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Endemic Q Fever in New South Wales, Australia: A Case Series (2005–2013)

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Abstract. Q fever is endemic in Australia, and during the period 2005–2013 our laboratory diagnosed 379 cases in New South Wales. To evaluate clinical symptoms, epidemiology, mode of diagnosis, antibody profiles, and treatment, a subset of 160 (42%) Q fever cases were analyzed in detail following the return of a questionnaire by the patient's doctor and from their laboratory reports. Overall, 82% patients were male and predominantly middle aged. The majority of patients (89%) had animal contact among which 63% were with cattle, 11% with sheep, and 7% with kangaroos. Clinical symptoms were nonspecific: myalgia (94%), fever (91%), headache (80%), acute fatigue (64%), and arthralgia (55%). Most cases (93%) were acute, and serology (immunofluorescence) was the main diagnostic modality. Positive real-time polymerase chain reaction results were useful in the diagnosis of both acute and chronic Q fever, as was the isolation of *Coxiella burnetii* in cell culture. Doxycycline was the antibiotic most commonly used.

BACKGROUND

Q fever was first recognized as a new disease in Australia in 1937[1] followed by detection of *Coxiella burnetii* in the United States.[2] This infection has been recognized worldwide, with the exceptions of New Zealand[3] and French Polynesia.[4] It is a zoonosis with protean clinical manifestations and difficult to diagnose clinically, even with a definite history of animal contact. Laboratory investigations (mainly serology) are usually required to confirm the diagnosis of Q fever.

The purpose of the current study was to investigate a series of 379 symptomatic and laboratory confirmed cases in New South Wales (NSW), Australia, during 2005–2013. This study supplements earlier investigations into Q fever in other states of Australia: Powell[5] and Derrick[6] in Queensland, and Spellman[7] and Buckley[8] in Victoria.

METHODS

The Australian Rickettsial Reference Laboratory, a diagnostic and research laboratory, diagnoses Q fever following referral from doctors. All sera were collected from symptomatic patients seeking a diagnosis. Serology was undertaken by immunofluorescence, using both phase 2 and phase 1 *C. burnetii* antigens, and fluorescein-labeled antihuman serum (KPL, Gaithersburg, MD) used at 1/100 dilution to detect three immunoglobulin classes and total antibody to this bacterium. Patient sera were diluted to 1/25, and if positive, a doubling dilution series (1/25 to 1/3,200) of each serum was prepared.[9] *Coxiella burnetii* Nine Mile strain phase 1 (NMI) was grown in wild-type mice following intraperitoneal inoculation and antigen harvesting from the spleen 7 days later. *Coxiella burnetii*, Nine Mile phase 2 /Clone 4 was grown in VERO cells, using RPMI-1640 medium (Life Technologies, Grand Island, NY), with 10% bovine fetal calf serum at 35°C in 5% CO_2 for 2–3 weeks. Each antigen preparation was diluted to a suitable concentration (~50–100 *C. burnetii* per high-power field) before spotting into glass slides and fixing with methanol.

Q fever DNA amplification was undertaken by two separate real-time polymerase chain reactions (qPCR) targeting two genes unique to *C. burnetii*, *com1* and *htpAB*. A threshold cycle reading at or below 40, for both genes, was considered to be positive.[10] *Coxiella burnetii* culture was undertaken by inoculating the patient serum into VERO cells and showing subsequent increases in qPCR positivity on a standard area of scraping of the cell monolayer.

In the case of negative serology, a request was made for follow-up serum to detect any subsequent seroconversion. In the case of a seropositive patient, a request was also made for a follow-up serum to look for any significant changes in antibody titers. A one page questionnaire was sent to all doctors whose patient had a confirmed case of Q fever. Of the 379 questionnaire sent, 160 (42%) were returned.

A case of Q fever was defined as: 1) culture of *C. burnetii*, 2) a positive qPCR assay from a patient sample (usually serum), or 3) positive serology. For diagnosing acute Q fever, the latter required a single serum with a positive phase 2 IgM (> 100) or positive phase 2 IgM plus positive phase 2 IgG (> 100) without positive phase 1 antibodies. For diagnosing chronic Q fever, a single serum with a positive phase 1 IgG (≥ 800) plus positive phase 1 IgA (≥ 200) was required. A second serum showing changes in antibody classes and titers over time gave a more convincing serological diagnosis. In all serologically defined cases, the clinical features were consistent with Q fever.

RESULTS

Of the 379 patients sequentially diagnosed with Q fever during 2005–2013, 82% were male. Of the 124 patients on whom the animal contact question was answered, 89% had animal contact, mostly with cattle (63%) although there were some cases that were associated with sheep, kangaroos, and other animals (Figure 1).

