

## Waning population immunity prior to a large Q fever epidemic in the south of The Netherlands

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### SUMMARY

Historical survey data suggest that the seroprevalence of antibodies against *Coxiella burnetii* in the general population of The Netherlands decreased from more than 40% in 1983 to 2·4% in 2007, just before the start of the large 2007–2010 Q fever epidemic. To assess whether the sharp decline in seroprevalence was real, we performed a cross-sectional study using historical samples. We tested samples using a contemporary commercial indirect immunofluorescence assay. In plasma samples from the south of The Netherlands from 1987, we found an age- and sex-standardized seroprevalence of 14·4% (95% confidence interval 11·2–18·3). This was significantly lower than a 1983 estimate from the same area (62·5%), but significantly higher than 2008 (1·0%) and 2010 (2·3%) estimates from the same area. The study suggests that there was a steady and sharp decline in Q fever seroprevalence in the south of The Netherlands from 1987 to 2008. We assume that seroprevalence has decreased in other parts of The Netherlands as well and seroprevalence surveys in other European countries have shown a similar declining trend. Waning population immunity in The Netherlands may have contributed to the scale of the 2007–2010 Q fever epidemic. For a better understanding of the infection dynamics of Q fever, we advocate an international comparative study of the seroprevalence of *C. burnetii*.

**Key words:** *Coxiella*, epidemiology, infectious disease epidemiology, Q fever, zoonoses.

### INTRODUCTION

Q fever is a globally occurring zoonosis caused by *Coxiella burnetii*, an intracellular Gram-negative bacterium. The main reservoirs for Q fever in humans are

domestic ruminants, such as cattle, goats and sheep. Transmission to humans mainly occurs through inhalation of contaminated aerosols, released when infected animals give birth [1].

From 2007 to 2010, The Netherlands faced increasingly large annual and seasonal outbreaks of Q fever. More than 4000 acute human Q fever cases were notified, making this the largest recorded Q fever epidemic worldwide to date [2]. Although no outbreaks

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of Q fever had been reported in The Netherlands prior to 2007, historical data suggest high endemicity in preceding decades. In sera of blood donors from various regions of The Netherlands in 1968 and 1983, high seroprevalences were found [3], significantly higher than the 2.4% seroprevalence in the general population of The Netherlands in 2006–2007, just prior to the first Q fever outbreak [4].

However, comparison of findings from different surveys may be problematic, due to discrepancies in the populations surveyed and the laboratory methods used. For humans, population-based seroprevalence studies routinely measure IgG phase II antibodies. Indirect immunofluorescence assay (IFA) is regarded as the reference test for serological detection of Q fever. However, performing IFA is labour intensive and there is no uniform consensus for the cut-off titres. For large studies, ELISA is a convenient test as it can be easily automated. However, ELISA may be less sensitive than IFA [5].

We analysed the seroprevalence dynamics of Q fever before and during the recent Dutch epidemic. We used historical and recently acquired seroprevalence data, taking into account the dilemma of using different diagnostic tests and cut-off definitions.

## MATERIALS AND METHODS

### Study design and study area

We performed a cross-sectional study using historical samples from 1987, analysed with a commercial IFA. The results were compared with historical results from 1983 to assess the accuracy of the historical data. Furthermore, the results were compared with recently acquired data from 2008 and 2010. All samples and data were collected from the same geographical area, i.e. South Limburg, the most southern part of the province of Limburg, The Netherlands (Fig. 1).

This region had 606 000 inhabitants in 2014 [data from Statistics Netherlands (CBS)] combining both urban and rural areas. With 917 population/km<sup>2</sup>, it is one of the most densely populated regions of The Netherlands. The largest cities in the region are Maastricht, Heerlen and Sittard. In 2009, the South Limburg region faced a large outbreak of Q fever around the municipality of Voerendaal originating from a dairy goat farm [6].

