OXFORD

QJM: An International Journal of Medicine, 2016, 661–668

doi: 10.1093/qjmed/hcw041
Advance Access Publication Date: 1 April 2016
Original Paper

ORIGINAL PAPER

The natural history of acute Q fever: a prospective Australian cohort

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Summary

Background: A detailed description of the natural history of acute Q fever, caused by infection with *Coxiella burnetii*,
Aim: To significantly increase understanding of the illness.
Design: Subjects with provisional acute Q fever ($n = 115$) were recruited from primary care in rural Australia, and followed prospectively by interview and blood collection including for serological confirmation. A nested series of subjects with prolonged illness (cases), and those without (controls), were investigated in detail.
Methods: Total phase I and phase II anti-*C. burnetii* antibodies were detected by complement fixation test; and IgG, IgM and IgA phase I and phase II titres by immunofluorescence. Flow cytometric analysis was conducted to enumerate circulating T cells subsets, B cells, monocytes and natural killer cells.
Results: Serological testing confirmed acute Q fever in 73 subjects (63%). The acute illness featured fever, headache, sweats, fatigue and anorexia; and varied widely in severity, causing an average of 8 days in bed and 15 days out of work or other role in the first month of illness. The illness course varied from 2 days to greater than a year. No cases of chronic, localized Q fever infection, such as endocarditis, were identified. Neither severe nor prolonged illness were associated with persistence of *C. burnetii* DNA, altered patterns of *C. burnetii*-specific IgG, IgM or IgA antibody production, or altered leucocyte subsets.
Conclusions: The severity of acute Q fever alone predicted prolonged duration. Further studies are warranted to better understand the pathophysiology of prolonged illness after acute Q fever.

Background

Q fever is a highly infectious zoonotic disease which is endemic worldwide, caused by *Coxiella burnetii* infection, a rickettsia-like Gram-negative intracellular pathogen commonly carried by livestock, including cattle, sheep and goats.[1] Excretion of infectious particles into blood and body fluids results in formation of airborne dust-like particles,[2] which transmit infection via inhalation of very low infective doses (<10 organisms).[3] Acute Q fever and its chronic sequelae are among the most serious infective hazards for occupational health. In Australia, annual laboratory notification rates of 2 per 100 000 are likely to be significant under-estimates, reflecting those who do not present to care, or are misdiagnosed as influenza or similar illnesses.[4–6]

Received: 1 October 2015; **Revised (in revised form):** 24 February 2016

The clinical manifestations of acute Q fever range from asymptomatic infection to severe disease, and may be complicated by pneumonia, hepatitis and cardiac manifestations. The organism may also persistent to establish chronic, localized infection, such as endocarditis or osteomyelitis.[7] We previously reported the outcomes of a prospective cohort study of acute Q fever showing that ~10% had ongoing illness (but not ongoing infection) with significant disability lasting 6 months or more,[8] referred to as post-Q fever fatigue syndrome (QFS).[9]

Here, we describe a systematic and prospective record of the natural history of the illness, and levels of disability, in a substantive cohort of patients with serologically confirmed infection. We describe predictors of the severity and course of illness, and variations in the pattern of antibody production and cellular activation.

Design and Methods

Subjects were enrolled in the Dubbo Infection Outcomes Study,[8] having presented to primary care with a febrile illness, and a provisional diagnosis of acute Q fever made via *C. burnetii* IgM ELISA. Acute and convalescent sera were subsequently assayed against phase I and phase II antigens, where seroconversion or 4-fold change in IgG titre confirmed the diagnosis. Written, informed consent was obtained. Human research ethics approvals were provided by University of New South Wales and the local Area Health Service.

Demographics and clinical history were recorded, as well as symptom pattern and severity, functional impairment and health care utilization at regular intervals over 12 months.[8] The symptom profile was described using the Somatic and Psychological HEalth REport (SPHERE) questionnaire.[10] The functional impact of the illness was recorded via the Brief Disability Questionnaire.[11]

All symptom data were reassigned to 'days post illness onset', and collated into time intervals: 0–3, 4–6, 7–12, 13–24, 25–36 and 37–52 weeks. Designation of significant ongoing illness (caseness) was based on consistently positive scores on the SOMA subscale of the SPHERE, which correlates with disability due to illness.[10] Illness duration was designated as the number of days from onset to resolution of caseness on this subscale.

