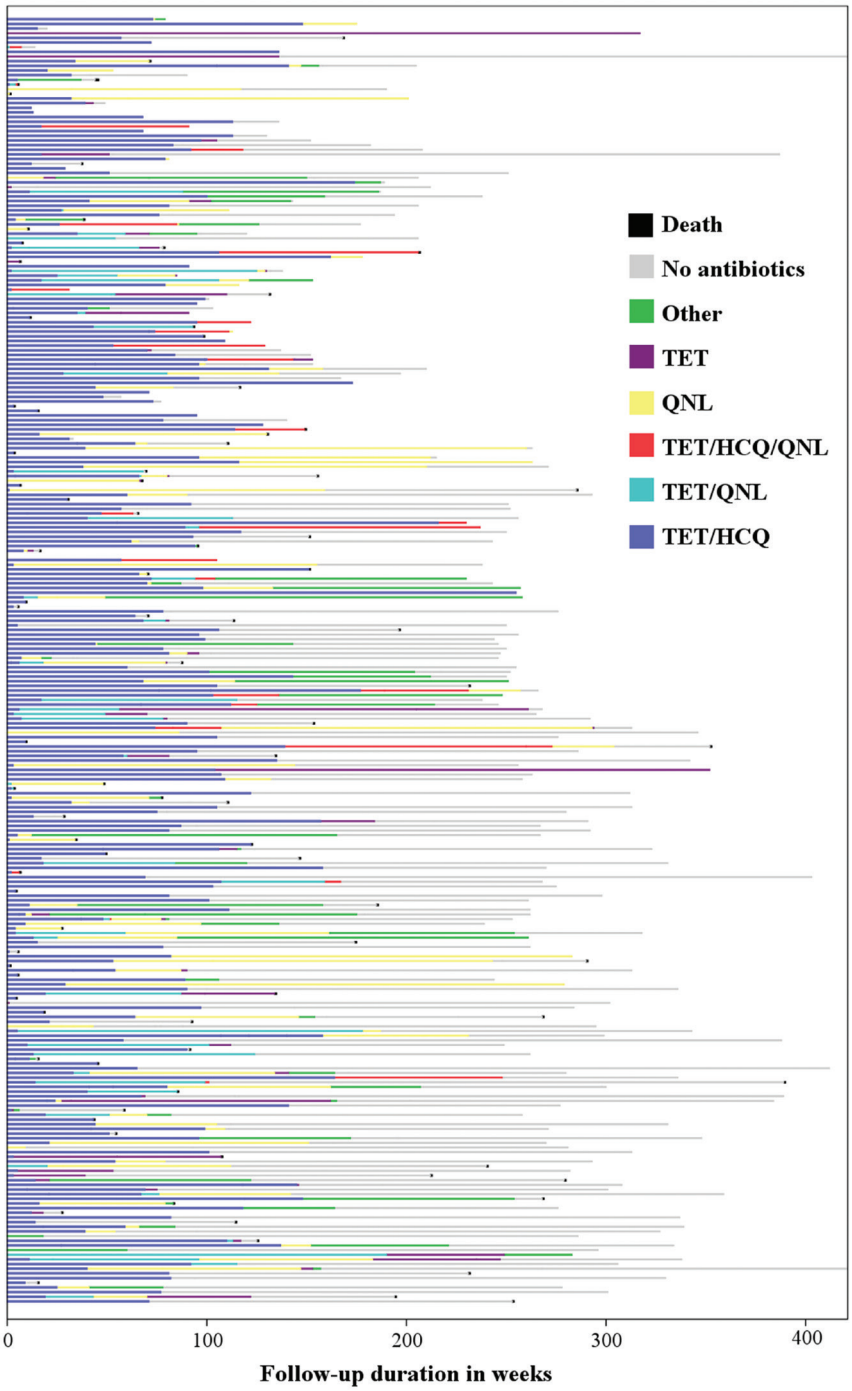
References

1. Tissot-Dupont H, Raoult D. Q fever. Infect Dis Clin North Am, 2008; 22(3): 505-14.
2. Kampschreur LM, Delsing CE, Groenwold RH, Wegdam-Blans MC, Bleeker-Rovers CP, de Jager-Leclercq MG *et al*. Chronic Q fever in the Netherlands 5 year after the start of the Q fever epidemic: results from the Dutch chronic Q fever database. J Clin Microbiol, 2014; 52(5): 1637-43.
3. Clark WH, Lennette EH. Antibiotics in Q fever. Ann N Y Acad Sci, 1952; 55(6): 1004-18.
4. Baylon H, Bloch F, Giroud P, Coumel H. The cardiovascular manifestations and the evolution of rickettsial diseases. Bull Mem Soc Med Hop Paris, 1952; 68(1-2): 67-72.
5. Kristinsson A, Bentall HH. Medical and surgical treatment of Q-fever endocarditis. The Lancet, 1967; 2(7518): 693-7.
6. Levy PY, Drancourt M, Etienne J, Auvergnat JC, Beytout J, Sainty JM et al. Comparison of different antibiotic regimens for therapy of 32 cases of Q fever endocarditis. Antimicrob Agents Chemother, 1991; 35(3): 533-7
7. Maurin M, Benoliel AM, Brongrand P, Raoult D. Phagolysosomal alkalinization and the bactericidal effect of antibiotics: the *Coxiella burnetii* paradigm. J Infect Dis, 1992; 166(5): 1097-102.
8. Raoult D, Houpikian P, Tissot Dupont H, Riss JM, Arditi-Djiane J, Brouqui P. Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydroxychloroquine. Arch Int Med, 1999; 159(2): 167-73.
9. 9. Botelho-Nevers E, Fournier PE, Richet H, Fenollar F, Lepidi H, Foucault C *et al. Coxiella burnetii* infection of aortic aneurysms or vascular grafts: report of 30 new cases and evaluation of outcome. Eur J Clin Microbiol Infect Dis, 2007; 26(9): 635-40.
10. Anderson A, Bijlmer H, Fournier PE, Graves S, Hartzell J, Kersch GJ *et al*. Diagnosis and Management of Q fever – United States 2013: Recommendations from CDC and the Q fever Working Group. MMWR Recomm Rep, 2013; 62(RR-03): 1-30.
11. Keijmel SP, van Kasteren ME, Blokx WA, van der Meer JW, van Rossum M, Bleeker-Rovers CP. Cutaneous hyperpigmentation induced by doxycycline:a case series. Neth J Med, 2015; 71(1): 37-40.
12. Kampschreur LM, Hagenaars JC, Wielders CC, Elsman P, Lestrade PJ, Koning OH *et al*. Screening for *Coxiella burnetii* seroprevalence in chronic Q fever high-risk groups reveals the magnitude of the Dutch Q fever outbreak. Epidemiol Infect, 2013; 141(4): 847-51.

13. Wegdam-Blans MC, Kampschreur LM, Delsing CE, Bleeker-Rovers CP, Sprong T, van Kasteren ME et al. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Infect, 2012; 64: 247-59.
14. Wegdam-Blans MC, Kampschreur LM, Nabuurs-Franssen MH, Renders NHM, Delsing CE, Bijlmer HA. Richtlijnen: Nederlandse consensus chronische Q-koorts. Tijdschr Infect, 2011; 6: 71-3.
15. Eldin C, Melenotte C, Mediannikov O, Ghigo E, Million M, Edouard S *et al*. From Q fever to *Coxiella burnetii* infection: a paradigm change. Clin Microbiol Rev, 2017; 30(1): 115-90.
16. Dutch coordination for infectious diseases control (LCI). LCI guideline Q fever. Available at: http://www.rivm.nl/Documenten_en_publicaties/Professioneel_Praktisch/Richtlijnen/Infectieziekten/LCI_richtlijnen/LCI_richtlijn_Q_koorts. Accessed 4 May 2017.
17. Mansur AJ, Grinberg M, da Luz PL, Bellotti G. The complications of infective endocarditis: a reappraisal in the 1980's. Arch Intern Med, 1992; 152(12): 2428-32.
18. Malhotra A, Rayner J, Williams TM, Prendergast B. Infective endocarditis: therapeutic options and indications for surgery. Curr Cardiol Rep, 2014; 16: 464-70.
19. Smeds MR, Duncan AA, Hartlander-Locke MP, Lawrence PF, Lyden S, Fatima J, et al. Treatment and outcomes of aortic endograft infection. J Vasc Surg, 2016; 63: 332-40.
20. Morelli S, Carmenini E, Caporossie AP, Aguglia G, Bernardo ML, Gurgo AM. Spondylodiscitis and infective endocarditis: case studies and review of the literature. Spine, 2001; 26(5): 499.500.
21. Imazio M, Gaita F, LeWinter M. Evaluation and treatment of pericarditis: a systematic review. JAMA, 2015; 314(14): 1498-1506.
22. Packages available at: Cran.R-project.org. Accessed 1 September 2016.
23. Million M, Thuny F, Richet H, Raoult D. Long-term outcome of Q fever endocarditis: a 26-year personal survey. Lancet Infect Dis, 2010; 10(8): 527-35.

Supplementary figure 1. Individual treatment timelines and time of death.



*TET=tetracycline. QNL=quinolone. HCQ = hydroxychloroquine.

Supplementary table 1. Overview of patients with PCR-positive relapse or chronic Q fever-related mortality after stop of treatment.

ID	Sex	Classification	Focus of infection	Complications	Reason stop	Duration treatment	Duration TET/HCQ or TET/QNL*†	PCR-positive relapse / chronic Q fever-related	Time of event after stop antibiotics*	Cause of death
1	M	Proven	Vascular	Yes	Side-effects	45	45	CQF-related mortality	1	Side effects
2	M	Proven	Vascular & endocarditis	Yes	Sufficient response	155	65	CQF-related mortality	42	Surgical complications (valve repair)
3	F	Proven	Endocarditis	Yes	Side-effects	21	21	CQF-related mortality	160	Cardiac complication
4	M	Proven	Vascular	Yes	Unknown	96	94	CQF-related mortality	1	Surgical complications (vascular prosthesis)
5	M	Proven	Vascular	Yes	Unknown	5	5	CQF-related mortality	1	Surgical complications (vascular prosthesis)
6	M	Proven	Endocarditis	Yes	Side-effects	103	54	PCR-positive relapse	6	n/a
7	F	Proven	Vascular	No	Side-effects	1	1	PCR-positive relapse	6	n/a
8	M	Probable	Endocarditis	No	Sufficient response	81	60	CQF-related mortality	54	Insufficient treatment and active disease, cause unknown
9	M	Proven	Vascular	Yes	Side-effects & Refusal patient	10	10	CQF-related mortality	5	Insufficient treatment and active disease, clinical deterioration
10	M	Proven	Endocarditis	Yes	Sufficient response	15	15	CQF-related mortality	101	Cardiac complication
11	M	Proven	Vascular	Yes	Refusal patient	18	12	CQF-related mortality	11	Acute aneurysm
12	F	Proven	Vascular & endocarditis	Yes	Other	77	67	CQF-related mortality	60	Acute aneurysm
13	F	Proven	None	No	Sufficient response	71	0	PCR-positive relapse	53	n/a
14	M	Proven	Vascular	Yes	Side-effects	206	56	PCR-positive relapse	3	n/a
15	M	Proven	Vascular	Yes	Sufficient response	58	58	CQF-related mortality	112	Acute aneurysm
16	M	Proven	Vascular	No	Unknown	35	1	CQF-related mortality	37	Acute aneurysm
17	M	Proven	Endocarditis	Yes	Sufficient response	118	118	PCR-positive relapse	38	n/a
18	M	Probable	Vascular	No	Sufficient response	84	44	CQF-related mortality	34	Acute aneurysm
19	M	Proven	Vascular	Yes	Side-effects	42	33	CQF-related mortality	22	Insufficient treatment and active disease, cause unknown
20	M	Proven	Vascular	Yes	Refusal patient	78	2	CQF-related mortality	1	Insufficient treatment and active disease, cause unknown
21	M	Proven	Other	Yes	Side-effects	134	87	CQF-related mortality	1	Sepsis e.c.i., no other pathogen

**in weeks, truncated to whole weeks. †TET=tetracycline. HCQ = hydroxychloroquine. QNL=quinolone.*

Chapter 6

The effect of measuring serum doxycycline concentrations on clinical outcomes during treatment of chronic Q fever

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Abstract

Background

First choice treatment for chronic Q fever is doxycycline plus hydroxychloroquine. Serum doxycycline concentrations (SDC) >5 µg/mL were associated with a favorable serological response, but the effect on clinical outcomes is unknown.

Objectives

To assess the effect of measuring SDC during treatment of chronic Q fever on clinical outcomes.

Methods

We performed a retrospective cohort study, to assess the effect of measuring SDC on clinical outcomes in patients treated with doxycycline and hydroxychloroquine for chronic Q fever. Primary outcome was the first disease-related event (new complication or chronic Q fever-related mortality); secondary outcomes were all-cause mortality and PCR-positivity. Multivariable analysis was performed with a Cox proportional hazards model, with shared-frailty terms for different hospitals included.

Results

We included 201 patients (mean age 68 years, 83% male): in 167 patients (83%) SDC were measured, 34 patients (17%) were treated without SDC measurement. First SDC was >5 µg/mL in 106 patients (63%), all with 200 mg doxycycline daily. In patients with SDC measured, dosage was adjusted in 41% (n=68), concerning an increase in 64 patients. Mean SDC was 4.1 µg/mL before dosage increase, and 5.9 µg/mL afterwards. SDC measurement was associated with a lower risk for disease-related events (HR0.51, 95%CI 0.26-0.97, p = 0.04), but not with all-cause mortality or PCR-positivity.

Conclusion

SDC measurement decreases the risk for disease-related events, potentially through more optimal dosing or improved compliance. We recommend measurement of SDC and striving for SDC >5 µg/mL and <10 µg/mL during treatment of chronic Q fever.

Introduction

Q fever is caused by the intracellular Gram-negative bacterium *Coxiella burnetii*. After primary infection, approximately 60% of patients remain asymptomatic. The remaining patients develop illnesses, such as flu-like symptoms or pneumonia.[1] A small proportion of patients develop chronic Q fever after primary infection.[1] In contrast to acute Q fever, chronic Q fever is a potential life-threatening disease. Most often, it leads to endocarditis, infection of vascular prostheses or arterial aneurysms.[1] The recommended first line treatment for chronic Q fever is doxycycline combined with hydroxychloroquine for at least 18 months, or alternatively doxycycline combined with a quinolone.[2-5] Culturing of *C. burnetii* is not routinely performed in practice, since it is difficult and only allowed in laboratories with a biohazard safety level 3 facility.[6-8] Therefore, monitoring of treatment effect mainly depends on measurement of antibody titers, monitoring of PCR for *C. burnetii* on blood or tissue, and follow-up of the patients' clinical condition.[1]

To assess adequacy of doxycycline dosage and compliance to therapy, measurement of serum doxycycline concentrations (SDC) is a potential tool. In two clinical studies, SDC >5 µg/mL were associated with favorable serological response, and higher SDC to MIC ratios were associated with rapid decline in phase I IgG antibodies to *C. burnetii* in another study.[9-11] This can be explained by doxycycline being more effective in higher concentrations, or resistance leading to decreased effectiveness in patients with lower SDC.[12-14] In two reports, resistance has been reported in 6-23% of isolates. [9,14]

The effect of measuring SDC on clinical endpoints such as complications and mortality and PCR-positivity, has not been studied before. By evaluating data from a nationwide cohort of chronic Q fever patients, we aimed to assess the effect of treatment with dosage based on SDC, as performed in clinical practice, for these clinical endpoints in chronic Q fever patients treated with doxycycline and hydroxychloroquine.

Materials and methods

Data from the Dutch national chronic Q fever database were used: in this database, detailed data regarding treatment of patients with proven or probable chronic Q fever diagnosed since the start of the Dutch Q fever outbreak (January 1st 2007) are recorded. With these data, we retrospectively assessed the effect of measuring SDC in patients that were treated with doxycycline and hydroxychloroquine for at least 12 weeks. Since this is retrospective study and action taken by clinicians on SDC results is not standardized, we could not study the effect of SDC values on clinical outcomes: clinicians intervene based on SDC values and may aim for higher values in patients with more severe disease, which leads to bias. Therefore, we study the effect of a treatment strategy in which dosage adjustment and coaching regarding compliance is based on SDC.

Data collection and inclusion of patients

Design of the Dutch national chronic Q fever database was approved by the Medical Ethical Committee of the University Medical Center in Utrecht. This database contains complete follow-up data of all chronic Q fever patients ≥18 years of age in the Netherlands from January 1st 2007 to May 1st 2016, from 28 hospitals in the Netherlands (see appendix 1). Diagnosis and classification of chronic Q fever was based on the Dutch chronic Q fever consensus group criteria.[15] Clinicians identified patients based on a positive PCR for *C. burnetii* on serum or tissue and/or *C. burnetii* phase I IgG ≥1:1024. Patients with a serological profile and clinical condition matching acute Q fever were excluded. The Dutch chronic Q fever consensus group criteria discriminate between proven, probable and possible chronic Q fever. For this study, only patients with proven or probable chronic Q fever were included. Possible chronic Q fever patients were not included since the presence of clinical relevant infection in possible chronic Q fever patients is questionable and these patients do not have an indication for treatment: therefore evaluation of treatment-related aspects and outcomes is impossible. The observation period for all patients ended on May 1st 2016.

Laboratory testing

Microbiological testing consisted of an indirect fluorescent-antibody assay (IFA) for phase I and II IgG against *C. burnetii* on plasma or serum (Focus Diagnostics, Inc., Cypress, CA, USA or Fuller Diagnostics, LLC., Anchorage, AK, USA). Titration of antibody levels was carried out at different hospital sites with dilutions on a binary scale with a cut-off of 1:32. Furthermore, PCR for *C. burnetii* DNA on serum or plasma and if applicable on tissue samples was performed (NucliSENS easyMAG; bioMérieux, Marcy l'Etoile, France). All SDC measurements were performed in the apothecary laboratory of the Jeroen Bosch Hospital in 's-Hertogenbosch, with exception of one measurement of a patient from the Laurentius Hospital in Roermond. SDC were analyzed by use of HPLC. Measurement of doxycycline was performed after extraction from serum samples. In brief, 500 µL serum was mixed with 500 µL internal standard

(2.5 mg/L dantrolene) and 2 mL monosodium phosphate-sulfite buffer. After gently mixing for 30 seconds, 8 mL dichloromethane was added for extraction. The mixture was vortexed for 1 minute and centrifuged at 3200G for 5 minutes. The organic phase was transferred to a clean tube and evaporated. The residue was dissolved in 200 µL of mobile phase (i.e. distilled H2O + 200 µL triethylamine + 1250 µL phosphoric acid 85%). The sample was passed on the chromatograph column (flowrate 0.6 mL/min). The HPLC system was Htachi equipped with an DAD-detector set at 348 nm. The column was a Lichrosper 100-5 RP-18e, 120 x4mm, from Knauer.

Definitions

The primary outcome of this study was the first disease-related event (a new complication of chronic Q fever or chronic Q fever related-mortality) during treatment or within one year after end of treatment with doxycycline plus hydroxychloroquine. The hazard for occurrence of the first event was studied in case of multiple consecutive events, since multiple events within individuals are not independent of each other. Secondary outcomes were [1] all-cause mortality during treatment or within one year after end of treatment with doxycycline plus hydroxychloroquine and [2] PCR-positivity during treatment, defined as a new positive PCR having been negative for at least 3 months or a persistent positive PCR for more than 6 months during treatment with doxycycline plus hydroxychloroquine. PCR-positivity was used as outcome because presence of *C. burnetii* is considered proven in case of a positive PCR.[1] The cut-off period of one year after end of treatment was chosen because complications and disease-related mortality most often occur far within this timeframe.[16]

Conditions considered complications of chronic Q fever were rupture or dissection of aneurysm; acute symptomatic aneurysm; arterial fistula; endoleak of vascular prosthesis; spondyl(odisc)itis or osteomyelitis; (cardiac) abscess; cerebrovascular accident(hemorrhagic or ischaemic)/transient ischaemic attack; cardiac arrest or tamponade during pericarditis. Cause of death was reviewed by two investigators in all cases (CB en SR) and classification of the relationship between death and chronic Q fever was performed by reaching consensus. Death was defined as related to chronic Q fever in case of active disease AND a cause of death related to chronic Q fever. Active disease was defined as *C. burnetii* phase I IgG ≥1:1024 or positive PCR on serum or tissue. Cause of death related to chronic Q fever was defined as sepsis/feverish episode with no other cause; brain infarct or hemorrhage during endocarditis or due to cerebral aneurysm; arterial fistula; ruptured/dissected aneurysm; heart failure; fatal arrhythmia or cardiac arrest during endocarditis; surgical complications; side effects of antibiotic therapy; clinical deterioration during active disease with no other cause; chronic Q fever as cause of death proven by autopsy; unknown cause in the presence of chronic Q fever-related complications or unknown cause without adequate chronic Q fever treatment.

Side effects and severity of side effects, reasons for discontinuing and/or switching and dosage adjustments of antibiotics were reported. Furthermore, SDC values were reported to evaluate if clinicians aim at SDC values >5µg/mL, as is currently recommended in literature by experts.[9,10]

Statistical methods

Data were retrieved from electronically stored patient records, or paper records if applicable, and stored anonymously in a Microsoft Access 2010 database. Continuous data were compared by independent samples t-test (Welch method) or ANOVA (if > 2 categories). For ANOVA with significant results, post-hoc analysis was performed by Tukey's range test. In univariable analysis, survival curves were compared with a Log-rank or Tarone-Ware test as appropriate. Multivariable analysis was performed with a Cox proportional hazards model. Covariates were selected based on previously identified predictors for the outcomes.[16] For disease-related events, age, presence of prosthetic material before diagnosis of chronic Q fever and PCR-positivity during disease were encountered as covariates in the model. Age and presence of prosthetic material before diagnosis of chronic Q fever were encountered as covariates in the model for all-cause mortality and PCR-positivity during treatment. For all-cause mortality, cause-specific HR were calculated. For first disease-related event and PCR-positivity, subdistribution HR were calculated to account for right-censoring. Left-censoring was accounted for by stratification for the presence of complications before start of treatment. Since the effect of measuring SDC on primary and secondary outcomes may vary per hospital, leading to correlation of patients from the same hospital, a random effect for hospital was included by fitting shared-frailty terms in the model (assuming a Gaussian distribution of the frailty parameter). If patients were treated in multiple hospitals consecutively, the hospital in which the patient was diagnosed was selected. An additional analysis was performed to assess whether intensity of patient care explained the results, since the effect of measuring SDC could be a proxy for intensity of patient care. The ratio of number of phase I IgG antibody titer measurements and follow-up duration in weeks was considered a proxy for intensity of patient care. We compared this ratio for patients with treatment based on SDC and patients without treatment based on SDC, and for patients with and without primary and secondary outcomes. Finally, we repeated Cox-regression analysis for primary and secondary outcomes, with adjustment for the ratio of number of phase I IgG antibody titer measurements and follow-up duration in weeks in the model. The Cox-regression models were fitted with the "cmprsk" and "survival" packages in R studio, version 3.2.2.17 The proportional hazard assumption was verified with both formal tests and graphically, using Schoenfeld residuals. Level of significance was set at a p-value of <0.05. Descriptive data were generated in SPSS, version 21.0. Figures were made in R studio, version 3.2.2.

Results

Of 439 chronic Q fever patients in the database, we identified 201 eligible patients with proven or probable chronic Q fever treated with doxycycline and hydroxychloroquine for at least 12 weeks (figure 1). In 167 patients (83%), SDC were measured. The remaining 34 patients (17%) were treated without measurement of SDC. Doxycycline and hydroxychloroquine was the first treatment for chronic Q fever in 188 patients (94%). Characteristics of all patients and number of primary and secondary outcomes in total and per subgroup are summarized in table 1.

Figure 1. Flowchart of inclusion

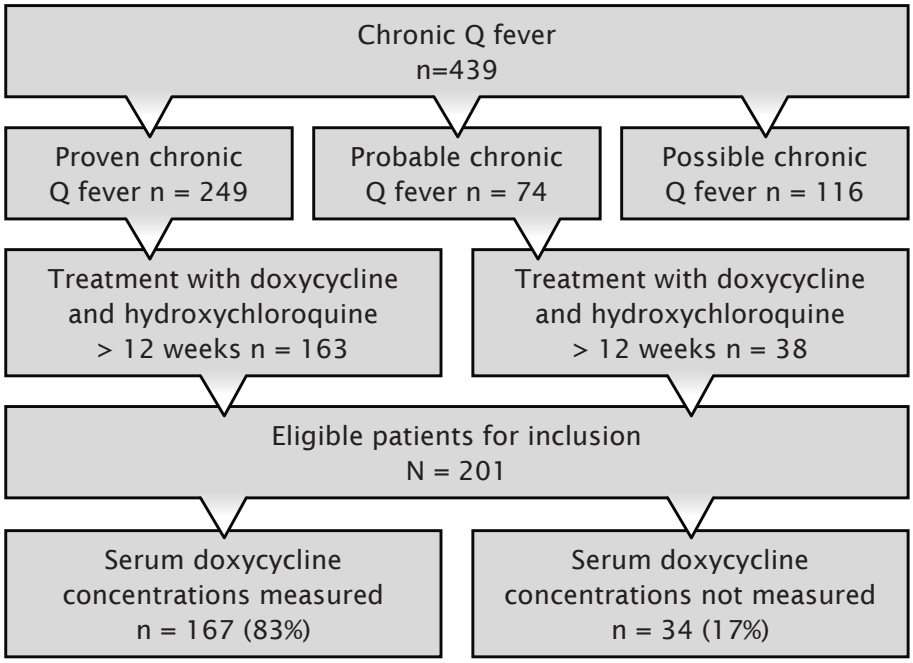


Table 1. Characteristics of proven and probable chronic Q fever patients treated with doxycycline and hydroxychloroquine for at least 12 weeks.

Patient characteristics	All	SDC[a]	No SDC
Number of patients (%)	201	167 (83)	34 (17)
male gender (%)	167 (83)	138 (83)	29 (85)
mean age in years (sd)	68 (11)	68 (11)	71 (13)
mean total follow-up in weeks (sd)	204 (98)	213 (95)	159 (102)
Treatment			
median maximum dosage of doxycycline (IQR)	200 (200-300)	200 (200-300)	200 (200)[b]
mean total treatment duration in weeks (sd)	118 (66)	122 (65)	95 (71)
mean duration DH treatment in weeks (sd)[c]	77 (44)	82 (44)	54 (36)
DH as first treatment (%)[d]	188 (94)	157 (94)	31 (91)
Presence of complications before treatment	66 (33)	52 (31)	14 (41)
Classification			
proven (%)	163 (81)	137 (82)	26 (76)
probable (%)	38 (19)	30 (18)	8 (24)
Focus of infection			
vascular (%)	102 (51)	84 (50)	18 (53)
endocarditis (%)	48 (24)	40 (24)	8 (24)
combined (%)	33 (16)	28 (17)	5 (15)
other / no focus (%)	18 (9)	15 (9)	3 (9)
Clinical outcomes			
all-cause mortality (%)[e]	28 (14)	23 (14)	5 (15)
mean time to death in weeks (sd)	90 (50)	88 (49)	101 (56)
chronic Q fever-related mortality (%)[e]	16 (8)	13 (8)	3 (9)
mean time to death in weeks (sd)	90 (47)	83 (44)	117 (64)
complications (%)[e]	41 (20)	28 (17)	13 (38)
mean time to complication in weeks (sd)	63 (51)	65 (50)	59 (56)
disease-related event (%)[ef]	49 (24)	36 (22)	13 (38)
mean time to event in weeks (sd)	65 (50)	67 (48)	59 (56)
PCR-positivity (%)[g]	20 (10)	19 (11)	1 (3)
mean time to PCR-positivity in weeks (sd)	39 (17)	39 (18)	31[h]

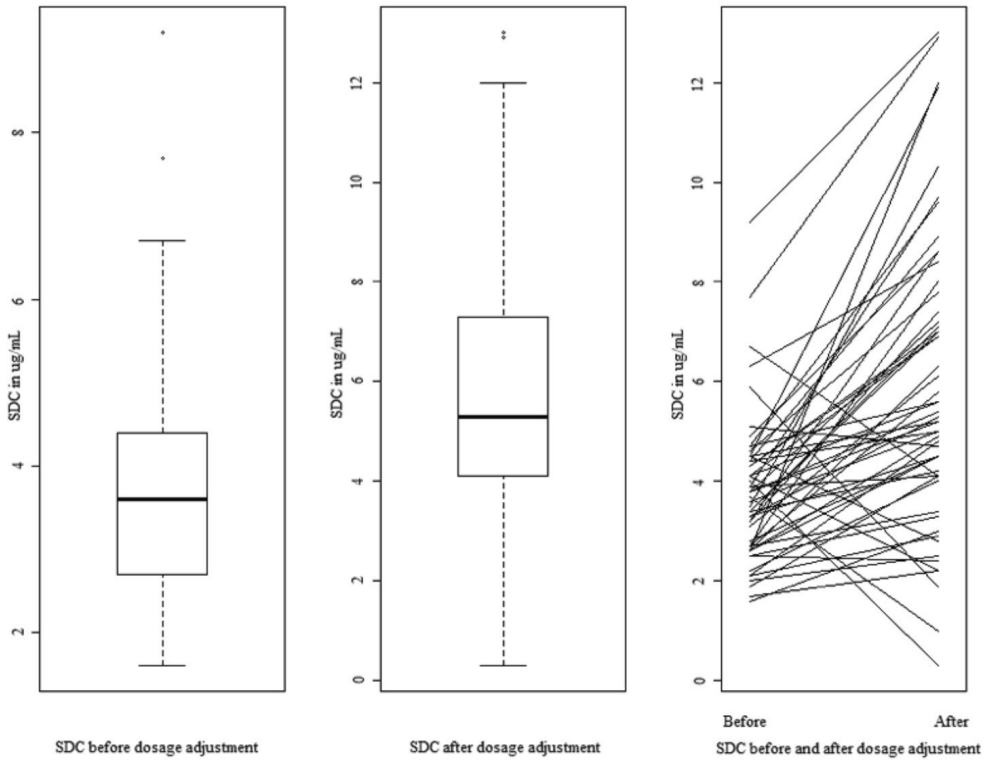
[a]SDC = serum doxycycline concentration. [b]interquartile range of patients used 200 mg as maximum dosage, therefore single value displayed. [c]DH = doxycycline and hydroxychloroquine. [d]defined as first treatment started or treatment with other antibiotics prior to start of doxycycline and hydroxychloroquine less than 4 weeks, [e]during treatment or within one year after stop of treatment. [f]disease-related event: complication or chronic Q fever-related mortality. [g]during treatment. [h]no standard deviaton shown: only one input value.

