CrossMark

Epidem. Inf. (1987), **98**, 211–219
Printed in Great Britain

Q fever in the Netherlands: a sero-epidemiological survey among human population groups from 1968 to 1983

By J. H. RICHARDUS, A. DONKERS, A. M. DUMAS, G. J. P. SCHAAP,

Laboratory for Virology, Public Health Service of the City of Rotterdam, POB 70032, 3000 LP, Rotterdam, The Netherlands

J. P. W. M. AKKERMANS

Stichting Gezondheidsdienst voor Dieren in West Nederland, POB 87, 2800 AB Gouda, The Netherlands

J. HUISMAN

Department of Infectious Diseases, Public Health Service of the City of Rotterdam, POB 70032, 3000 LP, Rotterdam, The Netherlands

and H. A. VALKENBURG

Institute of Epidemiology, Erasmus University, POB 1738, 3000 DR, Rotterdam, The Netherlands

(*Accepted 15 September 1986*)

SUMMARY

A sero-epidemiological survey, using an indirect immunofluorescence test for IgG against *Coxiella burnetii* (phase II), was carried out in the Netherlands. Serum samples taken in 1968, 1975, 1979 and 1983 were tested. Occupational groups with a supposedly high risk of infection (veterinarians, residents of dairy farms and taxidermists) showed a significantly higher percentage of seropositives than defined controls. The percentage of seropositive amateur wool spinners was significantly higher than that of the controls from the same region. Since 1968 there has been no increase in the percentage of infected persons, indicating that, contrary to earlier assumptions, Q fever has been endemic in The Netherlands for a long time already. The increase in numbers of notified cases of overt Q fever is considered to be the result of the recent introduction of a sensitive indirect immunofluorescence test for IgM antibodies against *C. burnetii*. Antibody percentages in all age classes between 1 and 64 years were much alike, suggesting that most infections occur in early childhood. This is in accordance with the finding that 35% of our patients are younger than 3 years. The possibility of infection related to childbirth and lactation is discussed.

INTRODUCTION

Q fever is a zoonosis caused by *Coxiella burnetii* and was until recently considered to be a rare disease in the Netherlands. The number of notified cases increased from 2 in 1979 to 19 in 1981, with an average of 25 in the years thereafter (Richardus

Downloaded from https://www.cambridge.org/core. IP address: 18.204.48.199, on 20 Nov 2019 at 00:36:12, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0950268800061938

et al. 1984). In most cases (75%) the source of infection was assumed to be in the Netherlands as no contact could be traced to foreign sources (Richardus *et al.* 1984, 1985) and serological evidence of infection was found among Dutch dairy cattle (Schaap & Akkermans, 1981). In 1979 the Virological Laboratory of the Public Health Service of Rotterdam (regional virus laboratory for Zuid Holland and Zeeland) introduced, to supplement the existing complement fixation test (CFT), an indirect immunofluorescence test (IFT) for IgM against *C. burnetii* (phase II). The presence of these antibodies is considered indicative of a recent infection and the method proved to be more sensitive than the CFT (Schaap & Donkers, 1981).

The question arises whether the increase in the number of diagnosed cases is the result of the recent introduction of Q fever into the Netherlands or of the use of a more sensitive test in a stable epidemiological situation. To clarify this problem we undertook a sero-epidemiological investigation among different groups of the Dutch population. The survey was designed so as to provide data on (*a*) the prevalence of IgG antibodies at the time of investigation and over the preceding 15 years, (*b*) the presence or absence of enzootic spread of coxiella infections in the Netherlands deduced from the percentage of seropositives for IgG antibodies in certain categories of occupations and (*c*) possible geographic differences in the distribution of infection.

MATERIALS AND METHODS

High-risk group

A total of 432 persons considered to be at high risk of contracting an infection with *C. burnetii* because of close contact with animals or animal products were studied. The group consisted of the residents of dairy farms (94), veterinarians (260), taxidermists (33) and amateur wool spinners (45). Blood samples were all obtained between October 1982 and September 1983. The farm residents came from 18 farms and 221 of the veterinarians worked with large animals while 39 worked only with pets.