A case-control series was chosen from those with serologically confirmed acute Q fever for laboratory analysis. Cases who remained ill for 6 months or more completed specialist medical and psychiatric evaluations to exclude alternative explanations for ongoing symptoms, including medical assessment for signs of chronic, localized Q fever infection. At least two, age and sex-matched control subjects who recovered from the symptomatic acute Q fever within 3 months were chosen for each case. Longitudinally collected samples were tested for: total phase I and phase II anti-*C. burnetii* antibodies by complement fixation test; as well as IgG, IgM and IgA phase I and phase II antibody titres via indirect fluorescent-antibody test. Flow cytometric analysis of leucocyte sub-populations was conducted on stored peripheral blood mononuclear cells including for total (CD3), helper (CD4) and cytotoxic (CD8) T cells subsets, B cells (CD19), monocytes (CD14, CD16), natural killer (NK) cells (CD16, CD56) and activation status (CD69, HLA DR) (all antibodies BDBioscience).

Coxiella DNA was sought using sensitive reverse transcriptase-polymerase chain reaction (PCR) assays directed against both *Com1* and *IS111a* as described.[12] The Com1 assay included forward (5′ AAAACCTCCGCG TTGTCTTCA 3′) and reverse (5′ GCTAATGATACTTTGGCAGCGTATTG 3′) primers—both from Invitrogen. The probe (5′ AGAACTGCCCATTTTTGGCGG CCA 3′) (Biosearch Technologies) was dual labelled with fluorophore (5′ 6-FAM) and quencher (3′ BHQ-1). The IS1111a assay included forward (5′ GTTTCATCCGCGGTGTTAAT 3′) and reverse (5′ TGCAAGAATACGGACTCACG 3′) primers, and a dual-labelled probe (5′ 6-FAM-CCCACCGCTTCGCTCGCTAA-BHQ-1 3′). In excess of 25 000 monocytes were represented in each PCR reaction. Both assays detected a minimum of 10 target copies per reaction; in the IS111a assay this could correspond to as little as a single genome copy for sample.[12]

Statistical analyses were performed using SPSS (v15; IBM, Armonk, NY, USA). Principal components analysis (PCA) derived the severity index from the self-report questionnaire data as described.[13] Pearson correlations were sought between illness severity and disability. Time course analyses (Kaplan–Meier) assessed the impact of illness severity on disease duration.

Results

There were 115 subjects enrolled who presented to primary care with a febrile illness, and a provisional diagnosis of acute Q fever made via *C. burnetii* IgM ELISA, including 94 males (82%) with a median age of 38 years (range 16–73). The diagnosis was confirmed in 73 (64%) by testing of acute and convalescent sera. The case-control series comprised six subjects who suffered from prolonged illness, and 15 matched control subjects.

The median age for the serologically confirmed acute Q fever group was 39 years (range 16–73), including 62 men (85%). Sixty-three (86%) reported working as a shearer, grazier, abattoir worker or other rural position, whereas 62 (85%) reported being exposed to livestock in the 6 weeks prior to symptom onset. Twenty-three subjects (32%) were hospitalized during the acute illness. Pneumonia was identified in only two subjects (3%), and biochemical hepatitis was present in 47 subjects (64%). In the first month, the illness caused substantial disability with a mean of 8 days in bed (standard deviation, SD 6.8), and 15 days out of usual work or other role (SD 10.5). In a subset of patients ($n = 13$) with detailed longitudinal health care utilization data, the acute illness resulted in an average of three visits (range 0–10) to a general practitioner, and an average of five visits (range 0–15) to any health care professional.

