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

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SUMMARY

The Netherlands experienced an unprecedented outbreak of Q fever between 2007 and 2010. The Jeroen Bosch Hospital (JBH) in 's-Hertogenbosch is located in the centre of the epidemic area. Based on Q fever screening programmes, seroprevalence of IgG phase II antibodies to *Coxiella burnetii* in the JBH catchment area was 10·7% [785 tested, 84 seropositive, 95% confidence interval (CI) 8·5–12·9]. Seroprevalence appeared not to be influenced by age, gender or area of residence. Extrapolating these data, an estimated 40 600 persons (95% CI 32 200–48 900) in the JBH catchment area have been infected by *C. burnetii* and are, therefore, potentially at risk for chronic Q fever. This figure by far exceeds the nationwide number of notified symptomatic acute Q fever patients and illustrates the magnitude of the Dutch Q fever outbreak. Clinicians in epidemic Q fever areas should be alert for chronic Q fever, even if no acute Q fever is reported.

Key words: Coxiella burnetii, Q fever, seroprevalence, zoonoses.

Q fever is a zoonosis, which occurs in worldwide outbreaks, and is caused by the intracellular Gram-negative bacterium *Coxiella burnetii*. Most important animal reservoirs are goats, sheep and cattle, although infection of birds, pets and arthropods have also been described. When infected, mammals shed *C. burnetii* in urine, faeces, milk and especially birth products. Humans become infected from inhalation of contaminated aerosols. Most people become infected with *C. burnetii* because of windborne spread of bacteria, which can travel over several kilometres [1–4]. Initial infection results in 50–60% of patients in asymptomatic seroconversion. Acute Q fever, a mild influenza-like illness sometimes complicated by pneumonia or hepatitis, develops in 40–50% of infections [1, 2]. Reportedly, 1–5% of patients develop chronic Q fever, with endocarditis and vascular

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infection of an aortic aneurysm or central vascular reconstruction as the most common manifestations. Risk factors predisposing to chronic Q fever are pre-existent cardiac valvulopathy, vascular grafts and aneurysms, immunosuppression and pregnancy [2, 5, 6].

The Netherlands experienced an unprecedented outbreak of acute Q fever between 2007 and 2010 with over 4000 notified symptomatic cases (168 in 2007, 1000 in 2008, 2354 in 2009, 506 in 2010 and 81 in 2011; data from the National Institute for Public Health and the Environment). Since initial infection is often asymptomatic, this figure is probably an underestimation. Although, the acute Q fever epidemic has subsided following government measures at the end of 2009, a rising number of chronic Q fever cases are currently seen [3, 7]. As municipal screening for *C. burnetii* antibodies has not been performed, the magnitude of the Dutch Q fever outbreak, and the number of patients potentially at risk for chronic Q fever, remains unknown.

In May 2009, amidst the epidemic, *C. burnetii* IgG seroprevalence in blood donors in the area with the highest reported Q fever incidence in The Netherlands was assessed. This survey showed that 12·2% of blood donors were seropositive for *C. burnetii* IgG phase II antibodies [7]. Another study assessed *C. burnetii* IgG phase II seroprevalence at 9·0% in pregnant women in serum samples obtained between June 2007 and May 2009 [8]. Here, we set out to estimate the number of *C. burnetii*-infected persons in the catchment area of the Jeroen Bosch Hospital (JBH), which is located in the centre of the epidemic region, using seroprevalence rates obtained after the epidemic had ceased. These rates were extracted from two programmes for early detection of unnoticed chronic Q fever in high-risk patients offering subsequent appropriate medical intervention to identified patients.

First, a call/recall screening programme in high-risk patients with an aortic aneurysm or central vascular reconstruction was initiated in November 2009 in the catchment areas of the JBH and the neighbouring Bernhoven Hospital. Second, a screening programme in high-risk patients with a history of cardiac valve surgery was conducted between November 2010 and January 2011 in the JBH catchment area. A regional medical ethics committee [Medisch-Ethische Toetsing Patienten en Proefpersonen (METOPP)] waived the need for informed consent as far as testing of high-risk groups for chronic Q fever was concerned. The JBH catchment area comprises 11 municipalities with a total of 379 100 inhabitants as of 31 December 2010. Of these, 27·4% resided in the city of 's-Hertogenbosch, and the others in rural areas (data from Statistics Netherlands).

Screening was performed on sera obtained by venepuncture. Regardless of patients having past Q fever or chronic Q fever, we defined seropositivity as any IgG titre against *C. burnetii* phase II antigens (IgG phase II) of $\geqslant$1:128 as measured by immunofluorescence assay (IFA; Focus Diagnostics, USA). We observed that IgG phase II can be detected during acute Q fever, chronic Q fever and past Q fever and are the longest circulating antibodies during the immune response to *C. burnetii*. These antibodies are absent only during the very early stage of acute Q fever [9]. Although the manufacturer defines seropositivity as a titre of $\geqslant$1:16, we selected a higher cut-off to prevent overestimation of the number of *C. burnetii*-infected persons as a result of false positivity or cross-reactivity in the serological assay [2, 10].

We additionally gathered information on sex, age, and residence in urban or rural areas. To study the influence of age on seroprevalence, the screened population was divided in two groups with a 35-year age span. One group consisted of patients born between 1915 and 1949, while the other group consisted of patients born between 1950 and 1984. We compared prevalence of seropositivity in the different groups and used χ^2 tests to assess significance of findings. Significance level was set at $P \leqslant 0{\cdot}05$.