Control group

Controls were assumed to be at lower risk because of less contact with animals and were considered to be representative of the general Dutch population. For the main control study 359 blood donors aged from 20 to 64 were selected randomly from the Rotterdam blood bank in 1983 in such a way that a minimum of 25 samples from each 5-year-age class was obtained. As there were difficulties in collecting blood samples from the healthy 1- to 19-year-olds, sera taken from 77 patients with serologically confirmed rubella in 1983 were used – skin rash is rarely seen in Q fever. The age and sex distributions in both the high risk and control groups are presented in Tables 1 and 2.

In addition sera from other groups of non-high risk persons were studied. These were from donors from the blood banks at Groningen (250) and Maastricht (248) taken in 1983; rubella patients (90) aged 1–19 years taken in 1979; healthy children (120) (Institute of Epidemiology, Erasmus University, Rotterdam) again 5–14 years from Zoetermeer taken in 1975 and healthy adults (180) aged 45 years or older from Sluis, Vleuten and Haaksbergen taken in 1968 (see Fig. 1).

Downloaded from https://www.cambridge.org/core. IP address: 18.204.48.199, on 20 Nov 2019 at 00:36:12, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0950268800061938

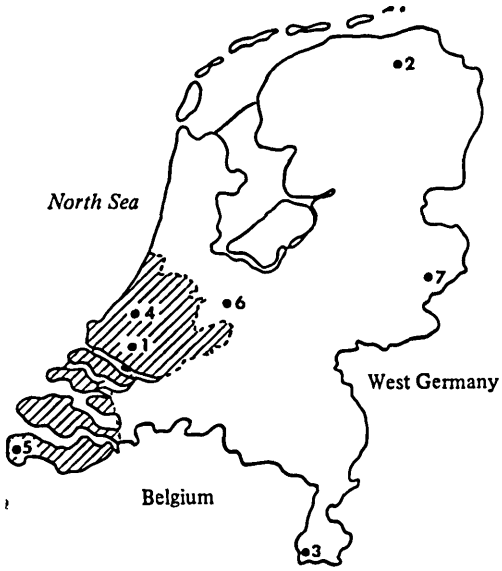

Fig. 1. The Netherlands. 1, Rotterdam; 2, Groningen; 3, Maastricht; 4, Zoetermeer; 5, Sluis; 6, Vleuten; 7, Haaksbergen. Shaded area: provinces Zuid Holland and Zeeland, region served by the Virological Laboratory, Rotterdam.

Immunofluorescence test

Antibodies were demonstrated by means of an indirect IFT for specific IgG antibodies against *C. burnetii* (phase II) (Richardus, 1985). A commercially available coxiella suspension (Institute Virion Ltd., Switzerland) was used. A 25 μl suspension in a suitable dilution was spread evenly in each well of microprint stock slides (Cl-100, Cel-line Associates Inc., USA), dried at 20 °C for 30–60 min and fixed in acetone 4 °C for 10 min. Slides were incubated with serum samples at 37 °C for 1 h, washed three times with PBS over 10 min, and incubated with rabbit anti human IgG-FITC (Nordic Immunology, Tilburg, The Netherlands) at 20 °C for 30 min in the dark. Finally, they were washed again as described above. After mounting in buffered glycerol (pH 8·0) slides were examined at a magnification of 400 times with incident light under a binocular Leitz Laborlux fluorescence microscope fitted with a Ploemopak 3-Lambda illuminator, a I-2 filter system a 50× water-immersion objective (Leitz, Wetzlar, FRG) and a HBO-50 mercury lamp (Osram, Berlin, Munich. FRG). IgG antibodies at a titre of $\geqslant 16$ were considered as indicative of a previous infection.

RESULTS

Controlled study

The results are presented in Tables 1 and 2. In the control group there was no significant difference in the proportion of seropositives between males and females in the younger age group, or between males in the younger age group and males in the older age group, or in the successive age group classes in either group. The difference between positives in the males and the females in the 20- to 64-year-old group was statistically significant ($P < 0{\cdot}05$).