The PCA-derived severity index accounted for 46% of the variance of the data, and featured typical dimensions including fever, headache, sleep disturbance, musculo-skeletal pain and fatigue. Scores were normalized around a mean value of zero, which divided the sample into two groups suffering from either 'high' or 'low' severity of acute Q fever (mean split). A comparison of these two groups is provided in Table 1. The index correlated with 'days in bed' ($P = 0.010$) and 'days out of usual work or other role' ($P = 0.036$) (Pearson correlations). Regression analysis determined that age ($P = 0.634$) and gender ($P = 0.053$) were not associated with illness severity.

The duration of the post Q fever illness was compared in subjects with high or low acute illness severity (Figure 1). Individuals with high acute illness severity had a significantly longer duration of symptomatic illness, lasting a mean of 227 days, in comparison to individuals with low severity who had a mean of 70 days of illness ($\chi^2 = 21.927$, $P = 0.000$). The prolonged illness caused ongoing disability with a mean of 9 days out of usual role in the last month (SD 8.9) and an average of 1 day in bed (SD 1.0).

The most frequent ongoing symptoms included a feeling of feverishness (but without documented fever), headache, joint pain, fatigue (waking up tired, weak muscles, heavy arms and

legs, tired after rest), and psychological symptoms (frustrated, irritable, easily annoyed). The proportion of the group reporting these symptoms at each time interval are shown in Figure 2.

The serological response to acute Q fever followed the typical pattern of primary infection with a rapid increase in phase II IgM antibody titres, followed by a gradual increase in the phase I IgM, but to levels significantly lower than the phase II antibodies (Figure 3). IgG responses took several months to peak, with phase II antibody titres again much higher than phase I titres. Cases with prolonged illness had similar patterns of antibody induction in comparison to controls. In some subjects ($n=2$ cases, $n=5$ controls), phase I and phase II-specific IgA titres became detectable at later time points, but these responses were not associated with the course of symptomatic illness (data not shown).

There was an increase in activated (HLA-DR+) CD8+ T lymphocytes early in the infection, decreasing over time. The proportions of total and activated NK and B cells did not change significantly (data not shown). There were no significant differences in the relative proportions of activated CD4+ or CD8+ T cells between cases with prolonged illness and subjects who recovered promptly (Figure 4A–D).

Of 104 samples tested, nine were positive for *C. burnetii* in one or both assays, and were found in both quickly resolving, and prolonged, illness groups. Eight positive samples were from 0- to 6-week time points, suggesting that infection of circulating leucocytes is more common early in the illness. By contrast, *C. burnetii* DNA was detectable in only one late time point sample (12 months) in a subject prolonged illness (in whom clinical and laboratory investigations for chronic localized Q fever were negative), making it unlikely that persistent infection in circulating leucocytes underpins the sometimes prolonged illness course.

Table 1. Characteristics of Q fever-associated disability which associate with 'high' and 'low' severity in the acute illness

Disease parameter	High severity mean (SD)	Low severity mean (SD)	Severity correlation[a] *P*
Fatigue score at enrolment	8.6 (1.9)	3.5 (2.2)	
Number of days in bed in the last month	10.0 (8.3)	6.2 (5.3)	0.010
Number of days out of role in the last month	17.3 (10.7)	12.5 (10.4)	0.036

[a]Pearson correlation with severity score.

