

The occurrence of *Coxiella burnetii* in North-Western England and North Wales

A report from five laboratories of the Public Health Laboratory Service*

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INTRODUCTION

Marmion & Stoker (1958) discussing the epidemiology of Q fever in Great Britain conclude that raw milk is the main source of infection, whilst contact with sheep, especially in the spring during lambing and shearing time, accounts for a proportion of the cases, the placenta and wool sometimes being heavily contaminated (Stoker *et al.* 1955, Welsh *et al.* 1958). Antibody to *C. burnetii* occurs twice as commonly amongst those exposed to sheep and cattle or materials from them compared with those not so exposed (Marmion & Stoker, 1958). There is, however, no evidence that tick bites transmit the disease to man although *Haemaphysalis punctata* found on sheep and cattle may be infected (Stoker & Marmion, 1955). Henderson (1967) investigating 1052 human sera has found a complement-fixing titre of 1/8 to 1/64 or higher in 20% of persons and is of the opinion that *C. burnetii* is of high infectivity but low pathogenicity. These and similar observations have led workers in five Public Health Laboratories in the North-West—Chester, Conway, Liverpool, Manchester and Preston—to make a retrospective survey of patients diagnosed serologically as suffering from Q fever during the 5 years 1962–6. Information has also been collected on patients during the same period whose serum titres suggested that infection had occurred at some time in the past. These results are compared with those found in persons occupationally exposed to infection, veterinary surgeons, abattoir workers and farm families, as well as with some from samples of the general population.

Since cow's milk is a known source of *C. burnetii* infection a survey has been made between 1965 and 1967 in the seven counties of Anglesey, Caernarvon, Merioneth, Flint, Denbigh, Cheshire and Lancashire of samples of milk predominantly from farms which produce milk for sale untreated (Fig. 1).

METHODS

Serological test for antibodies to Coxiella burnetii

Sera were tested for the presence of antibody to *C. burnetii* by the complement fixation test (C.F.T.) of Bradstreet & Taylor (1962) with minor modifications in the different laboratories. The antigen used routinely throughout the studies was

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phase 2 of the Nine Mile strain. An antigen prepared from uninfected yolk sacs was used as a control. Sera were tested in doubling dilutions starting at 1/8 or 1/10.

Screening of milk for Coxiella burnetii infection

Between 50 and 300 ml. of milk were centrifuged, the amount varying in the different laboratories, and the deposit resuspended in 2 ml. of gravity cream. One ml. of the mixture was inoculated intramuscularly into the thigh of a guinea-pig which was killed 6 weeks later and the serum examined for *C. burnetii* antibody by the C.F.T.

RESULTS

Patients

Sera from 7046 patients were examined for *C. burnetii* antibodies. Forty-one were considered to be suffering from recent infection at the time the samples were taken.

Q fever cases

A positive diagnosis of Q fever was made in the illness under investigation when two specimens of sera taken from a patient at intervals of generally about 10 days, but varying from 4 days to 2 years, were titrated in parallel and showed a fourfold or greater change in complement-fixing titre. A titre of 1/128 was considered to be highly suggestive of recent infection when only a single sample was available for testing or when at least one of a pair of samples attained this level, although the fourfold change was not shown. (Fourfold changes in individual patients varied from as little as < 1/20–1/40 to as high as 1/1280–1/5120.)

Thirty-one persons showed a significant rise in titre and were diagnosed as suffering from Q fever, and ten had titres suggestive of recent infection (Table 1). Thirty-nine were resident in the seven counties whose milk supply was investigated and their place of residence is indicated by an × on the map (Fig. 1). The total population for these counties in 1965 was 7,183,190 (Registrar General's report). Thirty-three patients were male and eight female. The number of patients varied little during each of the 5 years, but the incidence was higher in the spring, April accounting for 13 cases.

The age of 40 of the 41 patients was known, 28 (70%) being between 20 and 60 years. Of the 7 who were less than 20 years 4 were babies in the first 2 years of life. Eleven patients gave a relevant history of possible exposure to *Coxiella*. Six were at occupational hazard. These were a butcher, a poultry specialist, a constructional engineer and a scaffolder both of whom had been concerned in the demolition of farm buildings, an excavator driver who became ill 1 week after his return from a stay in the country during which he had been present at milking and had drunk raw milk, and a wool sterilizer. Five persons in addition to the excavator driver mentioned above gave a definite or possible history of drinking raw milk. One of these, a school-teacher, invariably drank raw milk, but an extensive investigation of the herd from which the milk came was completely negative for *C. burnetii* infection.