On 31 May 2011, a total of 932 patients had entered the two screening programmes, of which 785 patients lived in the JBH catchment area. Of these, 84 patients had an IgG phase II titre of $\geqslant$1:128, resulting in a seroprevalence rate of 10·7% [95% confidence interval (CI) 8·5–12·9]. There was no significant difference in seroprevalence between the two screening programmes (Table 1). Extrapolating these figures, the population estimate for *C. burnetii* antibody prevalence in the JBH catchment area is 40 600 persons (95% CI 32 200–48 900). In the years 2007 to 2010, only 644 patients with symptomatic acute Q fever living in the JBH catchment area were notified (data from Municipal Health Services). There was no significant difference between seroprevalence in the two age groups (born 1915–1949 and 1950–1984). Similarly, there was no significant difference in seroprevalence between males and females. With regard to geographical distribution, there was no significant difference in seroprevalence between patients living in

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

Characteristic	Vascular screening	Seroprevalence, % (95% CI)	Valvular screening	Seroprevalence, % (95% CI)	All patients	Seroprevalence, % (95% CI)	*P* value†
Screened population	276	11·2 (7·5–14·9)	509	10·4 (7·8–13·1)	785	10·7 (8·5–12·9)	0·723‡
Year of birth							0·265
1915–49 (1924–47)*	261	11·1 (7·3–14·9)	415	9·6 (6·8–12·5)	676	10·2 (7·9–12·5)	
1950–84 (1950–71)*	15	13·3 (0·0–30·5)	94	13·8 (6·9–20·8)	109	13·8 (7·3–20·2)	
Gender							0.694
Male	227	9·3 (5·5–13·1)	265	11·3 (7·5–15·1)	492	10·4 (7·7–13·1)	
Female	49	20·4 (9·1–31·7)	244	9·4 (5·7–13·1)	293	11·3 (7·6–14·9)	
Geographical location							0.483
Urban	92	16·3 (8·8–23·9)	180	9·4 (5·2–13·7)	272	11·8 (7·9–15·6)	
Rural	184	8·7 (4·6–12·8)	329	10·9 (7·6–14·3)	513	10·1 (7·5–12·7)	

CI, Confidence interval.

* Range (2·5th–97·5th percentiles of all patients).

† *P* values calculated for each characteristic of all patients, unless otherwise indicated.

‡ *P* value comparing seroprevalence in vascular screening and valvular screening.

the city and patients residing in more rural areas (Table 1).

In the Netherlands, the estimated seroprevalence of *C. burnetii* phase II IgG in 2006–2007, just before the outbreak, was 2·4% (using IFA with an IgG phase II cut-off titre of $\geqslant$1:32), although this study was largely conducted in municipalities which were not affected by the recent Q fever outbreak [11]. Using a more conservative cut-off titre of $\geqslant$1:128 for *C. burnetii* phase II IgG, we found a seroprevalence of 10·7% in the JBH catchment area, indicating the epidemic nature of the recent outbreak. We calculated that the estimated number of *C. burnetii*-infected persons in the JBH catchment area is 40 600 persons (95% CI 32 200–48 900), which is 50- to 75-fold higher than the number of notified patients. Since our catchment area comprises only 11 of the 57 municipalities recognized as high-incidence Q fever areas by the National Institute for Public Health and the Environment, the nationwide number of *C. burnetii*-infected persons in The Netherlands will be considerably higher. This suggests that the percentage of asymptomatic cases might also be considerably higher than the 50–60% reported previously [1, 2]. These figures indicate that a large group of patients is potentially at risk for development of chronic Q fever. Clinicians in endemic areas should be aware of chronic Q fever in patients with risk factors, like pre-existent cardiac valve disease, aortic aneurysm or vascular prosthesis, even when there is no history of acute Q fever [2, 5].

Currently, there is no standard cut-off titre for use in seroprevalence studies of *C. burnetii* antibodies. We choose a high IgG phase II cut-off titre to prevent overestimation of the number of *C. burnetii*-infected persons. However, it seems more likely that this conservative cut-off resulted in underestimation. IgG phase II titres $<$1:128 and even negative titres have been observed after 1-year follow-up of acute Q fever patients in the Dutch outbreak [12]. This makes it feasible that IgG phase II titres $<$1:128 in persons living in a (previously) epidemic area might well reflect past Q fever. If our cut-off titre was set at $\geqslant$1:64, 117 (14·9%) out of 785 patients would have been considered seropositive indicating an even greater magnitude of the Dutch Q fever outbreak. This figure is in line with the reported seroprevalence of 12·2% in Dutch blood donors, and 9·0% in pregnant women, using an IgG phase II cut-off titre of $\geqslant$1:64 [7, 8]. In our opinion, use of a high cut-off titre also allowed for selection of relatively recent infections since IgG phase II titres decrease with time following acute infection.

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

There are conflicting reports on age- and gender-related differences in seroprevalence rates of *C. burnetii* antibodies. In Zimbabwe, a seroprevalence rate of 37% was noted without age- or gender-related differences [13]. In the USA, seroprevalence for persons aged $\geqslant$20 years was 3·1%, increased with age and was higher for men [14]. We explain the absence of age- and gender-related differences in our survey as a consequence of emergence of Q fever in an epidemic situation. This differs from endemic situations like in Zimbabwe and the USA in which infection might be more related to occupational exposure. However, especially in the younger age group, our sample size was relatively small and, therefore, the absence of age-related differences in seroprevalence needs to be viewed with caution. We found no difference in seroprevalence between rural and urban populations. In this context, it is notable that the only city in our catchment area, 's-Hertogenbosch, is relatively small (39·98 km^2) and that windborne spread reportedly can transport *C. burnetii* up to 18 km into metropolitan areas [4].

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

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DECLARATION OF INTEREST

None.

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