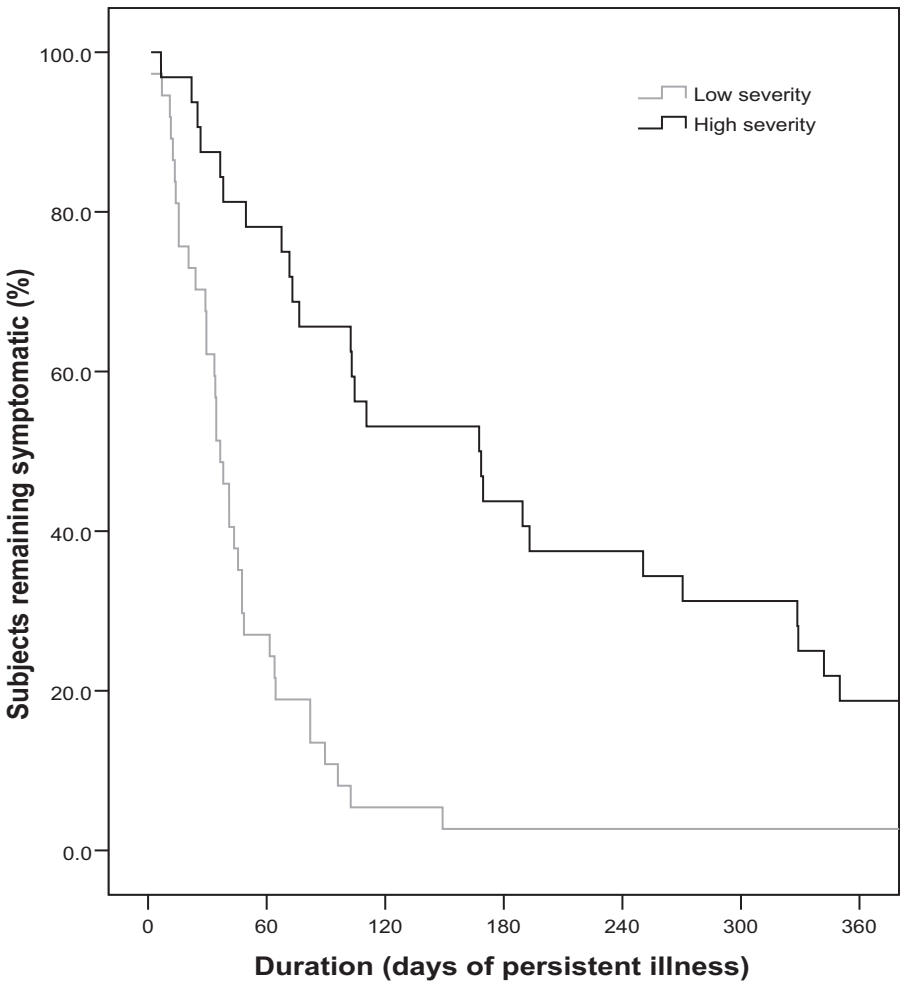

Figure 1. Severity predicts duration of illness. A severity factor score derived by PCA was used to divide the cohort by mean split into those suffering high ($n=32$) and low ($n=37$) severity of acute Q fever. Duration of illness was calculated from the number of days each subject remained symptomatic following the self-reported symptom onset date.