SDC measurement

In 167 patients, 652 SDC were measured (median 3 SDC per patient, interquartile range 2-5). The first SDC was >5 µg/mL in 106 patients (63%), all with a dosage of 200 mg doxycycline per day. In 145 patients (87%), at least one SDC of >5 µg/mL was measured.

Doxycycline dose was adjusted in 68 patients (41%) in whom SDC were measured: increased in 55 patients (81%), decreased in 4 patients (6%) and both increased and decreased in 9 patients (13%). In patients with increased dosage and SDC measured before and afterwards (n = 59), mean SDC before the first increase was 4.1 µg/mL and mean SDC after the first increase was 5.9 µg/mL (figure 2).

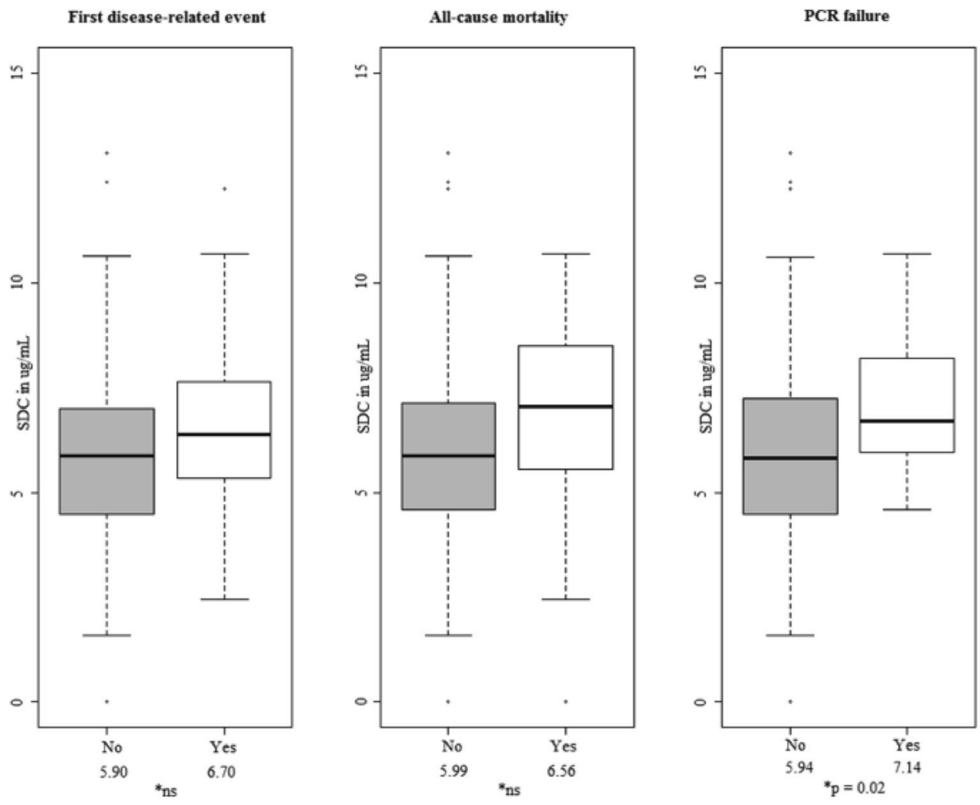
Figure 2. Mean SDC before dosage adjustment and mean SDC after dosage adjustment



SDC = serum doxycycline concentration. In case of multiple adjustments, first adjustment selected. Only cases with increase in dosage shown. Cases with no afterwards or before measurement not shown in these graphs.

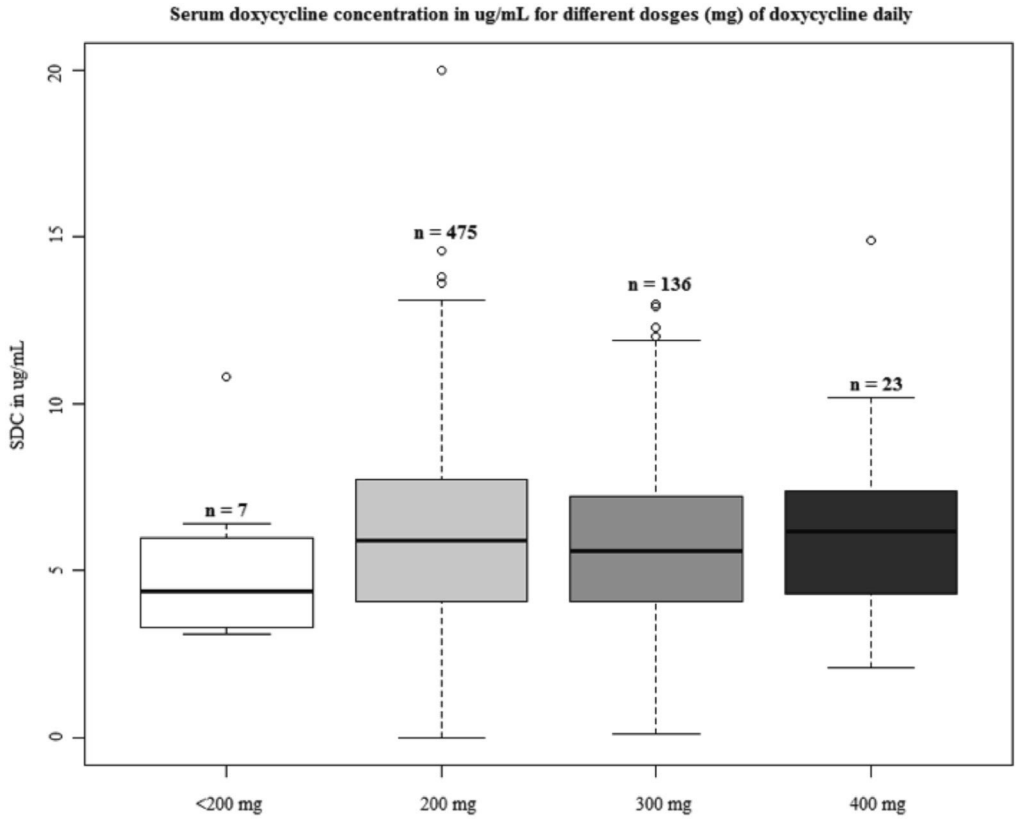
Mean SDC was studied for different subgroups of patients. In both patients with- and without primary or secondary outcomes, overall mean SDC was >5 µg/mL (figure 3). Mean SDC values were higher for patients with the outcomes, compared to those without the outcomes. For disease-related events and all-cause mortality, this difference was not significant. For patients with PCR-positivity, this difference was significant (p = 0.02). Furthermore, during treatment with different doxycycline dosages, overall mean SDC was >5 µg/mL with no significant differences in SDC between dosages (p = 0.70). All measured SDC values with corresponding doxycycline dosages are displayed in figure 4, with multiple measurements within individuals included. The number of patients with and without measurement of SDC diagnosed in time is shown in figure 5: no evident increase in the proportion of patients with SDC measurements in time was observed. In patients without SDC measurement, doxycycline dose was increased in 1 patient (3%); all other patients used 200 mg doxycycline per day.

Figure 3. Differences in SDC for patients with and without primary and secondary outcomes



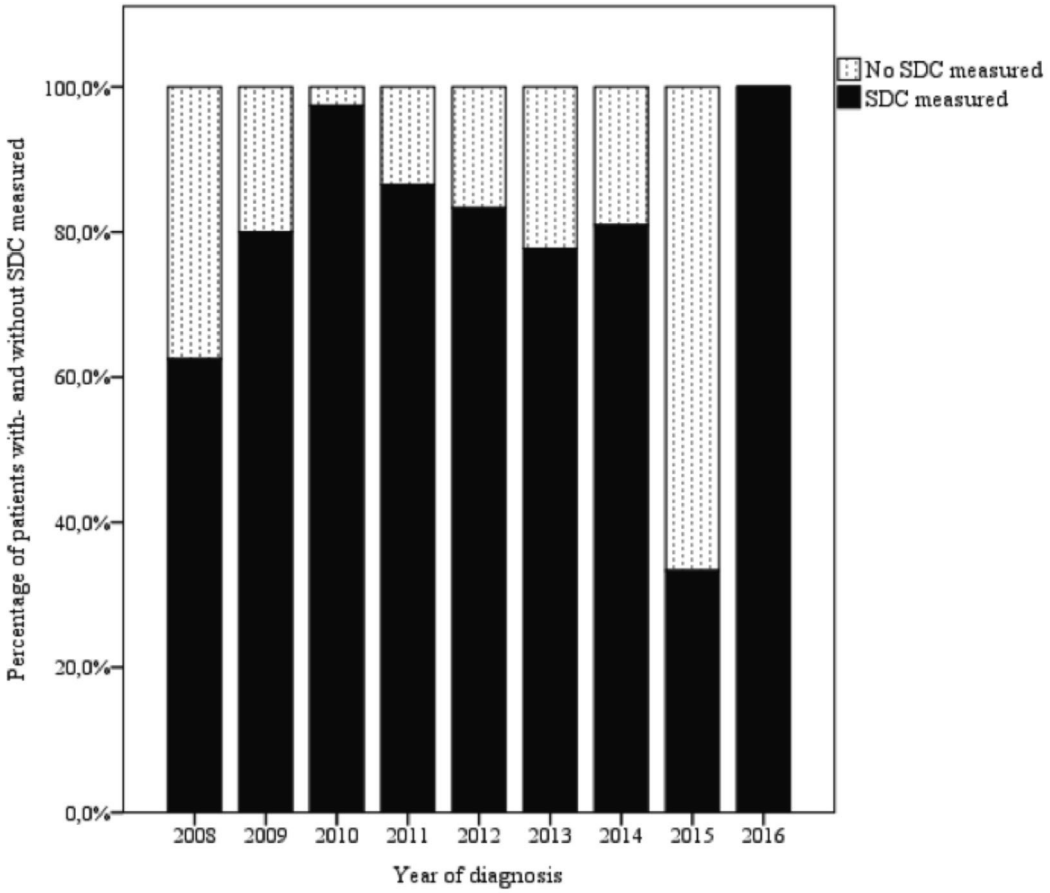
SDC = serum doxycycline concentration. Mean serum doxycycline concentration in µg/mL and statistical significance (t-test) of difference shown below figure. Dosage unknown during 11 SDC measurements in one patient.

Figure 4. All measured serum doxycycline concentrations in µg/mL, with corresponding dosages of doxycycline in mg/day



SDC = serum doxycycline concentration. Multiple SDC measurements within individuals all included. Dosage unknown during 11 SDC measurements in one patient.

Figure 5. Percentage of patients with or without measurement of SDC diagnosed in time

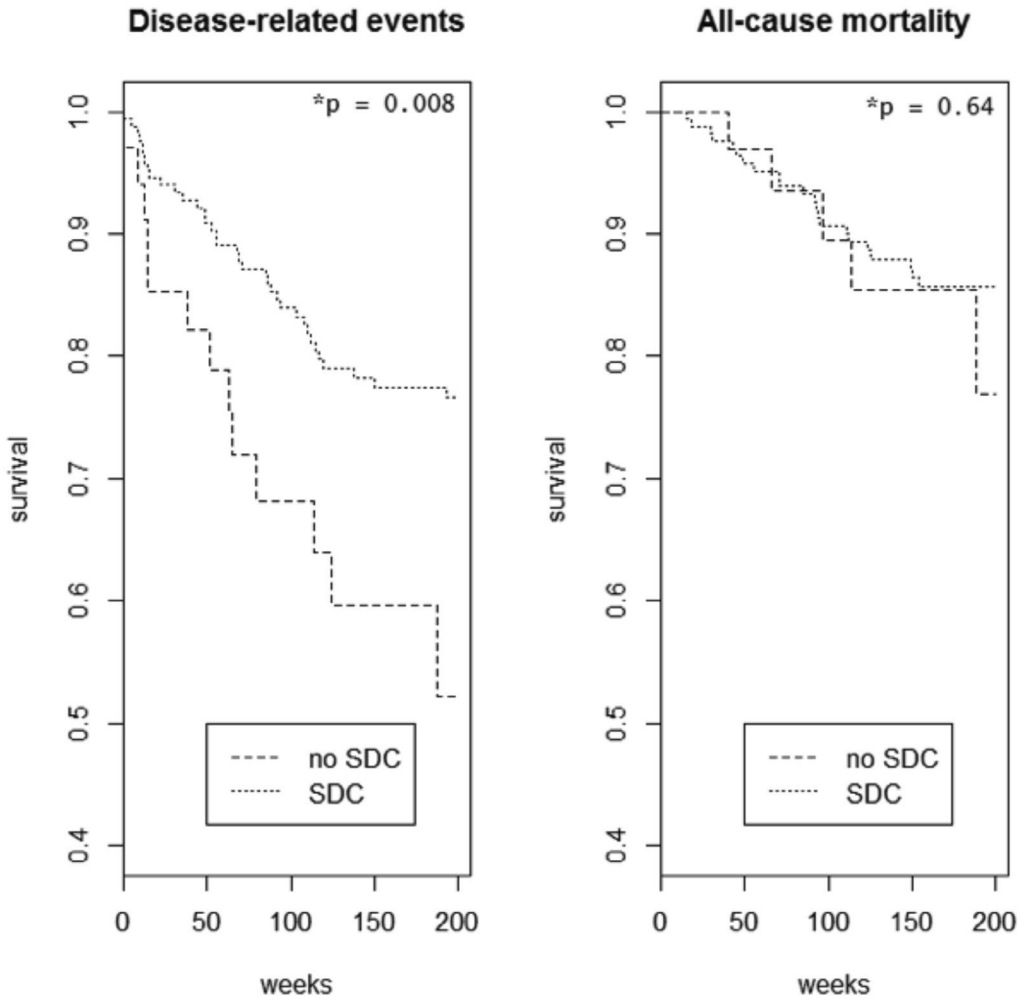


SDC = serum doxycycline concentration. Total number of patients diagnosed in time: 8 in 2008, 20 in 2009, 39 in 2010, 67 in 2011, 18 in 2012, 18 in 2013, 21 in 2014, 9 in 2015, 1 in 2016.

SDC in relation to primary and secondary outcomes

The primary outcome (first disease-related event) occurred in 36 patients (22%) with SDC measurement and in 13 patients (38%) without SDC measurement. As for secondary outcomes, all-cause mortality occurred in 23 patients (14%) with SDC measurement and 5 patients (15%) without SDC measurement. PCR-positivity occurred in 19 patients (11%) with SDC measurement and 1 patient (3%) without SDC measurement. Kaplan-Meier survival curves for disease-related events and all-cause mortality are shown in figure 6. In univariable survival analysis, there was a significant difference in disease-related events between patients with SDC measured and patients without SDC measured, with a lower risk for the outcome for those with SDC measured ($p = 0.008$). No significant differences in all-cause mortality were observed between patients with and without SDC measured ($p = 0.64$).

Figure 6. Kaplan-Meier curves for all-cause mortality and disease-related events (unadjusted for covariates): comparison of patients with and without treatment based on serum doxycycline concentrations (SDC)



In multivariable analysis, treatment based on SDC was associated with a significantly lower risk for disease-related events (HR 0.51, 95% CI 0.26-0.97, p = 0.04). It was not associated with a lower risk for all-cause mortality during treatment or within one year after end of treatment (HR 0.93, 95% CI 0.35 – 2.51, p = 0.89) or PCR-positivity during treatment (HR 5.87, 95% CI 0.73 – 46.98, p = 0.10), see table 2.

Table 2. HR for association between measurement of serum doxycycline concentrations and occurrence of primary and secondary outcomes.

Outcome	SDC[a]	No SDC	(S)HR[b] (95%CI) for SDC measurement	p-value
Number of patients	167	34	-	-
Disease-related events[cd]	36 (22)	13 (38)	0.51 (0.26 - 0.97)	0.04
All-cause mortality[ce]	23 (14)	5 (15)	0.93 (0.35 - 2.51)	0.89
PCR-positivity[cd]	19 (11)	1 (3)	5.87 (0.73 - 46.98)	0.10

[a]SDC = serum doxycycline concentration. [b]subdistribution hazard ratio. [c]in univariable analysis non-significant differences (Chi-square test, Fisher exact test or independent samples t-test as appropriate). For disease-related events: presence of prosthetic material prior to occurrence of complications and age significant covariates. PCR-positivity non-significant covariate. For all-cause mortality and PCR failure: age and presence of prosthetic material prior to occurrence of complications non-significant covariates. [d]subdistribution HR. [e]cause-specific HR.

Additional adjustment for intensity of patient care

The ratio of number of phase I IgG antibody titer measurements and follow-up duration in weeks was significantly higher in patients with SDC measured (0.071 versus 0.057, p = 0.02). The ratio was significantly higher in patients that died (of all-causes) in comparison to patients that survived (0.093 versus 0.021, p = 0.001). No differences in this ratio were found between patients with and without disease-related events (0.076 versus 0.067, p = 0.11) or PCR-positivity during treatment (0.077 versus 0.068, p = 0.21). In Cox-regression analysis, correction for the ratio of number of phase I IgG antibody titer measurements and follow-up duration in weeks did not significantly change the HR estimates and CI for primary and secondary outcomes.

Side effects

Patients treated based on SDC reported side effects in 78% (n = 131), in patients without measurement of SDC side effects occurred in 62% (n = 21), see table 3. Mean maximum dosages were not significantly different in patients experiencing side effects and in patients stopping due to side effects in comparison to those who did not (mean 232 versus 233, p = 0.97 and mean 228 versus 235, p = 0.36). Moreover, there was no significant difference in the mean SDC value for those with side effects (mean 6.18 µg/mL) compared to those without side effects (mean 5.76 µg/mL, p = 0.37).

Table 3. Reasons for stop and switch and severity of side effects

Reason for stop/switch	All	SDC[a]	No SDC
n (%)	201	167 (83)	34 (17)
Side effects (%)	80 (40)	63 (38)	17 (50)
Deceased (%)	14 (7)	12 (7)	2 (6)
Adequate treatment finished (%)	58 (29)	52 (31)	6 (18)
Insufficient clinical response (%)	28 (14)	25 (15)	3 (9)
Not stopped at end of observation period (%)	28 (14)	19 (11)	9 (26)
Other (%)	30 (15)	26 (16)	4 (12)
Severity of side effects			
Side effects	152 (76)	131 (78)	21 (62)
Side effects – hospitalization (%)	9 (4)	7 (4)	2 (6)
Side effects – permanent damage (%)	14 (7)	11 (7)	3 (9)
Side effects – life threatening (%)	3 (1)	3 (2)	-
Side effects – potentially lethal (%)	2 (1)	2 (1)	-

[a] *SDC = serum doxycycline concentrations.*

Discussion

Treatment based on serum doxycycline concentrations (SDC) was associated with a lower hazard for disease-related events compared to treatment with a fixed doxycycline dose. The effect may be explained through better treatment accomplished by optimized dosage of doxycycline, or by better adherence to medication in patients with measurement of SDC. We hypothesized that treatment based on SDC could be a proxy for intensity of patient care, which may explain favorable outcomes for patients in whom treatment was based on SDC. Indeed, the ratio of number of phase I IgG antibody titer measurements and follow-up duration in weeks was higher in patients treated based on SDC. Adjustment for this ratio did not lead to different conclusions. This could be due to the fact that both parameters represent exact the same underlying variable. However, the ratio was associated with all-cause mortality while SDC measurement was not. Therefore, the distribution of this variable is different from SDC measurement and we must conclude that increased intensity of patient care cannot explain the observed effect entirely.

Measurement of SDC was not associated with all-cause mortality or PCR-positivity during treatment. The lack of an association between treatment based on SDC and all-cause mortality or PCR-positivity may be due to lack of power: we only observed 28 deaths within one year of stopping doxycycline plus hydroxychloroquine and 20 cases with PCR-positivity during treatment.

In earlier studies, SDC >5 μg/mL proved to be beneficial in terms of serological response. Here, we provide the first study on the effect of treatment based on SDC on clinical endpoints, which makes this a unique study. Due to the retrospective

origin of this study, it was not possible to draw conclusions on the classical dose-response relation. Clinicians titrate doxycycline dosage up to SDC of >5 µg/mL, in accordance with recommendations in literature, or address incompliance in order to improve SDC.[9-10] The fact that no differences in SDC were observed under different doxycycline dosages supports the hypothesis that clinicians in practice aim at values > 5 µg/mL. This leads to bias when assessing the correlation between SDC values and clinical outcomes: in patients with the most severe disease, clinicians will perhaps aim for higher SDC values. Therefore, higher SDC values will be associated with worse outcomes, probably due to reversed causality: in patients with most severe disease SDC are titrated up to higher values. Therefore, we decided to assess the effect of a treatment strategy in which dosage and coaching of patients with regard to compliance is based on SDC, instead of evaluating the actual SDC values.

Besides the problems with assessing the relation between SDC values and clinical outcomes, there may be a risk of bias caused by confounding by indication when studying the effect of measuring any SDC too. Clinicians may measure SDC more often in patients with more pronounced disease or more complications. However, we observed an effect in the opposite direction: in patients with no measurement of SDC, complications occurred more frequently than in patients with SDC measured. The strength of the effect and relation between SDC measurement and the other outcomes may be underestimated by confounding by indication.

Another potential issue is that there is 'learning effect' among clinicians: the more patients they have treated in time, the more experience they gained and the better they are able to manage these patients. Theoretically, this may result in an increase of the proportion of patients in which SDC was measured in time. However, we did not observe an evident effect of time on the proportion of patients with SDC measured (although the numbers in the group of patients without measurement of SDC are small), leading to less favorable treatment outcome for these patients.

Finally, for this analysis we only included patients treated with the combination of doxycycline and hydroxychloroquine. Therefore it is uncertain if these results are generalizable to patients treated with doxycycline monotherapy or doxycycline combined with other antibiotics.

Altogether, SDC measurement seems beneficial during treatment for chronic Q fever. Therefore, we recommend measuring of SDC during treatment of chronic Q fever with doxycycline and hydroxychloroquine. We advise to aim for SDC >5 µg/mL since clinicians strived for SDC values >5 µg/mL in this study, and this strategy was successful. No literature is available on upper target levels during treatment of chronic Q fever. From clinical experience, we advise to strive for values >5 µg/mL but <10 µg/mL. The recommendation on the upper target range is not evidence based: there is no literature on toxic SDC values during prolonged treatment with doxycycline.

In conclusion, treatment based on SDC was associated with a lower hazard for disease-related events, but not for all-cause mortality or PCR-positivity. Intensity

of patient care could not entirely explain the association we found, suggesting that SDC-based dosing itself decreases the risk for disease-related events in these patients, potentially through more optimal dosing or through improved compliance. We therefore recommend measurement of SDC and to strive for SDC >5 μg/mL and <10 μg/mL during treatment of chronic Q fever.

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References

1. Eldin C, Melenotte C, Mediannikov O et al. From Q fever to *Coxiella burnetii*: a paradigm change. Clin Microbiol Rev 2017; 30: 115-90.
2. Maurin M and Raoult D. Q fever. Clin Microbiol Rev 1999; 12: 518-553.
3. Raoult D, Houpikian P, Tissot Dupont H et al. Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydroxychloroquine. Arch Intern Med 1999; 159: 167-73.
4. Million M, Raoult D. Recent advances in the study of Q fever epidemiology, diagnosis and management. J Infect 2015; 71: S2-S9.
5. Anderson A, Bijlmer H, Fournier PE et al. Diagnosis and Management of Q fever – United States 2013: Recommendations from CDC and the Q fever Working Group. MMWR Recomm Rep 2013; 62: 1-30.
6. Samuel JE, Hendrix LR. Laboratory maintenance of *Coxiella burnetii*. Curr Protoc Microbiol 2009; Chapter6C.
7. Vincent GA, Graves SR, Robson JM et al. Isolation of *Coxiella burnetii* from serum of patients with acute Q fever. J Microbiol Methods, 2015; 119: 74-8.
8. Omsland A, Hackstadt T, Heinzen RA. Bringing culture to the uncultured: *Coxiella burnetii* and lessons for obligate intracellular bacterial pathogens. PLoS Pathog 2013; 9: e1003540.
9. Rolain JM, Mallet MN, Raoult D. Correlation between serum doxycycline concentrations and serologic evolution in patients with *Coxiella burnetii* endocarditis. J Infect Dis 2003; 188: 1322-5.
10. Lecaillet A, Mallet MN, Raoult D *et al*. Therapeutic impact of the correlation of doxycycline serum concentrations and the decline of pahse I antibodies in Q fever endocarditis. J Antimicrob Chemother 2009; 63: 771-4.
11. Rolain JM, Boulos A, Mallet MN et al. Correlation between ratio of serum doxycycline concentration to MIC and rapid decline of antibody levels during treatment of Q fever endocarditis. Antimicrob Agents Chemother 2005; 49: 2673-6.
12. Rouli L, Rolain JM, El Filali A et al. Genome sequence of *Coxiella burnetii* 109, a doxycycline-resistant clinical isolate. J Bacteriol 2012; 194: 6939.
13. 13. Vranakis I, De Bock PJ, Papadioti A et al. Quantitative proteome profiling of *C. burnetii* under tetracycline stress conditions. PLoS One 2012; 7: e33599.
14. Rolain JM, Lambert F, Raoult D. Activity of telithromycin against thirteen new isolates of *C. burnetii* including three resistant to doxycycline. Ann N Y Acad Sci 2005; 1063: 252-56.
15. Wegdam-Blans MC, Kampschreur LM, Delsing CE et al. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Infect 2012; 64: 247-59.
16. van Roeden SE, Wever PC, Kampschreur LM *et al*. Chronic Q fever-related complications and mortality: data from a nationwide cohort. In: ECCMID 2017 eLibrary material of the 27th European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria, 2017. Abstract OSO906, https://www.escmid.org/research_projects/eccmid/past_eccmids/. European Society of Clinical Microbiology and Infectious Diseases, Basel, Switzerland.
17. The Comprehensive R Archives Network. “cmprsk” and “survival”. Cran.R-project.org

Appendix 1 - List of participating hospitals

Participating hospitals: Amphia Hospital in Breda, Atrium Medical Center in Heerlen, Bernhoven Hospital in Uden, Bravis Hospital in Roosendaal, Canisius-Wilhelmina Hospital in Nijmegen, Catharina Hospital in Eindhoven, Diakonessenhuis in Utrecht, Elkerliek Hospital in Helmond, Erasmus Medical Center in Rotterdam, Hospital Gelderse Vallei in Ede, Gelre Hospital in Apeldoorn, Groene Hart Hospital in Gouda, Jeroen Bosch Hospital in 's -Hertogenbosch, Leids University Medical Center in Leiden, Izore Laboratory in Leeuwarden, Isala Clinic in Zwolle, Laurentius Hospital te Roermond, Maasstad Hospital in Rotterdam, Maxima Medisch Centrum in Eindhoven, Meander Medical Center in Amersfoort, Medisch Spectrum Twente in Enschede, Onze Lieve Vrouwe Gasthuis in Amsterdam, Radboud university medical center in Nijmegen, Reinier de Graaf Hospital in Delft, Rijnstate Hospital in Arnhem, Sint Elisabeth Hospital in Tilburg, Sint Antonius Hospital in Nieuwegein and University Medical Center Utrecht in Utrecht.

Hospitals providing cooperation but without chronic Q fever patients: Admiraal de Ruyter Hospital in Goes, Albert Schweitzer Hospital in Dordrecht, Bronovo Hospital in The Hague, Diaconessenhuis in Leiden, St. Fransiscus Gasthuis in Rotterdam, St. Jansdal in Harderwijk, Vlietland Hospital in Schiedam.

Part III

Coxiella burnetii and the risk of non-Hodgkin lymphoma

Chapter 7

Case report: *Coxiella burnetii* vascular infection and lymphoma in the Netherlands

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Abstract

Objectives and design

Non-Hodgkin lymphoma has been linked to infection with *Coxiella burnetii*, potentially through overproduction of IL-10 during infection with *C. burnetii*.

Material and methods

Description of a case report.

Results

We describe a patient with retroperitoneal non-Hodgkin lymphoma and vascular infection with *C. burnetii*. Immunofluorescence staining and fluorescence in situ hybridization targeting specific *C. burnetii* 16S rRNA were performed on the retroperitoneal lymphoma tissue sample obtained at diagnosis of NHL. Both were strongly positive for the presence of *C. burnetii*.

Conclusions

This case provokes questions regarding a potential association between *C. burnetii* and NHL, and underlines the importance of further exploration of this association.