Downloaded from https://www.cambridge.org/core. IP address: 18.204.48.199, on 20 Nov 2019 at 00:36:12, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0950268800061938

Table 1. *The presence of IgG antibodies against* C. burnetii *(phase II) in the sera of control groups from Rotterdam (1–19 years, rubella patients 1983; 20–64 years, blood donors 1983*

Age (years)	Male			Female		
	n	IgG	(%)	*n*	IgG	(%)
1	1	0	(44)	5	1	(25)
2	1	1		3	1	
3	1	1		5	0	
4	6	2		7	3	
5–9	8	2	(25)	14	4	(29)
10–14	7	3	(43)	7	1	(14)
15–19	9	1	(11)	3	1	(33)
Total	33	10	(30±8)*	44	11	(25±7)
20–24	25	8	(32)	25	5	(20)
25–34	50	18	(36)	36	5	(14)
35–44	53	14	(26)	30	5	(17)
45–54	50	14	(28)	30	3	(10)
55–64	30	11	(37)	30	3	(10)
Total	208	65	(31·3±3·2)	151	21	(13·9±2·8)
			$P < 0{\cdot}05$			

* Proportion plus/minus standard errors.

All the high-risk groups had significantly larger proportions of individuals seropositive for *C. burnetii* antibodies than the controls. The highest proportion (83·7 %) was amongst the veterinarians caring for large domestic animals, but there was no statistically significant difference between the groups, the veterinarians for pets, the taxidermists and the female wool spinners having 77 %, 70 % and 58 % respectively.

Other groups

The proportion of positive donors in the two other centres was higher than in Rotterdam but there were no real differences between successive year classes (Table 3). In donors from Groningen like those from Rotterdam, males had a significantly higher incidence of seropositivity than females; this was not so in Maastricht.

In Table 4 the 1- to 19-year-olds in Rotterdam in 1979 and 1983 are compared with 5- to 14-year-olds in Zoetermeer in 1975. Amongst the former the proportion of seropositive males and females in both the years surveyed was much the same. In the latter the proportions in both groups were higher and more females than males were positive ($P < 0{\cdot}05$). In all groups successive age classes did not differ substantially.

In Table 5 the presence of antibodies in the sera of the over-45-year-olds in the three towns in 1968 is presented for comparison with the results from the three blood donor centres in 1983. In two of the towns the males again were more often positive than females but overall the incidence in both sexes differed very little between the years.

Downloaded from https://www.cambridge.org/core. IP address: 18.204.48.199, on 20 Nov 2019 at 00:36:12, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0950268800061938

Table 2. *The presence of IgG antibodies against* C. burnetii (*phase II*) *in the sera of residents of dairy farms and veterinarians for large domestic animals, compared with control groups from Rotterdam (1983)*

Age (years)	Resident of dairy farms			Veterinarians*			Control groups: 1–19 year (male and female)			Control groups: 20–64 year (male)		
	n	IgG	(%)	*n*	IgG	(%)	*n*	IgG	(%)	*n*	IgG	(%)
0	1	1		—	—	—	0	0		—	—	—
1	3	2		—	—	—	6	1		—	—	—
2	2	1	(63)	—	—	—	4	2	(31)	—	—	—
3	0	0		—	—	—	6	1		—	—	—
4	2	1		—	—	—	13	5		—	—	—
5–9	14	10	(71)	—	—	—	22	6	(27)	—	—	—
10–14	8	6	(75)	—	—	—	14	4	(29)	—	—	—
15–19	7	5	(71)	—	—	—	12	2	(17)	—	—	—
20–24	5	2	(40)	—	—	—	—	—	—	25	8	(32)
25–34	16	11	(69)	67	56	(84)	—	—	—	50	18	(36)
35–44	14	12	(86)	108	93	(86)	—	—	—	53	14	(26)
45–54	7	4	(57)	32	26	(81)	—	—	—	50	14	(28)
55–64	9	4	(44)	14	10	(71)	—	—	—	30	11	(37)
65	6	5	(83)									
Total	94	64	(68±5)†	221	185	(83·7±2·5)	77	21	(27±5)	208	65	(31·3±3·2)

* In 100 sera of veterinarians for large domestic animals CFT was also performed: 37 were positive (titre 1 : ⩾ 4).
† proportion plus/minus standard error.

