Eur. J. Clin. Microbiol. Infect. Dis., 1995, 14: 421–427

Validation of an Enzyme Immunoassay for Serodiagnosis of Acute Q Fever

D. Waag[1]*, J. Chulay[2], T. Marrie[3], M. England[1], J. Williams[4]

An enzyme immunoassay was validated for the serodiagnosis of acute Q fever. Minimum positive tests were determined for both serial dilutions and a single dilution of patient sera. To establish the specificity of the test, 152 serum samples were tested from individuals with no evidence of past *Coxiella burnetii* infection. Diagnostic titers were set at ≥ 128 for the IgM and IgG responses to phase I, at ≥ 512 for the IgM response to phase II and at ≥ 1,024 for the IgG response to phase II *Coxiella burnetii*. These titers gave a false-positive rate of ≤ 1 %. Alternatively, testing a single dilution of sera (1:128) gave specificities ranging from 97.3 to 98.7 %. Tests with the greatest sensitivities, using serially diluted early convalescent-phase sera, were the IgM (84 %) and IgG (80 %) responses to phase II *Coxiella burnetii*. At a single serum dilution, 92 % of early convalescent sera had a positive IgG response to phase II *Coxiella burnetii*. With a high specificity and good sensitivity, the EIA can be used to diagnose acute Q fever with a single convalescent serum specimen. The duration of a positive response was greater than five years.

Q fever, caused by the intracellular bacterium *Coxiella burnetii*, is generally an acute and self-limited febrile illness that rarely causes a chronic debilitating disease. Although domestic livestock usually acquire and transmit *Coxiella burnetii*, domestic pets can also be a source of infection (1–4). Infection is most commonly acquired by breathing infectious aerosols. Less frequent portals of entry include ingestion of infected milk (5) and parenteral acquisition caused by the bite of an infected tick (6). *Coxiella burnetii* infection may be asymptomatic or characterized by fever, pneumonia, headache, myalgias, arthralgias, cough, asthenia, hepatitis and occasionally splenomegaly and meningoencephalitis (7, 8). The differential diagnosis of acute Q fever presents unique problems for the clinician because there are few characteristic signs or symptoms that distinguish it from other febrile illnesses, such as influenza and pneumonia caused by mycoplasma or chlamydia infection.

Because cultivating *Coxiella burnetii* or working with the native microorganism can be hazardous to laboratory personnel, the diagnosis of Q fever is usually based on serological testing. Although specific cellular immune responses may be suppressed in cases of acute Q fever, humoral immune responses appear to continue unabated during infection (9, 10). Due to the relative ease of assaying serum samples for antibodies, serological profiles of patients with Q fever have been established (10). Thus, clinicians frequently encounter situations where a presumptive diagnosis of acute Q fever is considered likely enough to warrant treatment.

While the complement fixation assay (CFA) is generally regarded as the most specific serological assay for Q fever, the indirect fluorescent antibody assay (IFA), the microagglutination assay (MAA) and the enzyme immunoassay (EIA) can provide positive results earlier in the course of an infection (11). Recent results showed good correlation between the IFA and EIA when testing sera from acute Q fever patients and patients diagnosed with other bacterial diseases (12). Determination of antibodies against phase I and phase

[1] Pathogenesis and Immunology Branch, Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702–5011, USA.
[2] Department of Infectious Diseases and Immunology, Burroughs Wellcome Company, 3030 Cornwallis Road, Research Triangle Park, North Carolina 27709, USA.
[3] Victoria General Hospital, Halifax, Nova Scotia, Canada B3H 2Y9.
[4] Division of Vaccines and Related Products Application, Office of Vaccine Research and Review, Food and Drug Administration, Rockville, Maryland 20852, USA.

II *Coxiella burnetii* may help distinguish between acute and chronic Q fever (10).

Although a fourfold rise in the convalescent serological titer against the suspected microorganism is generally accepted as indicating a causal relationship with a particular illness, we are frequently asked to confirm a diagnosis of Q fever based on titers from a single convalescent-phase serum sample. We have analyzed convalescent-phase sera from cases of acute Q fever and from a population of Q fever–susceptible individuals with no evidence of past or present infection with *Coxiella burnetii* to establish serological criteria for clinical diagnosis of acute Q fever using a single serum specimen.