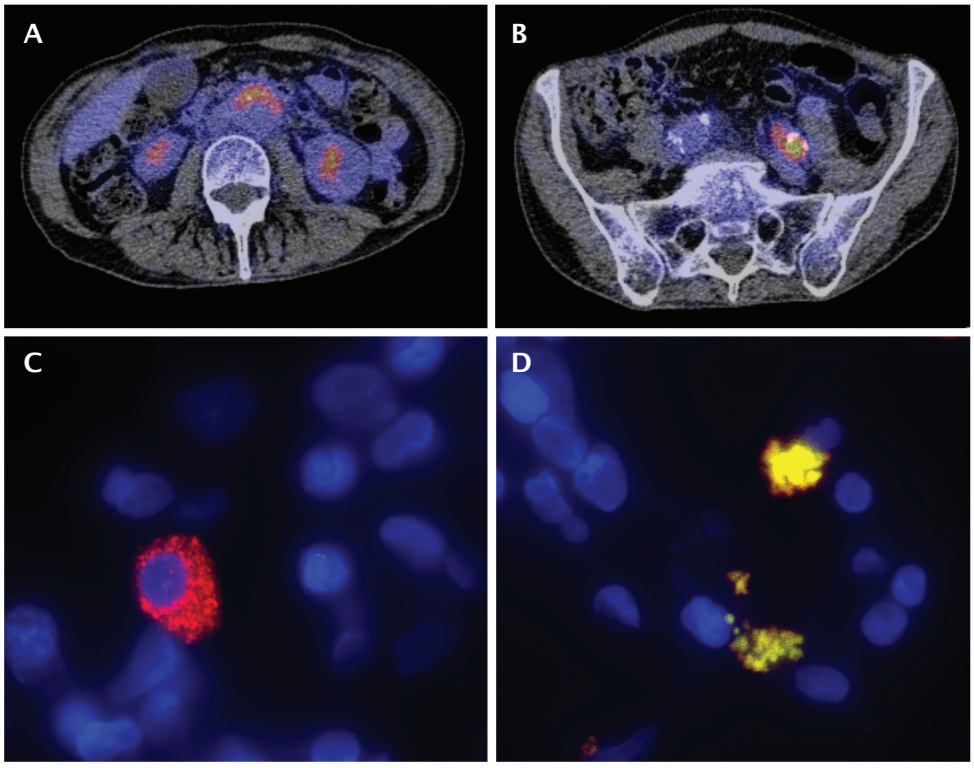
Case report

A 58 year-old male patient, with a history of a vascular bypass because of occlusion of the infrarenal aorta in September 2010 and rheumatoid arthritis for which he used etanercept weekly, underwent abdominal ultrasound during routine follow-up in September 2012. He mentioned lower abdominal pain since a few weeks but did not report any night sweats, fever, weight loss, rash or other complaints. He did not have any contact with animals, but lived in the Netherlands in an area were Q fever had been highly epidemic between 2007 and 2010.[1] Laboratory results on presentation are shown in table 1. On abdominal ultrasound, bilateral hydronephrosis with extensive retroperitoneal masses were observed. A subsequent Computed Tomography scan and Positron Emission Tomography (PET) scan showed extensive retroperitoneal, intra-abdominal, mediastinal, cervical, and axillary lymphadenopathy, a pulmonary mass and a lesion in the right adrenal. In addition, the abdominal aorta and left iliac artery showed increased ^{18}F-FDG uptake, which was not further analyzed. The PET-scan was made as part of the standard work-up procedure of suspected non-Hodgkin lymphoma (NHL), in accordance with Dutch guidelines.[2,3] Biopsy of the retroperitoneal masses and a bone marrow biopsy revealed an Ann Arbor stage IV B-cell NHL, with initially indefinite histopathological classification of the subtype of NHL. Second opinion of the pathology specimens in an academic hospital confirmed the presence of a B-cell NHL, with a marginal zone lymphoma as the most likely histopathological subtype. Chemo-immunotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) was initiated and etanercept was discontinued. After discontinuation of etanercept, the patient experienced two flares of his rheumatoid arthritis for which he received short courses of prednisone. Follow-up PET scan after three cycles of chemotherapy showed regression of all lymphoma locations, with exception of the pulmonary lesion, adrenal mass, and lower cervical lymph nodes. The increased ^{18}F-FDG uptake in aorta and left iliac artery was unchanged. Because of unresponsiveness to chemotherapy of the pulmonary lesion a second malignancy, possibly metastatic lung carcinoma, was suspected and further analysis was planned. Meanwhile, chemotherapy was discontinued indefinitely, while awaiting analysis of the suspected lung lesion. Shortly after the last PET scan, the patient was diagnosed with pulmonary embolism for which heparin was started. Because of the use of anticoagulants and difficult accessibility of the pulmonary lesion, the diagnosis could not be confirmed pathologically. A follow-up PET scan was performed in February 2013, on which all NHL localizations were persistent in regression. However, the ^{18}F-FDG uptake in aorta and left iliac artery was strongly increased and the pulmonary lesion and pathological cervical lymph nodes were unchanged. The adrenal lesion was slightly larger. Patient was referred for second opinion and after due consideration did not want any further diagnostics and treatment of his pulmonary lesion. Analysis of the increased uptake in aorta and left iliac artery was performed in June 2013. Repeated PET scanning revealed highly increased uptake of ^{18}F-FDG of the abdominal aorta and left iliac artery, and a lesion suspect for a small abscess near the left iliac artery (figure 1). As infection of the vascular bypass was suspected, blood cultures and serology for *Coxiella burnetii* were performed. Blood cultures were negative, but phase I and II IgG antibodies for *C. burnetii* were both repeatedly positive with a maximum phase I IgG antibody titer of 1:4096 (Indirect

Fluorescent-antibody Assay, Focus Diagnostics, Inc., Cypress, CA, USA). Phase I and II IgM antibodies against *C. burnetii* were negative. Polymerase chain reaction on serum was performed twice but both samples tested negative. Vascular infection with *C. burnetii* was diagnosed according to both the Dutch chronic Q fever consensus group criteria and the criteria formulated by Eldin *et al.*, and treatment with doxycycline (200 mg once daily) and hydroxychloroquine (200 mg three times daily) was started in July 2013.[4,5] No signs of endocarditis were present on physical examination or PET-CT, but an echocardiogram was not performed. In absence of an echocardiogram the presence of a concomitant endocarditis could not be excluded with certainty.[4,6] After start of treatment, the patient experienced severe gastro-intestinal side effects and refused further therapy in September 2013. In March 2014, he reported repeated melena and rectal bleeding. An aortoduodenal fistula was suspected, but the patient did not want any further diagnostic interventions. His clinical condition deteriorated and he died that same month.

We performed immunofluorescence staining (IF) and fluorescence in-situ hybridization (FISH) targeting specific *C. burnetii* 16S rRNA on the retroperitoneal lymphoma tissue sample obtained at diagnosis of NHL in 2012, which were both strongly positive, figure 1. We refer to a previous article for technical details on IF and FISH for *C. burnetii*.[7] FISH is a relatively novel diagnostic method for detection of *C. burnetii*, with few studies using FISH targeting *C. burnetii* performed. The technique was applied in one clinical study and one case-report only.[7,8] Melenotte et al were able to detect *C. burnetii* in four out of seven lymphoma biopsies with FISH. All samples had positive immunofluorescence as confirmation, but negative immunohistochemistry.[7] Both FISH and IF are highly sensitive new diagnostic techniques to detect bacteria in situ, that may be superior to immunohistochemistry.

Figure 1. Composite figure of PET-CT, immunofluorescence (IF) and fluorescence in-situ hybridization (FISH).



(A) Highly increased ^{18}F-FDG uptake in the aortic wall. (B) Highly increased ^{18}F-FDG uptake in the left iliac artery. (C) Microscopic image (original magnification x100) of immunofluorescence staining (IF) of retroperitoneal lymphoma tissue of a patient with vascular chronic Q fever in which nuclei are stained blue (4',6-diamidino-2-phenylindole, DAPI), while perinuclear Coxiella burnetii is stained red.(D) Microscopic image (original magnification x100) of fluorescence in-situ hybridization (FISH) of the same tissue in which nuclei are stained blue (4',6-diamidino-2-phenylindole, DAPI), while C. burnetii, organized in perinuclear vacuoles, is stained yellow. Yellow signal results of the co-localization of the universal probe EUB (red) and specific 16S rRNA C. burnetii probe (green). For both (C) and (D): Leica DMI6000 B microscope was used.

The presence of *C. burnetii* in the NHL tissue indicates that the infection was already present at the time of diagnosis of NHL in September 2012. At the moment of presentation in September 2012, our patient did not report any weight loss or fever. Nevertheless, he developed clear signs of inflammation shortly after admission (see table 1). A potential explanation for the absence of fever at presentation may be the fact that our patient was immunocompromised. Absence of weight loss may be due to the fact that self-reporting of symptoms is not very accurate. In the Dutch national chronic Q fever database with data of 439 patients with persistent or chronic Q fever from the Netherlands, only 14% of patients had fever at the moment of presentation and only 28% of patients reported weight loss at presentation.[9] The absence of these symptoms illustrates that patient with a chronic infection can present with atypical symptoms.

Table 1. Laboratory results at the day of presentation.

Laboratory measurement	Result	Normal range	Units of measurement
Hemoglobin	7.2	8.5 – 11.0	mmol/L
Thrombocytes	179	150 – 400	10^9/L
Leukocytes*	9.2	4.0 – 10.0	10^9/L
Creatinine*	551	60 – 110	µmol/L
C-reactive protein	14	0 – 8	mg/L
Aspartate aminotransferase*	19	0 – 34	U/L
Alanine aminotransferase*	13	0 – 44	U/L
Gamma-glutamyltransferase*	<10	0 – 54	U/L
Alkaline phosphatase*	81	43 – 115	U/L
Lactate dehydrogenase*	246	0 – 247	U/L

**In the days following initial presentation, these laboratory values changed. Maximum values during admission were: leukocyte count 35.6· 10^9/L, C-reactive protein 186 mg/L, aspartate aminotransferase 35, alanine aminotransferase 49, alkaline phosphatase 177, gamma-glutamyltransferase 146, lactate dehydrogenase 400.*

It has been hypothesized that there is a causal relationship between persistent infection with *C. burnetii* and development of B-cell NHL.[7] This patient presents similar as the index case of the series reporting a link between Q fever and B-cell lymphoma: both patients had a vascular focus of infection and lymphoma located closely to the focus of infection. [7] A potential pathophysiological pathway is overproduction of IL-10 during infection with *C. burnetii*, which could play a role in the development of B-cell NHL. [7,10] However, both diseases have a considerable diagnostic delay. It cannot be ruled out that *C. burnetii* infects monocytes and macrophages in tumorous tissue and that this patient developed NHL before infection. Furthermore, both diseases have common risk factors, such as immunocompromised state.[11,12] Our patient was severely immunocompromised, which is a risk factor for development of both the lymphoma and the vascular infection with *C. burnetii*.

In this report, we describe a single case of NHL after Q fever. Naturally, no hard conclusions with regard to the association between *C. burnetii* and NHL and its causality can be drawn based on one single case. However, this case provokes further questions regarding the potential association between *C. burnetii* infection and NHL and its causality and potential diagnostic tools for detection of *C. burnetii* in tissues. Furthermore, it underlines the importance of exploration of the association and exploration of the necessity of active surveillance for lymphoma in areas where Q fever is or was endemic.

Acknowledgements

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References

1. 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. FMS Immunol Med Microbiol 2012;64(1):3-12.
2. Dutch society for Hematology (Nederlandse Vereniging voor Hematologie). Protocol on non-Hodgkin lymphoma. Derived from: http://www.hematologienederland.nl/non-hodgkin-lymfomen, accessed 24 July 2017.
3. Hematology department University Medical Center Groningen. Protocol on non-Hodgkin lymphoma. Derived from: https://hematologiegroningen.nl/protocollen/, accessed 24 July 2017.
4. Wegdam-Blans MC, Kampschreur LM, Delsing CE et al. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Infect 2012;64(3):247-59.
5. Eldin C, Melenotte C, Mediannikov O, Ghigo E, Million M, Edouard S et al. From Q fever to *Coxiella burnetii* infection: a paradigm change. Clin Microbiol Rev 2017;30(1):115-90.
6. Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 2000 Apr;30(4):633-8.
7. Melenotte C, Million M, Audoly G et al. B-cell non Hodgkin-lymphoma linked to *Coxiella burnetii*. Blood 2016;127(1):113-21.
8. Kumpf O, Dohmen P, Ertmer M et al. Rapid molecular diagnosis of infective aortic valve endocarditis caused by *Coxiella burnetii*. Infection 2016; 44(6):813-17.
9. Unpublished data from the national Dutch chronic Q fever database. Generated July 25th 2017.
10. Levy Y, Brouet JC. Interleukin-10 prevents spontaneous death of germinal center B cells by induction of the bcl-2 protein. J Clin Invest 1994;93(1):424-28.
11. Raoult D. Host factors in the severity of Q fever. Ann N Y Acad Sci 1990;590:33-8.
12. Shankland KR, Armitage JO, Hanchock BW. Non-Hodgkin lymphoma. Lancet 2012;380(9844):848-57.

Chapter 8

Exposure to *Coxiella burnetii* and the risk of non-Hodgkin lymphoma: a retrospective population-based analysis in the Netherlands

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Abstract

Background

An association between *Coxiella burnetii* and non-Hodgkin lymphoma (NHL) has been suggested. After a large Q fever epidemic in the Netherlands (2007 – 2010), we hypothesized that the incidence of NHL would be increased during and after the epidemic in high Q fever incidence compared to low Q fever incidence areas.

Methods

We performed a retrospective population-based analysis and calculated relative risks (RR) of NHL during one-year periods before (2002 - 2006), during (2007 - 2010), and after the epidemic (2011 - 2013) for intermediate and high Q fever incidence areas compared to low Q fever incidence areas, and calculated the RR of NHL in chronic Q fever patients compared to the general population.

Findings

During the study period 48,760 NHL were diagnosed. The yearly incidence rate of NHL ranged from 21·4 to 26·7 / 100,000 PY, with the highest incidence in 2010. A statistically significant association with NHL was found for high Q fever incidence areas compared to low Q fever incidence areas in 2009 (RR 1·16, 95% CI 1·02–1·33, p = 0·03), but not in any other year or for intermediate Q fever incidence areas. Among 439 chronic Q fever patients, five developed NHL yielding a crude absolute risk of 301·0 / 100,000 PY and a RR of 4·99, 95% CI 2·07–11·98, p < 0·01 compared to the general Dutch population.

Interpretation

These findings do not lend support to the hypothesis that Q fever has a relevant etiologic role in the development of NHL. Several limitations, inherent to the design of this study, may lead to both underestimation or overestimation of the studied association .

Funding

This project was funded by foundation Q-support and Institut Mérieux.

Introduction

Non-Hodgkin lymphoma (NHL) are among the most frequently diagnosed cancers in the western world.[1] The etiology of NHL is believed to be multifactorial and has only been clarified partially. Previously identified factors that contribute to a higher risk of NHL are alcohol consumption, smoking, immunocompromised state, auto-immune diseases and environmental factors such as exposure to chemicals.[1,2] In addition, multiple viral and bacterial pathogens have been associated with NHL, such as Human Immunodeficiency Virus (HIV), Epstein-Barr virus (EBV), *Helicobacter pylori*, and *Borrelia burgdorferii*.[3-6]

Recently, an increased incidence of NHL after infection with *Coxiella burnetii*, the causative agent of Q fever, has been reported.[7] A potential explanation for this association is the existence of a causal relationship between infection with *C. burnetii* and development of NHL, for example through increased IL-10 production during infection.[7-9]

The Netherlands faced a large Q fever epidemic with multiple seasonal outbreaks between 2007 and 2010, with an estimated 40,000 – 50,000 infected individuals.[10,11] Globally, infections with *C. burnetii* are reported in outbreak settings, as ongoing endemic disease or sporadically.[12-18] From a public health perspective, it is highly relevant to further clarify the risk of NHL after exposure to *C. burnetii* in comparison to unexposed individuals. The Dutch Q fever epidemic provides the unique opportunity to assess the association between NHL and exposure to *C. burnetii* in a large population. We hypothesized that if there is an association between exposure to *C. burnetii* and the occurrence of NHL, an increased incidence of NHL would be observed in high Q fever incidence areas during and shortly after the epidemic in the Netherlands.

Methods

To study the potential association between *C. burnetii* and NHL, we performed a retrospective population-based analysis. The incidence of NHL in areas with high and intermediate Q fever incidence was assessed for the years during and after the Q fever epidemic, with low Q fever incidence areas and previous years as reference. Since the duration between exposure and onset of disease is unknown, the risk was assessed per year. Furthermore, the hypothesis was that the incidence of NHL would be increased in patients with chronic Q fever. Therefore, we compared the incidence of NHL in chronic Q fever patients to the incidence of NHL in the general population.

Inclusion of patients and data collection

To assess the risk of NHL after exposure to *C. burnetii*, the incidence of NHL in the entire general Dutch population between 01-01-2002 and 31-12-2013 was studied. The number of inhabitants on July 1st of each year, per five year age-category, gender,

and geographic areas (four-digit zipcode) was derived from Statistics Netherlands. The number of newly diagnosed NHL per year, per five year age-category, gender, and geographic areas were retrieved from the Netherlands Cancer Registry (NCR), managed by IKNL. The IKNL systematically reviews all medical charts in each hospital and registers all new cases of malignancy in the Netherlands. The numbers of reported acute Q fever cases per four-digit zipcode areas were obtained from the National Institute For Public Health and Environment (RIVM). The RIVM registers all reported acute Q fever cases: reporting of laboratory confirmed acute Q fever cases to the RIVM is obligatory by law in the Netherlands. Since data are completely anonymized, untraceable to individuals, and patients in this study were not subject to any interventions, no approval of a Medical Ethical Committee was requested or obtained.

Additionally, the risk of NHL in chronic Q fever patients was studied for which data from the Dutch national chronic Q fever database were used. In this database, clinical, microbiological, and radiological data of all known proven, probable, and possible chronic Q fever patients >18 years of age in the Netherlands, diagnosed after 01-01-2007 and defined according to the definitions formulated by the Dutch chronic Q fever consensus group, are stored.[19] Design of this database, to which 35 hospitals contribute, was approved by the Medical Ethical Committee of the University Medical Centre in Utrecht. Details on design and content of this database were described in a previous publication.[20]

Definitions

Exposure to *C. burnetii* was studied using a proxy parameter. Due to privacy reasons, it was impossible to directly link reported *C. burnetii* infections to the NCR data. Therefore, we compared three areas with different intensity of exposure, based on number of reported cases of acute Q fever between 1-1-2007 and 31-12-2010. The proxy parameter consists of three Q fever incidence areas, representing a certain level of exposure:

1. 'low Q fever incidence areas' (four-digit zipcode areas with no reported acute Q fever cases);
2. 'intermediate Q fever incidence areas' (four-digit zipcode areas with one reported acute Q fever case);
3. 'high Q fever incidence areas' (four-digit zipcode areas with > one reported acute Q fever case).

The primary outcome of this study was the risk of NHL in the Dutch population for different groups of exposure to *C. burnetii* based on Q fever incidence in the area of residence. The secondary outcome of this study was the risk of NHL in chronic Q fever patients, as compared to the population.
NHL was defined according to the International Classification of Diseases for Oncology (ICD-O) 10 classification of tumours of haematopoietic and lymphoid tissues (appendix 1).[21]

Statistics

Data from Statistics Netherlands, Netherlands Cancer Registry and RIVM were registered in Microsoft Excel (Windows version 2010). Data analysis was performed in R studio (version 3.2.2) and SPSS (version 21.0) was used for generating figures. We hypothesized that if there is an association between exposure to *C. burnetii* and the occurrence of NHL, an increased incidence of NHL would be observed in high Q fever incidence areas during and shortly after the epidemic in the Netherlands and an increased incidence of NHL would be observed among chronic Q fever patients. No power calculation was performed, since this concerns a population-based analysis, making a power calculation redundant. Since multiple NHL can occur within one individual, patients were not censored after developing their first NHL. For reporting of multiple primary cancers, the International Rules for Multiple Primary Cancers (formulated by the International Agency For Research on Cancer of the World Health Organization) were applied by the Netherlands Cancer Registry.[22] Crude absolute risks (or incidence rates) were calculated for each studied year and for chronic Q fever patients per 100,000 person-years (PY). We used a Poisson regression model to calculate adjusted incidence rate ratio's, which can be interpreted as relative risks. For the primary outcome, the relative risks (RR) for NHL in high and intermediate Q fever incidence areas during and after the Dutch Q fever epidemic, compared to low Q fever incidence areas and previous years, were calculated by studying the interaction between time (per year) and area (low, intermediate and high Q fever incidence). The low Q fever incidence area per year served as a reference category. For the secondary outcome, relative risks for NHL were calculated for patients with chronic Q fever, compared to the general population which served as a reference category. For both primary and secondary outcomes, analyses were adjusted for age and gender. The natural logarithms of the number of PY at risk for the outcome were included in the models as an offset. Model diagnostics were performed by checking residual plots and influential observations. Overdispersion was assessed by calculating phi (variance vs. mean). The phi for the model calculating the risk for any NHL after exposure to *C. burnetii* was 1·04 and the phi for the model calculating the risk for NHL after chronic Q fever was 1·07 (both indicating good fit and very minimal overdispersion). The phi for the model for the risk of B-CLL or small lymphocytic B-cell NHL was 0·77 (indicating slight underdispersion). Residuals were slightly increasing towards higher values; there were no problematic influential observations. We reported relative risks (incidence rate ratio's), with 95% confidence intervals (Wald) and p-values. P-values of <0·05 were considered statistically significant. A sensitivity analysis was performed to calculate the RR for NHL among chronic Q fever patients, excluding one patient in which the focus of infection was unclear.

Role of the funding source

The funding sources funded the staff wages of the primary investigator, S.E. van Roeden, in order to be able to perform data collection, management, analysis and writing of this article. The funding source did not play a role in study design, collection of data, data analysis, data interpretation, writing of this manuscript and decision on

where to submit the paper for publication. SR, FH, CD, SH, ML and LK had access to (a part of) the raw data. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Missing data

For all chronic Q fever patients, follow-up data were available from medical records. Registration data are, as far as the possibility of hospital registration reaches, complete.

Results

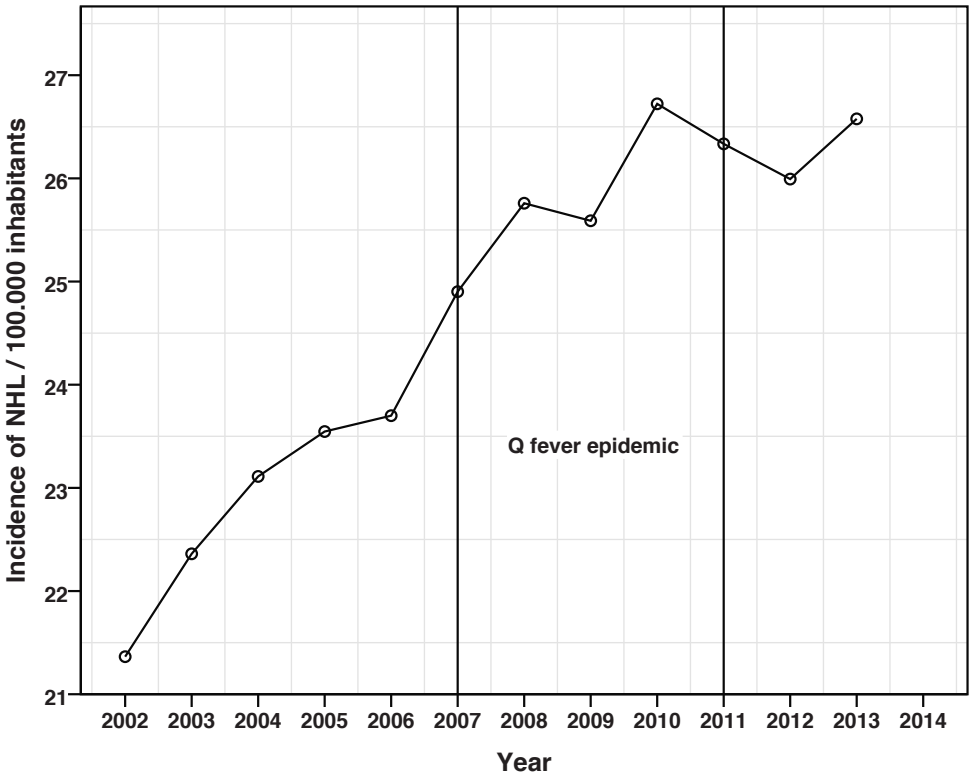
In total, 197,545,500 PY were observed during 12 years of follow-up (mean population size: 16·5 million inhabitants), with 71% living in low Q fever incidence areas (139,684,940 / 197,545,500 PY), 16% living in intermediate Q fever incidence areas (32,054,848 / 197,545,500 PY) and 13% living in high Q fever incidence areas (25,805,713 / 197,545,500 PY) during the Q fever epidemic (table 1). During 2002 - 2013, 48,760 persons were diagnosed with NHL. The number of NHL consisted of 11,588 cases of B-CLL or small lymphocytic B-cell NHL, 11,351 cases of indolent NHL, 18,917 cases of aggressive NHL, 2,212 cases of NK- and T-cell NHL, 2,049 cases of cutaneous NHL, and 2,643 cases of lymphoblastic lymphoma. The yearly incidence rate of NHL ranged from 21·4 / 100,000 PY to 26·7 / 100,000 PY, with the highest incidence in 2010, and significantly increased over time (Pearson correlation coefficient 0·949, $p < 0·01$) between 2002 and 2013, see figure 1. Furthermore, the incidence of NHL was higher in men than in women, and the incidence of NHL was strongly age-dependent (figure 2).

Table 1. Laboratory results at the day of presentation.

Laboratory measurement	Result	Normal range	Units of measurement
N (in person-years)	48,760	197,545,500	25
Male gender (%)	27,842 (57)	97,753,690 (50)	28
Median age (IQR)	69 (59-79)	39 (23 - 59)	-
Area of Q fever endemicity			
Low endemic (%)	34,879 (72)	139,684,940 (71)	25
Intermediate endemic (%)	7,713 (16)	32,054,848 (16)	24
High endemic (%)	6,168 (13)	25,805,713 (13)	24

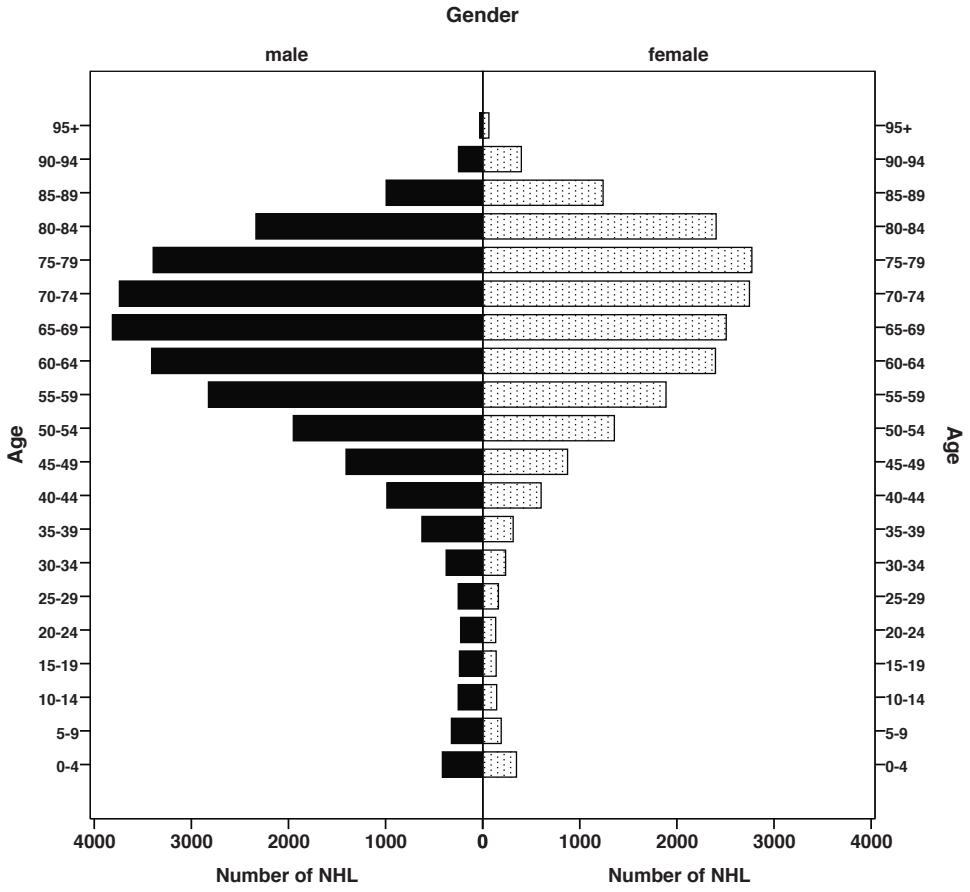
NHL = non-Hodgkin lymphoma.