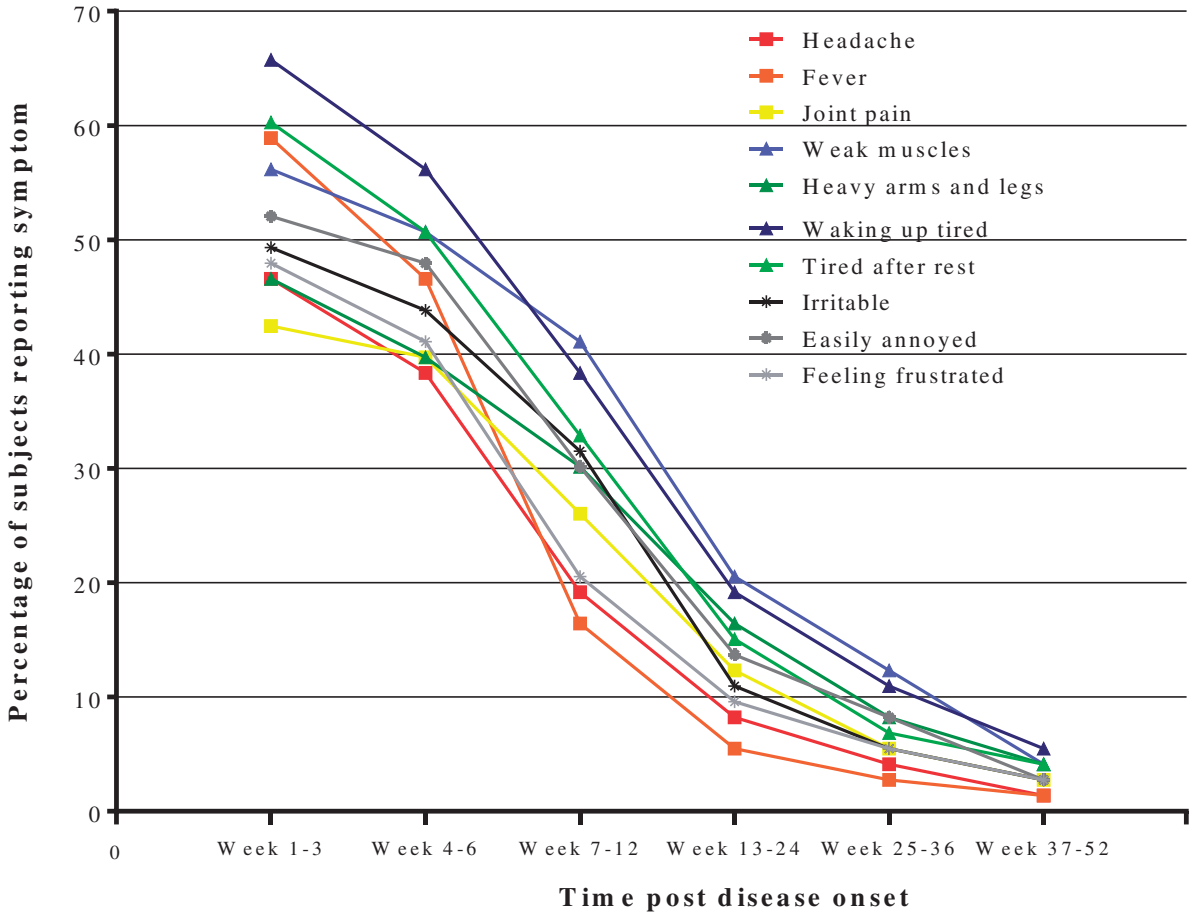

Figure 2. Persistence of symptoms following acute Q fever. Data collected from longitudinal self-report data from the SPHERE questionnaire ($n = 73$) for the 10 most frequently reported symptoms at enrolment.

Discussion

This study provides a systematic documentation of the natural history of serologically confirmed acute Q fever in subjects without chronic, localized infection and an examination of markers of host immune responses and persistent pathogen nucleic acid detection in the case of prolonged illness course.

Although 115 subjects were provisionally diagnosed with acute Q fever on the basis of a suggestive clinical illness and detection of *C. burnetii* IgM, 42 diagnoses were subsequently unconfirmed as IgG seroconversion or 4-fold changes in IgG antibody titres were not found. This affirms the recommendation that a definitive diagnosis of acute Q fever requires testing of longitudinally collected sera to detect changes in IgG antibody production.[14] False-positive Q fever IgM antibody results have been found in subjects suffering from other infectious diseases which may present similarly, including those resulting from *Mycoplasma pneumoniae*, *Bordetella pertussis* or *Legionella* sp infections[15,16] and also acute viral infections, notably with Epstein-Barr virus.[17] *C. burnetii* IgM ELISAs have considerably lower specificity than equivalent tests for IgG.[18] High pre-existing titres of *C. burnetii*-specific IgG may also interfere and cause false positive IgM test results.[19]

In this cohort, contact with livestock was the predominant risk factor for contraction of Q fever, reflected by an over-representation of men from occupational exposure. A subset of subjects (15%) did not report any contact with livestock in the 6 weeks prior to illness onset, suggesting alternative exposure mechanisms, perhaps via inhalation of windborne contaminated dust. This incidence argues for a broadening of the existing vaccination program in Australia, which targets those with occupational exposure only, to the general rural population.[20]

Windborne transmission has been implicated in other studies of Q fever outbreaks, where there has been no proximate contact between infected animals or their waste products and the humans who become infected.[21,22] In this context, illness onset was more common in spring and summer months, which may relate to the timing of lamb and calf births. Interestingly, an analysis of the yearly rainfall during the recruitment period described here, which included several years of drought, revealed a strong reciprocal relationship between falling annual rainfall with rising Q fever incidence (Lloyd A *et al.*, in preparation).