Materials and Methods

Positive Patient Sera. A total of 51 serological specimens were tested from patients diagnosed with Q fever by fourfold or greater rises in IFA serological titers. Of these, 40 serum samples were collected over four months from 16 patients during the course of an investigation of a Q fever outbreak on Prince Edward Island, Canada, which occurred in the fall of 1986 (13). Of the remaining 11 samples, nine convalescent-phase sera from persons diagnosed with acute Q fever were obtained from the Centers for Disease Control (CDC; Atlanta, USA). Two convalescent sera obtained from laboratory-infected persons were provided by The Salk Institute (Swiftwater, PA, USA).

Depending on the number of days after the onset of acute Q fever symptoms that sera were collected, all of the serological specimens were categorized as early convalescent phase (days 19–30), intermediate convalescent phase (days 67–75) or late convalescent phase (days 98–107). Twenty-five early convalescent phase, 14 intermediate convalescent phase and 12 late convalescent phase sera were used in this analysis. No more than one serum specimen from each patient was included in each group. No samples were available in the intervening time intervals.

Patient sera from the Canadian acute Q fever outbreak were also obtained 223 to 251 days (n = 7) and 2,051 to 2,062 days (n = 8) after the onset of acute Q fever. These samples were used to evaluate the EIA as an epidemiological screening technique for determination of previous cases of acute Q fever.

Negative Patient Sera. In addition to sera from the well-defined cases of acute Q fever described above, 152 serum samples were obtained from persons presumably susceptible to Q fever who had no evidence of a previous *Coxiella burnetii* infection. The CDC provided 39 convalescent control sera from patients with febrile illnesses other than Q fever. Of those 39 samples, ten sera were from individuals diagnosed with ehrlichiosis (*Ehrlichia chaffeensis*), five had spotted fever (*Rickettsia rickettsii*), five had murine typhus (*Rickettsia typhi*), nine had legionnaires' disease (*Legionella*) and ten were from normal controls. In addition, the CDC also provided six acute serum specimens having IFA titers of < 16 from six patients with acute Q fever.

Thirteen acute serum samples (one sample per patient) from the Canadian outbreak of 1986 had been collected fewer than eight days after onset of Q fever and tested negative for antibodies against *Coxiella burnetii* by the IFA. Five serum samples were obtained from five individuals who later developed Q fever (4 serum samples were from The Salk Institute and 1 was from the United States Army Medical Research Institute of Infectious Diseases [USAMRIID]). Two of these samples may have been drawn during the incubation period of acute Q fever; they were obtained two and five weeks before clinical diagnosis of acute Q fever. The remaining three samples were obtained from healthy individuals 6 to 14 months before acute Q fever was diagnosed.

Eighty-nine serum samples from healthy individuals were obtained from volunteers at USAMRIID. These individuals consisted of males and females between the ages of 18 and 55 who had no clinical history of Q fever. They also had a negative skin test reaction to *Coxiella burnetii* antigens and had no evidence to suggest previous exposure to *Coxiella burnetii* antigens as measured by the lymphocyte proliferation assay.

Positive and negative control sera (1 each) were included in each daily EIA. No effort was made to exclude individual sera from analysis on the basis of patient age, sex or geographic location.

Enzyme Immunoassay. An EIA was used to test serum specimens for the presence of specific IgA, IgG and IgM antibodies to *Coxiella burnetii.* Serum specimens were tested for reactivity to *Coxiella burnetii* phase I Nine Mile strain, clone 7, whole cells; phase II Nine Mile strain, clone 4, whole cells; and phase I Ohio strain lipopolysaccharide (LPS) (14). The phase I and phase II viable *Coxiella burnetii* were purified from hen yolk sacs by density gradient centrifugation (15). Microorganisms were suspended in phosphate-buffered saline (PBS) containing 0.25 M sucrose and were killed by irradiation (2.1 Mrads) while on dry ice. Formalin was added to a final concentration of 1 %, and the suspension was stirred continuously at 4°C for 48 h. The formalin was removed by dialysis against sterile water (3.5 kDa molecular mass cutoff) for five days at 4°C with daily changes of water. *Coxiella burnetii* LPS was prepared from irradiated and formalin-treated cells as previously described (14).