Table 1. *Cases diagnosed as Q fever*

Age	Sex	Diagnosis	Day of disease on which specimens collected		Reciprocal of titres	
			First	Second	First	Second
19	M	Pneumonitis	9	19	< 20	160
37	M	Pneumonia	10 days*		10	1280
43	M	Pneumonia	7	12	< 5	160
?	M	Pneumonia	4	22	< 20	160
55	F	Pneumonia	8	15	< 10	80
21	M	Meningism	5	14	20	160
53	M	Pneumonia	19	44	1280	5120
56	M	Pneumonia	24	38	256	256
42	M	Meningism	2	12	< 10	640
45	M	Meningism	4	14	< 10	640
22	F	Meningism	7	17	160	640
68	M	Meningism	5	15	20	160
71	F	Pneumonia	21	34	640	640
35	M	Meningism	5	17	< 20	80
37	M	Pneumonia	6	16	20	80
73	M	Pneumonia	12	42	< 20	40
38	M	Endocarditis	212	—	128 phase 2 512 phase 1	—
45	M	Pneumonia	14	80	80	320
25	F	Influenza	14 days*		20	80
73	M	Pneumonia	11	26	10	320
57	M	Influenza	35	700	640	40
45	F	Pneumonia	18	49	32	256
50	F	Pyrexia	42	52	128	1024
44	M	Pneumonia	7	14	20	640
47	F	Pneumonia	6	20	20	80
30	M	Pneumonia	7	19	20	160
4/12	M	Pneumonia	6	21	< 20	> 320
22	M	Pneumonia	3	70	16	128
28	M	Meningitis	15	24	< 8	> 128
27	M	Pneumonitis	14	21	256	256
62	M	Pneumonia	16	26	160	320
52	M	Pericarditis	4	24	640	640
7	M	Lymphadenopathy	6	37	40	160
2/12	M	Pneumonia	9	27	< 20	80
11/12	M	Lymphadenopathy	19	—	> 320	—
43	M	Pneumonia	8	15	80	> 320
1½	M	Meningism	7	20	160	160
56	M	Pneumonia	6	10	< 8	256
24	M	Meningism	1	17	8	64
23	F	Meningism	14	42	128	128
2½	M	Cerebellar ataxia	?	?	8	64

* Interval between collection of first and second specimen, the dates of which are not known.

Clinical manifestations

Pulmonary. Twenty-five patients were variously described as suffering from pneumonia, virus pneumonia, atypical pneumonia, pneumonitis or influenza, some with consolidation of the lung, with or without pleurisy. Headache was specifically mentioned in three of these 25, one of whom had photophobia and neck stiffness.