The main presenting clinical features were nonspecific (Figure 2) with nothing pointing to Q fever as the correct diagnosis. There were no pathognomonic symptoms or signs to assist the doctor in making the diagnosis of Q fever. In the 12 patients with chronic Q fever, two had endocarditis and one had osteomyelitis. Rash and neurological symptoms were absent from the 12 chronic Q fever cases. Overall, 67% of patients had hepatitis (based on abnormal liver

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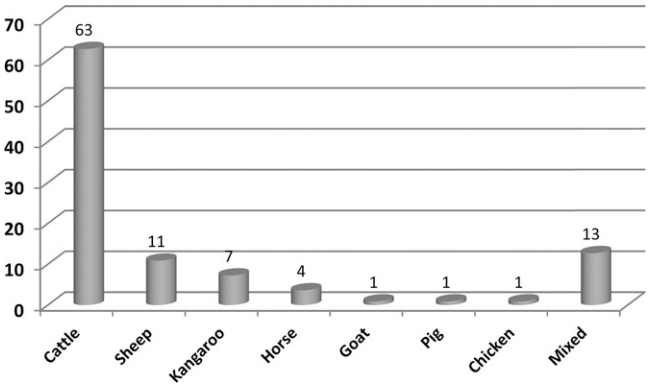

FIGURE 1. Distribution of Q fever patients with specific animal contact (%) among patients in the animal contact group (n = 110).

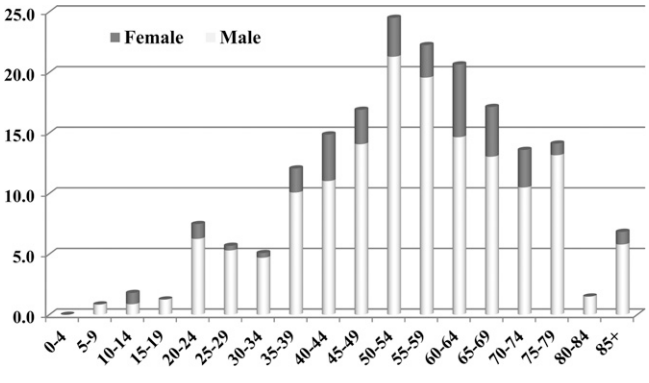

FIGURE 3. Age and sex distribution of 379 Q fever patients per 100,000 populations per annum.

function tests), 37% had respiratory symptoms (usually mild, e.g., cough), and 23% had both hepatitis and respiratory symptoms.

In prevalence by age, there were very few cases diagnosed in the 0- to 19-year-age group (Figure 3) but from that age onward the likelihood of infection increased in each age cohort up to 50–54 years, and then started to fall again. The age of major risk of Q fever in NSW was between 45 and 69 years.

Q fever diagnosis by immunofluorescence serology (Table 1) shows the total number of sera tested of the 160 patients, 51 of whom seroconverted. Among the 160 patients, only 40 cases were confirmed using a single serum. The remaining 120 cases provided a further one or more sera to confirm the diagnosis. Of the 51 patients who seroconverted, some seroconverted to only phase 2 antigen (N = 13), this being the first antibody to be produced in acute Q fever, but many (N = 38) seroconverted to the both phase 1 and phase 2 antigens. No patient seroconverted to only phase 1 antigen. Representative examples of antibody changes in the serum of acute and chronic patients (three patients each) are shown in Table 2. The order of antibody appearance following acute infection was: phase 2 IgM, phase 2 IgG, phase 1 IgM, phase 1 IgG, phase 2 IgA, and phase 1 IgA. The difference between patients with chronic Q fever and patients with acute Q fever were negative IgM (both phase 2 and phase 1), positive phase 1 IgA, and usually higher phase 1 IgG titers in chronic Q fever than in acute Q fever. Patient number 199 was initially diagnosed as acute Q fever with high phase 2 IgM titers but during 5 years, the antibody pattern changed to chronic Q fever with the presence of high phase 1 IgG and IgA titers (Table 2).

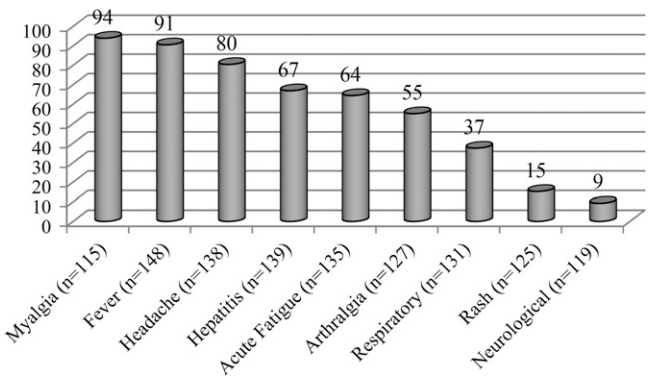

FIGURE 2. Distribution (%) of clinical symptoms of Q fever patients.