Figure 1. Incidence rate of non-Hodgkin lymphoma per year



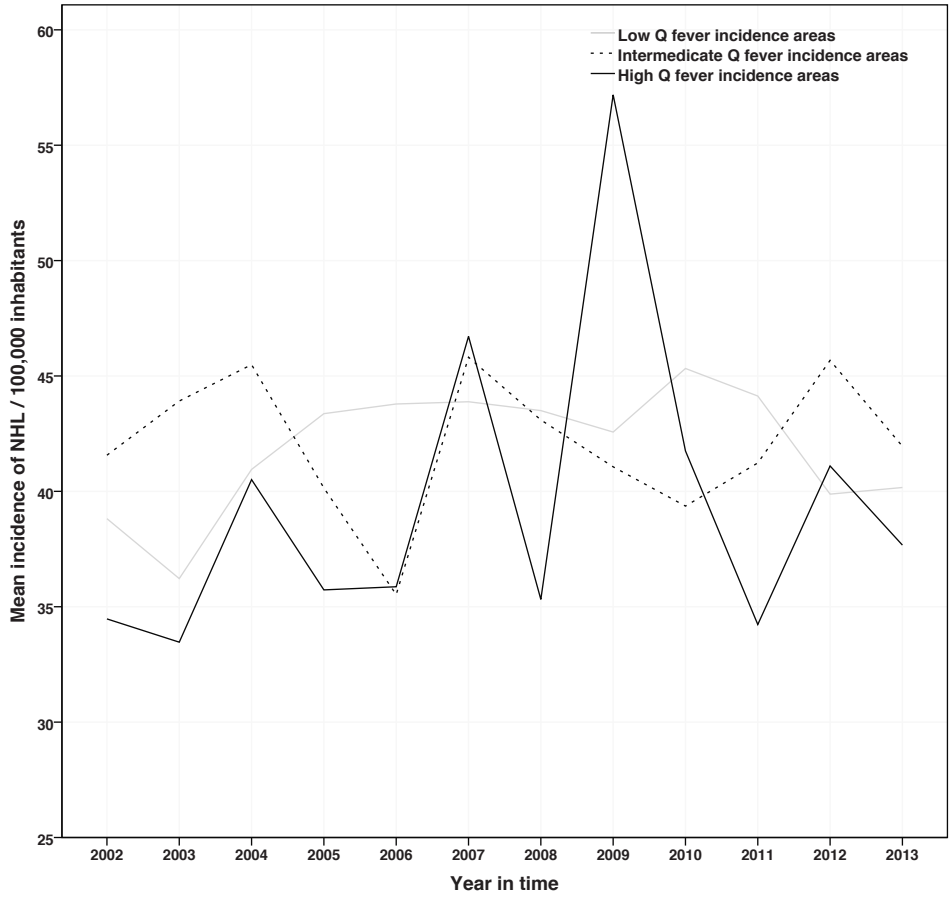
**incidence rate per year for the entire population, not weighed by age or gender.*

Figure 2. Number of diagnosed non-Hodgkin lymphoma per five year age-category and gender.



The mean incidence of NHL for different Q fever incidence areas was highest in high Q fever incidence areas in 2009 (see figure 3). When comparing relative risks (RR) of NHL in high and intermediate Q fever incidence area to low Q fever incidence area for 12 individual years, an increased incidence was observed in one of 22 analyses. The RR of NHL in this year in high Q fever incidence areas, compared to low Q fever incidence areas, was 1·16, 95% CI 1·02 – 1·33, p = 0·03 (see table 2). The crude absolute risk of NHL in high Q fever incidence area in 2009 was 27·7 / 100,000 PY, while it was 25·3 / 100,000 PY for low Q fever incidence area in 2009, resulting in a risk difference of 2·4 / 100,000 PY. When exploring the interaction between Q fever incidence area and time for all different subtypes of NHL, the RR for B-CLL or small lymphocytic B-cell NHL was 1·41, 95% CI 1·07 – 1·85, p = 0·01, in 2009 in high Q fever incidence areas, compared to low Q fever incidence areas (see table 3). The risk of indolent NHL, aggressive NHL, NK- and T-cell NHL, cutaneous NHL, and lymphoblastic lymphoma was not significantly increased in high Q fever incidence area in 2009 (supplementary table 1).

Figure 3. Interrupted time series: mean* incidence rate of non-Hodgkin lymphoma per geographic area in time.



**mean incidence rates of all incidence rates for NHL per five-year age and gender category is shown, leading to 'weighing' of the incidence rate for sex and age distribution in the population. Since the incidence rate is highly variable among specific subgroups (depending on age and sex), the mean incidence rate differs from the overall incidence rate shown in figure 1.*

Table 2. Relative risk for non-Hodgkin lymphoma (all subtypes) per area in time.

Factor	Number of diagnosed NHL	Relative risk	95% Confidence intervals	p-value
Interaction time and area (all including 2002 & Low incidence areas are reference categories)				
2003 * Intermediate	614	1·12	0·99 – 1·28	0·07
2003 * High	458	1·06	0·92 – 1·23	0·41
2004 * Intermediate	635	1·12	0·99 – 1·28	0·07
2004 * High	485	1·08	0·94 – 1·25	0·28
2005 * Intermediate	539	0·89	0·78 – 1·02	0·09
2005 * High	489	1·02	0·88 – 1·17	0·79
2006 * Intermediate	599	1·00	0·88 – 1·13	0·94
2006 * High	469	0·98	0·85 – 1·12	0·73
2007 * Intermediate	623	0·98	0·86 – 1·11	0·77
2007 * High	502	0·99	0·86 – 1·14	0·86
2008 * Intermediate	664	1·01	0·89 – 1·14	0·92
2008 * High	509	0·96	0·84 – 1·11	0·59
2009 * Intermediate	671	1·05	0·93 – 1·19	0·44
2009 * High	597	1·16	1·02 – 1·33	0·03
2010 * Intermediate	706	1·03	0·91 – 1·16	0·67
2010 * High	549	0·99	0·87 – 1·14	0·92
2011 * Intermediate	690	1·02	0·90 – 1·15	0·79
2011 * High	565	1·03	0·90 – 1·19	0·63
2012 * Intermediate	727	1·09	0·96 – 1·23	0·18
2012 * High	540	1·00	0·87 – 1·15	0·96
2013 * Intermediate	713	1·04	0·92 – 1·18	0·54
2013 * High	586	1·06	0·93 – 1·21	0·40

NHL = non-Hodgkin lymphoma. Significant predicting covariates in model: female gender, age and time. Non-significant predicting covariates in model: geographic area. Number of NHL diagnosed for the reference categories: 2002 low incidence area n = 2,499; 2002 intermediate incidence area n = 532; 2002 high incidence area n = 419; 2003 low incidence area n = 2,556; 2004 low incidence area n = 2,643; 2005 low incidence area = 2,815; 2006 low incidence area n = 2,806; 2007 low incidence area n = 2,954; 2008 low incidence area n = 3,063; 2009 low incidence area n = 2,962; 2010 low incidence area n = 3,185; 2011 low incidence area n = 3,141; 2012 low incidence area n = 3,088; 2013 low incidence area n = 3,167. Table 3. Relative risk for B-cell chronic lymphocytic leukemia or small lymphocytic B-cell non-Hodgkin lymphoma per area in time

Table 3. Relative risk for B-cell chronic lymphocytic leukemia or small lymphocytic B-cell non-Hodgkin lymphoma per area in time.

Factor	Number of diagnosed B-CLL / SL NHL	Relative risk	95% Confidence intervals	p-value
Interaction time and area (all including 2002 & Low incidence areas are reference categories)				
2003 * Intermediate	144	0·95	0·73 – 1·23	0·67
2003 * High	112	1·04	0·77 –1·39	0·81
2004 * Intermediate	148	0·92	0·71 –1·18	0·50
2004 * High	105	0·91	0·68 –1·23	0·54
2005 * Intermediate	137	0·88	0·68 –1·14	0·34
2005 * High	119	1·07	0·80 –1·43	0·66
2006 * Intermediate	163	0·99	0·77 –1·28	0·97
2006 * High	125	1·06	0·79 –1·41	0·69
2007 * Intermediate	151	0·86	0·66 –1·11	0·24
2007 * High	130	1·02	0·77 –1·36	0·87
2008 * Intermediate	158	0·85	0·66 –1·09	0·20
2008 * High	115	0·85	0·64 –1·14	0·28
2009 * Intermediate	169	1·00	0·78 –1·29	0·99
2009 * High	173	1·41	1·07 –1·85	0·01
2010 * Intermediate	160	0·90	0·70 –1·16	0·43
2010 * High	150	1·16	0·88 –1·53	0·29
2011 * Intermediate	164	0·93	0·72 –1·20	0·59
2011 * High	135	1·05	0·79 –1·40	0·71
2012 * Intermediate	160	0·98	0·76 –1·26	0·85
2012 * High	111	0·93	0·69 –1·25	0·62
2013 * Intermediate	158	1·05	0·81 –1·36	0·70
2013 * High	108	0·98	0·73 – 1·32	0·92

B-CLL =B-cell chronic lymphocytic leukaemia or small lymphocytic B-cell B-cell NHL. Significant predicting covariates in model: female gender, age and time. Non-significant predicting covariates in model: geographic area. Number of B-CLL diagnosed for the reference categories: 2002 low incidence area n = 578; 2002 intermediate incidence area n = 136; 2002 high incidence area n = 96; 2003 low incidence area n = 645; 2004 low incidence area n = 683; 2005 low incidence area = 656; 2006 low incidence area n = 690; 2007 low incidence area n = 739; 2008 low incidence area n = 782; 2009 low incidence area n = 707; 2010 low incidence area n = 740; 2011 low incidence area n = 733; 2012 low incidence area n = 683; 2013 low incidence area n = 625.

In the Dutch national chronic Q fever database, 439 patients were identified with a total follow-up duration of 1,661 PY (median per person 4·3 years, IQR 2·0 - 5·4 years) of whom five developed NHL after diagnosis of chronic Q fever: three were diagnosed with B-CLL, one had a mantle cell lymphoma, and one had a B-cell NHL of inconclusive subtype (table 4). The crude absolute risk of NHL among chronic Q fever patients was 301·0 / 100,000 PY. The RR for NHL in chronic Q fever patients when compared to the general population was 4·99 (95% CI 2·07 – 11·98, p < 0·01), adjusted for age and gender. Median time between diagnosis of chronic Q fever and diagnosis of NHL was eight months (interquartile range 3 - 26 months). When only evaluating chronic Q fever patients with B-CLL, median time between diagnosis of chronic Q fever and NHL was six months. One patient had no clear focus of infection, but was classified as probable chronic Q fever patients based on immunocompromised state (table 4). When we excluded this patient from our analysis, as a sensitivity analysis, the RR for NHL in chronic Q fever patients was 3.99 (95% CI 1.50 – 10.63).

Table 4. Characteristics of chronic Q fever patients with subsequent NHL.

ID	Gender	Age (years)	Classification of chronic Q fever	Focus of chronic Q fever	Type of NHL	Year of diagnosis of NHL	Other risk factor(s) for NHL	Time between chronic Q fever and NHL (months)
1	Male	85	Proven	Endocarditis	B-CLL	2012	Smoking	3
2	Male	80	Probable	Pericarditis	Mantle cell lymphoma	2011	Smoking, alcohol†	6
3	Male	64	Probable	No focus ¶	B-CLL	2011	Smoking, alcohol†	8
4	Male	72	Proven	Vascular prosthesis	B-CLL	2012	Smoking, alcohol†	10
5	Male	76	Proven	Endocarditis + vascular prosthesis	Large B-cell NHL / marginal zone B-cell lymphoma*	2015	Smoking	42

*NHL = non-Hodgkin lymphoma. In none of the patients, acute Q fever was notified. * Pathology report inconclusive: large B-cell non-Hodgkin lymphoma or marginal zone B-cell lymphoma. †one patient used <1 unit daily, one patient used 3 units daily and one patients used 6 units daily. ¶ Patient diagnosed with probable chronic Q fever based on immunocompromised state due to hypogammaglobulinemia.*

To explore the possibility of detection bias as explanation for the increase in observed B-CLL or small lymphocytic B-cell NHL cases, we assessed the number of leukocyte differentiation analyses in the Jeroen Bosch Hospital (a large hospital located in the midst of the Q fever epidemic) and of Saltro (a large laboratory organisation with multiple facilities widespread in the Netherlands, mainly serving general practitioners). The number of leukocyte differentiation analyses performed in the

Jeroen Bosch Hospital or Saltro was not at its peak in 2009, compared to other years during or after the Dutch Q fever epidemic of 2007 - 2010 (supplementary table 2).

Discussion

In this national population-based analysis, the incidence of NHL gradually increased from 2002 until 2013, with no discernable change in this trend during or after the large Dutch Q fever epidemic in the Netherlands of 2007 until 2010. When comparing the relative risk of NHL in high and intermediate Q fever incidence area to low Q fever incidence area for 12 individual years, an increased incidence was observed in one of 22 analyses: in high Q fever incidence area in 2009. The effect estimate corresponds to an absolute risk difference of 2·3 episodes per 100,000 person years between high and low Q fever incidence area. Among 439 chronic Q fever patients, the risk of NHL was increased based on five patients that developed NHL, on average eight months (IQR 3 - 26 months) after diagnosis of chronic Q fever, yielding a crude absolute risk of NHL among chronic Q fever patients of 301·0 / 100,000 PY and a RR of 4·99 (95% CI 2·07 – 11·98, p < 0·01). The observed effects may be due to chance (type I error), since an increased incidence was observed in one of 22 analyses only.

If *C. burnetii* were to play a pathophysiological role in the etiology of NHL, we hypothesized that this would lead to an increased risk of NHL after exposure to *C. burnetii*. The association however, was only observed in 2009 and absent in the years thereafter. Since the majority of patients were diagnosed with Q fever in 2009 and the risk for NHL is increased in 2009, only a very short timeframe between infection and development of NHL could explain this finding. In a previous study, the time between exposure to *C. burnetii* and NHL in patients with acute or chronic Q fever was on average eight months (range -4 months to 21 months).[7] In the current study, the moment of infection with *C. burnetii* was unknown for all patients developing NHL, including those with chronic Q fever. Therefore, we could not assess intervals between primary infection and NHL development, but the median time between diagnosis of chronic Q fever and development of NHL in this study was eight months. In another study, the average time between primary and secondary infection was six months.[23] For other pathogens, e.g. *H. pylori*, EBV and HIV, time between exposure and NHL development is even longer, varying up to 4 – 14 years.[3-6] The average time between primary *C. burnetii* infection and development of NHL will therefore be longer than eight months, making it unlikely that the excess risk in 2009 is explained by the number of notified infections in 2009. In the current study, we were able to detect an effect up to nine years after start and four year after control of the Q fever epidemic, and it seems unlikely that increased incidence of NHL would occur after such a period.

Furthermore, the risk for NHL was not increased in intermediate Q fever incidence areas. The absence of an exposure-response relationship in the intensity of exposure and the risk of NHL based on reported incidence of acute Q fever, does not plead for a causal relationship. However, one could consider continuous and prolonged exposure to *C. burnetii* in chronic Q fever patients as more 'intense' than in the

setting of acute Q fever, and therefore assume an exposure-response relationship based on the higher risk after chronic Q fever compared to the risk after exposure to *C. burnetii* in the general population (which is based on the number of notified acute Q fever cases).

Our study has several limitations. One of these is detection bias, which may explain the increased incidence of NHL in high Q fever incidence areas in 2009. In that year, the Q fever epidemic was at its peak with 2,354 notified patients.[23] Patients diagnosed with Q fever or presenting with symptoms suggestive for Q fever may have undergone laboratory testing more often. B-CLL or small lymphocytic B-cell NHL may be detected by measuring a simple leukocyte differentiation, which could explain the increase in NHL that was mainly due to an increase in the number of B-CLL or small lymphocytic B-cell NHL diagnoses.[24] We found no evidence to support this hypothesis in exploring the number of leukocyte differentiation laboratory measurements during and after the Q fever epidemic in two large laboratories, as we did not find a peak in the number of leukocyte differentiation analyses in 2009. As NHL is not self-limiting, such patients would have been diagnosed 'earlier' with NHL, theoretically leading to a decreased incidence in the years after the epidemic (since all cases have been detected before), which was not observed. Also among chronic Q fever patients detection bias could explain the increased incidence because these patients undergo extensive diagnostic investigation upon diagnosis and during follow-up, including PET-CT and laboratory investigations, potentially leading to a higher detection rate of hematological malignancies.[19]

Naturally, the association between NHL and Q fever in 2009 may be based on reversed causality: patients with (yet undetected) NHL may be immunocompromised, leading to more severe symptoms resulting in a higher likelihood of being diagnosed with Q fever. However, since the number of notified acute Q fever cases is considered a proxy for the number of actual infected (both symptomatic and asymptomatic) individuals and we did not directly link notified infection to the outcome, the association is based both on notified and unnoticed cases. Therefore, it is unlikely that the association between acute Q fever and NHL in this study relies on an increased reported incidence of Q fever in immunocompromised hosts.[25,26] For chronic Q fever patients, we cannot exclude that NHL was present (but not yet detectable) in these cases before or at the moment of diagnosis of chronic Q fever.

By using the number of notified acute Q fever cases in a certain Q fever incidence areas as proxy parameter, it is uncertain if all individuals in those areas were actually exposed. This may influence the association and distort the results in both directions. Furthermore, we were not able to correct for other predictors for NHL, such as viral infections or immunodeficiency. For example, none of the chronic Q fever patients were HIV-positive, whereas in the general population HIV-positive individuals were included. In the Netherlands, approximately 40 HIV patients develop NHL each year.[27] This could lead to a minor underestimation of the risk of NHL in chronic Q fever patients. Moreover, we assessed the risk of NHL in chronic Q fever patients by comparing them to the general population. However, we did not account for exposure to *C. burnetii* in the setting of acute Q fever in the general population,

which could dilute the effect and lead to underestimation of the risk of NHL in chronic Q fever patients. The number of NHL for specific Q fever incidence areas could not be provided per year. Since the epidemic had the largest geographic spread and was most intense in 2009, the actual areas with high Q fever incidence in 2007, 2008, and 2010 were considerably smaller than defined, in contrast to 2009.[28,29] Therefore, some high Q fever incidence areas may have been misclassified in 2007, 2008, and 2010, potentially leading to dilution of the effect of exposure to *C. burnetii* on the risk of NHL in those years. Finally, data on subtype of NHL within subgroups were not available. Therefore, it is theoretically possible that an association for rare, specific subtypes (that are now grouped in one of the larger subgroups) may not have been detected. The association between *C. burnetii* and NHL in the previous report, by Melenotte et al, relied on the subtypes diffuse large cell B-cell NHL and follicular lymphoma which are by far the most common NHL.[1,7] Therefore, we are confident that any relevant association for these subtypes would have been detected with this study. However, within the subgroups of aggressive and indolent NHL (to which these type of NHL belong) no association was observed.

The Dutch Q fever epidemic provided the unique opportunity to assess the association between exposure to *C. burnetii* and NHL in a large population. For this study, the incidence of NHL in the entire Dutch population over twelve years was assessed. The NCR provides high quality data that is collected by systematically registering NHL in all Dutch hospitals based on medical charts. Furthermore, we assessed the risk of NHL in the setting of acute and chronic Q fever. Currently, 35 hospitals contribute to the Dutch national chronic Q fever database providing follow-up up to nine years after start of the epidemic. Due to the fact that we identified patients via microbiological laboratory results, we are confident that most known Dutch chronic Q fever patients are captured in the comprehensive database.

In conclusion, the current study of the incidence of NHL after the largest Q fever epidemic ever reported did not convincingly confirm an association between Q fever and NHL. Several limitations, inherent to the design of this study, may lead to both underestimation or overestimation of the studied association. Further research exploring potential pathophysiological mechanisms and studying exposure on an individual level may provide more definite answers.

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References

1. Shankland KR, Armitage JO, Hanchock BW. Non-Hodgkin lymphoma. Lancet 2012;380:848-57.
2. Czarnota J, Gennings C, Colt JS et al. Analysis of environmental chemical mixtures and non-Hodgkin lymphoma risk in the NCI-SEER NHL study. Environ Health Perspect 2015;123(10):965-70.
3. Hjalgrim H, Askling J, Rostgaard K et al. Characteristics of Hodgkin's lymphoma after Infectious Mononucleosis. NEJM 2003;349:1324-32.
4. Beral V, Peterman T, Berkelman R, Jaffe H. AIDS-associated non-Hodgkin lymphoma. The Lancet, 1991;337(8754):805-9.
5. Parsonnet J, Hansen S, Rodriguez L et al. *Helicobacter pylori* infection and Gastric lymphoma. NEJM 1994:330(18):1267-71.
6. Schöllkopf C, Melbye M, Munksgaard L et al. *Borrelia* infection and risk of non-Hodgkin lymphoma. Blood 2008;111(12):5524-29.
7. Melenotte C, Million M, Audoly G et al. B-cell non Hodgkin-lymphoma linked to *Coxiella burnetii*. Blood 2016; 127(1):113-21.
8. Honstettre A, Imbert G, Ghigo E et al. Dysregulation of cytokines in acute Q fever: role of interleukin-10 and tumor necrosis factor in chronic evolution of Q fever. J Infect Dis 2003; 187 (6): 956-962.
9. Levy Y, Brouet JC. Interleukin-10 prevents spontaneous death of germinal center B cells by induction of the bcl-2 protein. J Clin Invest 1994;93(1):424-428.
10. Kampschreur LM, Hagenaars JC, Wielders CC et al. Screening for *Coxiella burnetii* seroprevalence in chronic Q fever high-risk groups reveals the magnitude of the Dutch Q fever outbreak. Epidemiol Infect 2013;141(4):847-51.
11. 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; 17(3): 20058.
12. Georgiev M, Afonso A, Neubauer H et al. Q fever in humans and farm animals in four European countries, 1982 to 2010. Euro Surveill 2013;18(8):20407.
13. Anderson AD, Szymanski TJ, Emery MP et al. Epizootiological investigation of a Q fever outbreak and implications for future control strategies. J Am Vet Med Associ 2015;247(12):1379-86.
14. Bond KA, Vincent G, Wilks CR et al. One health approach to controlling a Q fever outbreak on an Australian goat farm. Epidemiol Infect 2015;23:1-13 (epub ahead of print).
15. Alonso E, Lopez-Etxaniz I, Hurtado A et al. Q fever outbreak among workers at a waste-sorting plant. PLoS One 2015;10(9):e0138817.
16. Bellini C, Magouras I, Chapuis-Taillard C et al. Q fever outbreak in the terraced vineyars of Lavaux, Switzerland. New Microbes New Infect 2014;2(4):93-9.
17. D'Amato F, Eldin C, Georgiades K et al. Loss of TSS1 in hypervirulent *Coxiella burnetii* 175, the causative agent of Q fever in French Guiana. Comp Immunol Microbiol Infect Dis 2015;41:35-41.
18. Akbarian Z, Ziay G, Schauwers W et al. Brucellosis and *Coxiella burnetii* Infection in Householders and Their Animals in Secure Villages in Herat Province, Afghanistan: A Cross-Sectional Study. PLoS Negl Trop Dis 2015;9(10):e0004112.
19. Wegdam-Blans MC, Kampschreur LM, Delsing CE et al. Chronic Q fever:

review of the literature and a proposal of new diagnostic criteria. J Infect 2012;64(3):247-59.
20. Kampschreur LM, Delsing CE, Groenwold RH et al. Chronic Q fever in the Netherlands 5 year after the start of the Q fever epidemic: results from the Dutch chronic Q fever database. J Clin Microbiol 2014;52(5):1637-43.
21. Fritz A, Percy C, Jack A et al. International Classification of Diseases for Oncology 3rd edition [Internet]. Geneva: World Health Organization; 2013 [updated 2013 Nov 28; cited 2016 June 20]. Accessed August 2017 at http://apps.who.int/iris/bitstream/10665/96612/1/9789241548496_eng.pdf.
22. IACR and WHO. International rules for multiple primary cancers (ICD-O third edition), 2004. Accessed August 2017 at http://www.iacr.com.fr/images/doc/MPrules_july2004.pdf.
23. Fenollar F, Fournier PE, Carrieri MP, Habib G, Messana T, Raoult D. Risks Factors and Prevention of Q Fever Endocarditis. CID 2001;33(3):312-16.
24. Hallek M, Cheson BD, Catovsky D et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the international workshop on chronic lymphocytic leukemia updating the national cancer institute – working group 1996 guidelines. Blood 2008;111(12):5446-56.
25. Madariaga MG, Rezai K, Trenholme GM et al. Q fever: a biological weapon in your backyard. Lancet Infect Dis. 2003 Nov; 3(11):709-21.
26. Tigertt WD, Benenson AS, Gochenour WS. Airborne Q fever. Bacteriol Rev 1961 Sep;25:285-93.
27. van Sighem AI, Boender TS, Wit FWNM et al. Monitoring reports 2007 - 2016. Human immunodeficiency virus (HIV) infection in the Netherlands. Amsterdam: Stichting HIV-Monitoring, 2016. Accessed on August 17th 2017 at: www.hiv-monitoring.nl.
28. National Institute for Public Health and the Environment, RIVM, Accessed Augustus 2017 at http://www.rivm.nl/Onderwerpen/Q/Q_koorts.
29. Dijkstra F, van der Hoek W, Wijers N. The 2007–2010 Q fever epidemic in The Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. FEMS Immunol Med Microbiol 2012; 64(1): 3-12.

Appendix 1

The definition of NHL includes: B-cell chronic lymphocytic leukaemia (B-CLL) or small lymphocytic B-cell B-cell NHL; all indolent NHL (including follicular lymphoma, lymphoplasmacytic lymphoma, Waldenström macroglobulinemia, hairy cell leukaemia, and other indolent NHL); all aggressive NHL (including B-cell prolymphocytic leukaemia, mantle cell lymphoma, Burkitt lymphoma, diffuse large cell B-cell NHL, other, and unspecified aggressive NHL); mature T and NK-cell NHL (including T- or NK-cell leukemia, T- or NK cell lymphoma); cutaneous NHL (including B- and T-cell cutaneous lymphoma); lymphoblastic leukemia or lymphoma (including B-cell acute lymphoblastic leukemia/lymphoma, T-cell acute lymphoblastic leukemia/lymphoma, precursor B-cell lymphoblastic leukemia/lymphoma, and precursor T-cell lymphoblastic leukemia/lymphoma).[21]

Supplementary table 1. Relative risks for NHL other than B-CLL or small lymphocytic B-cell NHL in high endemic area in 2009.

Factor	Relative risk interaction 2009 and high incidence area	95% Confidence intervals	p-value
Indolent NHL	1·19	0·89 – 1·59	0·25
Aggressive NHL	1·05	0·84 – 1·30	0·69
T and NK-cell NHL	0·91	0·47 – 1·76	0·78
Cutaneous NHL	1·09	0·58 – 2·06	0·79
Lymphoblastic lymphoma	1·20	0·61 – 2·07	0·72

**estimates and p-values are reported for the interaction term for 2009 and high incidence area specifically, to explore for which subtype of B-NHL the relative risk was increased in that year. These estimates are derived from full models, exploring the interaction term for all studied years and Q fever incidence areas.*

Supplementary table 1. Relative risks for NHL other than B-CLL or small lymphocytic B-cell NHL in high endemic area in 2009.

Year	Number of notified acute Q fever cases	General Practice laboratories	Jeroen Bosch Hospital laboratory
2006	13	unknown	unknown
2007	168	unknown	unknown
2008	1,000	165,645	62,681
2009	2,354	158,480	77,352
2010	504	153,104	79,888
2011	81	149,440	108,554
2012	66	142,382	90,976
2013	19	132,985	77,810
2014	28	132,097	74,719
2015	22	143,185	74,539
2016	12	145,574	79,161

**data on number of reported acute Q fever cases derived from the RIVM.[24] Data on the number of leukocyte differentiation analyses derived from the clinical laboratory of chemistry and hematology of the Jeroen Bosch Hospital (Jacqueline Leuvenink) and Saltro Laboratories (David Boss) in August 2017.*

Chapter 9

Coxiella burnetii in non-Hodgkin lymphoma tissue samples: innocent until proven otherwise?

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Abstract

Coxiella burnetii has been suggested as a potential cause of B-cell non-Hodgkin lymphoma (B-NHL), as *C. burnetii* was detected in B-NHL tissues. To further investigate this potential relationship, we hypothesized that among subjects previously exposed to *C. burnetii*, the bacterium is more frequently detectable in tissues of patients with B-NHL (cases) compared to patients without B-NHL (controls). We aimed to evaluate this hypothesis by assessing the presence of *C. burnetii* with polymerase chain reaction (PCR), immunofluorescence staining (IF) and fluorescent in-situ hybridization (FISH). Eligible patients were those previously exposed to *C. burnetii* (confirmed serologically) with tissue samples available for analysis. Negative (non-infected L929 cells, non-infected SCID mouse tissues, seronegative patient tissues) and positive control samples (*C. burnetii* infected L929 cells, *C. burnetii* infected SCID mouse tissues) were included. Samples were available for 13 cases and 16 controls. *C. burnetii* was demonstrated in tissues of 8/29 patients in total (28%), with either PCR, IF or FISH: in 5/13 cases (38%) and 3/16 controls (19%), p = 0.41. Negative and positive control samples were all negative and positive appropriately for all three diagnostic methods. In patients previously exposed to *C. burnetii* the bacterium was detected in tissue samples from subjects with and without B-NHL, without significant differences in the proportion positive samples. Therefore, we conclude that detection of *C. burnetii* in tissues of patients previously exposed to *C. burnetii* is a non-specific finding.

Introduction

Q fever is a zoonosis that causes clinically relevant disease in humans and animals.[1] After primary infection, individuals may develop a flu-like illness, pneumonia or hepatitis. A small proportion develops a chronic or persistent focalized infection, resulting in endocarditis, vascular infections, lymphadenitis or rarer manifestations.[1]

Apart from causing Q fever, *Coxiella burnetii* has been implicated as a potential causative agent in the development of non-Hodgkin lymphoma (NHL).[2] An increased incidence of NHL was reported in patients with Q fever infection. Moreover, *C. burnetii* was demonstrated in macrophages and plasmacytoid dendritic cells in NHL tissues. The hypothesis of a causal relationship between *C. burnetii* and NHL was postulated.[2] One of the potential mechanisms could be that increased interleukin-10 (IL-10) production during infection with *C. burnetii* induces the development of NHL.[3-6] It has been suggested that IL-10 plays a role in development of lymphoma, B-cell NHL (B-NHL) specifically, through stimulation of proliferation of B-cells and prevention of apoptosis.[7]

Another potential explanation for the presence of *C. burnetii* in B-NHL tissue samples is that *C. burnetii* also invades antigen-presenting cells in malignant tissues (e.g. tumor-infiltrating monocytes or macrophages) without playing a direct role in lymphoma development. In one previous study, latent presence of *C. burnetii* in various tissues up to five years after primary infection was reported.[8]

We hypothesized that if *C. burnetii* induces the development of B-NHL, the bacterium would be significantly more often present in B-NHL tissues than in tissues of patients without NHL. If *C. burnetii* is detected equally frequent in B-NHL tissues and tissues without B-NHL, the finding of *C. burnetii* in B-NHL tissues could be considered non-specific. To test this hypothesis, we investigated the presence of *C. burnetii* in tissues of patients previously exposed to *C. burnetii* both with and without B-NHL, with highly sensitive diagnostic techniques.

