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REVIEW

Q fever in the Netherlands from 2007 to 2010

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ABSTRACT

Since 2007, the Netherlands is faced with the largest outbreak of Q fever ever reported. In the last four years, over 4000 cases have been reported. The course of the epidemic and possible factors associated with this sudden surge in cases of Q fever is described and the preventive measures in the veterinary sector and the outbreak management of this unique epidemic are summarised. Finally, the latest data on clinical presentation and diagnostic and therapeutic dilemmas of Q fever in the Netherlands are reviewed.

KEYWORDS

Coxiella, Q fever, the Netherlands

INTRODUCTION

Since 2007, the Netherlands is faced with the world's largest outbreak of Q fever with over 4000 notified cases. Q fever is caused by *Coxiella burnetii*, a small, Gram-negative obligate intracellular bacterium, phylogenetically related to Legionellales. Transmission occurs through inhalation of infected aerosols. The reservoir consists mainly of dairy goats and sheep, but excretion of *Coxiella* has also been described in other cattle and rodents. Small outbreaks associated with parturient pets such as cats and dogs have also been reported.[1] The animals shed the bacterium in urine and faeces, and in very high concentrations in birth by-products. *C. burnetii* is extremely infectious as was illustrated by an experiment demonstrating that inhalation of a single bacterium could cause seroconversion in humans.[2] It was classified as a category B bioterrorism agent.

EPIDEMIOLOGY AND CONTROL OF Q FEVER IN THE NETHERLANDS

The epidemic in the Netherlands is concentrated in the southern part of the country, in the provinces of Brabant, Gelderland and Limburg, although increasing numbers are being reported from the Northern provinces. A seroprevalence study performed on samples that were collected in a national immunisation survey in 2006 (PIENTER-2) showed a low seropositivity rate for Q fever of 2.4% just before the outbreak, indicating that the epidemic in 2007 was indeed newly emerging and did not merely reflect heightened awareness of physicians for Q fever.[3]

Already in 2007, an association with intense goat farming in the region was suggested.[4] In 2008, a large human cluster of Q fever in an urban area was clearly linked to a dairy goat farm with a Q fever related abortion episode a few weeks before the first human cases presented.[5] The high relative risk (31.1 [95% CI 16.4 to 59.1]) to contract Q fever when living within a 2 km radius of a dairy goat farm compared with persons living more than 5 km away supported this hypothesis.[5] Although most cases seem to be related to dairy farming, transmission from non-dairy sheep has also been described in a small epidemic of at least 28 cases among patients and staff of a psychiatric institution.[6] Besides proximity to urbanisation, multiple other environmental factors are involved in the transmission of Q fever from infected farms. Hunink and colleagues showed a correlation between higher vegetation density and higher groundwater levels and lower transmission of Q fever from infected farms in various regions in the Netherlands.[7] Since *Coxiella* is extremely resistant to heat and desiccation, it can survive in the environment for many months.

Outbreaks of this magnitude are unprecedented. Recent European outbreaks in Germany and Switzerland were limited to a short epidemic without significant recurrences in the following years.[8,9] Up until 2007, an average of 20 cases of human Q fever were reported

yearly in the Netherlands. The reason for the surge in clinical problems in humans and animals is still unclear. Possible explanations include the increase of the dairy goat population (from 5000 in 1985 to over 375,000 in 2009). Moreover, the type of goat husbandry has changed with sometimes up to thousands of animals in one dairy farm. In contrast to other countries experiencing smaller Q fever epidemics, in the Netherlands these farms are often located in highly populated areas.

After the first year of the epidemic in the Netherlands (with 168 confirmed cases), a limited number of preventive measures were taken. However in 2008, there was a further increase in the number of reported human cases in the Netherlands. Preventive regulations implemented in autumn 2008 consisted of making Q fever a notifiable disease for the veterinary sector (for humans it has been notifiable since 1978), prohibiting the spread of manure from infected farms, and compulsory vaccination for all non-pregnant goats on dairy goat farms in the region. Although vaccination does not eliminate the disease, it is effective in reducing massive bacterial shedding from a heavily infected herd, thereby limiting the risk of environmental contamination.[10] Before 2010, however, there was a considerable shortage of veterinary vaccine and vaccination of all goats was not possible. Therefore, after the further increase in human cases in 2009 (by then with multiple reports of fatal cases, especially of chronic Q fever), drastic measures were taken. Farms were tested by means of polymerase chain reaction testing for *Coxiella* on bulk milk, and culling of more than 50,000 goats on 88 infected farms was started in December 2009. Furthermore, breeding on those farms was prohibited. Inhabitants of the area within 5 km of the contaminated farms were alerted regarding possible Q fever. A list of positive farms was made available to the public (http://www.vwa.nl/onderwerpen/dierziekten/dossier/q-koorts/kaart-met-overzicht-van-besmette-bedrijven). In 2010, fewer cases of Q fever have been reported compared with 2009. Although differences in weather circumstances could play a role in this decrease, it seems that the measures taken have had a positive effect on limiting transmission to humans.