Downloaded from https://www.cambridge.org/core. IP address: 18.204.48.199, on 20 Nov 2019 at 00:36:12, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0950268800061938

Table 3. *The presence of IgG antibodies against* C. burnetii (*phase II*) *in the sera of donors to bloodbanks in Rotterdam, Groningen and Maastricht*

	Rotterdam						Groningen						Maastricht					
	Male			Female			Male			Female			Male			Female		
Age (years)	*n*	IgG	(%)	(*n*	IgG	(%)	*n*	IgG	(%)	*n*	IgG	(%)	*n*	IgG	(%)	*n*	IgG	(%)
20–24	25	8	(32)	25	5	(20)	25	23	(92)	25	13	(52)	25	16	(64)	25	16	(64)
25–34	50	18	(36)	36	5	(14)	25	19	(76)	25	10	(40)	25	18	(72)	25	17	(68)
35–44	53	14	(26)	30	5	(17)	25	18	(72)	25	13	(52)	25	15	(60)	25	14	(56)
45–54	50	14	(28)	30	3	(10)	25	13	(52)	25	13	(52)	25	14	(56)	25	18	(72)
55–64	30	11	(37)	30	3	(10)	25	18	(72)	25	11	(44)	25	11	(44)	23	14	(61)
Total	208	65	(31·3 ±3·2)*	151	21	(13·9 ±2·9)	125	91	(72·8 ±4·0)	125	60	(48·0 ±4·5)	125	74	(59·2 ±4·4)	123	79	(64·2 ±4·3)
			$P < 0·05$						$P < 0·05$									

* Proportion plus/minus standard error.

Table 4. *The presence of IgG antibodies against* C. burnetii (*phase II*) *in the sera of children aged 5–14 years from Zoetermeer (1975) and in rubella patients aged 1–19 years from Rotterdam in 1983 and in 1979*

	1983						1979						1975					
	Males			Females			Males			Females			Males			Females		
Age (years)	*n*	IgG	(%)	*n*	IgG	(%)	*n*	IgG	(%)	*n*	IgG	(%)	*n*	IgG	(%)	*n*	IgG	(%)
1	1	0		5	1		5	0		1	0		—	—	—	—	—	—
2	1	1	(44)	3	1	(25)	1	1	(27)	1	1	(63)	—	—	—	—	—	—
3	1	1		5	0		2	2		1	0		—	—	—	—	—	—
4	6	2		7	3		3	0		5	4		—	—	—	—	—	—
5–9	8	2	(25)	14	4	(29)	15	5	(33)	13	2	(15)	30	11	(37)	30	18	(60)
10–14	7	3	(43)	7	1	(14)	8	0	(0)	2	0	(0)	30	11	(37)	30	24	(80)
15–19	9	1	(11)	3	1	(33)	15	5	(33)	18	5	(28)	—	—	—	—	—	—
Total	33	10	(30±8)*	44	11	(25±7)	49	13	(27±6)	41	12	(29±7)	60	22	(37±6)	60	42	(70±6)
															$P < 0·05$			

* Proportion plus/minus standard error.

Downloaded from https://www.cambridge.org/core. IP address: 18.204.48.199, on 20 Nov 2019 at 00:36:12, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0950268800061938

Table 5. *The presence of IgG antibodies against* C. burnetii (*phase II*) *in the sera of adults from Sluis, Vleuten and Haaksberger compared with control groups from Rotterdam, Groningen and Maastricht*

	1968						1983					
	Males			Females			Males			Females		
Residence	*n*	IgG	(%)	*n*	IgG	(%)	*n*	IgG	(%)	*n*	IgG	(%)
Sluis	30	18	(60)	30	12	(40)	—	—	—	—	—	—
Vleuten	30	10	(33)	30	11	(37)	—	—	—	—	—	—
Haaksbergen	30	20	(67)	30	12	(40)	—	—	—	—	—	—
Rotterdam	—	—	—	—	—	—	208	65	(31·3)	151	21	(13·9)
Groningen	—	—	—	—	—	—	125	91	(72·8)	125	60	(48·0)
Maastricht	—	—	—	—	—	—	125	74	(59·2)	123	79	(64·2)

DISCUSSION

Clearly infection with *C. burnetii* occurs far more frequently in the general population in the Netherlands than the number of clinically diagnosed cases would lead one to suspect; this accords with the earlier findings in the United Kingdom (Marmion *et al.* 1953). The higher incidence in males than in females seen in several of the groups was, however, not universal and remains unexplained. Regional differences are also apparent with a higher incidence generally in rural populations than in urban ones. In another study 61 % of the contacts, household or otherwise, of cases of clinical Q fever had specific *C. burnetii* IgG antibodies (Richardus, 1985). When tested for IgM antibodies 10 % of the contact group were positive compared with 0·6 % in the Rotterdam blood donors. This provides further evidence for the high rate of asymptomatic Q fever disease.