The EIA was similar to that described previously (13). Optimum dilutions of *Coxiella burnetii* antigens were previously determined by titration in carbonate-bicarbonate buffer, pH 9.6. Fifty µl of antigen per well (1.25 µg dry weight) were dispensed into selected rows of 96-well microtiter plates (Immunolon II, Dynatech, USA). The antigens were dried in the wells by incubating the plates at 37°C overnight in a dry air incubator. The plates were stored until use (up to 12 months) in plastic sleeves at room temperature. All wells of the antigen-coated microtiter plates were blocked before use with sodium carbonate-bicarbonate buffer containing 0.25 % gelatin (60 bloom) (250 µl/well), and the plates were incubated at 37°C for 1 h. The microtiter plates were washed five times with PBS-Tween (0.05 %) between each step, with a plate washer (Microplate II; Skatron, USA). Convalescent-phase test sera were diluted twofold in the microplate wells at dilutions ranging from 1:64 through 1:32,

768 with PBS-Tween containing 0.5 % gelatin as the diluent (final volume in wells was 50 µl). Normal sera were diluted from 1:16 through 1:32, 768. The plates were incubated for 1 h at 37°C and washed as described above. One hundred µl of class-specific goat anti-human immunoglobulin conjugated to alkaline phosphatase (Kirkegaard and Perry, USA) was added to each well. Plates were then incubated an additional 1 h at 37°C. The conjugate had been titrated against 10 ng of human mu, gamma or alpha heavy chain per well. The highest dilution of conjugate giving an optical density reading of 1.0 (at 405 nm) after incubation for 1 h at 37°C was used as the working concentration.

The plates were washed, and enzyme substrate (p-nitrophenylphosphate disodium, one 5 mg tablet/10 ml of buffer; Sigma, USA) in diethanolamine buffer (0.03 M, pH 9.8) was added in a volume of 100 µl. The plates were incubated for 1 h at 37°C. The enzyme reaction was stopped by adding 50 µl/well of 3 M sodium hydroxide. The color change was assessed in a MR600 microplate reader (Dynatech) at a test wavelength of 405 nm and a reference wavelength of 630 nm. Blanks were read from a well containing only enzyme substrate. Titration endpoints were determined by noting the highest serum dilution with a minimum difference of 0.05 absorbence units between the nonantigen- and antigen-containing wells. For statistical purposes, convalescent sera with endpoint titers of < 64 were assigned a titer of 32, and normal sera with endpoint titers of < 16 were assigned a titer of 8. Sera without endpoints were retested at dilutions through 1:262, 144. Cumulative titers (reciprocals of sample dilutions up to and including the endpoint titer) were determined, and the percentage of sera positive at a particular titer was plotted against that titer. For example, if a serum sample had an endpoint titer of 256, that sample was scored positive at titers of 64, 128 and 256. The IgA, IgG and IgM titers to phase I and phase II cellular antigens and *Coxiella burnetii* LPS were evaluated.

We tested an alternative serodiagnostic method which would conserve reagents and allow more serum samples to be tested per day. This would be important in epidemiological screening for Q fever prevalence or in acute Q fever epidemics. Sera were evaluated at a single 1:128 dilution. The optical density (OD) was determined, and the OD of the adjacent nonantigen-containing well was subtracted from the OD of the phase II Nine Mile– and from the phase I Nine Mile–containing well to give the ΔOD for the IgG and IgM responses.

Specificity. The specificity of the EIA was determined by analysis of the sera collected from 152 individuals who had a negative Q fever skin test, a negative history and a negative in vitro test (LPA) for Q fever. Serum specimens were included from patients with other established clinical diagnoses. By evaluating antibody titers, the specificity of the test was set at 99 % or greater by choosing diagnostic titers exceeded by ≤ 1 % of these sera.

The mean ΔOD at a serum dilution of 1:128 plus three standard deviations was determined for serum specimens from the 152 individuals, including those diagnosed with non-*Coxiella burnetii* bacterial diseases and those negative for Q fever. This value is the minimum diagnostic ΔOD. If the ΔODs of these serum specimens were normally distributed, 1 % of the specimens would statistically be expected to exceed the diagnostic ΔOD. The specificity of the test was empirically determined as the percentage of sera from 152 Q fever negative individuals exceeding the diagnostic ΔOD.

Sensitivity. The sensitivity of the EIA was determined by analysis of the 51 sera collected from individuals having a laboratory-confirmed diagnosis of acute Q fever. Sensitivity was calculated as the percentage of sera (early, intermediate or late convalescent, or sera obtained 8 months or 5.5 years after the onset of acute Q fever) meeting or exceeding the minimum diagnostic criteria (titer or ΔOD).