CLINICAL ASPECTS OF Q FEVER

Acute Q fever

Acute Q fever occurs two to six weeks after exposure depending on the infective dose. The infection remains asymptomatic in up to 60% of patients. Patients with symptomatic disease usually present with fever and mild flu-like symptoms. Because these symptoms are very non-specific, under-reporting is probably quite substantial. A case-control study investigating the first outbreak of Q fever in the Netherlands in 2007 showed that all patients with symptomatic infection experienced fever.[11] Headache and cough were reported by 92 and 85%, respectively. Smoking was found to be an important risk factor, as had been shown by others previously.[12,13] Male sex has also been identified as a risk factor for symptomatic disease.[14] In 2007 and 2008, the female-to-male ratio of acute Q fever was 1:1.7.[15] The mean age was 51 years. Although hospitalisation rates of 2% have been described in literature, hospital admission was much more frequent in the Netherlands. In 2007, almost 50% of Q fever cases were admitted.[11] This high percentage could have been influenced by active case finding in a retrospective study among hospitalised patients. In the subsequent years, admission rates stabilised to around 20%, still considerably higher than reported in literature.[16]

Chronic Q fever

Chronic Q fever develops in 1 to 2% of patients after acute Q fever. Some patients with chronic Q fever do not recall having had an acute infection.[17] It usually develops insidiously, months or even years after acute infection and patients often present with non-specific symptoms such as low-grade fever, night sweats and weight loss. In a large retrospective study from France, endocarditis was found to be the predominant manifestation of chronic Q fever, constituting 73% of chronic Q fever cases. Other manifestations were vascular infection (8%), chronic infection in pregnancy (6%), and chronic hepatitis (3%).[18] In the Netherlands however, a substantially higher percentage of chronic Q fever cases consists of patients with infected aneurysms and vascular prostheses. In a recent report, 12 out of 22 chronic Q fever patients in the Netherlands had vascular infection.[19] Four of these patients were diagnosed by screening 52 patients with an aortic aneurysm. The authors advocate screening all patients with symptomatic aortic aneurysms in a highly endemic region for chronic Q fever. Chronic Q fever is not systematically reported to the national health authorities in the Netherlands. It is estimated that around 40 to 50 cases of chronic Q fever have been diagnosed in the Netherlands in the past three years. Up to half of these cases have vascular infection. A nationwide database on chronic Q fever will be established to collect these data and facilitate epidemiological research.

Q fever and pregnancy

Literature on chronic Q fever during pregnancy is limited. A case series of 53 pregnant women diagnosed with Q fever showed obstetric complications in 81% of patients not treated with long-term cotrimoxazole therapy compared with 44% in patients who did receive cotrimoxazole.[20] An important pitfall in this observational study, as indicated by the authors, is the fact that serology for Q fever was

performed only after delivery in 28% of patients, often because of obstetric complications, creating a selection bias. Interpretation of these results is therefore difficult. Two large seroprevalence studies found no significant association between seropositivity for *Coxiella* and adverse pregnancy outcome.[21,22] A study among pregnant women in the area of the first outbreak in the Netherlands in 2007 showed evidence of recent infection in three out of 19 women (16%). This was significantly higher than in the surrounding regions.[23] A retrospective study in the highly endemic area in the southern part of the Netherlands showed no significant correlation of seropositivity for Q fever during early pregnancy and adverse pregnancy outcome.[16] To further investigate the effect of Q fever on pregnancy, a randomised controlled trial was started in the spring of 2010 evaluating the effect of a screen and treat policy of pregnant women in this area.

Post Q fever fatigue syndrome

Following acute Q fever, patients frequently report a long-lasting and debilitating fatigue. A study performed after an outbreak of acute Q fever in the United Kingdom showed 20% of patients suffered from chronic fatigue syndrome after ten years of follow-up, compared with 4% of controls.[24] A study among abattoir employees in Australia showed that 28% of patients with proven acute Q fever still fulfilled the CDC criteria of chronic fatigue syndrome at five years after infection compared with none of seronegative controls.[25] A case-control study among 54 patients who contracted acute Q fever in the first year of the epidemic in the Netherlands showed that after one year, over 50% still reported severe fatigue compared with 26% of controls.[26] The aetiology of this severe fatigue, referred to as QFS (Q fever Fatigue Syndrome), still remains to be elucidated. Cytokine dysregulation resulting from chronic immune stimulation and modulation by persistence of *Coxiella* or its antigens has been hypothesised.[27] Genotyping of patients suffering from QFS showed significant differences in HLA-DRB1*11 and interferon-γ intron 1 microsatellite compared with controls.[28,29] Some reports suggest persistence of *Coxiella* or antigenic non-viable cell residues in bone marrow.[30,31] Studies evaluating antibiotic treatment for QFS have shown conflicting results.[32,33] QFS leads to considerable morbidity and a high socioeconomic burden related to increased use of healthcare facilities and absence from work.