In 14 of the 160 patients, *C. burnetii* was isolated from the patient serum by VERO cell monolayer inoculation. This cohort consisted of 13 acute cases and one chronic case. Of these 14 cases for whom PCR was performed, only six were positive (43% sensitivity), showing that PCR was a valuable diagnostic technique only if positive. Given that two culture-positive cases were qPCR (false) negative, it is apparent that the qPCR assay lacked sensitivity compared with amplification by culture. However, qPCR is infinitely faster and when positive, of much greater use to the doctor. Serology alone (72%) was the most commonly used laboratory assay, followed by serology and PCR (22%). In 6% of patients' serology, PCR and culture of *C. burnetii* constituted the diagnosis (Figure 4).

Of the 135 patients in whom the doctor reported the use of antibiotics, 130 (96%) used doxycycline. Of these patients, 126 (97%) were reported as cured. The remainder were probably still fatigued due to the post-Q fever fatigue syndrome.

DISCUSSION

Australia could be considered the "home" of Q fever: the infection was first recognized as a new clinical entity among abattoir workers slaughtering pregnant cattle in Brisbane, Australia,[1] recognized as a probable *Rickettsia*,[11] its epidemiology was defined[12] and a clinical analysis was undertaken.[13] In the United States, the bacterium was detected in ticks[2] and shown to be a human pathogen.[14] In the intervening years, Q fever has been shown to be widespread in the world, an important zoonosis involving many vertebrate animal species and tick species, with recent refinements in diagnosis and treatment.[15,16] Unfortunately Australia is still the only country using a human Q fever vaccine.[17] A large French study[18] has provided benchmark data. But could the disease in Australia be different? There have been previous epidemiological studies of Q fever in Queensland,[5,6,12,13,19–22] Victoria,[7,8] and comparisons,[23,24] but there are limited data available on Q fever in NSW.[25–28] A seroprevalence study in the Hunter New England (northeast) region of NSW showed a seroprevalence range from 0.5% in the city (Newcastle) to

TABLE 1
Q fever diagnosis of patients by immunofluorescence serology

No. of sera and serology tests per patient	No. of patients tested	No. of patients who seroconverted	No. of patients who seroconverted to only P2 *Coxiella burnetii* antigens	No. of patients who seroconverted to P1 and P2 *C. burnetii* antigens
1	40	N/A	N/A	N/A
2	55	26	7	19
3	41	20	6	14
4	11	3	0	3
5	7	2	0	2
6	2	0	0	0
8	2	0	0	0
9	1	0	0	0
10	1	0	0	0
Total	160	51	13	38

N/A = not applicable; P1 = phase 1; P2 = phase 2.

a maximum of 22% in a rural area.[29] A 10-year summary (2001–2010) of the NSW official Q fever notification data[30] overlapped with the current study (2005–2013). In both studies, similar conclusions were noted, including the predominance of cases in rural NSW, in middle-aged males. The use of the Q fever vaccine as part of a government sponsored industry immunization program reduced the incident of Q fever over a decade from 4.5 to 2.8 cases per 100,000 persons per year.[30] The paucity of Q fever cases in children has been already reported in Australia,[31,32] but when it does occur, chronic osteomyelitis is the main clinical presentation.[33,34]

One important new finding from the current study is the role of macropods (especially kangaroos) in the epidemiology of Q fever in Australia. Several patients appear to have been infected via indirect contact with kangaroos, either via their feces or via their ticks. This link has been postulated previously.[35] Two species of kangaroos in western Queensland were shown to be seropositive for *C. burnetii*, and isolates of *C. burnetii* were made from their ticks, *Amblyomma triguttatum*, the ornate kangaroo's tick.[36] A patient in western Australia developed Q fever pericarditis after being bitten by kangaroo ticks.[37] Macropod seropositivity to *C. burnetii* in Queensland and western Australia has been reported.[38] Several Australian tick species have been shown to contain *C. burnetii* DNA[39] including *A. triguttatum* (from macropods) and *Haemaphysalis humerosa* (from bandicoots, which are known to be an important reservoir for *C. burnetii*). *Ixodes holocyclus*, the paralysis tick, which is the main human-biting tick in Australia and *Bothriocroton auruginans*, the wombat tick, also harbor *C. burnetii*. It is now beyond reasonable doubt that kangaroos and other macropods and their ticks carry *C. burnetii*. Kangaroo feces may pose a risk of Q fever especially if aerosolized by wind or lawn mowing. In the current study, 7% of patients remembered macropods contact.