Methods

Study design and population

The presence of *C. burnetii* in tissues of patients previously exposed to *C. burnetii* with and without B-NHL was assessed with different diagnostic techniques: polymerase chain reaction (PCR), immunofluorescence (IF) staining and fluorescent in-situ hybridization (FISH).

We collected tissues of patients previously exposed to *C. burnetii* with B-NHL (cases) and without B-NHL (controls). To identify cases, all B-NHL patients alive after 01-01-2007 diagnosed at the Jeroen Bosch Hospital in 's-Hertogenbosch, located in the heart of the Dutch Q fever outbreak of 2007 – 2010,9 were evaluated. All B-NHL patients ≥18 years of age, with *C. burnetii* serology performed in clinical routine care or with deep frozen serum available from prior venepunctures to perform serological testing, were evaluated. Patients were considered eligible if tissue samples were available. Patients with B-NHL deceased prior to 2007 were not evaluated, because Q fever was rare in the Netherlands before the outbreak and exposure to *C. burnetii* in these patients is highly unlikely.[10] In patients without serology performed in clinical routine with deep frozen serum available from prior venepunctures, serological testing was performed on a thawed serum sample. Tissues of all selected cases were assessed for presence of *C. burnetii*. If multiple tissues were available, the tissue on which the initial diagnosis was based was selected; if two tissue specimens from one patient were obtained simultaneously, both were selected.

Controls were selected by cross-checking clinical, pathology and microbiology records in the same hospital: all patients exposed to *C. burnetii* confirmed by serology with a tissue biopsy obtained between 2007 and 2015, without history or presence of any type of NHL, were considered eligible. To ensure comparability between cases and controls with regard to tissue type and time since exposure, controls were matched on tissue type and year of retrieval. If matching on year was not possible, the most nearby year was taken. If matching on tissue type was not possible, a lymph node biopsy was selected if available. If multiple eligible tissues were available, all were selected.

Data collection and storage

Design and performance of this study was approved and monitored by the Medical Ethical Committee Brabant in 2016. Waiver for informed consent procedure was obtained, since all tissues and data were collected in routine care and data and tissues were completely anonymised. Performing of serological testing and collection of clinical data from electronically stored medical records occurred between June 2016 and September 2016. Laboratory testing of tissue specimens occurred in December 2016 for B-NHL tissues and in June 2017 for control samples. All data were processed anonymously and stored and analysed in SPSS Version 21.0.

Laboratory diagnostics

Serum - Serological testing for *C. burnetii* consisted of indirect fluorescent-antibody assay (IFA; Focus Diagnostics, Inc., Cypress, CA, USA) for phase I and II IgM and IgG, performed by a trained microbiological technician from the Jeroen Bosch Hospital. Titration was performed with binary serial dilutions, with a detection cut-off titer of 1:32. Serum samples were retrieved from the central laboratory freezer, where samples are kept at a temperature of -20Cº, and thawed for serological assays.

Tissues - Positive control samples were prepared from L929 cells and tissues of a SCID mouse infected with the *C. burnetii* nine mile strain.[11] Negative control samples were prepared from non-infected L929 cells, tissues of a non-infected SCID mouse and tissues of patients without *C. burnetii* antibodies (a sample of a B-NHL patient as well as a sample of a patient without NHL). Diagnoses from histopathological reports generated in clinical routine were used.

Polymerase chain reaction

A laboratory-developed real time PCR for *C. burnetii* was performed on paraffin-embedded lymphoma tissue by a trained microbiological technician from the Jeroen Bosch Hospital.[12] All samples were analyzed in duplo. This real-time PCR targets the multicopy transposase gene (IS1111a element). A specimen of at least 2 square millimeters (mm^2) was used. Paraffin-embedded materials were dewaxed and pre-treated with proteinase K. DNA was extracted using easyMAG (NucliSENS, bioMérieux, Marcy l'Etoile, France). The DNA isolate was used as template for three PCRs: two for detection of *C. burnetii* and one for PhHV to ensure adequate DNA isolation and exclude inhibition.

Immunofluorescence

For IF, paraffin-embedded tissues were dewaxed by heating at 65ºC for 5 minutes, bathed in Clearify for 5 minutes, rehydrated in ethanol (three baths of 5 minutes in 100% ethanol, 95% ethanol and 70% ethanol respectively) and permeabilised in Phosphate Buffered Saline (PBS) for 5 minutes. Bovine Serum Albumin 3%/PBS was applied for blocking nonspecific sites and samples were incubated at 37ºC for 30 – 60 minutes. After three brief washes in PBS-Tween20 (0.1%), the first antibody (anti-*C. burnetii* rabbit IgG conjugated to Alexa Fluor 555 (red)) was applied (dilution 1/800) and samples were incubated at 37ºC for 1 hour. Samples were washed in three subsequent baths of PBS-Tween20 (0.1%) for 5 minutes, and the second antibody (goat anti-rabbit IgG conjugated to Alexa Fluor 555) was applied (dilution 1/800) and samples were incubated at 37ºC for one hour with again three subsequent baths of PBS-Tween20 (0.1%) for 5 minutes. After second antibody fixation, 4',6-diamidino-2-phenylindole (DAPI) staining (blue) was performed and slides were conserved at 4 ºC. The process was performed under protection from light.

Fluorescence in situ-hybridization

For FISH targeting specific *C. burnetii* 16S rRNA, paraffin-embedded tissues were first dewaxed by heating at 65ºC for 5 minutes, bathed in Clearify for 5 minutes, rehydrated in ethanol (three baths of 5 minutes in 100% ethanol, 70% ethanol and 50% ethanol respectively) and permeabilised in PBS-Tween20 (0.1%) for 5 – 10 minutes. Subsequently, samples were hybridized with RNA-probes. The probes, CB-440 (5'- CTTGAGAATTTCTTCCCC -3') and CB-189 (5'- CCGAAGATCCCCCGCTTTGC - 3') specifically target the *C. burnetii* 16S rRNA sequences (green). Furthermore, a probe for detection of bacterial 16S rRNA molecule (EUB-338, specific for most eubacteria, neon-red) and a non-specific probe (non-EUB-338, dark red) were added to exclude nonsense hybridization. A positive signal is yellow, as a result of the co-localization of the universal probe EUB (red) and the specific 16S rRNA *C. burnetii* probe (green). The probes were diluted in hybridization solution (dilution 1/100), that consists of natriumchloride, formamide, 10% sodium dodecyl sulfate (SDS), tris-hydrochloride (pH 8.0) and H2O. Samples were hybridized at 65ºC for 10 minutes and at 37ºC overnight. After hybridization, samples were washed in Washing Buffer (consisting of natriumchloride, ethylenediamineetetraacetic acid, tris-hydrochloride (pH 8.0), 10% SDS and H2O) for 15 minutes and briefly rinsed with H2O. After drying, DAPI staining (blue) was performed and slides were conserved at 4 ºC. The process was performed under protection from light.

Definitions

Presence of *C. burnetii* in tissue samples - The primary outcome of this study was the proportion of positive tissue samples, thus samples in which *C. burnetii* was detected, with any of the three highly sensitive and specific diagnostic techniques.
B-cell non-Hodgkin lymphoma - The definition of B-NHL includes all types of mature B-cell lymphoma, including precursor B-cell leukemia and precursor B-cell lymphoma. Mature B-cell lymphoma was defined as diffuse large cell B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, Burkitt lymphoma, extranodal marginal zone B-cell lymphoma or mucosa-associated lymphoid tissue B-cell lymphoma, nodal marginal zone B-cell lymphoma, splenic marginal zone B-cell lymphoma, chronic lymphatic leukemia, hairy cell leukemia, B-cell prolymphocytic leukemia and lymphoplasmacytic lymphoma or Waldenström macroglobulinemia.[13]

Exposure to *C. burnetii* - Patients considered exposed to *C. burnetii* are those with positive serology (with or without matching the definition of chronic or persistent Q fever). Definition of positive serology not matching chronic or persisting Q fever is a positive phase II IgG titer (≥1:32), without fulfilling the criteria for chronic or persisting Q fever. Not all patients had undergone radiographical imaging, since we used data and samples that were generated in routine clinical care. Therefore, radiographical imaging data were available only if imaging had been performed in clinical practice. Chronic Q fever was defined according to the Dutch consensus guideline (possible, probable or proven chronic Q fever), which implies having at least a phase I IgG titer of ≥ 1:1024.[14] The diagnosis of proven or probable chronic Q fever according to these criteria with a defined focus of infection, is comparable to the definition of a

definite or possible persistent focalized infection according to the criteria formulated by Eldin et al.[1] Patients without antibodies against *C. burnetii* (seronegative patients) are considered unexposed to *C. burnetii*. All dates between 01-01-2007 and 31-12-2010 are considered to be during the Dutch Q fever outbreak.[10]

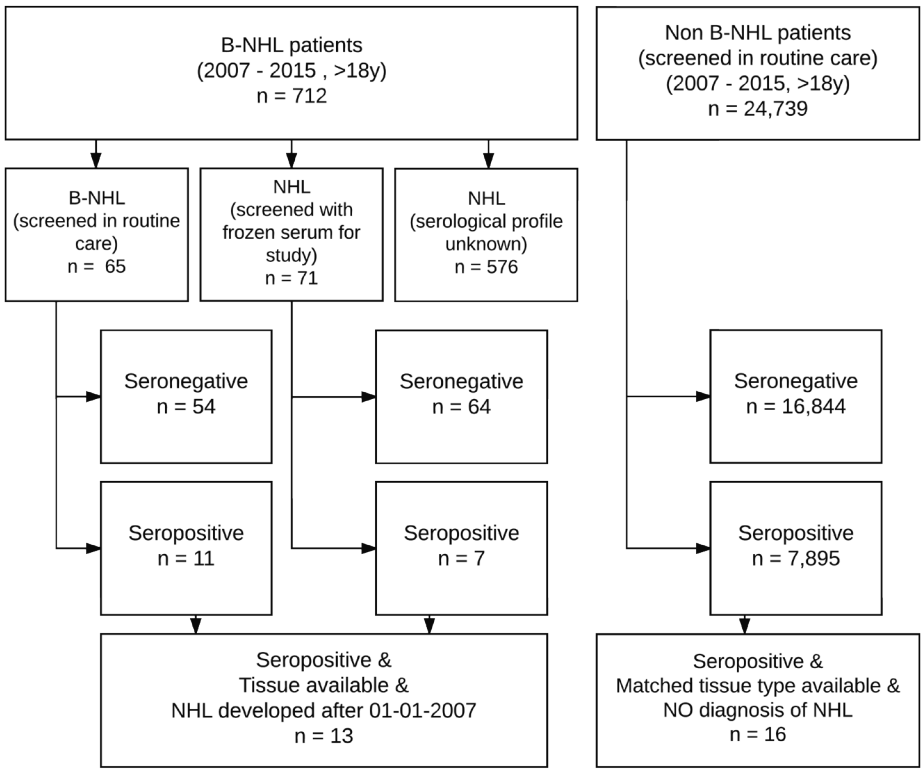
Statistics

Differences in proportions of positive tissues for cases and controls were calculated with a Chi-square or Fisher exact test, as appropriate. Calculations were performed in SPSS (Version 21.0). Univariable odds ratio's (OR) with 95% confidence intervals were calculated. P-values below 0.05 were considered to be statistically significant.

Results

In total, 136 B-NHL patients were evaluated for eligibility in this study (figure 1). See table 1 for baseline characteristics of all serologically evaluated B-NHL patients. Tissue samples were available from 13 cases and 16 controls, a description of cases and controls is given in table 2.

Figure 1. Flowchart of inclusion



**B-NHL = B-cell non-Hodgkin lymphoma. NHL = non-Hodgkin lymphoma*

Table 1. Baseline characteristics of all B-cell non-Hodgkin lymphoma patients.

	All	Exposed to	Not exposed
N (%)	136	18 (13)	118 (87)
Mean age (standard deviation (sd))	61 (15)	64 (18)	60 (14)
Male sex (%)	77 (57)	11 (61)	66 (56)
Residency in high endemic area (%)	124 (91)	17 (94)	107 (91)
Year of B-NHL diagnosis			
Pre-outbreak (%)	23 (17)	3 (17)	20 (17)
During outbreak (%)	20 (15)	1 (6)	19 (16)
After outbreak (%)	93 (68)	14 (78)	79 (67)
Serological diagnosis			
Exposed to *C. burnetii* (%)	18 (13)	18 (100)	–
Chronic Q fever (%)[σ]	2 (11)	2 (11)	–
Seronegative (%)	118 (87)	–	118 (100)
Subtype of lymphoma			
Follicular lymphoma (%)	34 (25)	7 (39)	27 (23)
Diffuse large cell B-cell lymphoma (%)	40 (29)	5 (28)	35 (30)
Mantle cell lymphoma (%)	16 (12)	2 (11)	14 (20)
B-acute lymphoblastic leukemia (%)	7 (5)	1 (6)	6 (5)
Marginal zone (including extranodal) lymphoma (%)	12 (9)	1 (6)	11 (9)
Small lymphocytic B-cell lymphoma (%)	5 (4)	–	5 (4)
Mucosa-associated lymphoid tissue lymphoma (%)	3 (2)	1 (6)	2 (2)
Burkitt lymphoma (%)	3 (2)	–	3 (3)
Waldenstrom (%)	3 (2)	–	3 (3)
Other (%)	13 (10)	1 (6)	12 (10)
Ann Arbor stage			
I-II (%)	52 (38)	9 (50)	43 (34)
III-IV (%)	76 (56)	8 (44)	68 (58)
n/a or unknown (%)	8 (6)	1 (6)	7 (6)
NHL risk factors			
Immunocompromised* (%)	11 (8)	2 (11)	9 (8)
Rheumatoid arthritis (%)	7 (5)	2 (11)	5 (4)
Prior chemo-/radiotherapy (for other malignancy) (%)	5 (4)	–	5 (4)
Use of alcohol[†] (%)	76 (56)	11 (61)	65 (55)
History of smoking[+] (%)	58 (43)	9 (50)	49 (42)

Q fever risk factor			
Valvulopathy worse than mild / valve prosthesis (%)	11 (8)	3 (17)	8 (7)
Aneurysm or vascular prosthesis (%)	5 (4)	2 (11)	3 (3)
Co-infections‡			
Eppstein-Barr virus (EBV) positive¶ (%)	4 (3)	–	4 (3)
EBV-positive lymphoma tissue sample ø (%)	4 (3)	–	4 (3)
Course of disease			
Deceased (%)	35 (26)	2 (11)	33 (28)
Treatment with chemo-/radiotherapy (%)	115 (82)	14 (78)	101 (86)
Stem cell transplant (%)	16 (12)	1 (6)	15 (13)

σ*see definitions. Of cases, 85% (n = 11) underwent PET-CT scanning and 31% (n=4) underwent echocardiography shortly before or after of diagnosis of NHL. Of controls, 25% (n = 4) underwent PET-CT scanning and 38% (n=6) underwent echocardiography shortly before or after biopsy for various reasons.*1 post renal transplant, 6 patients with immunosuppressive medication (DMARD, prednisone or biologicals or a combination), 1 hypogamma-globulinemia, 1 patient with both hypogammaglobulinemia and immunosuppressive medication (as previously defined), 1 MDS with severe leukopenia, 1 hemodialysis patient. †unknown for 18 patients.* ⁺*unknown for 8 patients. ‡No patients with known hepatitis B virus, hepatitis C virus or human immunodeficiency virus infection. Unknown HIV status for 77 patients, unknown HCV status for 70 patients, unknown HBV status for 74 patients. 4 patients with past HBV infection.* ¶*Active infection with detectable viral load. Past infection in 61 patients, unknown status for 63 patients.* ø *Epstein-Barr encoding region (EBER) in situ hybridization was standardly performed for all immunocompromised patients, all CD30+ lymphoma, all large cell lymphoma, plasmablastic lymphoma, Burkitt lymphoma, lymphomatoid granulomatosis and NK or T-cell lymphoma.*

Table 2. Description of cases (B-NHL patients) and controls (without any NHL)

	Cases	Controls
Number of patients (%)	13 (45)	16 (55)
Number of tissues (%)	14 (47)	16 (53)*
Mean age (sd)	65 (18)	59 (19)
Male sex (%)	9 (64)	10 (63)
Malignant tissue (%)	14 (100)	4 (25)
Type of tissue		
Lymph node (%)	6 (43)	8 (50)
Bone marrow (%)	2 (14)	3 (19)
Gastro-intestinal (%)	2 (14)	2 (13)
Spleen (%)	1 (7)	1 (6)
Lung (%)	1 (7)	1 (6)
Other (%)	2 (14)	1 (6)
Timing of biopsy		
Retrieved during epidemic (%)	1 (7)	3 (19)
Retrieved after epidemic (%)	13 (93)	13 (81)
Serological profile		
Patient with past Q fever infection (%)	11 (79)	12 (75)
Patient with chronic Q fever† (%)	2 (14)	4 (25)
Vascular focus of infection (%)	1 (7)	2 (13)
Combined endocarditis and vascular (%)focus	1 (7)	–
No focus of infection (%)	–	2 (13)
Endocarditis (%)	–	–

**biopsy findings: 2 patients with lung carcinoma, 1 mamma carcinoma, 1 renal cell carcinoma, 2 myelodysplastic syndromes, 2 sarcoidosis, 2 lymphadenopathy observed during vascular surgeries with chronic inflammation, 1 splenectomy performed during vascular surgery because of a complication without pathology, 1 lymphnode after hemistrumectomy (benign pathology), 1 lymphnode found in breast biopsy, 1 stomach biopsy, 1 coecum biopsy, 1 bone marrow biopsy without any pathological diagnosis.† 4 patients with proven chronic Q fever (three with a vascular focus of infection and one with both endocarditis and a vascular focus of infection, 1 patient with probable chronic Q fever (no focus of infection; probable based on immunocompromised state) and 1 patient with possible chronic Q fever (no focus of infection and no risk factors).*

C. burnetii was demonstrated in tissues of 8 patients (28%) with PCR, IF or FISH: in 5/13 cases (38%) and 3/16 controls (19%), p = 0.41 (OR for positivity in cases: 2.71, 95% confidence interval (CI) 0.50 – 14.54). Tissues of 7 patients were positive with IF and FISH: 5 cases (38%) and 2 controls (13%). There was complete agreement between IF and FISH. PCR was negative for all cases and positive in 2 controls (13%). Of 2 PCR positive control tissues, one was IF and FISH positive (table 3). Negative and positive control samples were all negative and positive appropriately, for all three diagnostic methods.

When assessing the number of positive tissues among chronic Q fever patients, 3/6 were positive (50%): 1 case (50%) and 2 controls (50%), p = 0.99, OR 1.00 with 95% CI 0.03 – 29.81. When assessing the number of positive tissues among past Q fever patients, 5/23 (22%) were positive: 4 cases (36%) and 1 control (8%), p = 0.16, OR 6.29 with 95% CI 0.58 – 68.42.

Table 3. Result of polymerase chain reaction (PCR), immunofluorescence (IF) and fluorescent in-situ hybridization (FISH) on case and control tissue samples.

	All	Cases	Controls
Number of patients (%)	29	13 (45)	16 (55)
Number of samples (%)	30	14 (47)	16 (53)
Number of patients positive with any test* (%)	8 (28)	5 (38)	3 (19)
Number of patients with positive PCR (%)	2 (7)	0†	2 (13)
Number of patients with positive IF (%)	7 (24)	5 (38)	2 (13)
Number of patients with positive FISH (%)	7 (24)	5 (38)	2 (13)

**Either PCR, IF or FISH or a combination of those positive. †1 missing due to repeated inhibition during PCR*

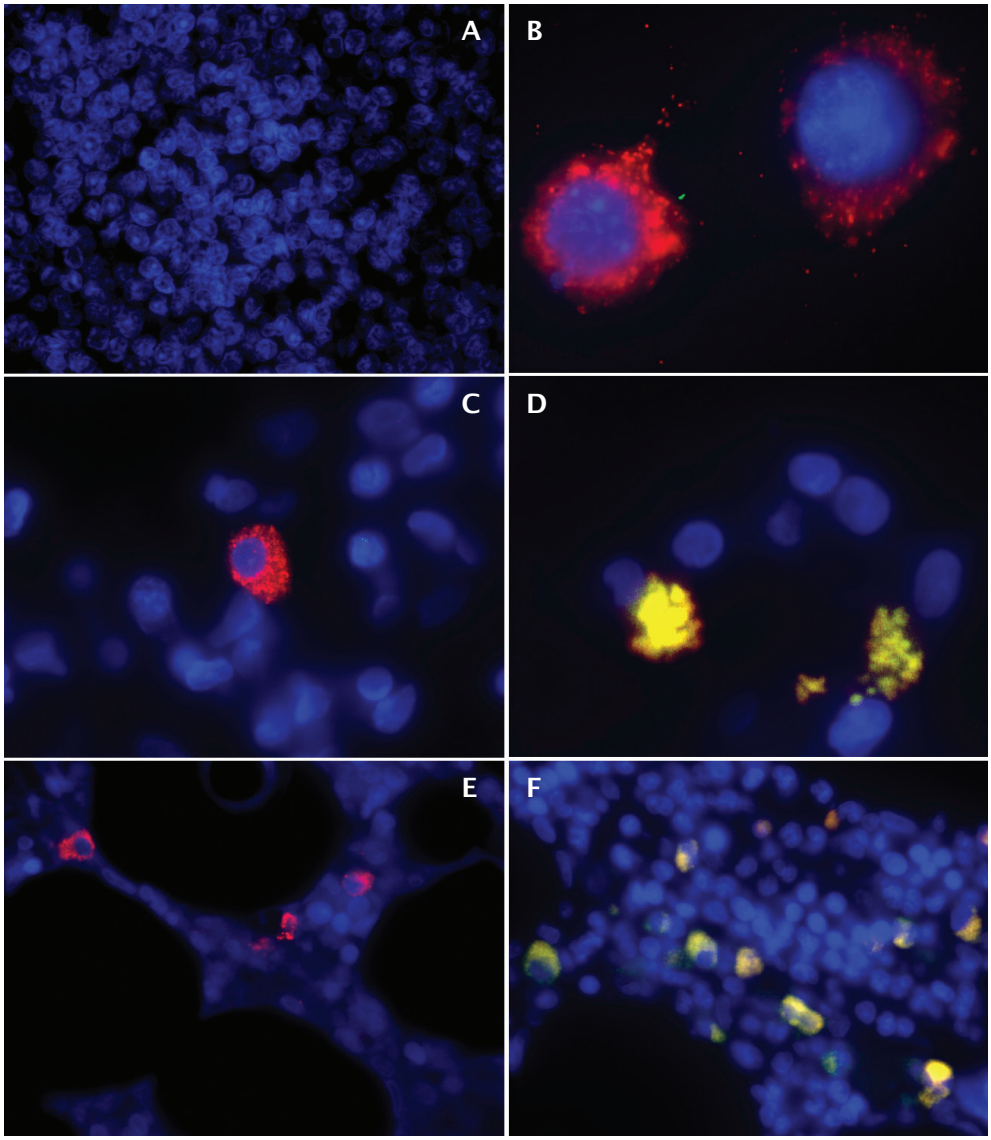
In table 4, specification of pathology diagnoses, tissue types and results of PCR, IF and FISH are shown for all case and control matched tissues. In figure 2, microscopic photographs of IF and FISH results are shown for negative controls, positive controls and a selection of positive samples.

Table 4. Overview of all tissues (of both cases and controls).

Matched ID and		Pathology diagnosis†	Tissue type¶	Serological	PCR	IF	FISH
1	Case	DLBCL	Spleen	Past	-	-	-
2	Case	MZL	Retroperitoneal mass	Chronic	-	+	+
3	Case	FL	LN	Past	-	-	-
4	Case	B-ALL	BM	Past	-	-	-
5	Case	Indefinite subtype B-NHL	Lung	Chronic	-	-	-
6	Case	DLBCL	LN	Past	-	-	-
7	Case	FL	LN	Past	-	+	+
8	Case*	FL	LN	Past	-	-	-
8	Case*	FL	BM	Past	-	+	+
9	Case	FL	LN	Past	-	-	-
10	Case	MCL	Nasopharynx	Past	-	-	-
11	Case	MALT lymphoma	Caecum	Past	-	+	+
12	Case	MCL	Stomach	Past	-	+	+
13	Case	DLBCL	LN	Past	-	-	-
1	Control	No pathology	Spleen^{+}	Past	-	-	-
2	Control	Chronic inflammation	Retroperitoneal LN^{+}	Chronic	+	+	+
2	Control	Lung carcinoma	LN	Past	-	+	+
3	Control	No pathology	LN	Chronic	-	-	-
4	Control	MDS	BM	Chronic	-	-	-
4	Control	No pathology	BM	Past	-	-	-
5	Control	Lung carcinoma	Lung	Past	-	-	-
6	Control	Chronic inflammation	LN^{+}	Chronic	+	-	-
6	Control	No pathology	LN	Past	-	-	-
7	Control	Mamma carcinoma	LN	Past	-	-	-
8	Control	Sarcoidosis	LN	Past	-	-	-
8	Control	MDS	BM	Past	-	-	-
9	Control	Renal cell carcinoma	LN	Past	-	-	-
11	Control	No pathology	Caecum	Past	-	-	-
12	Control	No pathology	Stomach	Past	-	-	-
13	Control	Sarcoidosis	LN	Past	-	-	-

*†DLBCL: diffuse large cell B-cell lymphoma. MZL: marginal zone lymphoma. FL: follicular lymphoma. B-ALL: B-acute lymphoblastic leukemia.MCL = mantle cell lymphoma. MALT: mucosa-associated lymphoid tissue lymphoma.MDS = myelodysplastic syndrome. ¶LN = lymph node. BM = bonemarrow. $^{+}$obtained during abdominal vascular surgery. *two tissues from one patient.*

Figure 2. Microscopic photographs of immunofluorescence and fluorescent in-situ hybdridization.



(A) *Microscopic image of immunofluorescence staining (IF) of negative control tissue (patient with sarcoidosis, never exposed to Coxiella burnetii) in which nuclei are stained blue (4',6-diamidino-2-phenylindole (DAPI)) and no C. burnetii is detected* ***(B)*** *Microscopic image of IF of positive control sample (L929 cells infected with C. burnetii) in which nuclei are stained blue (DAPI), while perinuclear C. burnetii is stained red.* ***(C)*** *Microscopic image of IF of retroperitoneal lymphoma tissue of a patient with vascular chronic Q fever in which nuclei are stained blue (DAPI), while perinuclear C. burnetii is stained red* ***(D)*** *Microscopic image of fluorescence in-situ hybridization (FISH) of the same tissue as* ***(C)*** *in which nuclei are stained blue (DAPI), while C. burnetii, organized in perinuclear vacuoles, is stained yellow. The yellow signal results of the co-localization of the universal probe EUB (red) and the specific 16S rRNA C. burnetii probe (green).* ***(E)*** *Microscopic image of IF of a bone marrow biopsy showing follicular non-Hodgkin lymphoma in a patient with past Q fever in which nuclei are stained blue (DAPI), while perinuclear Coxiella burnetii is stained red* ***(F)*** *Microscopic image of FISH of the same tissue as* ***(E)*** *in which nuclei are stained blue (DAPI), while C. burnetii, organized in perinuclear vacuoles, is stained yellow. The yellow signal results of the co-localization of the universal probe EUB (red) and the specific 16S rRNA C. burnetii probe (green). In all cases, a Leica DMI6000 B microscope was used and original magnification of images was x100.*

Discussion

Overall, *C. burnetii* was detected in tissues of 38% of cases and 19% of controls. The difference in proportion positive samples between cases and controls was not significant. When assessing past Q fever patients only, 36% of cases and 8% of controls were positive, which difference was also not significant. Therefore, we conclude that the presence of *C. burnetii* in tissue samples is not specific for B-NHL.

When evaluating the Bradford Hill criteria for establishing a causal relationship, evidence supporting some criteria is limited.[15,16] The previously reported association between exposure to *C. burnetii* and development of NHL was found to be quite strong with a 25-fold increased risk.[2] However, the consistency of this finding is moderate. In a nationwide Dutch epidemiological study, the risk for was increased in one year only (RR1.16), and not in any other year.[17] Thus, the height of the excess in the two studies risk ranges between 1 and 25, and there is a considerable risk of selection and detection bias in both studies.[2,17] Furthermore, the specificity of the potential relation is low, since there are other factors that may explain both diseases.[18-21] Temporality between exposure and development of disease is very difficult to confirm definitely, because both diseases can have a considerable diagnostic delay: infection with *C. burnetii* may occur asymptomatically and B-NHL may be difficult to detect at early stages. An experiment would therefore have to be performed to ensure a temporal relationship. The argument of a biological gradient (or dose-response relation) seems to hold: the risk is higher in patients with a chronic or persistent infection compared to those that develop NHL after a primary infection.[2,17] Moreover, there is a plausible potential pathophysiological explanation for the association.[1,2] Confirmation of this pathophysiological mechanism, for example by performing an experiment, would be desirable. Finally, there is coherence between epidemiological and laboratory findings.[2,17] Altogether, there are arguments supporting causation. However, additional evidence with regard to the temporal relation, specificity of the potential relation and confirmation of the supposed pathophysiological pathway is required. Understanding why *C. burnetii* is latently present more often in NHL tissues and what the effect of the presence of the bacterium is in those tissues, is of vital importance and will provide more definite answers.