Studies in other countries have generally been carried out using the complement fixation test (CFT) and/or the capillary agglutination test (CAT). In the United Kingdom 2·1 % of a large group of blood donors had antibody (Marmion *et al.* 1953). In three surveys in the United States of America residents of dairy farms were compared with local control groups. In Maryland 15·3 % (15·2 % male, 15·5 % female) of the former were seropositive and 0·15 % of the latter (Wagstaff *et al.* 1965), in Milwaukee 28·5 % and 2·2 % respectively (Wisniewski & Krumbiegel, 1970), and in Montana the figures varied in different areas from 19·5 % to 38·2 % and from 0·3 % to 11·9 % (Luoto, Casey & Pickens, 1965). About 25 % of Swiss veterinarians were positive in contrast to 3·5 % of blood donors (Gelzer *et al.* 1983). In our study the proportion of seropositives were already much higher both in the groups at risk and in 'normal' controls than in any of those quoted above. We attribute this to the greatly increased sensitivity of the IgG IF test. Studies on the comparative sensitivities of a number of tests for *C. burnetii* antibody were made by Luoto, Casey & Pickens (1965) on the sera of 53 farm residents. They found the CFT was the least sensitive (5·7 % seropositive) and the radio-iodine precipitin reaction the most sensitive (77·4 %). The CAT, skin test, agglutination resuspension and neutralization test in mice were of intermediate sensitivity. Though we have not made comparative studies, the IF test seems to be of the same order of sensitivity as the radio-iodine test.

Downloaded from https://www.cambridge.org/core. IP address: 18.204.48.199, on 20 Nov 2019 at 00:36:12, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0950268800061938

Because tests carried out (by CFT) in the 1950s on dairy cattle, abattoir workers and patients with atypical pneumonia failed to reveal the presence of any man or animal with *C. burnetii* antibodies it was assumed that Q fever was not endemic in the Netherlands (Jansen, 1953; Wolff & Kouwenaar, 1954; Dekking & Zanen, 1958). That notified cases increased in number in 1979 was thought to reflect a true rise in the incidence of infection. Our data, however, shows that from 1968 onwards the proportion of seropositive persons in the population has remained at a fairly constant level and this furthermore applies to children, adolescents and adult members of the population. The apparent rise was due in part to the introduction of the sensitive IF IgM test for diagnosis and, as a result, in part to greater clinical awareness of the possible presence of the disease. Clearly Q fever has been endemic in the Netherlands for a long time.

Our study indicates that the majority of people acquire their antibodies at an early age, and the 1- to 4- and 5- to 9- year-old age group have the same incidence as do adults. Taylor, Kingston & Rizk (1959), working in Egypt and the Sudan, found that the peak age of positive tests (CFT) was the second year of life, when 47 % of the children had antibodies. Of the 51 serologically confirmed cases of Q fever diagnosed in our laboratory during a $4\frac{1}{2}$-year period, 18 patients (35 %) were less than 3 years of age (Richardus *et al.* 1985), 4 (8 %) were between 3 and 19 and the rest (57 %) over 20. How young children acquire the infection is not understood. The inhalation of dust or consumption of unpasteurized milk seem unlikely in western environments to cause such a high incidence of infection. Circumstances might suggest the mother as the source and features supporting the possibility of transmission *in utero*, during delivery and during the lactation period have been recorded (Fiset, Wisseman & El Batawi, 1975; Syrucek, Sobelavsky & Gutvirth, 1958; Kumar, Yadav & Kakkar, 1981). Mother and child pairs' serological studies might provide some useful data.

Part of this study was performed under grant no. 28–923 of the 'Praeventiefonds', The Hague, the Netherlands.

