

## Research note

Fluorescence *in situ* hybridization for detecting *Coxiella burnetii* in tissue samples from chronic Q fever patients

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## ABSTRACT

**Objective:** Detection of the intracellular bacterium *Coxiella burnetii*, causative agent of chronic Q fever, is notoriously difficult. Diagnosis of and duration of antibiotic treatment for chronic Q fever is partly determined by detection of the bacterium with polymerase chain reaction (PCR). Fluorescence *in situ* hybridization (FISH) might be a promising technique for detecting *C. burnetii* in tissue samples from chronic Q fever patients, but its value in comparison with PCR is uncertain. We aim to assess the value of FISH for detecting *C. burnetii* in tissue of chronic Q fever patients.

**Methods:** FISH and PCR were performed on tissue samples from Dutch chronic Q fever patients collected during surgery or autopsy. Sensitivity, specificity, and overall diagnostic accuracy were calculated. Additionally, data on patient and disease characteristics were collected from electronic medical records. **Results:** In total, 49 tissue samples from mainly vascular walls, heart valves, or placentas, obtained from 39 chronic Q fever patients, were examined by FISH and PCR. The sensitivity and specificity of FISH compared to PCR for detecting *C. burnetii* in tissue samples from chronic Q fever patients was 45.2% (95% confidence interval (CI), 27.3% – 64.0%) and 84.6% (95% CI, 54.6% – 98.1%), respectively. The overall diagnostic accuracy was 56.8% (95% CI, 42.2% – 72.3%). Two *C. burnetii* PCR negative placentas were FISH positive. Four FISH results (8.2%) were deemed inconclusive because of autofluorescence.

**Conclusion:** With an overall diagnostic accuracy of 57.8%, we conclude that FISH has limited value in the routine diagnostics of chronic Q fever. **Sheila B. Buijs, Clin Microbiol Infect 2022;28:1502.e1–1502.e5**

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## Introduction

Q fever is caused by the obligate intracellular bacterium *Coxiella burnetii*. After primary infection, 1–5% of patients develop chronic Q fever: a persistent infection with *C. burnetii* of vascular walls, vascular prostheses, and/or heart valves [1]. In chronic Q fever

patients, the presence of *C. burnetii* in serum, blood, or tissue samples when available is routinely tested with polymerase chain reaction (PCR). When detected, antibiotic treatment is indicated, and prolonged detection has implication for the duration of treatment [2]. However, detection rates of *C. burnetii* PCR in tissue samples ranged between 78% and 100% [3–5].

**Abbreviations:** PCR, polymerase chain reaction; FISH, Fluorescence in situ hybridization; FFPE, Formalin-fixed, paraffin-embedded; Ct, Cycle Threshold; IgG, Immunoglobulin G.

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Fluorescence *in situ* hybridization (FISH), a diagnostic technique based on the binding of fluorescence-labelled target-specific DNA oligonucleotide probes to the RNA or DNA of pathogens [6], might be a promising technique for detecting *C. burnetii*. However, only case reports with mostly heart valves, and small studies in veterinary and human samples are available [4,7,8]. Therefore, we aimed to further examine the diagnostic value of FISH in tissue samples of chronic Q fever patients from the Dutch chronic Q fever cohort. We compared FISH results to PCR in a varied selection of tissue samples.

## Methods

### Study population and tissue samples

Tissue samples from Dutch patients with chronic Q fever were collected for this study. Patients were classified as having proven,

probable, or possible chronic Q fever based on the Dutch consensus guideline [9]. Data on diagnosis, focus of infection, and course of the disease was collected from the patients' electronic medical records. Tissue samples were obtained during routine care, i.e. during operation, biopsy, or autopsy, and were available in formalin-fixed, paraffin-embedded (FFPE) blocks.

### Polymerase chain reaction (PCR) and fluorescence *in situ* hybridization (FISH)

Laboratory-developed real-time PCR, targeting the multicopy IS1111 repetitive element of *C. burnetii*, was performed on all tissue samples in clinical practice [10]. All samples were analysed in duplicate.

FISH was performed on 3 µm thick tissue sections. Four oligonucleotide DNA-probes targeting 16S ribosomal RNA and the rRNA