Results

Enzyme Immunoassay Specificity. IgM, IgG and IgA antibody titers to *Coxiella burnetii* phase I, phase II and phase I LPS antigens were determined for 152 sera from patients with bacterial diseases other than Q fever and from individuals with no history or laboratory evidence suggestive of past *Coxiella burnetii* infection. Not more than 1 % of these normal sera were positive for phase II antigen at dilutions of 1:512, 1:1,024 and 1:128 for IgM, IgG and IgA, respectively (Table 1). The phase I antigen was less reactive, and ≤ 1 % of normal sera were positive at dilutions of 1:128,

Table 1: Summary of EIA endpoint titers to support a clinical diagnosis of acute Q fever.

Antibody/antigen	Diagnostic titer (n = 152)	False-positive results (%)	Sensitivity		
			Early (n = 25)	Intermediate (n = 14)	Late (n = 12)
IgA/II	128	1	44	93	83
IgG/II	1,024	0	80	93	100
IgM/II	512	1	84	57	58
IgA/I	64	0	4*	14*	17*
IgG/I	128	1	24	71	83
IgM/I	128	1	48	57	50

*Too few samples were tested at lower dilutions to evaluate the sensitivity of the assay at lower antibody titers.

Table 2: Geometric mean endpoint titers of IgA, IgG and IgM to *Coxiella burnetii* phase I, phase II and lipopolysaccharide.[a]

Antibody/antigen	Normal[b] (SD)	Early[c] (SD)	Intermediate[d] (SD)	Late[e] (SD)
IgA/I	8 (0)	32 (1)	39 (2)	36 (1)
IgA/II	9 (1)	105 (4)	464 (3)	683 (6)
IgA/LPS	8 (0)	32 (0)	32 (0)	32 (0)
IgG/I	10 (2)	53 (2)	297 (5)	724 (7)
IgG/II	39 (4)	1,938 (5)	12,173 (4)	23,170 (3)
IgG/LPS	9 (1)	32 (0)	32 (0)	34 (1)
IgM/I	9 (2)	132 (4)	156 (4)	102 (3)
IgM/II	21 (3)	1,144 (4)	594 (5)	456 (3)
IgM/LPS	8 (1)	40 (2)	37 (2)	32 (0)

[a] Human convalescent-phase sera with endpoint titers < 64 were assigned a titer of 32, and normal sera with an endpoint titer < 16 were assigned a titer of 8.
[b] Sera collected from individuals with no evidence of prior Q fever.
[c] Sera collected 19–30 days after onset of acute Q fever.
[d] Sera collected 67–75 days after onset of acute Q fever.
[e] Sera collected 98–107 days after onset of acute Q fever.
SD: geometric standard deviation.

Table 3: Sensitivity of the EIA using diagnostic ΔOD value to evaluate antibody response to *Coxiella burnetii* antigens in convalescent sera from patients with acute Q fever which met or exceeded the diagnostic ΔOD at a 1:128 dilution of serum.

Antibody/antigen	Percent sensitivity		
	Early	Intermediate	Late
IgG/I	52	86	100
IgG/II	92	93	100
IgM/I	48	71	50
IgM/II	88	71	43

1:128 and 1:64 for IgM, IgG and IgA, respectively. Less than 1 % of normal sera reacted positively to *Coxiella burnetii* LPS at dilutions of 1:64 for IgM and IgG (data not shown). No signal was detected in the IgA response to LPS, even at the lowest dilution of sera (1:16; data not shown). We chose, as diagnostic titers, reciprocals of those dilutions where a false-positive rate would not be expected to exceed 1 % (Table 1).

Using diagnostic ΔOD values of 0.134, 0.036, 0.098 and 0.033 for the IgG response to phase I and phase II antigen and the IgM response to phase I and phase II antigen, respectively, 97.3, 98.7, 98.7 and 98.0 % of the 152 Q fever negative sera were identified as negative. Statistically, we expected a 1 % false-positive rate. However, the actual false-positive rate was between 1.3 and 2.7 % for the four tests that we performed.

Enzyme Immunoassay Sensitivity. Sera obtained at various times after the onset of acute Q fever were tested for antibodies reactive with *Coxiella burnetii* phase I, phase II and LPS antigens. Geometric mean IgA and IgG titers to phase II antigen and IgG titers to phase I antigen progressively increased after onset of acute Q fever (Table 2). Mean IgM titers to phase II *Coxiella burnetii* were highest when testing early convalescent sera, while the IgM response to phase I *Coxiella burnetii* peaked when intermediate convalescent sera were tested.