### Study population

The samples for 1987 were available from the biobank of the Monitoring Project on Cardiovascular Risk



**Fig. 1.** Map of South Limburg region with the municipality of Maastricht highlighted.

Factors. This project was conducted in The Netherlands from 1987 to 1991. More than 36 000 persons aged 20–59 years were screened for cardiovascular risk factors, using questionnaires and blood samples [7]. The survey was conducted in the municipalities of Amsterdam, Doetinchem and Maastricht. Stored plasma samples from this survey were available for further research and had not been tested previously for Q fever or other infectious diseases. A random selection of plasma samples from 1987 was taken from the Maastricht subgroup. Assuming an estimated seroprevalence of 50% with a confidence level of 95%, 385 samples would be required for adequate power. The samples had been stored at  $-35^{\circ}\text{C}$  in the biobank at the centres for Nutrition and Health (CVG) and Prevention and Health Services Research (PZO) at the National Institute for Public Health and the Environment (RIVM). The use of the anonymous plasma samples was approved by the Scientific Advisory Group of the PZO.

For the 1987 samples, antibodies against *C. burnetii* were determined in plasma by IFA (Focus Diagnostics, USA) measuring IgG against *C. burnetii* phases I and II according to the manufacturer's instructions. Seropositivity was defined as an IgG phase II titre  $\geq 1:32$ . The plasma samples were analysed at RIVM.

### Comparison populations

Data from 1983 were available from the Maastricht subgroup in a seroprevalence survey performed by Richardus *et al.* [3]. In this survey, plasma samples

Table 1. Prevalence of IgG antibodies against phase II of *Coxiella burnetii* in samples from Maastricht and South Limburg

Year	Male		Female		Total		Adjusted seroprevalence* (95% CI)
	N	Pos, n (%)	N	Pos, n (%)	N	Pos, n (%)	
1983	125	74 (59.2)	123	79 (64.2)	248	153 (61.7)	62.5 (56.3–68.3)
1987	176	26 (14.7)	204	31 (15.2)	380	57 (15.0)	14.4 (11.2–18.3)
2008	181	2 (1.1)	666	6 (0.8)	847	7 (0.8)	1.0 (0.5–1.9)
2010	290	6 (2.1)	343	11 (3.2)	633	17 (2.7)	2.3 (1.4–3.8)

CI, Confidence interval.

\* Seroprevalence standardized for age distribution and gender, based on population data from Statistics Netherlands (CBS).

from 248 not randomly selected blood donors were screened using an in-house developed IFA. Seroprevalence data for 2008 and 2010 were available from a survey that was conducted in 2010 in response to the large outbreak of Q fever in South Limburg [6]. In this study, 847 samples from 2008 of adults vaccinated for healthcare-related hepatitis B risk and 633 samples from 2010 of persons attending the regional sexual health clinic from January to April 2010 were screened for antibodies against *C. burnetii*, using a commercial ELISA (Institute Virion/Serion GmbH, Germany). Positive and intermediate results were confirmed using an IFA (Fuller Laboratories, USA) at a cut-off value of 1:16, according to the manufacturer's instructions.

### Statistical analysis

The samples for 1987, 2008 and 2010, were divided into the same five age groups that were used in the 1983 survey of Richardus *et al.* [3]. For each year, the results were directly standardized for age group and gender, using demographic data for The Netherlands for the respective year as published by Statistics Netherlands. For the years 1983, 1987 and 2008 (the years without recent outbreaks), the seroprevalence figures by age were also visualized using error bar diagrams, representing the confidence interval (CI) of the mean seroprevalences. For data analysis SPSS Statistics v. 21 (IBM Corp., USA) was used.

## RESULTS

Of the 385 selected plasma samples from 1987, 380 were suitable for analysis. The samples were from 176 (46%) male and 204 (54%) female participants. Of the 380 samples, 57 tested positive for antibodies against *C. burnetii*. When standardized for age and gender, this resulted in a seroprevalence of 14.4% (95% CI 11.2–18.3).

The 1983 study included 125 (50%) male and 123 (50%) female participants [3]. Of the 248 samples, 153 tested positive for antibodies against *C. burnetii*, resulting in an age- and sex-standardized seroprevalence of 62.5% (95% CI 56.3–68.3).