The most common end-organ complication of acute Q fever was biochemical hepatitis, occurring in almost half of cases, whereas pneumonia occurred in only two subjects, consistent with an earlier Australian report.[23] In the south of France, hepatitis complicating acute Q fever is more common than pneumonia, while in The Netherlands, Canada and the Basque region of Spain, pneumonia is a more common manifestation of infection.[24–27] The biological basis of these varied manifestations is unknown, but may result either from strain variation in *C. burnetii* or genetic factors of the host population.

In this cohort, the acute Q fever illness was often severe, evidenced by a high rate of hospitalization, substantive disability

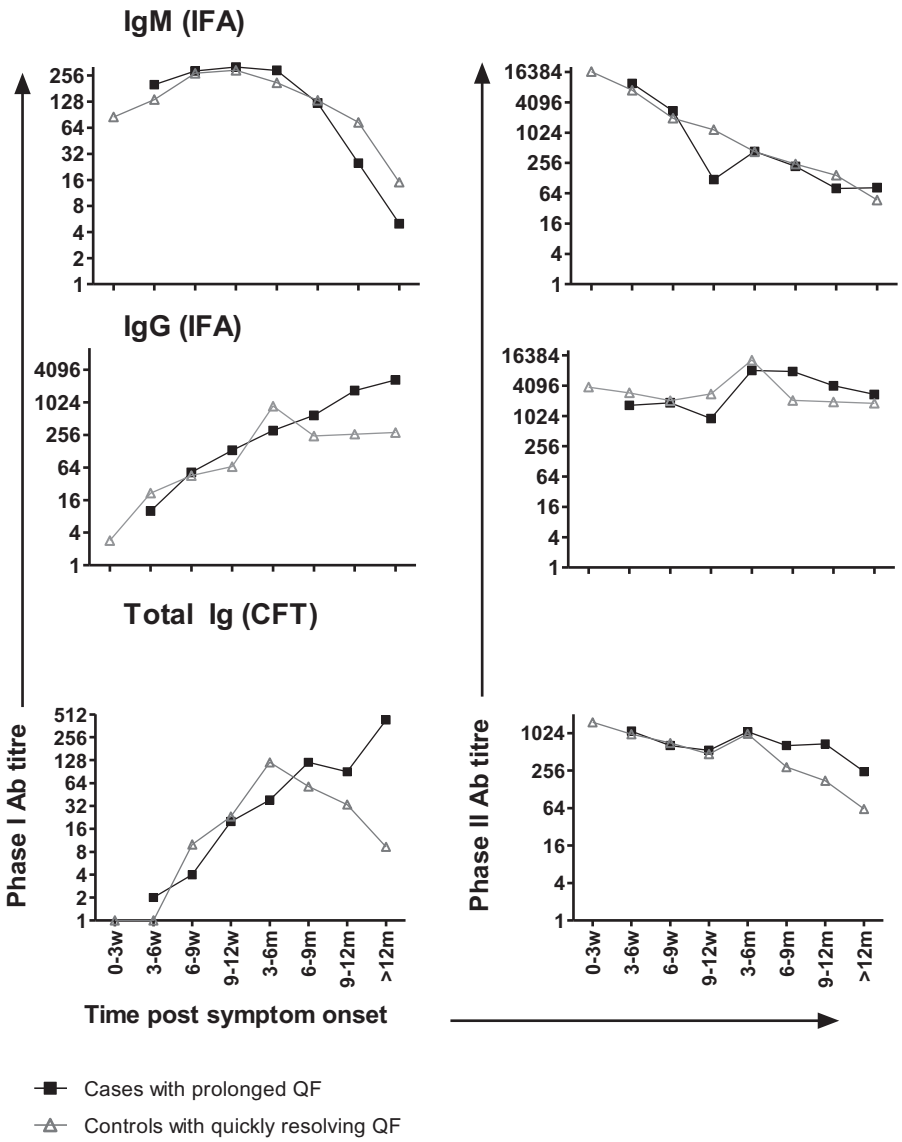

Figure 3. Mean antibody titers in subjects with quickly resolving ($n = 15$) or prolonged ($n = 6$) symptoms following acute Q fever. Phase I specific antibody (left column) is consistently present at lower titres than phase II specific antibody (right column). IgM and IgG titres were determined by immunofluorescence assay (IFA), and total Ig was determined by complement fixation test (CFT).

and significant health care utilization following diagnosis in the majority of subjects. The disability and health care needs continued for many months in a significant minority of subjects who reported fatigue and other physical or psychological symptoms of illness lasting 6 months or longer.