At a diagnostic titer of 512, 84, 57 and 58 % of early, intermediate and late convalescent-phase sera, respectively, were positive for IgM anti-phase II antibody. Table 1 shows the sensitivity of the IgG, IgM and IgA responses of Q fever convalescent-phase sera to phase II and phase I *Coxiella burnetii* antigens. The sensitivity of the IgM response to phase II *Coxiella burnetii* was highest when early convalescent-phase sera were tested. In contrast, sensitivities of the IgG and IgM responses to phase I *Coxiella burnetii* were highest when the late and intermediate convalescent-phase sera, respectively, were tested. Sera reacted poorly with *Coxiella burnetii* LPS antigen; a maximum of 25 % of sera from acute Q fever patients was positive (data not shown).

When the early, intermediate and late convalescent-phase sera were evaluated by diagnostic ΔOD, the sensitivity of the test ranged from 43 to 100 % (Table 3). The most sensitive test for measuring a positive response with early convalescent-phase sera was the IgG response to phase II *Coxiella burnetii*, where 92 % of the specimens tested positive.

Table 4: Comparison of the sensitivity of the titer and diagnostic ΔOD methods to identify laboratory-confirmed cases of acute Q fever 8 months (n = 7) and 5.5 years (n = 8) after disease onset.

Antibody/antigen	Percent sensitivity			
	Titer		ΔOD	
	8 months	5.5 years	8 months	5.5 years
IgG/I	86	100	86	100
IgG/II	100	100	100	100
IgM/I	14	0	29	0
IgM/II	43	25	43	13

Sera from patients who developed acute Q fever symptoms approximately 8 months and 5.5 years previously were tested for antibodies reactive to phase I and phase II *Coxiella burnetii*. The ability to detect a high percentage of individuals previously diagnosed with acute Q fever suggests that the EIA is a good epidemiological screening tool. All the serum specimens collected 8 months and 5.5 years after the onset of acute Q fever had a positive IgG response to phase II *Coxiella burnetii* (Table 4). However, only a minority of these specimens were positive for IgM antibodies to *Coxiella burnetii* antigens.

Discussion

A useful serological assay for *Coxiella burnetii* infection can identify persons who have (had) Q fever (sensitivity), while correctly excluding persons who do not have Q fever (specificity). In general, as the sensitivity of the serological test increases (by lowering the qualifying diagnostic titer), the specificity of the assay tends to decrease, which produces more false positives. Our objective in validating this serological assay was to determine diagnostic titers to specific antigens that maximized both sensitivity and specificity. A disease where the signs and symptoms are not characteristic (such as Q fever), occurring in a setting where the probability of a Q fever–like illness actually being Q fever is rather low, demands that the specificity of the serological assay be high to reduce the number of false-positive results. This ensures that useful information is provided to clinicians evaluating a case.

A recent study showed that the specificity and sensitivity of the Q fever EIA was equivalent to the IFA when used to evaluate sera from confirmed cases of acute Q fever and other bacterial diseases (12). In addition, the IFA gave good sensitivity (50 and 70 % of acute Q fever cases, respectively) when testing sera three and four weeks after the onset of clinical acute Q fever (16). Therefore, use of either test is based largely on personal preference. Because the EIA can be automated, the ability to test large numbers of sera in an epidemiological survey or a disease outbreak would favor this assay. While others report the ability to diagnose Q fever by complement fixation or IFA two weeks after onset (17–20), we did not have serum samples that allowed us to evaluate the sensitivity of our EIA at that time.

We chose to compare two methods for determining a positive test, first by generating a diagnostic titer by serial dilution of the specimen where no more than 1 % of the sera from individuals with no evidence of *Coxiella burnetii* infection tested positively. As an alternative, a test format involving a single dilution of serum may be sufficiently sensitive and specific, be more convenient and conserve reagents.