The lack of a significant difference between the proportion positive samples in cases and controls may be due to a lack of power. Nevertheless, we must conclude that finding *C. burnetii* in tissues is not specific for B-NHL tissues, even if the difference in proportions would have been significant. Among patients (both cases and controls) without chronic or persistent Q fever but only previously exposed to *C. burnetii*, the bacterium was demonstrated in over 22% of tissues. Positive tissues were obtained in 2011, 2012, 2014 and 2015 while the Dutch Q fever outbreak occurred between 2007 and 2010, indicating that *C. burnetii* can be detected many years after primary infection in antigen-presenting cells in different types of tissues in the absence of chronic or persistent infection. This is in line with a previous study, which reported latent presence of *C. burnetii* in various tissues up to five years after primary infection.[8] The finding of *C. burnetii* in lymph nodes, bone marrow and gastro-intestinal tissues

demonstrates the extensive infiltration of the bacterium in lymphatic tissues. It is obvious that the bacterium can be detected during active infections like acute or chronic Q fever). However, after clearance of a primary infection and in absence of a chronic infection, it is remarkable that *C. burnetii* remains latently present. When comparing the proportion of positive samples of cases and controls with past Q fever, samples of cases were more often positive compared to controls although this difference was also not significant.

Different criteria for diagnosis of chronic Q fever have been developed.[1,14] For this Dutch cohort, the Dutch chronic Q fever consensus group criteria were used. When applying the criteria for diagnosing persistent focalized *C. burnetii* infection, formulated by Eldin et al., three (instead of two) cases would potentially classify as having a persistent focalized *C. burnetii* infection. In the control group, three (instead of four) would potentially classify as having a persistent focalized infection. This demonstrates that the definition used for identification of patients influences the description of these study patients. However, since we selected all patients that were exposed to *C. burnetii* previously (with either a past or chronic infection), the main outcome of interest would not have changed by using either definition.

This is the second study (with exception of one additional case report) in which the value of IF and FISH to demonstrate *C. burnetii* in human samples was explored.[2,22] The agreement between IF and FISH was 100% in this study and in the previous study.[2] In the current study, PCR was performed additionally on all tissue samples. IF and FISH were able to detect *C. burnetii* in 6 PCR-negative tissues. Contradictory, PCR was positive in one sample on which IF and FISH were both negative. This may be caused by the fact that this patient was treated with antibiotics at the time of biopsy retrieval for treatment of chronic Q fever. The cycle threshold-values in this case were 39.26 and 41.19, which are marginal positive values. It is possible that due to extensive bacterial and cellular degradation FISH and IF were both negative: both techniques require a specific positive signal in the perinuclear compartment or intracellular vacuoles. Moreover, the difference may rely on sampling error: the bacterial load may vary for different samples. Since microscopic findings with IF and FISH are highly specific, and especially because an additional probe targeting non-specific bacterial DNA was used during FISH to detect false positivity, it is very unlikely that positive IF and FISH are caused by false positivity of these techniques.[23] The higher detection rate with IF and FISH suggests that these methods may be more sensitive for detection of *C. burnetii* in paraffin-embedded tissues.

After the index study, this was the first study to compare presence of *C. burnetii* in both B-NHL tissues and tissues of patients without B-NHL.[2] In the previous study, patients with lymphadenitis were explored as well.[2] In this study, we set out to explore the implication of finding *C. burnetii* in tissue in patients with chronic or past infection, in the absence of primary infection. The large Dutch Q fever outbreak provided the unique opportunity to explore presence of the association between *C. burnetii* and B-NHL, with a relatively large number of samples. We used the best available diagnostic techniques to ensure optimal sensitivity and specificity and to provide insight in the value of these novel diagnostic techniques. Naturally, our

study has drawbacks as well. It is likely that our numbers were too small to detect a difference between patients with and without B-NHL. We observed differences between B-NHL-cases and controls, but none of the differences were significant. Moreover, this is a retrospective study. Therefore, diagnostic work-up for our patients was unstandardized, leading to variable timing of serology and absence of transthoracic echocardiography or PET-CT imaging in part of our patients. Additionally, although Q fever has a worldwide distribution, it remains a relatively rare disease.1 Therefore, the absolute risk for B-NHL after exposure to *C. burnetii* remains small (in a large cohort of 1,468 Q fever patients, 7 patients developed B-NHL).[2] Assuming a causal relationship, the contribution of *C. burnetii* on the total number of diagnosed B-NHL may be limited, since many other factors contribute to the incidence of B-NHL. Nevertheless, from a scientific point of view and for a selected group of patients, it is very relevant to explore the causation of this association. Furthermore, all patients in our study were infected during the Dutch Q fever outbreak, with the Dutch Q fever outbreak strain. It is possible that other *C. burnetii* strains behave differently, leading to different clinical sequelae. Therefore, these results will have to be validated in other populations. Another potential issue is the fact that we used certain diagnostic techniques (FISH and IF) that are very sensitive and specific on theoretical grounds, but clinical data on sensitivity and specificity of these techniques are very scarce. Finally, patients might still develop B-NHL over time, and the presence of any lymphoprolipherative disease has not been excluded systematically in the control groups. These considerations make the interpretation of the implication of detection of *C. burnetii* in tissues challenging.

In conclusion, *C. burnetii* was detected in 38% of B-NHL tissues of patients that were previously exposed to the bacterium. Although *C. burnetii* was detected in more B-NHL tissues than in tissues of control patients (19%), the difference was not significant. Therefore, we conclude that the finding of *C. burnetii* in tissues is a non-specific finding. Experimental research, such as animal experiments with induction of lymphomagenesis or micro-array on oncogenic proteins in antigen-presenting cells, may provide more definite answers.

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References

1. Eldin C, Melenotte C, Mediannikov O et al. From Q fever to *Coxiella burnetii* infection: a paradigm change. Clin Microbiol Rev 2017;30(1):115-90.
2. Melenotte C, Million M, Audoly G et al. B-cell non Hodgkin-lymphoma linked to *Coxiella burnetii*. Blood 2016;127(1):113-21.
3. Capo C, Zaffran Y, Zugun F et al. Production of interleukin-10 and transforming growth factor beta by peripheral blood mononuclear cells in Q fever endocarditis. Infect Immun 1996;64(10):4143-7.
4. Honstettre A, Imbert G, Ghigo E et al. Dysregulation of cytokines in acute Q fever: role of interleukin-10 and tumor necrosis factor in chronic evolution of Q fever. J Infect Dis. 2003;187(6):956-62.
5. Ghigo E, Capo C, Raoult D et al. Interleukin-10 stimulates *Coxiella burnetii* replication in human monocytes through tumor necrosis factor down-modulation: role in microbicidal defect of Q fever. Infect Immun. 2001;69(4):2345-52.
6. Meghari S, Bechah Y, Capo C et al. Persistent *Coxiella burnetii* infection in mice overexpressing IL-10: an efficient model for chronic Q fever pathogenesis. PLoS Pathog. 2008;4(2):e23.
7. Akdis M, Aab A, Altunbulakli C et al. Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: receptors, functions, and roles in diseases. J Allergy Clin Immunol 2016; Published online 2016 August 28. Available from: http://www.sciencedirect.com/science/article/pii/S0091674916307151.
8. Harris RJ, Storm PA, Lloyd A et al. Long-term persistence of *Coxiella burnetii* in the host after primary Q fever. Epidemiol Infect 2000;124(3):543-9.
9. Kampschreur LM, Hagenaars JC, Wielders CC et al. Screening for *Coxiella burnetii* seroprevalence in chronic Q fever high-risk groups reveals the magnitude of the Dutch Q fever outbreak. Epidemiol Infect 2013;141(4):847-51.
10. National Institute for Public Health and the Environment, RIVM, http://www.rivm.nl/Onderwerpen/Q/Q_koorts, Accessed August 2017.
11. Melenotte C, Lepidi H, Nappez C et al. Mouse model of *Coxiella burnetii* aerosolization. Infect Immun 2016; 84(7):2116-23.
12. 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; 17(2): 286-90.
13. 13. Swerdlow SH, Campo E, Harris NL et al. WHO classification of tumours of haemotopoietic and lymphoid tissues (4th edn.), IARC Press, Lyon, France (2008).
14. Wegdam-Blans MC, Kampschreur LM, Delsing CE et al. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Infect 2012;64(3):247-59.
15. Hill AB. The environment and disease: association or causation? Proc R Soc Med 1965;58(5):295-300.
16. Fedak KM, Bernal A, Capshaw ZA et al. Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology. Emerg Themes Epidemiol 2015;12:14.
17. van Roeden SE, van Houwelingen F, Donkers CMJ et al. Exposure to *C. burnetii* and the risk for non-Hodgkin lymphoma: a retrospective population based analysis. Revisions at Lancet Hematology, February 2018.

18. Shankland KR, Armitage JO, Hancock BW. Non-Hodgkin lymphoma. Lancet 2012; 380(9844): 848-857.
19. Kampschreur LM, Dekker S, Hagenaars JCJP et al. Identification of risk factors for chronic Q fever, the Netherlands. Emerg Infect Dis 2012; 18(4): 563-570.
20. Raoult D. Host factors in the severity of Q fever. Ann N Y Acad Sci 1990; 590: 33-38.
21. Schoffelen T, Kampschreur LM, van Roeden SE et al. *Coxiella burnetii* infection (Q fever) in rheumatoid arthritis patients with and without anti-TNFα therapy. Annals of Rheumatic Diseases, 2014; 73(7):1436-8.
22. Kumpf O, Dohmen P, Ertmer M et al. Rapid molecular diagnosis of infective aortic valve endocarditis caused by *Coxiella burnetii*. Infection 2016;44(6):813-17.
23. Kaittanis C, Boukhriss H, Santra S et al. Rapid and sensitive detection of an intracellular pathogen in human peripheral leukocytes with hybridizing magnetic relaxation nanosensors. PLoS One 2012;7(4):e35326.

Part IV

Transmission of *Coxiella burnetii* through transplantation

Chapter 10

Seroprevalence of *Coxiella burnetii* antibodies and chronic Q fever among post-mortal and living donors of tissues and cells from 2010 – 2015 in The Netherlands

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Abstract

Background

After a large Q fever outbreak in the Netherlands in the period from 2007 to 2010, the risk of Q fever transmission through tissue and cell transplantation from undiagnosed chronic Q fever cases became a potential issue.

Aim

We aimed to evaluate the risk of Q fever transmission through tissue and cell transplantation.

Methods

We performed a retrospective observational cohort study among 15,133 Dutch donors of tissues and stem cells from 2010 to 2015 to assess seroprevalence of *Coxiella burnetii* antibodies, to identify factors associated with presence of *C. burnetii* antibodies, and to assess the proportion of undiagnosed chronic Q fever cases.

Results

The study population consisted of 9,478 (63%) femoral head donors, 5,090 (34%) post-mortal tissue donors and 565 (4%) cord blood donors. Seroprevalence of *C. burnetii* antibodies gradually decreased after the outbreak, from 2.1% in 2010 to 1.4% in 2015, with a significant trend in time ($p < 0.001$). Of 301 seropositive donors, seven (2.3%) were newly detected with chronic Q fever (0.05% of all screened donors).

Conclusion

This study shows that seroprevalence of *C. burnetii* antibodies among donors of tissues and cells in the Netherlands after 2014 was similar to pre-outbreak levels in the general population. The proportion of newly detected chronic Q fever patients among donors of tissues and cells was smaller than 0.1%. This study may prompt discussion on when to terminate the screening programme for chronic Q fever in donors of tissues and cells in the Netherlands.

Introduction

Q fever is a zoonosis caused by the intracellular bacterium *Coxiella burnetii*, with a reservoir in a wide range of domesticated and wild animals. The bacterium can be spread via airborne transmission. The Netherlands faced the largest Q fever outbreak ever recorded from 2007 until 2010, resulting in over 4,000 reported and 40,000 estimated infected people. Affected areas were mainly located in the south and east of the Netherlands around infected goat farms [1,2].

Infected animals may be asymptomatic or can show symptoms such as infertility, stillbirth, abortion, endometritis or mastitis [3]. In most human cases (ca 60%), primary infection with *C. burnetii* elapses without symptoms. The remaining proportion of patients develops influenza-like symptoms or more serious conditions such as pneumonia or hepatitis. After acute infection, 1–5% of patients develop chronic infection [3]. The main manifestations of chronic Q fever are endocarditis and vascular infections such as infected aneurysms or vascular grafts [4-6]. Patients most at risk of chronic Q fever are those with underlying valvulopathy, prior valve surgery, aneurysms or vascular prosthesis, or immunocompromised individuals [7-10]. Since the Dutch Q fever outbreak, data from all chronic Q fever patients have been collected systematically for research purposes in the Dutch national chronic Q fever database.

Transmission of *C. burnetii* through tissue transplantation has not been described in literature. However, single cases of likely transmission through blood transfusion and possible transmission through bone marrow transplantation to an immunocompromised recipient have been reported [11,12]. Furthermore, transmission through transplantation (liver, thymus and lymph nodes) in animals has been observed [13]. After the acute phase, bacteria are usually not detectable in the blood, but they can persist in monocytes, bone marrow, spleen, prostate and liver [14]. An assessment showed that a potential risk of transmitting *C. burnetii* lies with transplantation of some tissues such as heart valves, musculoskeletal tissues and skin, although processing techniques may reduce the risk significantly [15].

Since the outbreak of Q fever ended, incidence of acute Q fever in the Netherlands has been low [2]. During a known infection (either acute or chronic), patients are excluded as potential tissue or cell donors in the Netherlands. Therefore, the risk of *C. burnetii* transmission through transplantation of tissues or cells originates mainly from patients with undiagnosed chronic Q fever. In order to reduce the risk of transmission of *C. burnetii* through transplantation and as advised by the Dutch Health Council, testing of all centrally tested donors of tissues and cells was started in 2010 in the Netherlands [16].

The aim of the current study was to assess the seroprevalence of *C. burnetii* antibodies, identify factors associated with the presence of *C. burnetii* antibodies in donors, and to determine the proportion of patients with a serological profile indicative of chronic Q fever among Dutch donors of tissues and cells after the Q fever outbreak in the Netherlands. The proportion of Dutch donors of tissues and cells with chronic Q fever newly detected during this study was compared with the prevalence of known chronic

Q fever on a national level. The rationale for this observational cohort study was to generate epidemiological data that may be used in decision-making with regard to continuation of the screening for *C. burnetii* antibodies in donors of tissues and cells.

Methods

We performed a retrospective observational cohort study among Dutch donors of tissues and cells (post-mortal donors, cord blood donors, bone and cartilage donors) between 2010 and 2015. Data on the prevalence of chronic Q fever in the Netherlands were retrieved from the Dutch national chronic Q fever database.

Patient selection and data collection for donors

The study included all post-mortal tissue donors who had at least one tissue approved at initial assessment, living donors of femoral heads of Sanquin Bone bank (located in the east of the Netherlands), living donors of femoral heads of BISLIFE (located in the west of the Netherlands) and umbilical cord blood donors from the Netherlands. Besides femoral head donors, cartilage and skullcap donors were included among donors from Sanquin Bone bank. We report the numbers of cartilage and skullcap donors but because their number was very limited, the entire group of bone and cartilage donors was then further referred to as femoral head donors in this manuscript. The donors from these tissue and cell banks represented all post-mortal donors and all cord blood donors in the Netherlands. Bone and cartilage donors were near-complete because a very limited number of bone and cartilage donors are managed and tested in local hospitals. Donors of semen or bone marrow were not included in this study.

From one donor, multiple types of tissue can be retrieved and each type of tissue can result in multiple transplants for different recipients. As a donor is tested once, in this study, we refer to the number of donors, not the number of derived transplants. The year of screening was the year of initial donation. Tissues can be stored from a month (cornea) up to 5 years (skin, heart valves and musculoskeletal tissues) after retrieval, which may delay the use of tissues.

No approval of a medical ethical committee was obtained or considered necessary according to Dutch regulations. Screening of post-mortal tissue donors started on 1 November 2010. From June 2012, testing of solo cornea donors was abandoned as the risk of transmission of *C. burnetii* through cornea transplantation was considered very low. Testing of BISLIFE femoral head donors started in June 2012, followed by testing of Sanquin femoral head donors and umbilical cord blood donors from August 2012. All available screening data from 1 November 2010 until 1 January 2016 were included. Donor characteristics, such as age, sex, donated tissues and place of residence were recorded. For all donors who tested positive for *C. burnetii* antibodies and had IgG phase 1 titres≥1:1,024, additional information regarding clinical condition was gathered from hospital medical records, general practitioners,

next of kin, autopsy results (if autopsy had been done) and results of histological examination of remnant hearts (if heart valve donation had been performed). The cut-off value of IgG phase 1 titres ≥ 1:1,024 was based upon the diagnostic criteria for chronic Q fever where this titre is indicative of proven, probable or possible chronic Q fever according to the Dutch chronic Q fever consensus group criteria[6]. Besides a phase 1 IgG titre of ≥ 1:1,024, proven chronic Q fever required a definite endocarditis according to the Duke criteria, a positive PCR for *C. burnetii* on tissue, serum or plasma, or a vascular infection diagnosed with imaging studies [17]. Patients with probable chronic Q fever were those who did not meet the criteria for proven chronic Q fever but had a risk factor for chronic Q fever, symptoms consistent with a chronic infection or a focus other than endocarditis or vascular infection. Patients with possible chronic Q fever were those who only had serological evidence of the disease (i.e. a phase 1 IgG titre of ≥ 1:1,024), without fulfilling the definitions of a proven or probable chronic Q fever.

Patient selection and data collection for chronic Q fever patients

Data on chronic Q fever patients in the Netherlands were retrieved from the Dutch national chronic Q fever database (hosted by the University Medical Centre Utrecht). This database stores clinical, microbiological and radiological data of all known proven, probable and possible chronic Q fever patients older than 18 years in the Netherlands, defined as formulated by the Dutch chronic Q fever consensus group [6]. Registration started in February 2011, and the last update ended in May 2016; 35 hospitals in the Netherlands contributed to the initiative (Supplement 1). Design of this database was approved by the Medical Ethical Committee of the University Medical Centre in Utrecht. Clinicians identified patients based on a positive PCR on serum or tissue and/or a *C. burnetii* phase 1 IgG antibody titre of ≥ 1:1,024. Patients with a serological profile and clinical condition matching acute Q fever were excluded. Details on design and content of this database were described in detail in a previous publication [4]. For calculation of cumulative incidence of chronic Q fever, data on sex and age in the general population were retrieved from the Central Bureau for Statistics [18].

Definitions

Seropositivity was defined as presence of phase 2 IgG antibodies against *C. burnetii* at a titre ≥ 1:32. Seropositivity without fulfilling the definitions of chronic Q fever indicated a past Q fever infection (symptomatic or asymptomatic). Residency in a high-risk geographical area was defined as living in a four-digit postal code area where at least one Q fever patient had been reported in the 3 months preceding donation, living within a 5 km radius of a farm where *C. burnetii* had been detected in the bulk tank milk at the time of donation, or living in a three-digit postal code area where the Q fever incidence was higher than 20 per 100,000 inhabitants in any of the years 2007 to 2010. Ca 15% of the Dutch population live in an area where 87% of the Q fever cases were reported. The data on Q fever incidence were obtained from

the Dutch National Institute for Public Health and the Environment. The data on bulk tank milk-positive farms were obtained from the Dutch Food and Consumer Product Safety Authority website [19]. The 5 km radius from infected farms to the residence of each donor was determined by measuring the distance between both postal codes.

Laboratory detection of antibodies against *C. burnetii* or of *C. burnetii* DNA

Serum samples of living donors obtained at the time of donation, or for post-mortal donors within 24 hours after circulation stop, were screened for phase 2 IgG antibodies against *C. burnetii* using the CE-marked Serion enzyme immunoassay (EIA) (Serion, Clindia Benelux, Leusden, the Netherlands). The cut-off values for EIA (borderline) positivity were determined according to the manufacturer's instructions. Borderline reactive samples were considered positive. Confirmation of positive samples was performed by indirect fluorescent-antibody assay (IFA) for phase 1 and 2 IgG antibodies against *C. burnetii* (Focus Diagnostics, Cypress, United States (US)) with dilution on a binary scale, using a cut-off for positivity of 1:32. Laboratory testing of antibodies against *C. burnetii* for chronic Q fever patients from the Dutch national chronic Q fever database consisted of an IFA for phase 1 and 2 IgG against *C. burnetii* on plasma or serum (Focus Diagnostics, Inc., Cypress, US or Fuller Diagnostics, LLC., Anchorage, US). Titration of antibodies was carried out at different hospital sites with dilutions on a binary scale, with a cut-off of 1:32. Moreover, data on PCR for detection of *C. burnetii* DNA on serum or plasma or tissue were collected (NucliSENS easyMAG; bioMérieux, Marcy l'Etoile, France) [4].

Data analysis

All data on donors were stored and analysed in SPSS Statistics 23.0 (IBM SPSS Inc., Chicago, US). Data from the Dutch national chronic Q fever database were stored in Microsoft Access 2010, and exported via R (version i384, 3.1.1) to SPSS Statistics 21.0 for analysis. The numbers of false-positive tissue samples of post-mortal and living donors were compared by means of a Chi-squared test. To compare the mean age of seropositive and seronegative donors, an independent samples t-test was used. To identify factors associated with seropositivity, a binomial logistic regression (non-stepwise) was performed, with seropositivity as dichotomous outcome variable. The seroprevalence in time was studied with a Pearson correlation test. The number of newly detected chronic Q fever cases and prevalence of chronic Q fever in the population was not tested formally: numbers of patients were described. We considered p values ≤ 0.05 as statistically significant. Cumulative incidences were calculated for the population per 1,000,000 inhabitants.

Results

Donor characteristics

In total, 15,133 donors of tissues and cells were tested for presence of *C. burnetii* antibodies: 10,043 (66.4%) were living donors and 5,090 (33.6%) were post-mortal donors of whom at least one tissue was approved at initial assessment. Among living donors, 9,478 (94.4%) were femoral head donors (3,818 from the bone bank in the east and 5,660 from the bone bank in the west of the Netherlands) and 565 (5.6%) were cord blood donors. Of post-mortal tissue donors, 4,413 (86.7%) donated corneas, 2,535 (49.8%) donated skin, 993 (19.5%) donated cardiovascular tissues and 631 (12.4%) donated musculoskeletal tissues, with multiple tissue types donated by single donors. Donor characteristics are presented in Table 1.

Table 1. Sex, age and area of residency for all screened donors of tissues and cells, the Netherlands, 2010–2015 (n = 15,133)

Variable		Donors		Femoral head donors		Cord blood donors		Post-mortal donors	
		n	%[a]	n	%[a]	n	%[a]	n	%[a]
Number of patients		15,133	100	9,478	63	565	4	5,090	34
Sex	Male	7,122	47	3,829	40	NA		3,293	65
	Female	8,011	53	5,649	60	565	100	1,797	35
Mean age in years ±standard deviation		65±14		68±11		32±5		64±13	
Age group (in years)	0–10	27	<1	8[b]	<1	NA		19	<1
	11–20	88	1	46[c]	<1	1	<1	41	1
	21–30	329	2	46	<1	218	39	65	1
	31–40	499	3	51	1	329	58	119	2
	41–50	866	6	404	4	17	3	445	9
	51–60	2,404	16	1,422	15	NA		982	19
	61–70	5,022	33	3,502	37	NA		1,520	30
	71–80	4,685	31	3,071	32	NA		1,614	32
	81–90	1,202	8	917	10	NA		285	6
	91–100	11	<1	11	<1	NA		NA	
Residency in high-risk area	Yes	1,710	11	993[d]	10	33	6	684	13
	No	13,216	87	8,467	89	530	94	4,219	83
	Unknown	207	1	18	<1	2	<1	187	4

NA: not applicable.[a] Column percentages, except for the first row which shows row percentages.[b] Including eight skull-cap donors. [c] Including 16 skull-cap donors and five cartilage donors. [d] For the bank processing femoral heads in the eastern part of the Netherlands, 21.7% of donors lived in a high-risk area. For the bank processing femoral heads in the western part of the Netherlands, 3.0% of donors lived in a high-risk area (cord blood and post mortal donors were processed centrally).

Seroprevalence of *C. burnetii* antibodies and characteristics associated with seropositivity

Of 15,133 tested donors, 384 (2.5%) had a positive screening test (EIA); 209 positive and 175 borderline positive. For 353 positive samples (91.9%), a confirmation test (IFA) was done: 83 (23.5%) were negative and were thus considered false positives. For 31 donors with a positive screening test, no confirmation test results were available because the available plasma was not sufficient: these were considered to be true positive. Thus, 301 (2.0%) of all tested donors were considered to be seropositive. Samples of post-mortal donors were significantly more often false positive (n = 46; 0.9%) than tissues of living donors (n = 37; 0.4%; p < 0.001). Mean age did not differ between seropositive (66.4; standard deviation (SD) = 11.4) and seronegative donors (65.3; SD = 13.5; p = 0.14). Seroprevalence of *C. burnetii* antibodies was highest among male donors, donors aged 41–50 years, donors residing in a high-risk area and post-mortal donors. Male sex, residency in a high-risk area and post-mortal donations were independently associated with seroprevalence of *C. burnetii* antibodies in multivariable analysis (Table 2).

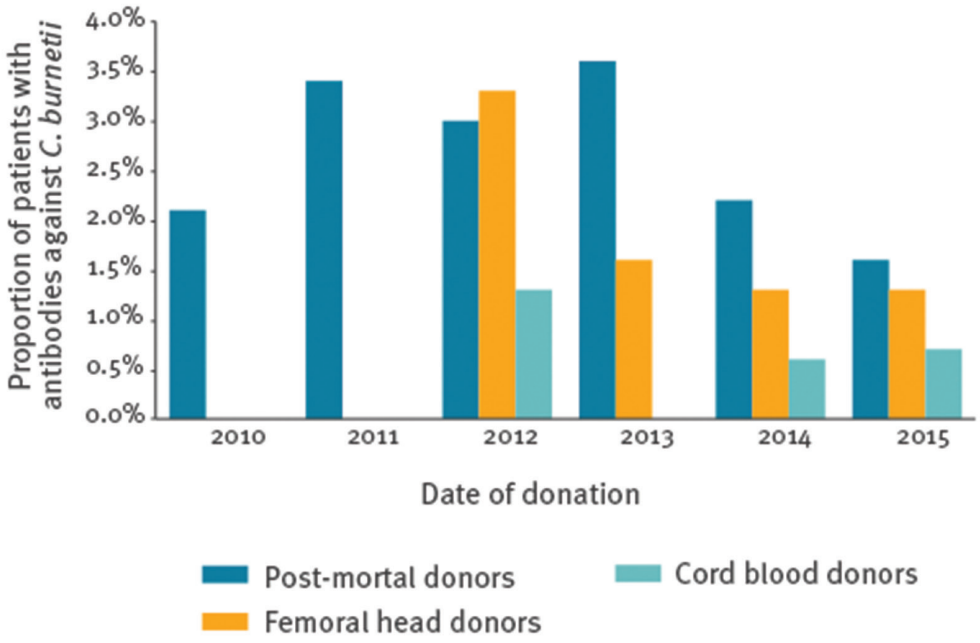
Table 2. Proportion of donors with antibodies against *Coxiella burnetii* per subgroup and characteristics associated with positive serology, the Netherlands, 2010–2015 (n = 15,133).

Variable		Proportion of seropositive patients in %	OR for seropositivity[a]	Femoral head donors
Overall		2.0	NA	NA
Sex	Male	2.7	1.79 (1.40 – 2.29)	p < 0.001
	Female	1.3	Ref	
Age group (in years)	0–10	0	1.08 (0.98 – 1.18)[b]	p = 0.11
	11–20	1.1		
	21–30	0.9		
	31–40	0.8		
	41–50	2.3		
	51–60	2.0		
	61–70	2.1		
	71–80	2.1		
	81–90	1.7		
	91–100	0		
Donor type	Post-mortal	2.8	1.56 (1.23 - 2.00)	p < 0.001
	Living	1.6	Ref	
Residency in high-risk area	Yes	3.7	1.99 (1.50 – 2.64)	p < 0.001
	No	1.8	Ref	

CI: confidence interval; NA: not applicable; OR: odds ratio; Ref: reference category for the variable.[a] Seropositivity in individual as dichotomous (yes/no) outcome. [b] OR per 10 years increase of age. When only evaluating donors screened after August 2012 (start date for screening of all tissue types), the estimates remained unchanged.

Among living donors, seroprevalence of *C. burnetii* antibodies was lowest in cord blood donors (3/565; 0.5%). Of the femoral head donors from the western part of the Netherlands, 1.3% (75/5,660) were seropositive. Of the femoral head donors from the eastern part of the Netherlands, 2.1% (79/3,818) were seropositive. Seroprevalence of *C. burnetii* antibodies among all donors gradually decreased in the years after the outbreak from 2.1% in 2010 (5/237), 3.4% in 2011 (53/1,580), 3.1% in 2012 (68/2,228), 1.9% in 2013 (77/4,072), 1.4% in 2014 (59/4,176) and to 1.4% in 2015 (39/2,840), with a significant trend in time (Pearson correlation: $r = -0.46$; $p < 0.001$), see Figure 1.

Figure 1. Seroprevalence of antibodies against *Coxiella burnetii* in post-mortal tissue donors, living femoral head donors and cord blood donors per year, the Netherlands, 2010–2015 (n = 15,133)



**Screening of femoral head donors and cord blood donors started in 2012.*

Chronic Q fever among donors and in the general population

Among all seropositive donors (n = 301), seven patients (2.3%) were newly detected with chronic Q fever during the screening for this study. This corresponds to a proportion of 0.05% people with chronic Q fever among the total of 15,133 donors. In the population in the Netherlands, 443 chronic Q fever patients were identified between January 2007 and May 2016. The cumulative incidence of chronic Q fever in the general population was low: the highest annual incidences were 11.0 per 1,000,000 for men and 5.7 per 1,000,000 for women in 2010, decreasing to 1.7 per 1,000,000 for men and less than 0.5 cases per 1,000,000 for women in

2015. The majority of chronic Q fever patients, both donors and non-donors, were men (74% of the total; n = 331). Furthermore, 75% of chronic Q fever patients (non-donor; n = 333) lived in high-risk areas, whereas none of the donors with chronic Q fever lived in a high-risk area (Table 3).