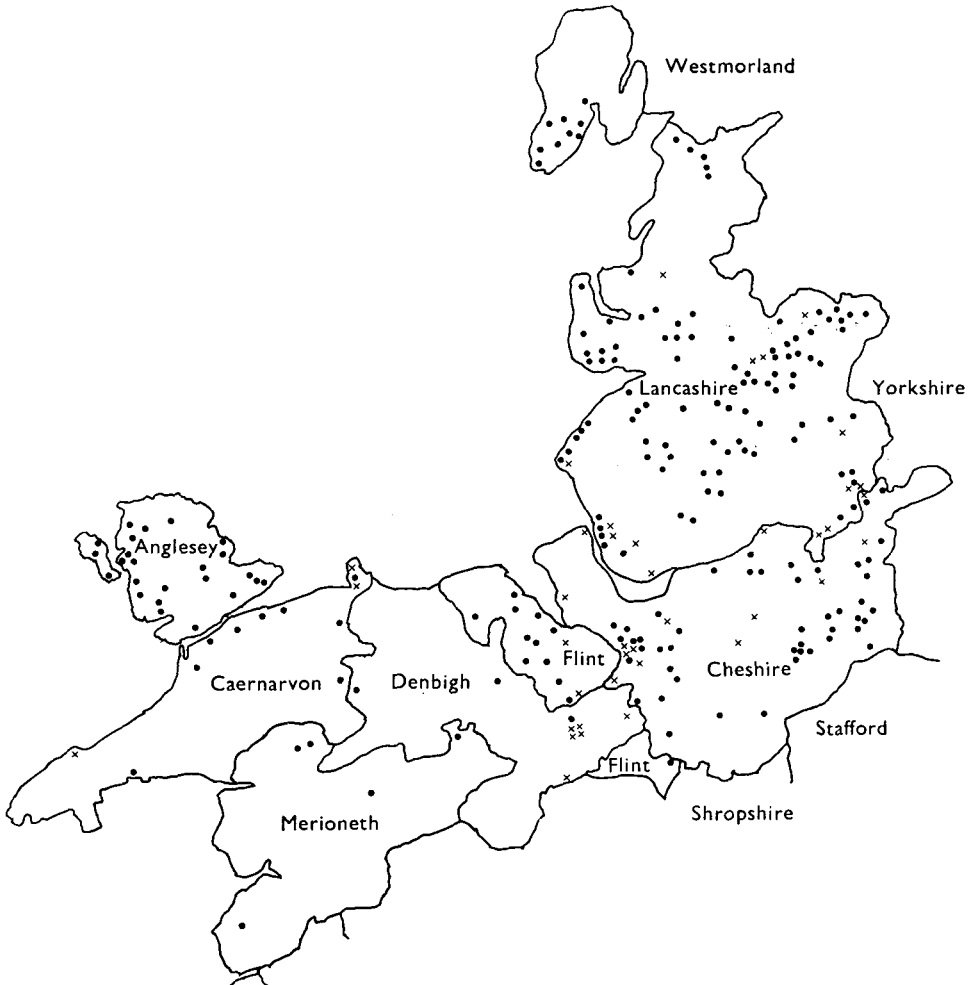


Fig. 1. Anglesey: 138 farms, 24 Q+; 247 samples, 43 Q+. Caernarvon: 148 farms, 9 Q+; 268 samples, 22 Q+. Merioneth: 123 farms, 5 Q+; 268 samples, 5 Q+. Denbigh: 27 farms, 3 Q+; 52 samples, 5 Q+. Flintshire: 52 farms, 12 Q+; 62 samples, 13 Q+. Cheshire: 232 farms, 47 Q+; 825 samples, 98 Q+. Lancashire: 1004 farms, 103 Q+; 2627 samples, 155 Q+. x, Location of cases by residence. ●, Location of infected farms.

Two patients were jaundiced, another had weakness of the face and foot drop. The wife and daughter of one patient diagnosed serologically as suffering from Q fever pneumonia also suffered from pneumonia about the same time but the sera were not examined.

Meningeal. In nine patients the main symptoms were headache, neck stiffness and pyrexia. No mention was made of chest involvement. One patient suffered from meningitis, the cerebrospinal fluid giving a white cell count of 88 with 97% lymphocytes; the chest was definitely stated to be clear.

Cardiac. The heart was affected in two cases. A man of 38 years became ill with endocarditis in August 1962. When his serum was examined in April 1963 the C.F.T. gave a titre of 1/128 to the phase 2 antigen. This serum was also titrated with phase 1 antigen (Stoker & Fiset, 1956) and was positive at a titre greater than 1/512. A mitral valvotomy was carried out in June 1964. The patient died in August 1965. The second patient, a man aged 54, suffered from pericarditis.

Miscellaneous. Two patients had lymphadenopathy, a third a pyrexia of 6 weeks duration, and a fourth cerebellar ataxia.

Evidence of infection at some time in the past

Patients whose sera gave a titre of at least 1/20 but less than 1/128 and did not, when paired sera were available, exhibit a fourfold change, were assumed to have been infected at some time.

Sera from 146 of 7005 patients, lacking evidence of recent infection, gave complement-fixing titres indicative of infection at some time in the past. In 79 the titre was 1/20; in 48, 1/32 or 1/40; in 17, 1/64 or 1/80; and in 2, 1/128. Although a single titre of 1/128 was the criterion for diagnosis of infection in the illness under study, two children with this titre are included in this group since the disease they were suffering from at the time was definitely proved to be other than Q fever.

Very little relevant information is available on these patients. Twenty-four had clinical syndromes resembling those found in Q fever, five had animal or agricultural contacts. Only one was known to have drunk raw milk.

Persons occupationally exposed to infection (Table 2)

Sera from veterinarians, abattoir workers, farm families and samples of the general population were screened by the C.F.T. at a dilution of 1/20 and those positive at this dilution were titrated.

