

A comparison of the rapid detection of *Coxiella burnetii* by real-time PCR and IF in a cohort of Australian Q fever patients.

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Introduction

Q fever occurs worldwide especially in areas with a high concentration of husbandry activities. Q fever in Australia usually occurs amongst abattoir workers and other related industries such as sheep shearing etc. Efforts by the government through implementation of a vaccination program and national notification system have markedly reduced the number of infections nation-wide. Infections do still occur with 310 cases reported in 2010. Unrecognized clinical and subclinical infections are estimated to be five times higher than the number reported.

Immunofluorescence assay (IF) is the gold standard in Q fever diagnostics due to its high sensitivity and rapid turnaround. However, a convalescent serum sample (12-20 days after acute sample) is needed in most cases to confirm diagnosis by either seroconversion or a four-fold increase in antibody titre. Polymerase chain reaction (PCR) has the advantage that results are often positive early in the course of the disease but the ability to detect the presence of the organism declines dramatically once antibodies start to form.

Method

A real-time PCR assay targeting a fragment of the IS1111 sequence was used to screen serum samples from confirmed Q fever patients (n=63) retrospectively. Samples were collected from within Australia over a 5 year period (2006-2010). Convalescent samples were collected 1 to 56 days following the acute specimen. Only convalescent samples corresponding to PCR-positive acute samples were tested (n=39).

DNA was extracted from serum specimens using a spin filter column based purification kit and assays were performed on a real-time PCR instrument.

Results

- *C. burnetii* DNA was detected by the real-time PCR assay in 49% (18/37) of the seropositive acute samples.
- Eighty-one percent (81%) of seronegative acute samples were PCR positive (21/26).
- Two out of three (2/3; 67%) convalescent samples collected less than 7 days after the acute specimen were PCR positive for *C. burnetii* DNA.
- Two out of twelve (2/12; 17%) convalescent samples collected between 7 to 14 days after the acute sample were positive for *C. burnetii* DNA.
- All convalescent samples collected > two weeks (n=24) were negative for *C. burnetii* DNA.

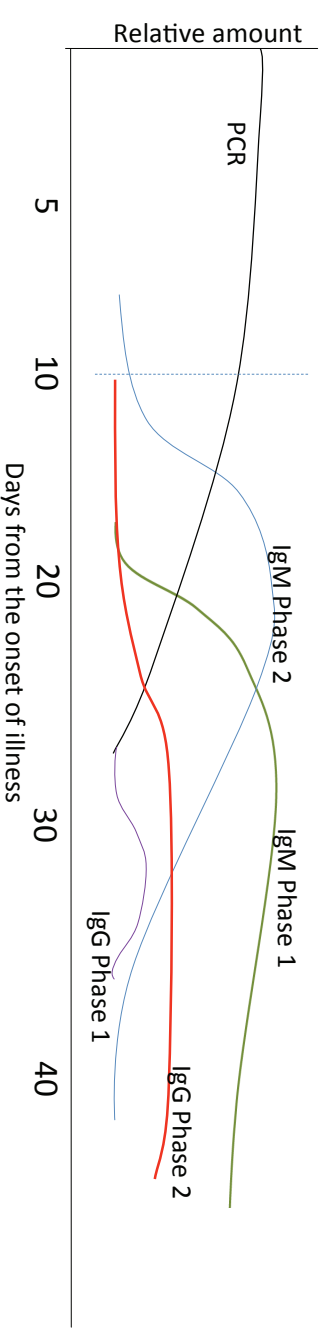


Figure 1– Idealized representation of DNA detection by PCR with relation to antibody levels based on clinical and laboratory experience at the IMVS, Adelaide and ARRL, Geelong. Acute samples on average are from Day 10.

Discussion

- Similar results were observed by Schneeburger *et al.* (2010) in samples from the Netherlands. However, only acute samples were available.
- Diagnosis of suspected Q fever cases in Australia with no detectable antibody titre in the acute sample can be performed by PCR.
- *C. burnetii* detection was increased from 59% (37/63) by IF to 92% (58/63) using both IF and PCR.
- Convalescent samples are usually obtained 12-20 days (recommendation to clinicians) after the acute sample. This usually equates to 20-30 days after onset of illness, thus DNA in serum at this time point is too low to be detectable by PCR (Fig. 1). However in practice, we received convalescent samples ranging from 1 to 56 days after the acute sample as observed in the study.

Conclusions

- Reinforces the important role that PCR analysis can play in early Q fever diagnosis from sero-negative acute serum samples received.
- IF on both acute and convalescent serum samples is still crucial in providing physicians with information of the disease in the patient.
- The variability of the number of days convalescent samples are collected post-acute sample may interfere with the interpretation of IF results as no significant increase in anti-*C. burnetii* antibodies would be observed if the convalescent sample is too close to the acute sample. PCR may help negate this problem by providing a definitive diagnosis if *C. burnetii* DNA is detected.
- PCR can dramatically increase sensitivity of diagnostic methods, but not entirely replace other tools in use.

References

Schneeburger PM, Hermans MH, van Hannen EJ, Schellekens JJ, Leenders AC, Wever PC. 2010. Real-time PCR of serum samples is indispensable for early diagnosis of acute Q fever. Clin Vaccine Immunol 17(2):286-290.



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