Table 3. Comparative presentation of donors newly detected with chronic Q fever in this study (n = 7) and chronic Q fever patients in the general population (n = 443), the Netherlands, 2010–2015.

Variable	Donors with chronic Q fever	Chronic Q fever patients in the general population
Total number	7	443
Male sex	5 (71%)	326 (74%)
Mean age (years) ± standard deviation	70.3 ± 10	65.8 ± 14
Residency in high-risk area	0	333 (75%)

**Donors with newly detected chronic Q fever are not included in column of chronic Q fever patients.*

Of screened donors with chronic Q fever, four were post-mortal tissue donors and three were living femoral head donors. In one post-mortal donor with newly diagnosed chronic Q fever, the donated cornea and skin were tested by PCR for presence of *C. burnetii*: both samples were negative. For two other post-mortal donors, histopathological data were available: no signs of endocarditis were present in one donor on examination of the heart after heart valve donation and the other donor had an aortic aneurysm with oesophageal fistula on autopsy. In the fourth donor, no additional evaluation was performed. Two of these living femoral head donors were shown to be PCR-negative for *C. burnetii* on blood (shortly after donation). In the third living femoral head donor, clinically relevant infection was confirmed and chronic Q fever treatment was started. Additional data of donors newly diagnosed with chronic Q fever are presented in Table 4.

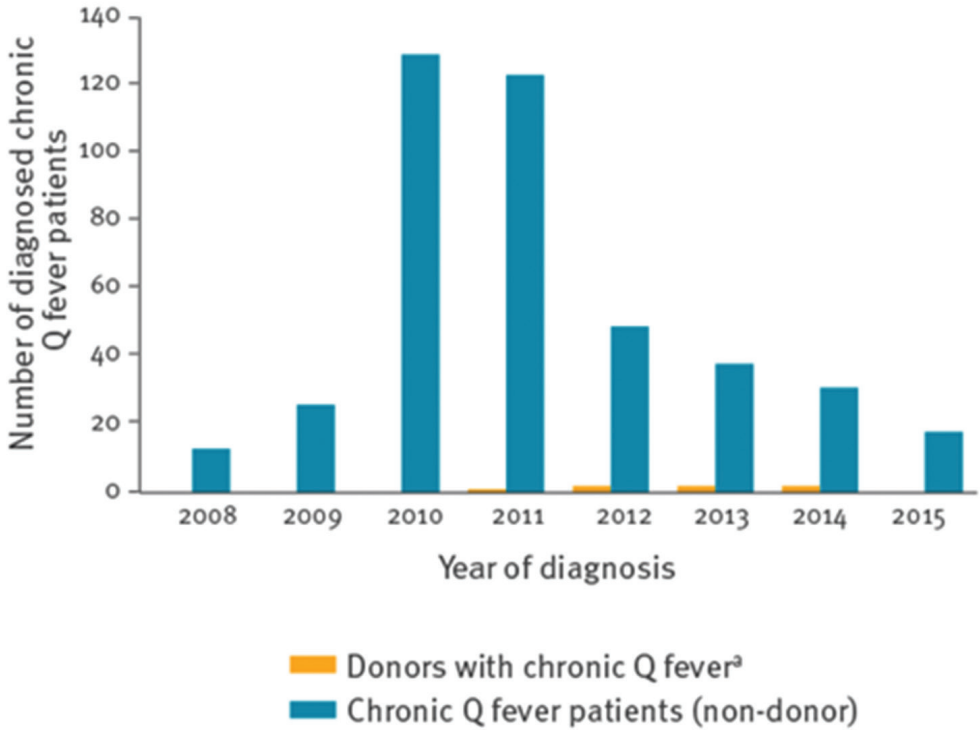
Table 3. Comparative presentation of donors newly detected with chronic Q fever in this study (n = 7) and chronic Q fever patients in the general population (n = 443), the Netherlands, 2010–2015.

Donor type	Sex	Age group (years)	Year	High-risk area	Risk factors for chronic Q fever	Phase 1 IgG titre	Phase 2 IgG titre
Post-mortal	Male	61-70	2011	No	No	1:4,096	1:4,096
Post-mortal	Female	51-60	2012	No	No	1:2,048	1:2,048
Post-mortal	Male	61-70	2012	No	Aortic aneurysm	1:4,096	1:4,096
Post-mortal	Female	71-80	2013	No	Aortic aneurysm	1:4,096	1:4,096
Living	Male	71-80	2013	No	Aortic aneurysm	1:2,048	1:1,024
Living	Male	71-80	2014	No	Aortic aneurysm	1:2,048	1:2,048
Living	Male	71-80	2014	No	No	1:4,096	1:4,096

**Donors with newly detected chronic Q fever are not included in column of chronic Q fever patients.*

In the general population, the number of newly diagnosed chronic Q fever patients decreased over time (Figure 2). For the majority of chronic Q fever patients, the moment of primary infection was unknown: in 139 patients, time between (serologically confirmed) acute and chronic infection was documented in the Dutch national chronic Q fever database. Mean duration between acute Q fever and chronic Q fever was 61 weeks (SD = 48; range: 0–224 weeks), 91% of all cases of chronic Q fever were diagnosed within two years of acute Q fever. Only four cases of chronic Q fever during pregnancy were reported in the Dutch chronic Q fever database (all with only placenta as focus of infection). In two of these cases, the acute episode was known: the interval between acute infection and chronic infection was 6–8 months. Three cases occurred in 2010 and one case in 2014. No chronic Q fever cases among cord blood donors were detected.

Figure 2. Newly detected chronic Q fever patients in the general population and among donors of tissues and cells, the Netherlands, 2008–2015 (n = 450).



** The number of donors with chronic Q fever before November 2010 is not known: the screening programme started in November 2010.*

Discussion

Seroprevalence of *C. burnetii* antibodies among donors of tissues and cells in the Netherlands is low, despite the large Q fever outbreak from 2007 to 2010. After the outbreak, seroprevalence in all donors of tissues and cells in the Netherlands peaked in 2011 with 3.4% and decreased significantly over time to 1.4% in 2015. This is similar to the seroprevalence of *C. burnetii* antibodies before the Dutch Q fever outbreak between 2007 and 2010: data from a routine nationwide seroprevalence survey for the National Immunisation Programme in the Netherlands in 2006 and 2007 showed the presence of *C. burnetii* antibodies in 1.5% of people [20]. The decrease in seropositivity among the population of donors of tissues and cells in the Netherlands is comparable with decreasing seroprevalence among blood donors in an outbreak area in the Netherlands, which was 12% in 2009 and 4% in 2012 [21].

Male sex and residency in a high-risk area were associated with seropositivity, which is in line with previous findings from a study among blood donors in high-risk areas and a study describing characteristics of acute Q fever patients [22,23]. Moreover, post-mortal donation was associated with increased seropositivity and increased false-positivity with the screening test (EIA). False positivity may be due to lower sample quality of post-mortal plasma caused by haemolysis, autolysis or bacterial contamination[24]. Therefore, a higher seropositivity in post-mortal samples than in samples of living donors may be explained by the nature and quality of the samples. Cord blood donors had much lower seroprevalence than the other donor groups, which may be explained by the fact that the group only consists of women and that most of the collection sites of the cord blood bank are located in the western part of the Netherlands representing a low-endemic area during the outbreak.

Only seven chronic Q fever patients were detected among screened donors (0.05%). Definite diagnostic classification of chronic Q fever was unknown for these donors, but according to the Dutch chronic Q fever consensus group criteria, four patients would have been diagnosed with at least probable chronic Q fever based on the presence of a risk factor [6]. In none of the donors, blood or tissues were found to be PCR-positive for *C. burnetii*. Remarkably, none of the donors with newly detected chronic Q fever lived in a high-risk area. This may be related to higher awareness among clinicians in high-risk areas and better detection of chronic Q fever patients in routine care (who are excluded from donation), resulting in fewer undiagnosed chronic Q fever patients among donors from high-risk areas.

The results of this study prompt a discussion on whether testing of all donors of high-risk tissues can or should be terminated. The overall number of newly detected chronic Q fever cases was low (<0.1%). Furthermore, the number of newly detected chronic Q fever patients and seroprevalence in the general population in the Netherlands decreased drastically in the years following the outbreak. The Q fever outbreak subsided more than 6 years ago, and 91% of registered chronic Q fever cases in the Netherlands was diagnosed within 2 years after primary infection. No cases of transmission during or after the outbreak have been reported, despite the fact that extensive and complete screening of all donors of tissues and cells started

2 years after the end of the outbreak. Altogether, it could be argued that screening can be stopped.

To our knowledge, the Netherlands is the only country in the world in which donors of tissues and cells are screened for Q fever, despite the fact that the disease is endemic in certain areas [25,30]. However, no guidelines are available specifying which level of risk of transmission of pathogens is acceptable for tissue or cell donors: factors such as type of pathogen, consequences of transmission and prevalence of the disease in the population are all factors that should be taken into account. To accurately estimate the possibility of transmission in the absence of screening in the Netherlands, the maximum possible duration between acute infection and chronic infection, the actual prevalence of chronic Q fever in the Netherlands (including undiagnosed cases) and the actual risk of transmission of Q fever after transplanting tissues or cells of an infected donor would have to be known.

Among patients registered in the Dutch national chronic Q fever database with a known episode of acute Q fever, more than 90% were diagnosed with chronic Q fever within 2 years after primary infection. However, it is questionable if the duration between primary and chronic infection of patients in whom primary infection was recorded is representative for patients in whom primary infection was not recorded. Furthermore, it is possible that incidental cases of chronic Q fever will occur, independently of the epidemic in the Netherlands (a limited number of primary cases are reported every year). Thus, even if the maximum possible interval of developing chronic Q fever after the epidemic has ended, transplantation free of transmission cannot be guaranteed.

The incidence of both acute and chronic Q fever decreases over time. Since the Q fever outbreak in the Netherlands ended, the number of reported primary Q fever cases has been low with an average of 20 cases per year [3]. The number of newly diagnosed chronic Q fever patients also decreased to 18 cases in 2015. However, from these data it is not possible to accurately estimate the number of undiagnosed chronic Q fever patients in the Netherlands. Moreover, data on the risk of transmission after transplantation of tissues or cells from an infected donor are not available. However, transmission of Q fever after tissue, investigations of femoral head or cord blood donation have not been described in literature.

Altogether, we may conclude that the risk of transmission of Q fever through undiagnosed chronic Q fever patients is very low, but probably not zero. Screening donors only from high-risk areas has been suggested as an alternative to screening all donors, but this seems to be inadequate since none of the newly detected chronic Q fever cases in this study lived in high-risk areas.

To our knowledge this is largest study investigating the outcome of screening donors of tissues and cells for Q fever in a standardised way. Since all post-mortal tissues, all cord blood donations and nearly all bone and cartilage donations are tested centrally, we gathered a near-complete national cohort. Our laboratory protocol was very effective and feasible in practice and may provide guidance for screening programmes globally in endemic or epidemic settings.

Naturally, it can be debated whether these donors were representative of the general Dutch population. However, since *C. burnetii* is highly infectious, the risk of Q fever and consecutive seropositivity is merely dependent on exposure to the pathogen and not on host characteristics [31]. The difference between men and women is believed to be caused by differences in occupational and behavioural exposure (e.g. outdoor smoking) [32]. Since 11.3% of the donors with known residence lived in high-risk areas, similar to the distribution of the general Dutch population, these data are likely to be representative of the general population.

Conclusion

This study shows that seroprevalence of *C. burnetii* antibodies among donors of tissues and cells in the Netherlands is low (1.4%) and similar to pre-outbreak levels. Furthermore, incidence of chronic Q fever in the general population and the numbers of newly detected chronic Q fever patients among donors of tissues and cells are low (<0.1%). This study may serve to prompt a discussion of when to terminate screening of donors of tissues and cells for chronic Q fever.

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References

1. Kampschreur LM, Hagenaars JC, Wielders CC, Elsman P, Lestrade PJ, Koning OH *et al*. Screening for *Coxiella burnetii* seroprevalence in chronic Q fever high-risk groups reveals the magnitude of the Dutch Q fever outbreak. Epidemiol Infect 2013;141(4):847-51.
2. National Institute for Public Health and the Environment, RIVM, http://www.rivm.nl/Onderwerpen/Q/Q_koorts, Accessed Sept 2015.
3. Eldin C, Melenotte C, Mediannikov O, Ghigo E, Million M, Edouard S et al. From Q fever to *Coxiella burnetii*: a paradigm change. ClinMicrobiol Rev 2017;30(1): 115-90.
4. Kampschreur LM, Delsing CE, Groenwold RH, Wegdam-Blans MC, Bleeker-Rovers CP, de Jager-Leclercq MG et al. Chronic Q fever in the Netherlands 5 year after the start of the Q fever epidemic: results from the dutch chronic Q fever database. J ClinMicrobiol 2014;52(5):1637-43.
5. Raoult D, Tissot-Dupont H, Foucault C, Gouvernet J, Fournier PE, Bernit E et al. Q fever: 1985-1998. Clinical and epidemiologic features of 1,383 infections. Medicine (Baltimore) 2000;79:109-23.
6. Wegdam-Blans MC, Kampschreur LM, Delsing CE, Bleeker-Rover CP, Sprong T, van Kasteren ME et al. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. J Infect 2012;64:247-59.
7. Raoult D. Host factors in the severity of Q fever. Ann N Y AcadSci 1990;590:33-8.
8. Fenollar F, Fournier PE, Carrieri MP, Habib G, Messana T, Raoult D. Risk Factors and Prevention of Q fever Endocarditis. Clin Infect Dis 2001 Aug 1;33(3):312-6.
9. Schoffelen T, Kampschreur LM, van Roeden SE, Wever PC, den Broeder AA, Nabuurs-Franssen MH *et al. Coxiella burnetii* infection (Q fever) in rheumatoid arthritis patients with and without TNF-α therapy. Ann Rheum Dis 2014.
10. Kampschreur LM, Dekker S , Hagenaars JCJP, Lestrade PJ,Renders NHM, de Jager-Leclerq MG et al. Identification of Risk Factors for Chronic Q Fever, the Netherlands. Emerg Infect Dis. 2012 Apr; 18(4): 563–570. doi:10.3201/eid1804.111478.
11. Raoult D, Marrie T: Q fever. Clin Infect Dis 1995;20:489-95.
12. Kanfer E, Farrag N, Price C, MacDonald D, Coleman J, Barrett AJ. Q fever following bone marrow transplantation. Bone Marrow Transplant 1988;3:165-6.
13. Criley JM, Carty AJ, Besch-Williford CL, Franklin CL.*Coxiella burnetii* infection in C.B-17 scid-bg mice xenotransplanted with fetal bovine tissue. Comp Med 2001;51:357-60.
14. Harris RJ, Storm PA, Lloyd A, Arens M, Marmion BP. Long-term persistence of *Coxiella burnetii* in the host after primary Q fever. Epidemiol Infect 2000;124: 543-9.
15. Van Wijk MJ, Hogema BM, Maas DW, Bokhorst AG. A Q fever outbreak in the Netherlands: consequences for tissue banking. Transfus Med Hemother 2011, 38:357–364.
16. Health Council of the Netherlands: Q fever: risk of transmission via blood or other body material; 2011. http://www.gezondheidsraad.nl/sites/default/files/201115EQfever.pdf.

17. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr, Ryan T et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 2000;30(4): 633-8.
18. Central Bureau for Statistics. Population and households: four-digit postal codes (age and sex), The Hague, CBS [updated 2014; cited 2016 April 25]. Available from: http://statline.cbs.nl/Statweb/?LA=nl.
19. Dutch Food and Consumer Product Safety Authority (NVWA). Adres locaties besmette bedrijven q-koorts (normlijst) [address locations of Q fever-infected farms (standard list)]. Utrecht: NVWA; 2015. Dutch. Available from: https://www.nvwa.nl/onderwerpen/dierziekten/documenten/dier/dierziekten/q-koorts/publicaties/q-koorts besmette-bedrijven-met-adresgegevens-normlijst.
20. Schimmer B, Notermans DW, Harms MG, Reimerink JH, Bakker J, Schneeberger P et al. Low seroprevalence of Q fever in the Netherlands prior to a series of large outbreaks. Epidemiol Infect 2011;16:1-9.
21. Slot, E., Hogema, B. M., Molier, M. and Zaaijer, H. L. Screening of blood donors for chronic *Coxiella burnetii* infection after large Q fever outbreaks. Transfusion 2014, 54:2867–2870. doi:10.1111/trf.12749.
22. Dijkstra F, van der Hoek W, Wijers N, Schimmer B, Rietveld A, Wijkmans CJ et al. The 2007–2010 Q fever epidemic in the Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. FEMS Immunol Med Microbiol 2012;64(1):3-12.doi: 10.1111/j.1574-695X.2011.00876.x.
23. De Lange MM, Schimmer B, Vellema P, Hautvast JL, Schneeberger PM, van Duijnhoven YT. *Coxiella burnetii* seroprevalence and risk factors in sheep farmers and farm residents in the Netherlands. Epidemiol Infect 2014;142(6):1231-44. doi: 10.1017/S0950268813001726.
24. Wilkemeyer I1, Pruss A, KalusU ,Schroeter J. Comparative infectious serology testing of pre- and post-mortem blood samples from cornea donors. Cell Tissue Bank 2012; 13(3):447-52.
25. Anderson AD, Szymanski TJ, Emery MP, Kohrs PH, Bjork AC, Marsden-Haug N et al. Epizootiological investigation of a Q fever outbreak and implications for future control strategies. J Am Vet Med Associ 2015;247(12):1379-86.
26. Bond KA, Vincent G, Wilks CR, Franklin L, Sutton B, Cowan R et al. One health approach to controlling a Q fever outbreak on an Australian goat farm. Epidemiol Infect 2015;23:1-13 (epub ahead of print).
27. Alonso E, Lopez-Etxaniz I, Hurtado A, Liendo P, Urbaneja F, Aspiritxaga I et al. Q fever outbreak among workers at a waste-sorting plant. PLoS One 2015;10(9):e0138817.
28. Bellini C, Magouras I, Chapuis-Taillard C, Clerc O, Masserey E, Peduto G et al. Q fever outbreak in the terraced vineyars of Lavaux, Switzerland. New Microbes New Infect 2014;2(4):93-9.
29. D'Amato F, Eldin C, Georgiades K, Edouard S, Delerce J, Labas N et al. Loss of TSS1 in hypervirulent *Coxiella burnetii* 175, the causative agent of Q fever in French Guiana. Comp ImmunolMicrobiol Infect Dis 2015;41:35-41.
30. Akbarian Z, Ziay G, Schauwers W, Noormal B, Saeed I, Qanee AH et al. Brucellosis and *Coxiella burnetii* Infection in Householders and Their Animals in Secure Villages in Herat Province, Afghanistan: A Cross-Sectional Study. PLoSNegl Trop Dis 2015;9(10):e0004112.

31. Madariaga MG, Rezai K, Trenholme GM,Weinstein RA. Q fever: a biological weapon in your backyard. Lancet Infect Dis. 2003 Nov; 3(11):709-21.
32. Karagiannis I, Schimmer B, van Lier A, Timen A, Schneeberger P, van Rotterdam B et al. Investigation of a Q fever outbreak in a rural area of the Netherlands. Epidemiol Infect, 2009 Sep:137(9);1283-94.

Supplement 1 – List of hospitals participating to the Dutch national chronic Q fever database

Participating hospitals:

Amphia Hospital in Breda, Atrium Medical Center in Heerlen, Bernhoven Hospital in Uden, Bravis Hospital in Roosendaal, Canisius-Wilhelmina Hospital in Nijmegen, Catharina Hospital in Eindhoven, Diakonessenhuis in Utrecht, Elkerliek Hospital in Helmond, Erasmus Medical Center in Rotterdam, Hospital Gelderse Vallei in Ede, Gelre Hospital in Apeldoorn, Groene Hart Hospital in Gouda, Jeroen Bosch Hospital in 's –Hertogenbosch, Leids University Medical Center in Leiden, Izore Laboratory in Leeuwarden, Isala Clinic in Zwolle, Laurentius Hospital te Roermond, Maasstad Hospital in Rotterdam, Maxima Medisch Centrum in Eindhoven, Meander Medical Center in Amersfoort, Medisch Spectrum Twente in Enschede, Onze Lieve Vrouwe Gasthuis in Amsterdam, Radboud university medical center in Nijmegen, Reinier de Graaf Hospital in Delft, Rijnstate Hospital in Arnhem, Sint Elisabeth Hospital in Tilburg, Sint Antonius Hospital in Nieuwegein and University Medical Center Utrecht in Utrecht.

Hospitals providing cooperation but without chronic Q fever patients: Admiraal de Ruyter Hospital in Goes, Albert Schweitzer Hospital in Dordrecht, Bronovo Hospital in The Hague, Diaconessenhuis in Leiden, St. Fransiscus Gasthuis in Rotterdam, St. Jansdal in Harderwijk, Vlietland Hospital in Schiedam.

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Part V

General discussion, summary and publication list

Chapter 11

General discussion

Introduction

In this thesis, different aspects of the prognosis and management of chronic Q fever have been addressed. In this chapter, the most important conclusions will be discussed and put in perspective.

The poor prognosis of proven chronic Q fever patients and importance of early diagnosis

Prognosis for proven chronic Q fever patients is poor in terms of complications and disease-related mortality: over 60% of patients developed complications and 25% died of chronic Q fever-related causes. Complications are serious and accompanied by high morbidity (chapter 2). In patients with proven chronic Q fever with a vascular focus of infection, 15% of patients develop arterial fistulae. This proportion is striking, since arterial fistulae are very rare (chapter 3). Complications are by far the strongest predictors for chronic Q fever-related mortality. The fact that over 60% of patients with complications presented with complications at time of diagnosis, suggests a considerable diagnostic delay in clinical practice. This is supported by the fact that patients with severe complications, such as arterial fistulae, were more often diagnosed with chronic Q fever in absence of a notified primary infection (chapter 3). Those with a primary infection are often monitored, which could aid in early recognition of chronic infection. The description of our cohort painfully visualized the poor prognosis of chronic Q fever patients, which may be partially caused by diagnostic delay. In the Netherlands, approximately 440 chronic Q fever patients were diagnosed during 10 years in 29 hospitals (chapter 2). This number demonstrates how rare chronic Q fever is: the average medical doctor does not encounter any chronic Q fever patients in his or her life, making the disease difficult to recognize. The complexity of the diagnosis complicates the recognition of the disease further.

The poor outcome in chronic Q fever patients has led to the discussion if a screening program for detection of chronic Q fever should be introduced. Currently, a cost-benefit analysis is made, partially based on our data. In the absence of a screening program, diagnosis of chronic Q fever relies on case finding. By publishing our data in international and Dutch guidelines, awareness amongst clinicians and patients will hopefully improve. Nevertheless, due to the rarity of the disease, recognition will remain difficult. Another potential tool to improve early detection of chronic Q fever is to optimize the current diagnostic possibilities. Diagnosis of chronic Q fever relies on a combination of tests and patient characteristics: all individual tests have limited sensitivity and specificity. There is high need for a sensitive and specific, unambiguous diagnostic test, in order to reduce the complexity of the diagnostic work-up of chronic Q fever. With optimization of diagnosis of chronic Q fever, diagnosis may be easier to interpret for clinicians and less time-consuming. This may be one of the key elements in improving prognosis of chronic Q fever patients. Therefore, in future research focus should be laid on improving diagnosis of chronic Q fever.

Treatment of chronic Q fever: do's and dont's

Treatment of proven or probable chronic Q fever should consist of a combination of doxycycline plus hydroxychloroquine, with doxycycline plus quinolones as potential alternative (chapter 5). Treatment with either doxycycline or quinolone monotherapy is not recommended, since both monotherapies were frequently switched due to insufficient response. Moreover, treatment with any of the combination antibiotic regimens for less than 18 months duration was associated with a higher risk of chronic Q fever-related mortality or PCR-positive relapse after stop of treatment (chapter 5). Therefore, we recommend treatment for all chronic Q fever patients with doxycycline plus hydroxychloroquine, or doxycycline plus quinolones as alternative option for at least 18 months. All proven chronic Q fever patients have an indication for treatment, because of their high risk for adverse outcomes (chapter 2).

For patients with probable chronic Q fever, the decision to start or withhold treatment is not so straightforward. The spectrum of probable chronic Q fever patients is more heterogeneous, making this the most difficult category of patients for making treatment decisions: a subset of these patients develops complications and is at risk for chronic Q fever-related mortality, while another subgroup of patients does not develop any adverse outcomes without treatment. The clinician's judgement on the patient's condition, patient's preferences and risk factors that were associated with adverse outcomes may aid clinicians in the decision on starting or withholding treatment (chapter 2). Clinicians apparently are very well capable of making this decision, since none of the untreated probable chronic Q fever patients died of disease-related causes.

Possible chronic Q fever patients do not have an indication for antibiotic treatment. In possible chronic Q fever patients, complications rarely occur and chronic Q fever-related mortality was not observed. The complications that were observed amongst possible chronic Q fever patients were probable a matter of definition, and not disease-related. Since the risk for adverse outcomes in possible chronic Q fever patients was unclear prior to the study in chapter 2, no advise or data on optimal management of these patients was available. In our cohort, 16% of patients with possible chronic Q fever were treated with antibiotics. However, despite absence of treatment in the majority of patients, prognosis was highly favorable in terms of complications and mortality. This suggests that no clinical relevant infection is present in these patients, and that their serological profile with high phase I IgG titers probably reflects an (over)adequate immune response instead of an ongoing or chronic infection with *C. burnetii* (chapter 2). Therefore, we can conclude that treatment is not warranted in patients with possible chronic Q fever.

During treatment of chronic Q fever with doxycycline and hydroxychloroquine, measuring serum doxycycline concentrations (SDC) should be performed. Measurement of SDC during treatment of chronic Q fever with doxycycline and hydroxychloroquine was associated with a lower hazard for disease-related events (chapter 6). The mechanism of improving clinical outcomes is unknown, but it seems plausible that SDC may influence prognosis by optimization of dosage in an

individual: SDC may vary within individuals and by adjustment of dosage, appropriate concentrations can be reached. Moreover, by measuring SDC, incompliance may be detected. Treatment of chronic Q fever is very extensive in terms of duration and intensity, which may lead to incompliance. SDC measurement is a tool to observe this, providing physicians the opportunity to intervene by discussing the importance of compliance with their patient (chapter 6).

A large proportion of patients experienced side effects during treatment with antibiotic combination regimens for chronic Q fever. Moreover, side effects where the most important reason to discontinue any of the three combination regimens (doxycycline plus hydroxychloroquine, doxycycline plus quinolones and doxycycline plus hydroxychloroquine plus quinolones) (chapter 5). This highlights the high toxicity and burden of treatment of chronic Q fever. Since the duration of treatment is very long and treatment is of vital importance, clinicians should pay attention to side effects, to prevent incompliance.

All studies describing treatment efficacy in chronic Q fever patients published so far, including ours (chapter 5), are observational studies. Therefore, confounding by indication is a major issue in all studies. Patients with relatively mild disease are more likely to be treated with less intensive regimens in clinical practice. When evaluating efficacy of less intensive treatment strategies from data generated in routine clinical care, results are probably biased and not generalizable to all chronic Q fever patients. Adjustment for confounders in analysis may not be sufficient to eliminate this bias. The fact that monotherapies were frequently switched because of insufficient effect and that treatment with short duration of combination therapy was associated with adverse outcomes, suggests that these therapies are inferior to combination therapy, despite the results of formal analysis. To get definite answers and reliable estimates on efficacy of different treatment regimens for chronic Q fever, large standardized studies (ideally randomized-controlled trials) are needed. Due to the scarcity of chronic Q fever patients and the long course of disease, this is probably only possible in an international collaborative setting. Therefore, the evidence provided in this thesis (chapter 5) may be the best evidence that will be available for the coming years.

Coxiella burnetii and non-Hodgkin lymphoma: innocent until proven guilty?

Altogether, we did not find convincing evidence for an association between infection with *C. burnetii* and non-Hodgkin lymphoma (NHL). Based on findings in this thesis, screening of Q fever patients for NHL or NHL patients for Q fever, is not recommended.

In summary, the incidence of NHL, and B-CLL or small lymphocytic B-cell NHL particularly, was increased (1.2-fold and 1.4-fold) in high Q fever incidence areas in 2009 only, but not in any other year (chapter 8). The relative risk of NHL in chronic Q fever patients was increased 5-fold. When exploring the presence of *C. burnetii* in tissues, the bacterium was detected in both cases and controls (chapter 9). In the first two analyses (chapter 8), there is a substantial risk of bias. The third analyses demonstrated that the finding of *C. burnetii* is not specific for B-NHL (chapter 9).

In the population-based analysis studying the risk of NHL in the Dutch population, there is a risk of detection bias (chapter 8). B-CLL may be detected with a simple laboratory measurement: it is possible that acute Q fever patients were evaluated more often by general practitioners with laboratory studies, and NHL was detected more often.3 Second, the significant increased relative risk may be due to a type I error: 1 of 22 p-values was statistically significant. After correction for multiple testing, this value would not have been significant anymore. Finally, there is a risk of misclassification in this study. Q fever incidence areas were categorized based in the incidence between 2007 – 2010. However, in practice, the incidence varied highly per year. This may lead to misclassification of the exposure (residency in a high Q fever incidence area), which could distort the results in both directions. Altogether, no convincing evidence in favour of an association was found in this study. Nevertheless, the existence of an association cannot be excluded definitively due to methodological drawbacks.

When interpreting the risk of NHL in chronic Q fever patients, detection bias is a major issue. All chronic Q fever patients are subjected to an extensive diagnostic work-up, including laboratory evaluation and PET-CT scanning.[4] Also, the temporal relationship remains difficult to establish: a diagnostic delay may occur both for diagnosis of chronic Q fever and NHL. Therefore, the increased risk for NHL in chronic Q fever patients does not provide definite answers either.