In patients with acute Q fever, phase 2 IgG appeared marginally sooner than phase 1 IgM. In patients with chronic Q fever, phase 1 IgA titers were generally higher than phase 2 IgA titers. The unusual acute-to-chronic case (no. 199) showed the stark serological differences between the two forms of the illness. The value the PCR in early Q fever diagnosis has already been reported in Australia,[40]

TABLE 2
Q fever immunofluorescence serological profiles of acute and chronic Q fever patients

		Antibody to phase 2				Antibody to phase 1			
		Coxiella burnetii antigen				*C. burnetii* antigen			
Infection type and patient number	Day of sample*	IgM	IgG	IgA	Total	IgM	IgG	IgA	Total
Acute Q fever									
174 (adult)	0	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	14	≥ 3,200	800	Neg	≥ 3,200	Neg	Neg	Neg	Neg
	44	400	≥ 3,200	400	≥ 3,200	200	200	200	200
176 (adult)	0	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	1	50	25	Neg	50	Neg	Neg	Neg	Neg
	15	≥ 3,200	≥ 3,200	Neg	≥ 3,200	100	Neg	Neg	100
267 (adult)	0	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	4	≥ 3,200	100	50	≥ 3,200	Neg	Neg	Neg	Neg
	11	≥ 3,200	≥ 3,200	50	≥ 3,200	200	Neg	Neg	200
Chronic Q fever									
271 (child: chronic osteomyelitis)	0	Neg	≥ 3,200	Neg	≥ 3,200	Neg	≥ 3,200	200	≥ 3,200
	198	Neg	≥ 3,200	Neg	≥ 3,200	Neg	≥ 3,200	200	≥ 3,200
	438	Neg	≥ 3,200	50	≥ 3,200	Neg	≥ 3,200	400	≥ 3,200
243 (adult: chronic endocarditis)	0	Neg	≥ 3,200	≥ 3,200	≥ 3,200	50	≥ 3,200	≥ 3,200	≥ 3,200
	446	Neg	≥ 3,200	≥ 3,200	≥ 3,200	Neg	≥ 3,200	≥ 3,200	≥ 3,200
	1,026	Neg	800	100	800	Neg	1,600	800	1,600
199† (adult: acute to chronic endocarditis)	0	≥ 3,200	Neg	Neg	≥ 3,200	Neg	Neg	Neg	Neg
	10	≥ 3,200	≥ 3,200	200	≥ 3,200	Neg	Neg	Neg	Neg
	1,825 (5 years)	Neg	1,600	Neg	1,600	Neg	≥ 3,200	≥ 3,200	≥ 3,200

Neg = negative.
*The day 0 sample would usually be within 1 week of onset of illness.
†Patient 199 was first diagnosed as acute Q fever, and 5 years later, represented with chronic Q fever.

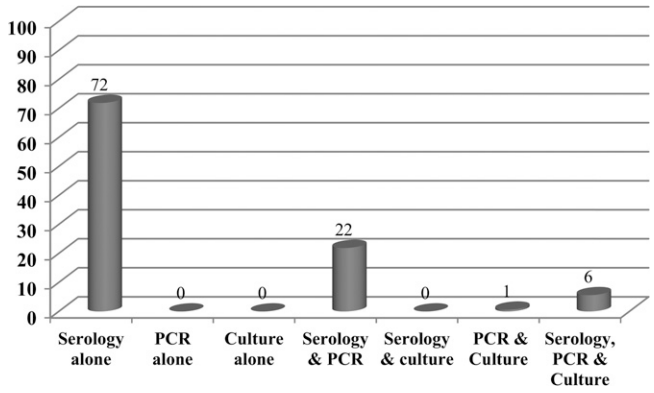

FIGURE 4. Distribution (%) of different laboratory technique(s) used for the diagnosis of Q fever patients (n = 160).

but the observation that culture was more sensitive than qPCR was an unexpected finding.

The myth that Australian Q fever patients do not have respiratory symptoms has also been overturned (Figure 2). Early studies on Q fever in Australia had shown that 69% of patients had a cough.[41] The current study has shown that hepatitis is more common than respiratory symptoms although many patients had both.

In summary, this study of 160 cases from a 379 case cohort of Q fever in NSW has demonstrated that much about this infectious disease in Australia is similar to elsewhere in the world, with a background endemicity associated with native wildlife (macropods in the case of Australia), and the main burden being due to livestock industries particularly cattle and sheep. Increased uptake of the Q fever vaccine by young adults (≥ 18 years), in rural Australia, should be encouraged.[28]

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