Due to the low sensitivity of the IgM and IgG antibodies in recognizing phase I *Coxiella burnetii* within one month of disease onset, tests for these antibodies provide little information to assist in the diagnosis of recent acute Q fever. The sensitivity of antibody responses to *Coxiella burnetii* LPS was too low to be useful in the diagnosis of acute Q fever. In contrast, IgM and IgG responses to phase II microorganisms were sensitive (84 and 80 %, respectively) and specific (≥ 99 %) using titrations of early convalescent-phase sera. When sera were scored positive or negative by diagnostic ΔOD, the EIA was 1 to 2 % less specific than when sera were evaluated by endpoint titration. However, the sensitivity was improved for this single dilution method. Eighty-eight and 92 % of sera from acute Q fever patients tested positive for IgM and IgG antibodies, respectively, to phase II *Coxiella burnetii*. The cost of greater sensitivity of the single dilution screening method was lower specificity compared to the titration method.

While our results showed that the IgG and IgM responses to phase II *Coxiella burnetii* were the most sensitive in diagnosing Q fever using early convalescent-phase sera, determining a significant antibody response to phase I *Coxiella burnetii* could help clinicians distinguish the acute from the chronic forms of the disease (10). Including phase I antigen in a test battery for acute Q fever may also help determine whether the infection is recent. Because a higher proportion of early convalescent-phase sera had titers for phase II than phase I microorganisms in this study, the presence of antibody to phase II *Coxiella burnetii* and the absence of antibody (IgG) to phase I *Coxiella burnetii* supports a diagnosis of acute Q fever infection occurring within the preceding six months.

If the diagnosis of acute Q fever (current or retrospective) is the primary objective and only a single test is to be used, we found that the most sensitive test is the IgG antibody response to phase II *Coxiella burnetii*. By the diagnostic ΔOD method, the IgG response to phase II was the most sensitive from early convalescence through a period 5.5 years after the onset of acute Q fever symptoms. By the titration method, this antibody-antigen combination was also the most sensitive test, except for sera drawn in early convalescence, when the IgM antibody response to phase II *Coxiella burnetii* was marginally better. Clinicians desiring a single test which is sensitive when using early convalescent sera and less sensitive for sera drawn later should measure the IgM response to phase II *Coxiella burnetii*.

We were surprised at the persistence of IgM antibodies to phase II antigen in cases of acute Q fever. Fifty-eight percent of late convalescent-phase sera had levels of IgM antibodies to phase II whole cells which we considered diagnostic (Table 1). Twenty-five percent (2/8) of sera collected from individuals involved in the Canadian outbreak had diagnostic IgM antibody levels to phase II but not to phase I *Coxiella burnetii* more than five years after the onset of acute Q fever (Table 4). These individuals currently have no evidence of chronic Q fever and are completely recovered. We did not test for rheumatoid factor in this study. However, in a different study which was blinded, we did not identify any false-positive results in sera from persons with a variety of bacterial diseases (12). Persistence of the IgM response to *Coxiella burnetii* has also been noted in some, but not all, serological studies using the IFA (18). With the IFA, the IgM response to phase I antigen persisted for 27 weeks, but the IgM response to phase II antigen was not detectable beyond 17 weeks. In another study, the IFA IgM response to phase I and phase II *Coxiella burnetii* disappeared in most patients after 10 to 12 weeks (9). However, 3 % of patients with acute Q fever still had significant IgM titers after 52 weeks. Other researchers report that specific complement fixation and IFA IgM titers from a majority of patients involved in an outbreak of acute Q fever in England persisted for more than six months (17). Therefore, with the EIA and IFA, a positive IgM (or IgG) titer to *Coxiella burnetii* may not necessarily indicate a current case of acute Q fever.

In summary, we determined empirically serological diagnostic titers for the serodiagnosis of acute Q fever by EIA. This assay showed a high level of specificity (≥ 99 %). Using these criteria, the sensitivity of the assay was good (80 to 84 %) for confirming cases of acute Q fever using early convalescent-phase sera. Screening the sera at a dilution of 1:128 for IgG and IgM responses to phase II *Coxiella burnetii* also provided a specific and sensitive evaluation for acute Q fever. The diagnostic ΔOD method is a very sensitive and efficient method for screening sera for past cases of Q fever. Readers wishing to use the EIA should similarly validate their test using their standardized diagnostic antigens because test reagents may differ among laboratories.

Acknowledgements

The authors thank the Viral and Rickettsial Zoonoses Branch of the Centers for Disease Control, Atlanta, USA, and the Government Services Division of The Salk Institute, Swiftwater, PA, USA, for providing serum samples which made these studies possible.

Disclaimer

The views, opinions and/or findings contained in this manuscript are those of the authors and should not be construed as an official United States Department of the Army position, policy or decision unless so designated by other documentation.