The role of antibody responses in the protective host response in Q fever is poorly understood, with most emphasis placed on antibody measurement simply as a diagnostic tool. IgG and IgM antibodies directed against phase II organisms appear early in infection and are maintained for several months following acute Q fever.[14,28] By contrast, IgA and IgG responses directed against phase I organisms are considered diagnostic of chronic Q fever,[19] although some dispute this.[14] In this cohort, antibody production profiles followed typical patterns with early high titre IgM responses and more delayed IgG responses of lower magnitude, with phase II-specific responses occurring earlier and at higher magnitude than phase I-specific responses. IgA was detected at later time points in a subset of subjects, although none had evidence of chronic, localized Q fever, and this IgA response was not associated with prolonged illness.

Similarly, no significant differences in circulating leucocyte sub-populations was found in subjects with quickly resolving or prolonged Q fever. Thus, it does not appear that QFS is associated with altered humoral or cellular responses to infection—at least as evidenced by these assays. By contrast, severe acute phase manifestations of the infection correlated strongly with a prolonged illness, confirming another study[29] and our earlier report of prolonged symptoms following Epstein-Barr virus (EBV) and Ross River virus (RRV) infections.[8] We have previously demonstrated that production of pro-inflammatory cytokines correlates with symptom severity in acute Q fever,[30] but not during the post-infective illness.[8] Accordingly, we hypothesize that the acute illness may trigger sensitization of the central nervous system[31] resulting in QFS.