Veterinarians

Blood was collected from 61 veterinarians, members of the Lakeland Veterinary Society and the Lancashire and Cheshire Veterinary Society. Fifty-two were in large-animal practice and their work was predominantly with cattle. Seventeen of 61 (28%) had Q antibody at a titre of 1/20 or higher.

Abattoir workers

Serum from 29 of 87 (33%) abattoir workers gave titres of 1/20 or higher. One aged 16 years had a titre of 1/320. He gave a history of a severe attack of 'influenza' 9 months earlier, 3 months after he began work.

Farm families

Twenty-four of 193 (13%) males and females had *C. burnetii* antibody detectable at a dilution of 1/20 or more. There is however a difference in incidence between men and women, 11 of 118 men (9.2%) and 13 of 75 women (17%) showing evidence of infection.

Table 2. *Comparison of serum titres in different sections of the community*

	Numbers of sera with reciprocal c.f. titre of:						Total
	< 20	20	32-40	80	160	320	
Veterinarians	44	13	4	0	0	0	61
Abattoir workers	58	18	10	0	0	1	87
Farm families							
Males	107	8	2	0	1	0	118
Females	62	7	5	1	0	0	75
Antenatal sera							
Liverpool	158	3	0	0	0	0	161
Preston	246	1	0	0	0	0	247
Blood donor sera from Manchester	84	2	1	0	0	0	87
Sera from N. Wales	148	2	0	0	0	0	150
Sera from Chester	50	1	1	0	0	0	52

Table 3. *Number of infected farms and milk samples found in 1965-67*

	Counties							Total	
	Anglesey	Caernarvon	Merioneth	Denbigh	Flint	Cheshire	Lancashire		
Farms selling untreated milk, 1965-7	94	185	157	162	174	c. 265	1530		
Farms tested	138*	148	123	27	52	232	1004	1724	
Farms positive	24	9	5	3	12	47	103	203	
Percentage positive	17	6	4	11	23	20	10	11.7	
Milk samples tested	247	268	268	52	62	825	2627	4349	
Samples positive	43	22	5	5	13	98	155	341	
Percentage positive	17	8	2	10	21	12	6	7.8	
			Slavin (1952)						
Farms tested	50	107	—	3	7	149	75	—	
Farms positive	—	—	—	—	—	20	8	—	
Percentage positive	—	—	—	—	—	13	11	—	

* Farms selling treated milk have been tested in some instances.

*General population (Table 2)**Antenatal patients*

Two series of antenatal patients were studied. Of 161 sera examined at Liverpool three (1.9%) contained antibody at a dilution of 1/20. Of 246 examined at Preston one (0.4%) contained antibody at this level.

Blood donors

Sera from 87 blood donors living in an area where milk was known to be consumed largely unpasteurized were tested at Manchester. Two sera fixed complement at a dilution of 1/20 and one at 1/32.

Miscellaneous

Sera came variously from waterworks employees, factory workers and patients under investigation for venereal disease. In North Wales 150 sera were examined, of which two contained antibody at 1/20. In Chester, out of 52 sera, one contained antibody at 1/20 and one at 1/40.

*Milk**Tests on milk samples*

In all, 4349 samples of milk from 1724 farms were examined in the counties of Anglesey, Caernarvon, Merioneth, Denbigh, Flint, Cheshire and Lancashire during the period 1965-7. Three hundred and forty-one samples (7.8%) from 203 farms (11.7%) were found to be infected (Table 3). The number of times milk from any one farm was examined varied widely. Some farms were tested repeatedly, others on one occasion only. Where frequent sampling was carried out it was often only after several examinations that a positive result was obtained. Similarly the proportion of farms sampled varied from county to county, but was fairly extensive other than in Denbigh and Flint. The positions of infected farms are shown by a dot on the map.

The rate of infection was highest in Flintshire with 23% of farms and 21% of samples positive, and lowest in Merioneth with 4% of farms and 2% samples positive.

The possibility of natural infection of guinea-pigs with *C. burnetii* can be excluded since no antibody could be demonstrated *post mortem* in 85 guinea-pigs inoculated with specimens other than milk, and small pools of sera from guinea-pigs inoculated with specimens other than milk are used regularly as a source of complement in the C.F.T. and no evidence of antibodies to *C. burnetii* has been found. Similarly the possibility of infection being spread between guinea-pigs sharing the same cage can be excluded. Of nine pairs of cage-mates examined, in each instance only one of the pair was found to have antibody to *C. burnetii*.