References

1. Kosatsky T: Household outbreak of Q fever pneumonia related to a parturient cat. Lancet 1984, ii: 1447–1449.
2. Langley JM, Marrie TJ, Covert A, Waag DM, Williams JC: Poker players' pneumonia: an urban outbreak of Q fever following exposure to a parturient cat. New England Journal of Medicine 1988, 319: 354–356.
3. Laughlin T, Waag D, Williams JC, Marrie T: Q fever: from deer to dog to man. Lancet 1991, 337: 676–677.

4. Pinsky RL, Fishbein DB, Greene CR, Gensheimer KF: An outbreak of cat-associated Q fever in the United States. Journal of Infectious Diseases 1991, 164: 202–204.
5. Huebner RJ, Jellison WL, Beck MD, Parker RR, Shepard CC: Q fever studies in Southern California. I. Recovery of *Rickettsia burnetii* from raw milk. Public Health Reports 1948, 63: 214–222.
6. Davis GE, Cox HR: A filter-passing infectious agent isolated from ticks. I. Isolation from *Dermacentor andersoni*, reactions in animals, and filtration experiments. Public Health Reports 1938, 53: 2259–2267.
7. Ferrante MA, Dolan MJ: Q fever meningoencephalitis in a soldier returning from the Persian Gulf War. Clinical Infectious Diseases 1993, 6: 489–496.
8. Marrie TJ: Acute Q fever. In: Marrie TJ (ed): Q fever: the disease. Volume 1. CDC Press, Boca Raton, FL, 1990, p. 126–160.
9. Dupuis G, Peter O, Peacock M, Burgdorfer W, Haller E: Immunoglobulin responses in acute Q fever. Journal of Clinical Microbiology 1985, 22: 484–487.
10. Peacock MG, Philip RN, Williams JC, Faulkner RS: Serological evaluation of Q fever in humans: enhanced phase I titers of immunoglobulins G and A are diagnostic for Q fever endocarditis. Infection and Immunity 1983, 41: 1089–1098.
11. Williams JC, Thomas LA, Peacock MG: Humoral immune response to Q fever: enzyme-linked immunosorbent assay antibody response to *Coxiella burnetii* in experimentally infected guinea pigs. Journal of Clinical Microbiology 1986, 24: 935–939.
12. Uhaa IJ, Fishbein DB, Olson JG, Rives CC, Waag DM, Williams JC: Evaluation of the specificity of the indirect enzyme-linked immunosorbent assay for diagnosis of human Q fever. Journal of Clinical Microbiology 1994, 32: 1560–1565.
13. Embil J, Williams JC, Marrie TJ: The immune response in a cat-related outbreak of Q fever as measured by the indirect immunofluorescence test and the enzyme-linked immunosorbent assay. Canadian Journal of Microbiology 1990, 36: 292–296.
14. Amano KI, Williams JC: Chemical and immunological characterization of lipopolysaccharides from phase I and phase II *Coxiella burnetii*. Journal of Bacteriology 1984, 160: 994–1002.
15. Williams JC, Peacock MG, McCaul TF: Immunological and biological characterization of *Coxiella burnetii*, phases I and II, separated from host components. Infection and Immunity 1981, 32: 840–851.
16. Dupont HT, Thirion X, Raoult D: Q fever serology: cutoff determination for microimmunofluorescence. Clinical and Diagnostic Laboratory Immunology 1994, 1: 189–196.
17. Guigno D, Coupland B, Smith EG, Farrell ID, Desselberger U, Caul EO: Primary humoral antibody response to *Coxiella burnetii*, the causative agent of Q fever. Journal of Clinical Microbiology 1992, 30: 1958–1967.
18. Hunt JG, Field PR, Murphy AM: Immunoglobulin responses to *Coxiella burnetii* (Q fever): single-serum diagnosis of acute infection, using an immunofluorescence technique. Infection and Immunity 1983, 39: 977–981.
19. Lennette EH, Clark WH, Jensen FW, Toomb CJ: Q fever studies. IV. Development and persistence in man of complement-fixing and agglutinating antibodies to *Coxiella burnetii*. Journal of Immunology 1952, 68: 591–598.
20. Murphy AM, Magro L: IgM globulin response in Q fever (*Coxiella burnetii*) infections. Pathology 1980, 12: 391–396.