DIAGNOSIS OF Q FEVER

Analysis of specimens from various infected dairy farms has shown that 14 different strains circulate in the Netherlands, but one is predominantly present in the highly endemic area (Roest HJ, unpublished data). Isolation of *Coxiella* from blood culture specimens of Q fever patients is difficult since it is an obligatory intracellular organism. In addition, chronic infection often resides in tissues (e.g., heart valves or vascular aneurysms) and shedding into peripheral blood occurs in very low concentrations. Culture of *Coxiella* requires very specific procedures and a biosafety level 3 laboratory, which is not available to most hospitals. Until now, culture of the pathogen has been successful in only one patient in the Netherlands, in whom *Coxiella* was cultured from a resected heart valve (Roest HJ, personal communication).

Diagnosis of acute Q fever is based on serology, the reference method being immunofluorescence assay (IFA). A seroconversion of a fourfold rise in antibody titre is diagnostic for acute Q fever.[34] An important drawback in diagnosis based on serology is that antibody production does not usually occur until a few weeks after onset of clinical symptoms. PCR on serum has been shown to have a high sensitivity (98%) for acute Q fever in seronegative patients and is a useful diagnostic tool for early diagnosis.[35] An algorithm for the diagnosis of acute Q fever in the Netherlands, designed by the Dutch working group on diagnostics of acute Q fever (an initiative of the National Institute for Public Health and the Environment (RIVM) and the Dutch Association for Medical Microbiology), has been published very recently.[36] There has been a substantial reduction of the diagnostic delay in the Netherlands from 82 days in 2007 to 20 days in 2009.[37] Because treatment has to be started before laboratory confirmation, physicians have to rely on clinical signs to guide the decision to start empiric therapy for Q fever. Antibiotic treatment of community acquired pneumonia in a high endemic region should include an agent active against *Coxiella burnetii*.[38]

Diagnosis of chronic Q fever remains difficult. Because the infection persists intracellularly, PCR on peripheral blood is not always positive. Imaging techniques can be useful to localise infection. Infected aneurysms or vascular prostheses can be identified by ^{18}F-fluorodexyglucose (FDG) positron emission tomography (PET) or CT. In case of endocarditis, however, diagnosis is often more complex. The original Duke criteria developed for diagnosing infective endocarditis include vegetations and positive blood cultures as major criteria. However, vegetations are often absent in Q fever endocarditis[18] and as mentioned above, *Coxiella* does not grow in conventional blood culture media. Therefore a revision of the Duke criteria has been proposed in which a serological profile compatible with chronic Q fever has been added to the major criteria.[39]

Serology is therefore an important tool in the evaluation of the development of chronic disease. *Coxiella burnetii* displays a unique antigenic variance in surface polysaccharides (phase 1 and phase 2 antigens). This can be used to distinguish between acute and chronic infection.

In acute infection, mainly phase 2 antibodies develop and convalescent sera show low titres of phase 1 antibodies, whereas chronic infection is characterised by high titres of phase 1 antibodies. Most literature on determination of cut-off values for establishing the diagnosis of chronic Q fever originates from the French National Reference Centre for Rickettsial Diseases (NRC) and this group has proposed a cut-off value for IgG to phase 1 proteins of 1:800 for the diagnosis of chronic Q fever.[40] This cut-off value was also adopted by the revised Duke criteria.[39] In the Netherlands, however, a substantial percentage of patients showed much higher titres of IgG1 during the first months after acute infection. There seems to be a considerable difference between the serological method used in the Netherlands (Focus diagnostics) and the method used in the NRC in France. This was recently illustrated by a case report of serological follow-up after acute Q fever, which showed high titres to IgG1 comparable with those found in Dutch patients but much lower when tested in the NRC in France.[41] In 2009, a provisional guideline was published in the Netherlands proposing a new cut-off value for IgG1 of 1:4096 (or an IgG1 equal to IgG2) (www.medischcontact.artsennet.nl). However, when using this algorithm, 40% of patients with proven chronic Q fever (signs and or symptoms compatible with chronic Q fever and persistently positive PCR on blood or positive PCR on resected valves or aneurysms) who presented at the Radboud Expertise Centre for Q fever do not fulfil these criteria (*table 1*). The Netherlands Society for Medical Microbiology (NVMM) is currently developing a new guideline for the diagnosis of chronic Q fever in the Netherlands.