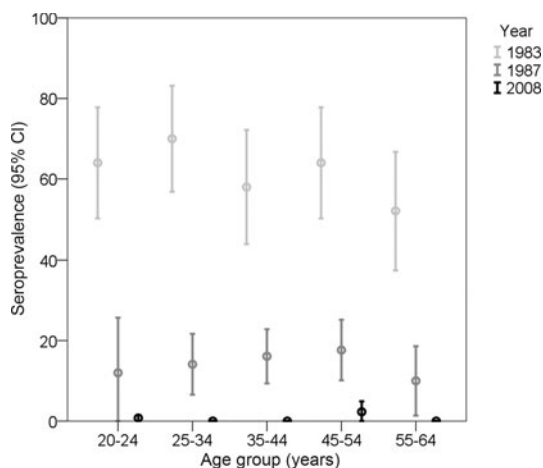
The 2008 samples included 181 (21%) male and 666 (79%) female participants. Of the 847 samples, seven samples tested positive for antibodies against *C. burnetii*, resulting in an age- and sex-standardized seroprevalence of 1.0% (95% CI 0.5–1.9).

The 2010 samples included 290 male (46%) and 343 (54%) female participants. Seventeen of the 633 samples tested positive for antibodies against *C. burnetii*, resulting in an age- and sex-standardized seroprevalence of 2.3% (95% CI 1.4–3.8).

Table 1 shows the crude and standardized seroprevalence results for all four populations. None of the surveys had statistically significant differences in seroprevalence between age groups or between males and females. A graphical comparison of the seroprevalence estimates from 1983, 1987 and 2008 is shown in Figure 2. The seroprevalence estimates for 1987 are significantly lower than the seroprevalence estimates from 1983 across all age groups. The seroprevalence in 1987 was significantly higher than the seroprevalence in 2008 for all age groups, except the 20–24 years age group (Fig. 2). The difference in seroprevalence between 2010 and 2008 was not statistically significant.

## DISCUSSION

The present study, using stored samples from 1987, confirms that seroprevalence of antibodies against *C. burnetii* was high in South Limburg in the 1980s, even though no Q fever outbreaks were described in that period. Between 1987 and 2008, only three cases of acute Q fever were notified in South Limburg (1999, 2002, 2007). During this period, the



**Fig. 2.** Comparison of seroprevalence of IgG phase II-specific antibodies to *Coxiella burnetii* (Q fever) in Maastricht in 1983, 1987, and 2008.

seroprevalence declined in this geographical area. Since 2008 the number of notifications increased with a peak of 238 cases in 2009 (Table 2). We suggest that waning immunity in the population may have contributed to the size of the Q fever outbreak that occurred in South Limburg in 2009.

In interpreting these findings, several limitations have to be considered. First, by comparing results from historical seroprevalence surveys, we must account for different methodologies and study populations. Original sera from the published 1980s' seroprevalence studies were no longer available and the in-house IFA employed at the time could not be reproduced. Therefore, we tested historical samples from 1987 using a contemporary commercial IFA. We used a cut-off of 1:32, whereas a cut-off of 1:16 was used in the 1983 survey. Assuming that the performance of both IFAs was otherwise comparable, a lower cut-off with increased sensitivity and decreased specificity may have led to more false-positive results and an overestimation of seroprevalence in 1983. Moreover, the samples from 1983 and 1987 were collected in different ways. Whereas the samples from 1987 were (retrospectively) randomly selected, the 1983 specimens were selected such that each age group would include 50 samples. However, no significant differences were found in the age/gender distribution for both 1983 ( $P = 0.999$ ) and 1987 ( $P = 0.856$ ). The plasma samples we used for retrospective testing were stored for many years at a temperature of  $-35^{\circ}\text{C}$ . The effect of this temperature on the survival of antibodies is unknown, but degradation of antibodies

**Table 2.** Number of notifications of acute Q fever in the South Limburg region, 1988–2014

Year	No. of notifications	Year	No. of notifications
1988*	0	2002	1
1989	0	2003	0
1990	0	2004	0
1991	0	2005	0
1992	0	2006	0
1993	0	2007	1
1994	0	2008	5
1995	0	2009	238
1996	0	2010	20
1997	0	2011	2
1998	0	2012	0
1999	1	2013	1
2000	0	2014	0
2001	0		

Data from RIVM-OSIRIS.

\* Surveillance data for acute Q fever is only available from 1988 onwards.

could have led to underestimation of the true seroprevalence.