When assessing the presence of *C. burnetii* in tissue samples of patients with and without NHL, the bacterium was detected in tissues of 38% of cases (with B-NHL) and 19% of controls (without B-NHL). The difference in proportion positive samples between cases and controls was not significant. When assessing past Q fever patients only, 36% of cases and 8% of controls were positive, which difference was also not significant. Although the lack of a statistically significant difference may be due to the relatively small sample size, we must conclude that the presence of the bacterium in tissues is not specific for B-NHL tissues.

Altogether, following the findings of Melenotte et al., we could not confirm that there is an association, let alone causal relation, between *C. burnetii* exposure and NHL.[5] When evaluating the Bradford Hill principles for establishing causal relations, the evidence supporting causation is not sufficient.[6] The consistency of the association is moderate. In our nationwide analysis, the risk for was increased in one year only (RR1.16), and not in any other year.[17] Thus, the height of the excess in the two studies risk ranges between 1 and 25, with a considerable risk of selection and detection bias in both studies (chapter 8).[6] Moreover, the increased risk in the general population in high Q fever incidence areas was observed in one year only, while we might expect a longer visible effect if there would truly be a causal relationship. The specificity of the potential relation is low, since there are other factors that may explain both diseases.[7] Temporality between exposure and development of disease is very difficult to confirm definitely, because both diseases can have a considerable diagnostic delay: infection with *C. burnetii* may occur asymptomatically and NHL may be difficult to detect at early stages. Therefore, an experiment would have to be performed to ensure a temporal relationship. The argument of a biological gradient

(or dose-response relation) seems to hold: the risk is higher in patients with a chronic or persistent infection compared to those that develop NHL after a primary infection (chapters 8 and 9). Moreover, there is a plausible potential pathophysiological explanation for the association.[5] Evaluation of this potential pathophysiological mechanism, for example by performing micro-array to unravel the genetic pathway of NHL after Q fever, could provide better insight. Finally, there is coherence between epidemiological and laboratory findings in the studies performed so far.[5]

After the largest Q fever outbreak recorded in history, we could not find convincing evidence for an association between *C. burnetii* and NHL. Due to methodological drawbacks, an association cannot be excluded definitively (chapters 8 and 9). Better understanding of the immunological and oncogenetic effects of presence of *C. burnetii* intracellular may lead to more definite conclusions. Studying the effect of (chronic) *C. burnetii* infection in more depth, for example by performing animal experiments with induction of lymphomagenesis or micro-array on oncogenic proteins in antigen-presenting cells, may shed more light on the potential relation between *C. burnetii* and NHL.

The risk for transmission of *Coxiella burnetii* through transplantations is low

Less than 0.1% of Dutch tissue and cell-donors has undetected chronic Q fever: only seven chronic Q fever patients were detected among 15,133 screened donors (0.05%) and none of them were PCR-positive on either tissue or blood (chapter 10). Because of this finding, the question if screening of tissue and cell-donors in the Netherlands should be continued was raised. The low number of newly detected chronic Q fever patients among donors corresponds with the decreasing number of newly diagnosed chronic Q fever patients registered in the Dutch national chronic Q fever database after the Q fever outbreak ended more than six years ago. The majority of chronic Q fever patients (>90%) in the database was diagnosed within two years after primary infection. No cases of transmission through transplantation during or after the outbreak have been reported, despite the fact that extensive and complete screening of all donors of tissues and cells started two years after the end of the outbreak. Moreover, transmission through tissue or cell-donation (other than bone marrow) has never been described in literature. Therefore, it could be argued that screening can be stopped. However, despite all evidence for a low risk of transmission, the risk is not zero. It is possible that incidental cases of chronic Q fever will occur, independently of or following the Dutch Q fever outbreak. No guidelines are available describing which level of risk of transmission of pathogens is acceptable for tissue or cell-donors: factors such as type of pathogen, consequences of transmission, prevalence of the disease in the population are all factors that can be taken into account. Recipients of transplant tissues, especially recipients of solid organs or cord blood cells, are highly susceptible for infections due to immunosuppressive medication. Therefore, transmission of Q fever could have catastrophic consequences. However, transplantation of tissue and cells will never be free of any risk of transmission of infectious diseases. It is highly subjective if this risk is acceptable, and if the costs of screening all tissue and cell donors outweigh the advantages of a higher level of

certainty and safety. Therefore, the decision on whether to stop screening of donors of tissues and cells in the Netherlands should be based on expert opinion after a discussion in which all advantages and disadvantages are carefully weighed.

References

1. Kristinsson A, Bentall HH. Medical and surgical treatment of Q-fever endocarditis. The Lancet, 1967; 2(7518): 693-7.
2. Levy PY, Drancourt M, Etienne J et al. Comparison of different antibiotic regimens for therapy of 32 cases of Q fever endocarditis. Antimicrob Agents Chemother, 1991; 35(3): 533-7.
3. Hallek M, Cheson BD, Catovsky D et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the international workshop on chronic lymphocytic leukemia updating the national cancer institute – working group 1996 guidelines. Blood 2008;111(12):5446-56.
4. Eldin C, Melenotte C, Mediannikov O et al. From Q fever to *Coxiella burnetii* infection: a paradigm change. Clin Microbiol Rev, 2017; 30(1): 115-90.
5. Melenotte C, Million M, Audoly G et al. B-cell non Hodgkin-lymphoma linked to *Coxiella burnetii*. Blood 2015; pii blood-2015-04-639617.
6. Hill AB. The environment and disease: association or causation? Proc R Soc Med 1965;58(5):295-300.
7. Shankland KR, Armitage JO, Hancock BW. Non-Hodgkin lymphoma. Lancet 2012; 380(9844): 848-857.

Chapter 12

Summary in English

Introduction

Q fever is caused by the Gram-negative intracellular bacterium *Coxiella burnetii*. It causes disease in humans and animals worldwide, with exception of New-Zealand. In The Netherlands, a large Q fever outbreak occurred between 2007 – 2010, with an estimated 40,000 – 50,000 individuals infected. After primary infection, approximately 40% of individuals develop an acute illness (acute Q fever), which most often results in a flu-like illness, pneumonia or hepatitis. Acute Q fever is self-limiting in most cases. However, 1-5% of primary infected individuals develop chronic Q fever, which is a more severe condition. Most important manifestations are endocarditis, infected arterial aneurysms or vascular prosthesis. Mortality rates up to 26% after 3 years of follow-up for specific subgroups of patients and severe complications during chronic Q fever, such as acute aneurysms and abscesses, have been described. Data on the incidence of complications, disease-related mortality and factors associated with these adverse outcomes are unavailable. In order to optimize recognition of chronic Q fever and complications, additional data are needed. Chronic Q fever is difficult to treat, and extensive and prolonged antibiotic treatment with doxycycline and hydroxychloroquine is recommended. All knowledge on treatment of chronic Q fever is derived from observational cohort studies, and the effect of treatment on occurrence of complications has not been studied before. Moreover, the effect of alternative treatment regimens has not been studied. Therefore, there is need for larger studies to study the efficacy of different antibiotic treatment strategies for chronic Q fever.

Besides a risk for complications and chronic Q fever-related mortality, it has been suggested that Q fever is associated with an increased risk for non-Hodgkin lymphoma (NHL). An increased incidence based on a cohort of Q fever patients with 7 NHL cases was reported, and the bacterium was demonstrated in NHL tissue in 4/7 cases. However, questions with regard to the validity of this study were raised. Therefore, the existence of an association must be confirmed and, in case of an association, its' causation requires further exploration.

Results

Part 1 of this thesis focused on the prognosis of chronic Q fever. **Chapter 2** describes the incidence of complications and chronic Q fever-related mortality per diagnostic classification. In patients with proven chronic Q fever, complications occurred in >60% and chronic Q fever-related mortality was 25%. In patients with probable chronic Q fever, 15% developed complications and 4% died of chronic Q fever. In patients with possible chronic Q fever, complications occurred in 2% and chronic Q fever-related mortality did not occur. The presence of prosthetic material prior to diagnosis of chronic Q fever and a positive serum PCR for *C. burnetii* were associated with occurrence of complications. Occurrence of complications was a strong predictor for chronic Q fever-related mortality. In **chapter 3**, the incidence of arterial fistula during chronic Q fever is described. In patients with proven, vascular chronic Q fever, 15% developed arterial fistulae. This is remarkable, since arterial fistulae are

very rare. Among patients with an arterial fistula, chronic Q fever-related mortality was 60%. Among proven chronic Q fever patients without an arterial fistula, chronic Q fever-related mortality was 21%. The ratio between primary and secondary fistula was 4:6, while previously reported ratio's in literature were 1:10. In **chapter 4**, long term quality of life (QOL) of proven or probable chronic Q fever patients treated for their infection was described. After a median follow-up duration of 5 years, QOL was impaired in 55% of patients. Treatment duration was the only factor associated with impaired QOL. Other patient- and disease-related factors were not associated with impaired QOL.

Part 2 of this thesis focusses on treatment of chronic Q fever. **Chapter 5** describes an analysis of antibiotic treatment of all proven and probable chronic Q fever patients included in the Dutch national chronic Q fever database. Doxycycline plus quinolones seems to be a viable alternative to doxycycline plus hydroxychloroquine, if treatment with doxycycline plus hydroxychloroquine cannot be tolerated, for example due to side effects. Doxycycline plus quinolones was not inferior in terms of complications, mortality or therapy failure. Treatment with either doxycycline or quinolone monotherapy is not advised, since these therapies were frequently discontinued due to insufficient response and treatment less than 18 months of any of the combination therapies was associated with a higher risk for PCR-positive relapse or chronic Q fever-related mortality after stop of treatment. In **chapter 6**, the effect of measuring serum doxycycline concentrations (SDC) is described. Measuring SDC was associated with a significant lower risk for complications and disease-related mortality, potentially through optimized dosing of doxycycline based on SDC, or through improved compliance.

In **Part 3** of this thesis, the association between Q fever and NHL is explored. In **chapter 7**, a case-report is presented describing a patient with NHL. In NHL tissue, *C. burnetii* was demonstrated intracellularly. He was diagnosed with underlying vascular chronic Q fever. Chapter 8 describes a population-based analysis in which the incidence of NHL in the entire Netherlands before, during and after the Dutch Q fever outbreak was studied for areas with low, middle and high Q fever incidence. The incidence of NHL was significantly higher in 2009 in high Q fever incidence areas, driven by the subgroup of B-cell chronic lymphocytic leukemia or small lymphocytic B-cell NHL, but not in any other year. In **chapter 8**, the incidence of NHL among chronic Q fever patients compared to the general Dutch population is analyzed. The incidence of NHL among chronic Q fever patients is increased significantly compared to the general Dutch population. **Chapter 9** describes an exploratory laboratory study, in which the presence of *C. burnetii* in both NHL tissues and control tissues (without NHL) is explored. All individuals were exposed to *C. burnetii* prior to retrieval of the tissue samples, which was confirmed serologically. In NHL patients, *C. burnetii* was present in tissues in 38%. In controls without NHL, *C. burnetii* was present in 19%. When assessing past Q fever patients only, 36% of cases and 8% of controls were positive, which difference was also not significant.

In **part 4** of this thesis (which consists of **chapter 10**), the number of newly detected chronic Q fever cases among Dutch tissue- and cell donors was described. Moreover,

the seroprevalence of *C. burnetii* antibodies was assessed. In total, 0.05% of donors were newly diagnosed with chronic Q fever. All resided in low Q fever incidence areas. Seroprevalence of *C. burnetii* antibodies gradually decreased after the Dutch Q fever outbreak to pre-outbreak levels.

Conclusions

Chronic Q fever is a life threatening condition, and is accompanied by high morbidity and mortality, especially for specific subgroups of patients. Long-term QOL is often impaired in patients that were treated for and survive chronic Q fever. Treatment of chronic Q fever should consist of a combination of doxycycline plus hydroxychloroquine, with doxycycline plus quinolones as a potential alternative. Treatment with either doxycycline or quinolones as monotherapy is not advised. SDC can be useful to verify if treatment dosage is adequate or to monitor compliance. Measuring SDC was associated with a lower hazard for complications or chronic Q fever-related mortality. There is a need for controlled studies, since all current available studies are observational and thus subject to confounding by indication. Based on the findings from this thesis, we conclude that there is insufficient evidence for an association between *C. burnetii* and NHL or a causal relationship between Q fever and NHL. Due to methodological drawbacks, the association cannot be excluded definitively. Additional research is needed to provide definite answers with regard to this potential association and its underlying mechanism. Finally, we conclude that the proportion of newly detected chronic Q fever patients through screening among Dutch donors of tissues and cells is very low, and seroprevalence of *C. burnetii* antibodies is back to pre-outbreak levels. Therefore, it is questionable if screening of Dutch donors of tissues and cells should be continued.

Chapter 13

Summary in Dutch
(samenvatting in het Nederlands)

Inleiding

Q-koorts wordt veroorzaakt door de Gram-negatieve intracellulaire bacterie *Coxiella burnetii*. De bacterie veroorzaakt wereldwijd (m.u.v. Nieuw-Zeeland) ziekte bij mens en dier. In Nederland trad een grote Q-koorts uitbraak op tussen 2007 en 2010, waarbij er naar schatting 40,000 – 50,000 mensen besmet raakten. Bij een deel van de geïnfecteerde personen treden symptomen op na primaire infectie en ontstaat acute Q-koorts met als meest voorkomende uitingsvormen griepachtige verschijnselen, pneumonie en hepatitis. Zo'n 60% van de geïnfecteerde personen blijft asymptomatisch na primaire infectie. Acute Q-koorts heeft een relatief gunstige prognose, met een gerapporteerde mortaliteit van <1%. Bij een klein deel van de patiënten (1-5%) persisteert de bacterie: zij ontwikkelen chronische Q-koorts in de jaren na primaire infectie. De belangrijkste manifestaties van chronische Q-koorts zijn endocarditis, geïnfecteerde aneurysmata en geïnfecteerde vaatprothesen of een combinatie ervan. In tegenstelling tot acute Q-koorts, is de prognose van chronische Q-koorts ongunstiger: complicaties komen frequent voor en een aanzienlijk deel van de patiënten overlijdt (tot 26% binnen 3 jaar). Er is weinig bekend over de exacte incidentie van complicaties en ziekte-gerelateerde mortaliteit, en over factoren die hiermee zijn geassocieerd, wat de herkenning van de ziekte moeilijk maakt. Het advies is om chronische Q-koorts langdurig te behandelen met doxycycline en hydroxychloroquine. Alle kennis met betrekking tot de behandeling van chronische Q-koorts is afkomstig uit kleine, observationele studies gericht op een beperkte selectie van antibiotica. Er is dan ook een sterke behoefte aan grotere studies, die de effectiviteit van verschillende antibiotica voor de behandeling van chronische Q-koorts inventariseren.

Eind 2015 werd gesuggereerd, op basis van een Franse studie, dat er een 25-voudig verhoogd risico zou zijn op non-Hodgkin lymfoom (NHL) na blootstelling aan *C. burnetii*. In een Frans cohort Q-koorts patiënten werd bij 7 patiënten NHL vastgesteld na- of rondom- Q-koorts, het voorkomen was hoger dan in de algemene populatie. Zij troffen de bacterie bij 4 patiënten eveneens aan in het NHL weefsel. De belangrijkste hypothese voor dit fenomeen was het bestaan van een oorzakelijke relatie tussen *C. burnetii* en NHL. Vanwege het zeer beperkte beschikbare bewijs en vraagtekens met betrekking tot de validiteit van de bevindingen van de Franse studie, is het van belang om het bestaan van een associatie tussen *C. burnetii* en NHL te bevestigen, en om het eventuele oorzakelijke verband van deze associatie te exploreren.

Resultaten

Deel 1 van dit proefschrift gaat over de prognose van chronische Q-koorts. **Hoofdstuk 2** beschrijft dat het voorkomen van complicaties en ziekte-gerelateerd overlijden sterk afhankelijk is van de diagnostische classificatie. Patiënten met bewezen chronische Q-koorts ondervinden in meer dan 60% van de gevallen complicaties en 25% van de patiënten overleed ten gevolge van chronische Q-koorts. Bij patiënten met waarschijnlijke chronische Q-koorts ontwikkelde 15% complicaties en 4% overleed ten gevolge van chronische Q-koorts. Van de patiënten met mogelijke

chronische Q-koorts ontwikkelde 2% complicaties, geen van de patiënten overleed ten gevolge van chronische Q-koorts. De aanwezigheid van kunstmateriaal vóór diagnose chronische Q-koorts en een positieve serum PCR op *C. burnetii* waren geassocieerd met complicaties. De aanwezigheid van complicaties was een zeer sterke voorspeller van chronische Q-koorts gerelateerde mortaliteit. In **hoofdstuk 3** wordt ingegaan op de cumulatieve incidentie van arteriële fistels. Bij 15% van de patiënten met een bewezen, vasculaire chronische Q-koorts infectie, trad arteriële fistelvorming op. Dit is opvallend, omdat arteriële fistels zeer zeldzaam zijn. Van de patiënten met een arteriële fistel overleed 60% ten gevolge van chronische Q-koorts, terwijl dit percentage 21% was bij patiënten zonder arteriële fistels. De ratio primaire en secundaire fistels was eveneens opvallend: in de literatuur wordt een ratio van 1:10 gerapporteerd, terwijl in dit hoofdstuk een ratio van 4:6 wordt gevonden. In **hoofdstuk 4** wordt de lange termijn kwaliteit van leven bij patiënten die behandeld zijn voor bewezen of waarschijnlijke chronische Q-koorts beschreven. Na een mediane follow-up duur van 5 jaar, was de kwaliteit van leven bij 55% van de patiënten klinisch relevant aangedaan. Behandelduur was geassocieerd met een verlaagde kwaliteit van leven, andere ziekte- en patiënt-gerelateerde factoren waren niet geassocieerd met de uitkomst.

In **deel 2** van dit proefschrift wordt ingegaan op de behandeling van chronische Q-koorts. **Hoofdstuk 5** beschrijft een analyse van de behandeling van alle patiënten met bewezen of waarschijnlijke chronische Q-koorts. Behandeling met doxycycline plus quinolonen lijkt een geschikt alternatief voor behandeling met doxycycline plus hydroxychloroquine, indien dit niet verdragen wordt vanwege bijvoorbeeld bijwerkingen. Behandeling met doxycycline of quinolonen als monotherapie wordt afgeraden, omdat deze behandelingen vaak gestaakt werden vanwege onvoldoende effect, en vanwege het feit dat er een hoger risico was op PCR-positiviteit of ziekte-gerelateerd overlijden na het staken van de behandeling bij patiënten die minder dan 18 maanden behandeld waren met combinatietherapie. In hoofdstuk 6 wordt het effect van het meten van doxycyclinespiegels op klinische uitkomsten geëvalueerd. Het meten van doxycycline spiegels is geassocieerd met significant minder ziekte-gerelateerde events (gedefinieerd als complicaties of ziekte-gerelateerde mortaliteit) bij chronische Q-koorts patiënten die behandeld worden met doxycycline en hydroxychloroquine, waarschijnlijk door betere therapietrouw (of het tijdig onderscheppen van therapie ontrouw) of door het bereiken van betere serumconcentraties na aanpassen van de dosering naar aanleiding van het meten van de spiegels.

Deel 3 van dit proefschrift gaat over de associatie tussen Q-koorts en non-Hodgkin lymfomen (NHL). In **hoofdstuk 7** wordt een casus beschreven met NHL, waarbij *C. burnetii* in NHL weefsel kon worden aangetoond. De patiënt bleek onderliggend chronische Q-koorts te hebben met een vasculair focus. **Hoofdstuk 8** beschrijft een populatie-brede analyse. De incidentie van NHL in heel Nederland voor, tijdens en na de epidemie werd bestudeerd voor gebieden met een lage, middelhoge en hoge Q-koorts incidentie tijdens de Nederlandse Q-koorts uitbraak van 2007 – 2010. Hierbij werd gevonden dat er een verhoogde incidentie van NHL in 2009 in gebieden met een hoge Q-koorts incidentie was. Deze verhoogde incidentie werd gedreven door de groep B-cel chronische lymfatische leukemie en kleincellige NHL.

In andere jaren was het risico op NHL niet verhoogd. Uit **hoofdstuk 8** blijkt eveneens dat chronische Q-koorts patiënten een hoger risico hebben op NHL in vergelijking met de algemene Nederlandse bevolking. **Hoofdstuk 9** beschrijft een exploratieve laboratoriumstudie, waarbij gekeken is of *C. burnetii* alleen aangetoond kan worden in NHL weefsels of ook in weefsels van patiënten zonder NHL. Alle patiënten waren blootgesteld aan *C. burnetii* vóór afname van de weefsels, wat werd bevestigd met serologisch onderzoek. Bij patiënten met NHL, werd in 38% *C. burnetii* aangetroffen in NHL weefsels. Bij controle patiënten zonder NHL (weefsels waren gematcht op type weefsel en jaar van afname) werd de bacterie bij 19% van de patiënten aangetroffen, dit verschil is niet significant. Wanneer patiënten met een chronische infectie buiten beschouwing gelaten worden en alleen patiënten met een doorgemaakte infectie bestudeerd worden, is *C. burnetii* aanwezig in 36% van de patiënten met NHL en 8% van de patiënten zonder NHL, dit verschil is niet significant.

In **deel 4** van dit proefschrift (bestaande uit **hoofdstuk 10**) wordt het aantal nieuw ontdekte chronische Q-koorts patiënten onder Nederlandse weefsel- en celdonoren beschreven. Ook wordt het voorkomen van antistoffen tegen *C. burnetii* beschreven en wordt gekeken welke factoren geassocieerd zijn met seropositiviteit. In totaal werd bij 0.05% van de donoren chronische Q-koorts vastgesteld. Het voorkomen van antistoffen tegen *C. burnetii* was vergelijkbaar met de situatie vóór de Nederlandse Q-koorts uitbraak. Deze gegevens roepen de vraag op of het screenen van alle Nederlandse weefsel- en celdonoren moet worden voortgezet, of dat de screening gestaakt kan worden.

Conclusies

Chronische Q-koorts is een ernstige, levensbedreigende aandoening die gepaard gaat met een hoog risico op complicaties en ziekte-gerelateerde mortaliteit, in het bijzonder voor specifieke subgroepen van patiënten. Patiënten die de ziekte overleven, hebben vaak een klinisch relevant verlaagde kwaliteit van leven op de lange termijn. Behandeling van chronische Q-koorts moet bestaan uit een combinatie van doxycycline en hydroxychloroquine, waarbij de combinatie van doxycycline en quinolonen een potentieel alternatieve optie is. Behandeling met doxycycline of quinolonen als monotherapie wordt afgeraden. Bij het monitoren van de effectiviteit van behandeling, kunnen serum doxycycline spiegels een waardevolle aanvulling zijn. Het meten van doxycycline spiegels is geassocieerd met minder complicaties en ziekte-gerelateerd overlijden, mogelijk door betere therapietrouw of een individueel geoptimaliseerde dosering. Er is een dringende behoefte aan gecontroleerde studies, omdat het risico op bias door 'confounding by indication' hoog is in observationele studies. Op basis van studies beschreven in dit proefschrift concluderen wij dat er onvoldoende bewijs is voor een associatie is tussen *C. burnetii* en NHL, of voor het bestaan van een oorzakelijk verband. Vanwege methodologische bezwaren, kan een relatie niet definitief uitgesloten worden. Er is meer onderzoek nodig om definitieve antwoorden te kunnen bieden met betrekking tot deze associatie. Tenslotte concluderen wij dat de proportie nieuw gediagnosticeerde chronische Q-koorts patiënten onder Nederlandse weefsel- en celdonoren, ontdekt door middel van

screening, bijzonder laag is. Het aantal patiënten dat is blootgesteld aan de bacterie (zonder chronische infectie), is gelijk ten opzichte van vóór de Nederlandse Q-koorts uitbraak. Het is dan ook twijfelachtig of het screenen van alle weefsel- en celdonoren gecontinueerd moet worden.

Part VI

Appendix

Curriculum vitae

Sonja van Roeden was born on December 23rd 1988 in Utrecht. She was raised in Paris and The Hague. After graduating from the Gymnasium Haganum in 2006, she started medical school at Utrecht University. During her study, her enthusiasm for internal medicine and, in particular, infectious diseases was encouraged. She started her scientific activities in 2011 at the Department of Internal medicine and infectious diseases at the University Medical Center Utrecht under supervision of dr. Linda Kampschreur, dr. Jan Jelrik Oosterheert and Prof. dr. Andy I.M. Hoepelman. After graduating in 2012 and a couple of months of travelling in India and South-East Asia, she started working as a resident (ANIOS) in internal medicine in March 2013 at the Diakonessenhuis in Utrecht under supervision of dr. Alex F. Muller and dr. Tom J. Tobé. In May 2014, she continued her training as a resident (AIOS) in the Diakonessenhuis. In October 2015, she fully focused on her PhD at the Department of internal medicine and Infectious diseases at the University Medical Center Utrecht under supervision of dr. Jan Jelrik Oosterheert, prof. dr. Andy I.M. Hoepelman and prof. dr. Marc J.M. Bonten and at the Department of Medical Microbiology and Infection Control of the Jeroen Bosch Hospital under supervision of dr. Peter C. Wever. Next to her PhD, she started and completed a post-graduate master epidemiology. In January 2018, she resumed her residency (AIOS) in internal medicine at the Diakonessenhuis in Utrecht. She is married to Vincent de Bruijne and mother of Fien de Bruijne (2018).

Publication list

Do-not-resuscitate, the next generation: VF-Only. **S.E. van Roeden**, J.J.M. van Delden. Journal of the American Geriatrics Society 2013;61(2):307-8.

Domperidone and the risk of sudden cardiac death (article in Dutch). **S.E. van Roeden**, G. Belle-van Meerkerk, S.A.C. van Tuyl, M.A. van de Ree, A.F. Muller. Nederlands Tijdschrift voor Geneeskunde 2013; 157(46):2189-93.

Coxiella burnetii infection (Q fever) in rheumatoid arthritis patients with and without anti-TNFα therapy. T. Schoffelen*, L.M. Kampschreur*, **S.E. van Roeden**, P.C. Wever, A.A. den Broeder, M.H. Nabuurs-Franssen, T. Sprong, L.A.B. Joosten, P.L.C.M. van Riel, J.J. Oosterheert, M. van Deuren, M.C.W. Creemers. Annals of Rheumatic Diseases 2014; 73(7):1436-8. *shared first authorship.

Clinical relevance of *C. pseudodiphtheriticum* in respiratory specimens. **S.E. van Roeden**, S.F. Thijssen, S. Sankatsing, G.J. Limonard. Infectious Diseases 2015;47(12):862-8.

Case-report: FDG-PET confirmed infected intracardial device (ICD) leads with *Abiotrophia* species. **S.E. van Roeden**, J.M. Hartog, V Bongers, S.F.T. Thijsen, S.U.C. Sankatsing. Case reports in Cardiology 2016;2016:6283581.

E-letter: B-cell non-Hodgkin lymphoma and chronic infection with *Coxiella burnetii*: cause or consequence? **S.E. van Roeden**, W. van der Hoek, P.W. Wever, L.M. Kampschreur, C.P. Bleeker-Rovers, J.T. van Dissel, J.J. Oosterheert. Blood 2015. Available online.

Case-report: *Coxiella burnetii* vascular infection and lymphoma in the Netherlands. **S.E. van Roeden**, C. Melenotte, M.H.A. Hermans, H.A.M. Sinnige, P.T.G.A. Nooijen, G. Audoly, A.I.M. Hoepelman, J.J. Oosterheert, D. Raoult, P.C. Wever. Infection 2018;46(1):131-4.

Treatment of chronic Q fever: clinical efficacy and toxicity of antibiotic regimens. **S.E. van Roeden**, C.P. Bleeker-Rovers, M.J.A. de Regt, L.M. Kampschreur, A.I.M. Hoepelman, P.C. Wever, J.J. Oosterheert. Clinical Infectious Diseases 2018;66(5): 719-26.

The effect of measuring serum doxycycline concentrations on clinical outcomes during treatment of chronic Q fever. **S.E. van Roeden**, C.P. Bleeker-Rovers, L.M. Kampschreur, M.J.A. de Regt, A. Vermeulen Windsant, A.I.M. Hoepelman, P.C. Wever, J.J. Oosterheert. Journal of Antimicrobial Chemotherapy 2018;73(4):1068-76.

Seroprevalence of *Coxiella burnetii* antibodies and chronic Q fever among post mortal and living tissue and cell donors from 2010 – 2015 in The Netherlands. **S. van Roeden**, E. Holsboer, J.J. Oosterheert, J. van Kats, J. van Beckhoven, B. Hogema, M. van Wijk. Eurosurveillance 2018;23(9).

Primary and secondary arterial fistulae during chronic Q fever. S. Karhoff*, **S.E. van Roeden***, J.J. Oosterheert, C.P. Bleeker-Rovers, N.H.M. Renders, G.J. de Borst, L.M. Kampschreur, A.I.M. Hoepelman, O.H.J. Koning, P.C. Wever. Journal of Vascular Surgery 2018; doi: 10.1016/j.jvs.2018.01.044 [Epub ahead of print]. *shared first authorship.

Exposure to *Coxiella burnetii* and the risk of non-Hodgkin lymphoma: a retrospective population-based analysis in the Netherlands. **S.E. van Roeden**, F. van Houwelingen, C.M.J. Donkers, S.J. Hogewoning, M.M.A. de Lange, W. van der Hoek, L.M. Kampschreur, M.J.M. Bonten, A.I.M. Hoepelman, C.P. Bleeker-Rovers, P.C. Wever, J.J. Oosterheert. Lancet Haematology 2018;5(5):e211-19.

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