TREATMENT OF Q FEVER

Comparative trials regarding the optimal antibiotic treatment for acute Q fever are sparse, often retrospective and sometimes show conflicting results. Doxycycline is the preferred choice, but the new-generation quinolones such as moxifloxacin are also active against *C. burnetii*.[42] Clarithromycin has also been shown to be effective[43] and co-trimoxazole is the treatment of choice in children younger than 8 years of age.[44] Although the national guidelines for treating community acquired pneumonia (CAP) issued by the Dutch Working Party on Antibiotic Policy (SWAB) and the Dutch College of General Practitioners (NHG) recommend doxycycline for first-line treatment of CAP, the alternative regimens in these guidelines do not routinely cover *C. burnetii*. Nevertheless, most general practitioners in the highly endemic region are aware of this problem and treat their patients with either doxycycline or moxifloxacin.[45] A survey among general practitioners in this region showed that 95% would consider Q fever as a possible pathogen when suspecting a pneumonia and would start empiric treatment with doxycycline.[46] The move away from doxycycline in the proposed update of the NHG guidelines for treatment of pneumonia by general practitioners seems inappropriate for endemic regions and may lead to increased numbers of chronic infections.

The optimal treatment of chronic Q fever consists of doxycycline and hydroxychloroquine.[47] The latter increases the intralysosomal pH and thereby achieves a bactericidal effect *in vitro* when combined with doxycycline, whereas doxycycline alone is only bacteriostatic.[48] Based on a retrospective study, a minimum duration of 18 months of combination therapy has been recommended with target levels of doxycycline of 5 mg/l.[47] This long-term therapy is associated with significant adverse effects and photosensitisation has been described in 100% of patients on long-term therapy.[47] Other frequently reported side effects include nausea, headache and dizziness. Regular evaluation by an ophthalmologist is recommended because of possible irreversible maculopathy due to hydroxychloroquine. Maintaining optimal adherence to therapy therefore requires intensive counselling and therapeutic drug monitoring.

Table 1. *Clinical and microbiological data from 10 patients with chronic Q fever presented to the Radboud Expertise Centre for Q fever*

Patient	Localisation of infection	IgG1 titre at diagnosis	IgG2 titre at diagnosis	PCR Q fever Blood	PCR Q fever Tissue
M, 75 y	Endocarditis	8192	32768	Positive	NA
M, 62 y	Aneurysm	2048	4096	Negative	Positive
M, 69 y	Endocarditis	65536	65536	Positive	Positive
M, 69 y	Endocarditis	16384	16384	Positive	NA
M, 57 y	Aneurysm	1024	4096	Negative	Positive
M, 68 y	Aneurysm	4096	4096	Negative	Positive
M, 75 y	Endocarditis	>4096	>4096	Positive	NA
M, 62 y	Aneurysm	1024	>4096	Positive	NA
M, 67 y	Aneurysm	2048	2048	Positive	NA
F, 60 y	Unknown	8192	4096	Negative	NA

PREVENTION OF Q FEVER IN HUMANS

The preventive measures taken in the veterinary sector have been aimed at reducing the spread of *C. burnetii* in the environment and thereby limiting the transmission to humans. Human vaccination is a different approach in preventing Q fever in individuals with a high risk of exposure to *Coxiella*. An effective whole-cell vaccine is available in Australia and has been extensively used in persons with high occupational risks such as abattoir employees. In this population, it has been proven to be highly effective.[49] Because administering this vaccine to patients with pre-existing immunity increases the risk of local and systemic inflammatory reactions, prior infection needs to be excluded by skin testing and serology. Recently, the Health Council of the Netherlands issued an advice on vaccinating patients with increased risk of chronic Q fever with this whole cell vaccine (http://www.gezondheidsraad.nl/nl/adviezen/vaccinatie-van-mensen-tegen-q-koorts-eerste-advies). The target population has been defined as patients with underlying cardiac conditions (the same category of patients who would also qualify for endocarditis prophylaxis according to current guidelines), as well as patients with a known (aortic) aneurysm or vascular prosthesis.

Since Q fever is highly endemic in the southern part of the Netherlands and infection can be asymptomatic, there is a possible risk of transmission through blood transfusion. Preliminary results indicate that in 2009 *C. burnetii* DNA was present in a small percentage of blood donations in this area (indicated by positive PCR on donated blood).[16,50] Sanquin, the Dutch blood supply foundation, has instituted screening of donated blood in the high-incidence area as a precautionary measure.

CONCLUSION

Although it appears that the epidemic of Q fever in the Netherlands is now subsiding, physicians are still faced with growing numbers of patients suffering from long-term sequelae of Q fever such as chronic infection and Q fever fatigue syndrome. Optimal management of these conditions is still unclear and further research is needed to improve diagnostic strategies, to evaluate treatment, and to prevent chronic infections.

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