DISCUSSION

Forty-one (0.57%) cases of *C. burnetii* infection were diagnosed in 7046 patients investigated serologically for the presence of viral and rickettsial antibodies during the years 1962-6 compared with 0.65% of 3500 patients in 1952 and 1953 (MacCallum, 1954). They showed the usual male-to-female predominance with the large majority occurring during active adult life (Clark, Lennette & Romer, 1951; Harvey, Forbes & Marmion, 1951; Stoker, 1954; Marmion & Stoker, 1958; Connolly, 1968). The incidence was highest in Spring (Clarke, Lennette & Romer, 1951; Marmion & Stoker, 1958). In just over a quarter of the patients there was evidence

of occupational hazard or a history of drinking raw milk. The remainder gave no relevant history.

A further 146 patients showed evidence of infection in the past and a proportion of these may have suffered from *C. burnetii* infection in the illness under investigation although their serological titres did not satisfy the criterion for a definite diagnosis. This may well have been so since, in the proved cases, in nine of those showing a fourfold or greater change in titre the higher titre was 1/80 or less. However, a single titre of this order cannot be unequivocally interpreted as diagnostic, since antibody may remain appreciably high for several months after the illness (Lennette *et al.* 1952). Indeed the serum of one abattoir worker screened in this survey gave a titre as high as 1/320 nine months after the probable acute illness.

The higher incidence of antibodies in those occupationally exposed to infection—veterinarians, workers in the abattoir and farm families—when compared with the general population reflected the findings of other workers (Marmion & Stoker, 1958; Connolly, 1968). In this study a higher percentage of veterinarians and workers in the abattoir showed evidence of infection than did members of farm families. This may be explained by the heavier exposure experienced by the two former groups, of which the veterinarians in particular handle the placenta, which may be grossly infected (Welsh *et al.* 1958), and workers in the abattoir are constantly exposed to the aerosols produced during killing and processing of animals. The greater incidence of antibody amongst the female members of farm families compared with the male was of special interest since clinical infection is far commoner in the male than in the female. Women on the farm usually look after the dairy and on producer-retailer farms are largely responsible for cleaning, filling and capping milk bottles and therefore have more contact with raw milk than the men, who do the actual milking but with modern machinery. This may result in constant exposure to small amounts of antigen and hence the development of antibody without manifestation of clinical disease. Stoker (1954) found that, in sera from healthy blood donors, the proportion of males to females with antibody was much less than the numbers with clinical disease would lead one to expect. He postulated that, perhaps owing to smaller dosages or to constitutional differences, women were less susceptible to clinical illness. In a short sharp outbreak described by Harvey, Forbes & Marmion (1951), despite an equal degree of exposure the proportions of males to females with clinical illness was 20 to 7. Whether the apparent increased incidence in blood donors tested at Manchester was due to drinking unpasteurized milk or other type of exposure is difficult to determine.

A higher rate of infection of milk was found in the present study when compared with that of Slavin (1952) except in Lancashire, where the percentage of infected farms was similar. He, however, used Henzerling antigen and sampling was on a smaller scale. In this study some farms were investigated only infrequently and more intense sampling might have revealed an even higher incidence of infection.

Despite the wide distribution of *C. burnetii* in milk and the heavy exposure to infection of some groups of the community, the fact remains that sporadic overt Q fever is not as commonly diagnosed as one might expect. This may be accounted

for by low pathogenicity of the organism, giving rise to subclinical infection only, by the under-use of diagnostic facilities, or by the mode of entry of the rickettsia to the body being sub-optimal for the establishment of disease.

SUMMARY

A retrospective survey of *Coxiella burnetii* infection in North-Western England and North Wales was made in five Public Health Laboratories for the years 1962–6 inclusive. Groups of the normal population and of persons occupationally exposed to infection were tested for the presence of antibody and the incidence of infected milk was investigated in seven counties.

Forty-one cases of *C. burnetii* infection were diagnosed in 7046 patients investigated serologically for the presence of viral and rickettsial antibodies. In just over a quarter of the patients there was evidence of occupational hazard or a history of drinking raw milk. The remainder gave no relevant history.

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