REFERENCES

DEKKING, F. & ZANEN H. C. (1958). Q-koorts in Nederland. *Nederlands Tijdschrift voor Geneeskunde* **102**, 65–8.

FISET, P., WISSEMAN, C. R. & EL BATAWI, Y. (1975). Immunologic evidence of human fetal infection with *Coxiella burnetii*. *American Journal of Epidemiology* **101**, 65–9.

GELZER, J., ABELIN, TH., BERTSCHINGER, H. U., BRUPPACHER, R., METZLER, A. E. & NICOLET, J. (1983). Wie verbreited ist Q-fieber in der Schweiz? *Schweizerische Medezinische Wochenschrift* **113**, 892–5.

JANSEN, J. (1953). Komt Q fever in Nederland voor? *Tijdschrift voor Diergeneeskunde* **78**, 838–41.

KUMAR, A., YADAV, M. P. & KAKKAR, S. (1981). Human milk as source of Q fever infection in breast-fed babies. *Indian Journal of Medical Research* **73**, 510–12.

LUOTO, L., CASEY, M. L. & PICKENS, E. G. (1965). Q fever studies in Montana. Detection of asymptomatic infection among residents of infected dairy premises. *American Journal of Epidemiology* **81**, 356–69.

MARMION, B. P., STOKER, M. G. P., MCCOY, J. H. & MALLOCH, R. A. (1953). Q fever in Great Britain – an analysis of 69 sporadic cases, with a study of the prevalence of infection in humans and cows. *Lancet* i, 503–10.

Downloaded from https://www.cambridge.org/core. IP address: 18.204.48.199, on 20 Nov 2019 at 00:36:12, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0950268800061938

RICHARDUS, J. H., SCHAAP, G. J. P., DONKERS, A., DUMAS, A. M. & HUISMAN, J. (1984). Q-koorts in Nederland; een beschrijving van 33 ziektegevallen waargenomen tussen 1979 en 1983. *Nederlands Tijdschrift voor Geneeskunde* **128**, 2253–8.

RICHARDUS, J. H., DUMAS, A. M., HUISMAN, J. & SCHAAP, G. J. P. (1985). Q fever in infancy: a review of 18 cases. *Pediatric Infectious Disease* **4**, 369–73.

RICHARDUS, J. H. (1985). Q-koorts in Nederland: Klinische, epidemiologische en immunologische aspekten. Thesis, Delft.

SCHAAP, G. J. P. & AKKERMANS, J. P. W. M. (1981). Q-koorts bij Nederlands melkvee. *Nederlands Tijdschrift voor Geneeskunde* **125**, 243–4.

SCHAAP, G. J. P. & DONKERS, A. (1981). Comparison between complement fixation test and determination of specific IgM in the diagnosis of Q fever. *Antonie van Leeuwenhoek* **47**, 474–5.

SYRUCEK, L., SOBELAVSKY, O. & GUTVIRTH, I. (1958). Isolation of *Coxiella burnetii* from human placentas. *Journal of Hygiene, Epidemiology, Microbiology and Immunology* II, 29–35.

TAYLOR, R. M., KINGSTON, J. R. & RIZK, F. (1959). Serological (complement-fixation) surveys for Q fever in Egypt and the Sudan, with special reference to its epidemiology in areas of high endemicity. *Archives Institut Pasteur (Tunis)* **36**, 529–56.

WAGSTAFF, D. J., JANNEY, J. H., CRAWFORD, K. L., DIMIJAN, G. G. & MEHSEN, J. J. (1965). Q fever studies in Maryland. *Public Health Reports* **80**, 1095–9.

WISNIEWSKI, H. J. & KRUMBIEGEL, E. R. (1970). Q fever in Milwaukee. III. Epidemiological studies of Q fever in humans. *Archives of Environmental Health* **21**, 66–70.

WOLFF, J. W. & KOUWENAAR, W. (1954). Een onderzoek naar het voorkomen van Q-koorts in Nederland. *Nederlands Tijdschrift voor Geneeskunde* **98**, 2726–32.

Downloaded from https://www.cambridge.org/core. IP address: 18.204.48.199, on 20 Nov 2019 at 00:36:12, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0950268800061938