The low cut-off that was used in 1983 and the unknown test qualities of the in-house IFA, suggest that the 1983 seroprevalence of 60% might have been overestimated. However, the seroprevalence of 14.4% we found for 1987 is still markedly high. By contrast, during the Dutch Q fever epidemic, in high-incidence areas seroprevalence surveys in pregnant women in 2008, blood donors in 2009, and persons with risk factors for chronic Q fever found a mean seroprevalence of 9.1%, 12.2% and 10.7%, respectively [8–10].

The seroprevalence estimate for 1987, based on the present analysis, is also significantly higher than the estimates based on samples from 2008 and 2010 from the same region. The samples from 2008 and 2010 were tested using a different IFA with a lower cut-off of 1:16, but this was according to the manufacturer's instructions. Further, the low seroprevalence estimates for 2008 and 2010 are comparable with the estimate in the study of Schimmer *et al.* [4], in which the IFA of Focus Diagnostics was used. In the Schimmer *et al.* study, the estimated seroprevalence increased from 1.5% to 2.4% after correction for false-negative ELISA results. Therefore, such a correction of the 2008 and 2010 results might have led to a small increase in seroprevalence.

We have no clear explanation for the high *C. burnetii* seroprevalence in the 1980s, without much clinical Q fever disease at the time. One possibility is that the

Table 3. *The seroprevalence of antibodies against Coxiella burnetii in other European countries*

Year [reference]	Study population	Testing assay (cut-off titre)	No. of samples	Positive samples <i>n</i> (%)
<b>Germany</b>				
1977 [18]	General population from the State of Hessen	CFT (>10)	1600	0 (0·0)
1983 [19]	Inhabitants of Seebron (State of Baden-Württemberg)	CFT ( $\geq$ 5)	715	136 (19·0)
1986 [20]	Blood donors from the State of Hessen	ih-ELISA	205	31 (15·1)
1987 [21]	Blood donors from the State of Hessen	ih-ELISA	207	32 (15·5)
2009*	General population from the State of Baden-Württemberg	cELISA + cIFA	1036	78 (7·5)
<b>France</b>				
1986 [22]	Blood donors from southern France	ih-IFA ( $\geq$ 32)	325	17 (5·2)
1988 [23]	Blood donors from Marseille	ih-IFA ( $\geq$ 100)	942	38 (4·0)
1996 [24]	Pregnant women from southern France	ih-IFA ( $\geq$ 100)	12 716	19 (0·2)
1996 [25]	General population of Briançon	ih-IFA ( $\geq$ 50)	785	42 (5·4)
2002 [26]	Tested patients without risk factors in the Chamonix Valley	ih-IFA ( $\geq$ 100)	578	31 (5·4)
<b>Spain</b>				
1983 [27]	Inhabitants of the Basque Country	CFT ( $\geq$ 8)	308	38 (12·3)
1985 [27]	Inhabitants of the Basque Country	CFT ( $\geq$ 8)	1180	199 (16·9)
1988 [27]	Inhabitants of Barcelona	CFT ( $\geq$ 8)	1016	91 (9·0)
1991 [28]	Inhabitants of the Basque Country	cIFA ( $\geq$ 20)	810	262 (32·3)
1993 [29]	Inhabitants of Soria	cIFA ( $\geq$ 80)	298	62 (20·8)
1994 [27]	Inhabitants of the province of Léon	cIFA ( $\geq$ 80)	406	165 (40·6)
1995 [30]	Population of Eastern Cantabria	cIFA ( $\geq$ 16)	595	289 (48·6)
2005 [31]	Random patients from Barcelona	cIFA ( $\geq$ 40)	216	33 (15·3)
<b>Crete</b>				
1985 [32]	Population of Anogia	cIFA ( $\geq$ 60)	231	80 (34·6)
1985 [32]	Population of Tymbaki	cIFA ( $\geq$ 60)	188	11 (5·9)
1987 [32]	Population of Anogia	cIFA ( $\geq$ 60)	231	88 (38·1)
1987 [32]	Population of Tymbaki	cIFA ( $\geq$ 60)	188	26 (13·8)
1998 [33]	Population of Anogia	cIFA ( $\geq$ 120)	238	100 (42·0)
2006 [34]	Healthy blood donors from Crete	cIFA ( $\geq$ 120)	493	240 (48·7)
<b>England and Wales</b>				
1966 [35]	Patients not exposed to infection	CFT ( $\geq$ 20)	7005	146 (2·1)
1987 [36]	General population around Taunton	CFT ( $\geq$ 20)	2773	52 (1·9)
1989 [37]	Subgroup of emergency service workers from Lancashire	ih-IFA ( $\geq$ 32)	395	43 (10·9)
1996 [38]	Rural population from Wales	ih-IFA ( $\geq$ 32)	265	21 (7·9)
2008 [39]	Pregnant women around London	cIFA ( $\geq$ 50)	438	20 (4·6)