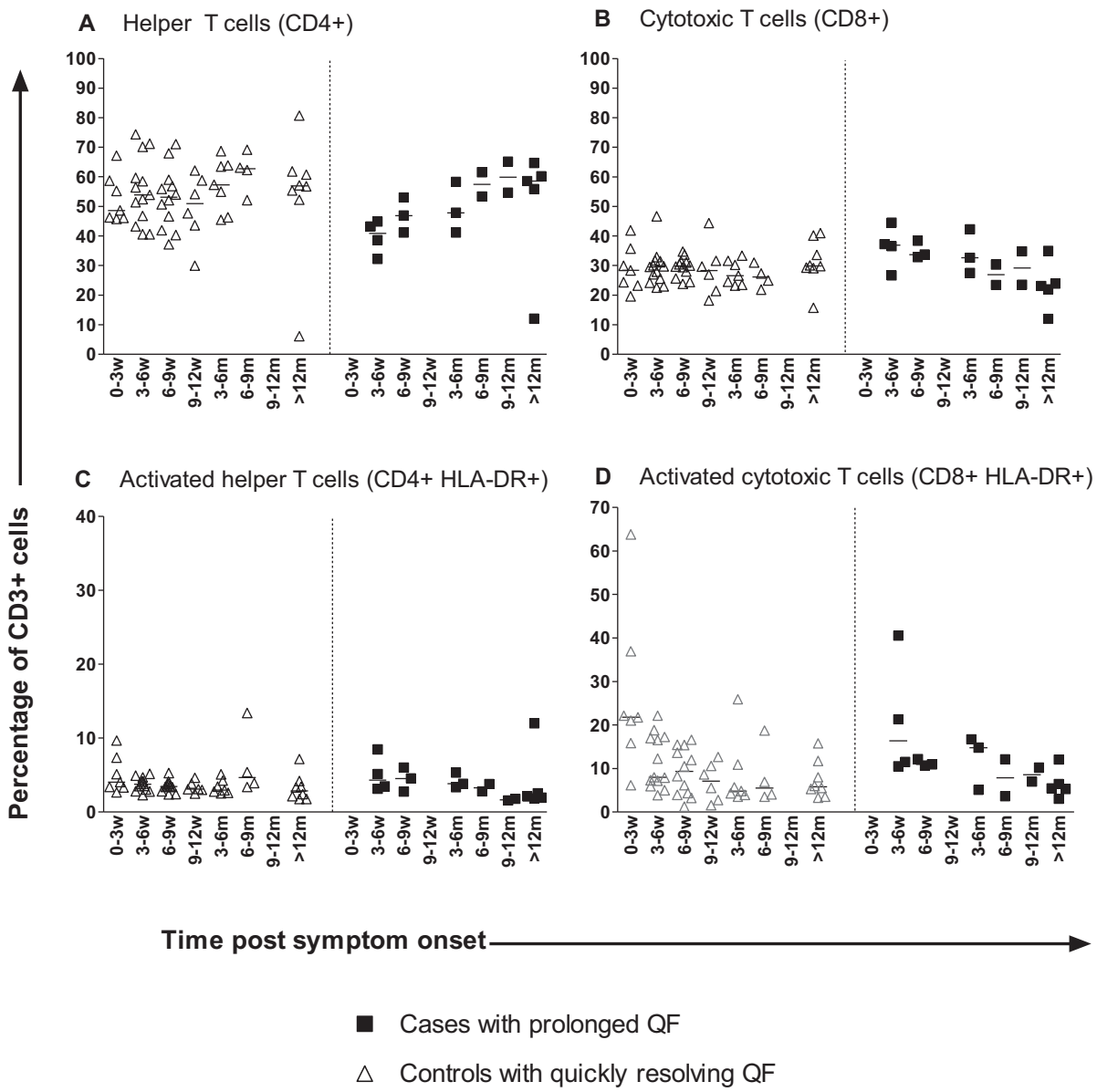

Figure 4. Longitudinal changes in leukocyte proportions and activation status. Flow cytometry was used to quantitate proportions of CD4+ helper T cells (A), CD8+ cytotoxic T cells (B), activated (CD69+) helper T cells (C) and activated (CD69+) cytotoxic T cells (D) as a proportion of total CD3+ cells in cases (■ $n = 6$) and control subjects (Δ $n = 15$).

Unlike previous reports[32,33] our study does not support the assertion of persistence of *C. burnetii* nucleic acids in association with QFS. *C. burnetii* DNA was detected in very few samples and without clear relationship to case or control status. Nevertheless, it remains possible that persistence of very few organisms or their antigenic remnants may still drive ongoing disease manifestations[34] or tissue-based infection (e.g. bone marrow or spleen) may harbour persistent organisms without infection in circulating leucocytes. This hypothesis is supported by the reported detection of *C. burnetii* DNA in 65% of bone marrow aspirate samples, but only 17% of circulating leucocyte samples, from patients with QFS using sensitive PCR techniques.[32] An uncontrolled pilot study of antibiotic treatment suggested benefit in QFS, suggesting that presence of viable organisms may contribute to the ongoing symptoms.[33] However, others have found evidence of *C. burnetii* DNA within bone marrow aspirates up to 12 years post-infection in a small proportion of subjects without ongoing illness.[35]

Conclusions

Q fever remains an important vaccine-preventable zoonotic infection worldwide. While disease severity and duration vary significantly, those with more severe acute illness have a significantly longer duration of illness. Further studies are warranted to better understand the pathophysiology of prolonged illness after acute Q fever.

Acknowledgements

This project was supported by a Cooperative Research Agreement with the Centers for Disease Control and Prevention (No U50/CCU019851-01), and by a Project Grant from National health and Medical Research Council of Australia (No 157902). A.R.L. is supported by an NHMRC Practitioner Fellowship (No. 1043067). The support of the general practitioners in the study regions and the subjects with acute Q fever who participated in this research are both gratefully acknowledged.

Funding

This project was supported by a Cooperative Research Agreement with the Centers for Disease Control and Prevention (No U50/CCU019851-01), and by a Project Grant from National health and Medical Research Council of Australia (No 157902). A.R.L. is supported by an NHMRC Practitioner Fellowship (No. 1043067).

Conflict of interest: None declared.

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