ih-IFA, in-house developed indirect immunofluorescence assay; cIFA, commercial IFA; ih-ELISA, in-house developed enzyme-linked immunosorbent assay; cELISA, commercial ELISA; CFT, complement fixation test.

\* Source: J. W. Brockman *et al.*, unpublished observations.

high seroprevalence was due to exposure to a *C. burnetii* cattle genotype that might have been less virulent than the *C. burnetii* goat genotype that caused the large 2007–2010 epidemic in The Netherlands [11–14].

Using PCR testing, DNA from the cattle *C. burnetii* genotype can be found in retail milk and a possible hypothesis is that a daily dose of fragments of *C. burnetii* in retail milk could cause a seroconversion and immunize people rather than making them ill. In this context it

would be interesting to study milk consumption patterns over time and changes that have taken place in milk pasteurization practices that in certain years may have been insufficient to kill *C. burnetii*. Another hypothesis is the spreading of the less virulent *C. burnetii* strain by dairy cattle farms. In The Netherlands, the number of dairy cattle declined while the dairy goat sector expanded rapidly after introduction of the European milk quota system in 1984 [15]. From 1984 to 2008, the number of cattle in

South Limburg did decrease from 73 366 to 36 011 (data from Statistics Netherlands). This could have resulted in a lower exposure to the cattle *C. burnetii* genotype.

As shown in animal experiments, antibodies against *C. burnetii* protect against the development of Q fever after exposure to viable *C. burnetii*, probably due to enhanced macrophage activity [16]. Therefore, the decreasing seroprevalence of antibodies against *C. burnetii* in the regional population of South Limburg could have played a role in the regional outbreak that occurred in the region in 2009. The question remains whether the results for South Limburg are representative for the entire Dutch population. The high seroprevalence found in 1983 in other regions of The Netherlands [3] and the lack of differences in seroprevalence between regions in 2007 [4], suggest that seroprevalence decreased in the entire country. This could have made the entire Dutch population vulnerable for the almost nationwide outbreak of Q fever in the years 2007–2010.

Assuming a high risk for exposure and infection in the 1980s, followed by a period of low exposure, one would expect an age-cohort effect with higher seroprevalence in the older age groups, which indeed was demonstrated in a 2006/2007 survey [4]. The age-cohort effect is further supported by the lack of an effect of age in the seroprevalence studies that were performed in the 1980s including our survey from 1987.

An interesting comparison can be made with the situation in the United States, where a national survey showed a seroprevalence of 3·1%, indicating that millions of people have been exposed to *C. burnetii* [17]. However, there are fewer than 100 notifications per year of human Q fever in the United States, suggesting gross underreporting and/or circulation of a less virulent (cattle) strain. Unfortunately, no historical seroprevalence results are known for the United States. For some European countries, historical data exist, as shown in Table 3. In some countries, a similar decrease in seroprevalence can be seen as for The Netherlands. However, the comparison of different seroprevalence surveys remains difficult, due to differences in the populations surveyed and developments in laboratory methods used since the 1980s. In contrast, in some high endemic areas, there seems to be an increase in seroprevalence.

## CONCLUSION

*C. burnetii* seroprevalence in South Limburg declined from the 1980s to 2008. This might indicate waning population immunity that could have contributed to

the 2009 Q fever outbreak in this region. A similar declining trend in the seroprevalence for other regions in The Netherlands, could possibly have contributed to the 2007–2010 epidemic. For a better understanding of the infection dynamics of Q fever and other infectious diseases, longitudinal studies over a longer period or repeated seroprevalence surveys with standardized serological methods will be necessary. We advocate a large multi-country study, with analyses performed by one reference centre.

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## DECLARATION OF INTEREST

None.

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