**Table 1**

Tissue sample characteristics with details of diagnostic testing

| #  | Tissue                                 | Therapy       | Phase I IgG   | PCR   | Mean CT value | Obtained during | FISH     |
|----|----------------------------------------|---------------|---------------|-------|---------------|-----------------|----------|
| 1  | Vascular wall                          | None          | 128           | Neg   | NA            | Autopsy         | Neg      |
| 2  | Tissue surrounding valvular prosthesis | None          | 16.384        | Pos   | 6.51          | Autopsy         | Pos      |
| 2  | Vascular wall                          | None          | 16.384        | Pos   | 23.47         | Autopsy         | Neg      |
| 2  | Tissue surrounding valvular prosthesis | None          | 16.384        | Pos   | 6.51          | Autopsy         | Pos      |
| 3  | Placenta                               | None          | 256           | Neg   | NA            | Delivery        | Pos      |
| 4  | Placenta                               | TMP/SMX       | 256           | Neg   | NA            | Delivery        | Pos      |
| 5  | Vascular wall                          | None          | 128           | Pos   | 23.72         | Postoperative   | Pos      |
| 5  | Vascular wall                          | None          | 128           | Pos   | 21.28         | Autopsy         | Pos      |
| 6  | Tissue surrounding vascular prosthesis | None          | >4.096        | Pos   | 34.2          | Autopsy         | Neg      |
| 6  | Lung                                   | None          | >4.096        | Pos   | 32.8          | Autopsy         | Pos      |
| 6  | Myocardium                             | None          | >4.096        | Pos   | 29.91         | Autopsy         | Pos      |
| 7  | Vascular wall                          | None          | 65.536        | Pos   | Missing       | Postoperative   | Inconcl. |
| 8  | Mitral valve                           | None          | 2.048         | Pos   | 16.36         | Postoperative   | Inconcl. |
| 9  | Gallbladder                            | None          | 1.024         | Neg   | NA            | Postoperative   | Neg      |
| 10 | Lymph node                             | Doxy/HCQ <3m  | 4.096         | Neg   | NA            | Biopsy          | Neg      |
| 11 | Vascular wall                          | Doxy/HCQ      | 8.192         | Pos   | 18.86         | Autopsy         | Inconcl. |
| 11 | Aortic valve                           | Doxy/HCQ      | 8.192         | Pos   | 21.19         | Autopsy         | Neg      |
| 12 | Skin                                   | None          | 4.096         | Neg   | NA            | Biopsy          | Neg      |
| 13 | Lung                                   | Doxy/HCQ <3m  | 1.024         | Neg   | NA            | Postoperative   | Neg      |
| 14 | Aortic valve                           | None          | 64            | Neg   | NA            | Postoperative   | Neg      |
| 15 | Vascular wall                          | None          | 16.384        | Pos   | 34.73         | Postoperative   | Neg      |
| 16 | Vascular wall                          | Doxy/rifa     | 8.192         | Pos   | 28.59         | Postoperative   | Pos      |
| 17 | Vascular wall                          | Doxy/HCQ <3m  | 4.096         | Pos   | 22.81         | Autopsy         | Neg      |
| 17 | Lung                                   | Doxy/HCQ <3m  | 4.096         | Pos   | 30.85         | Autopsy         | Neg      |
| 18 | Vascular wall                          | None          | 16.384        | Pos   | 17.98         | Postoperative   | Pos      |
| 18 | Vascular wall                          | Doxy/HCQ      | 131.072       | Pos   | 24.38         | Postoperative   | Neg      |
| 19 | Liver                                  | None          | 8.192         | Neg   | NA            | Autopsy         | Neg      |
| 20 | Vascular wall                          | None          | 16.384        | Pos   | 18.17         | Autopsy         | Neg      |
| 21 | Aortic valve                           | Doxy/HCQ/moxi | 8.192         | Pos   | 41.00         | Postoperative   | Pos      |
| 22 | Placenta                               | None          | 65.536        | Pos   | 41.31         | Delivery        | Pos      |
| 23 | Myocardium                             | Doxy/moxi     | 4.096         | Pos   | 35.93         | Postoperative   | Neg      |
| 24 | Vascular wall                          | None          | 32.768        | Pos   | 16.19         | Postoperative   | Neg      |
| 25 | Vascular wall                          | None          | 4.096         | Pos   | 24.97         | Postoperative   | Neg      |
| 25 | Vascular wall                          | Doxy/HCQ      | 4.096         | Pos   | 26.11         | Postoperative   | Pos      |
| 26 | Vascular wall                          | Doxy/cipro    | 4.096         | Pos   | 21.22         | Postoperative   | Neg      |
| 26 | Vascular wall                          | Doxy/cipro    | 4.096         | Pos   | 21.22         | Postoperative   | Neg      |
| 27 | Stomach                                | Doxy <3m      | Acute Q fever | Pos   | 34.04         | Biopsy          | Neg      |
| 28 | Vascular wall                          | None          | 16.384        | Pos   | 23.33         | Postoperative   | Neg      |
| 29 | Aortic valve                           | None          | 1.024         | Inhib | NA            | Postoperative   | Neg      |
| 30 | Myocardium                             | None          | 512           | Neg   | NA            | Biopsy          | Neg      |
| 31 | Vascular wall                          | Doxy/HCQ/moxi | 512           | Neg   | NA            | Postoperative   | Inconcl. |
| 32 | Vascular wall                          | None          | 512           | Neg   | NA            | Postoperative   | Neg      |
| 33 | Vascular wall                          | None          | 512           | Pos   | 33.0          | Postoperative   | Neg      |
| 34 | Tissue surrounding vascular prosthesis | Doxy/HCQ      | 1.024         | Neg   | NA            | Postoperative   | Neg      |
| 35 | Vascular wall                          | None          | 8.192         | Pos   | 31.67         | Postoperative   | Pos      |
| 36 | Vascular wall                          | None          | 16.384        | Pos   | 20.59         | Postoperative   | Neg      |
| 37 | Discus                                 | None          | 8.192         | Pos   | 34.33         | Postoperative   | Pos      |
| 38 | Tissue surrounding valvular prosthesis | Doxy/HCQ/moxi | 32.768        | Neg   | NA            | Postoperative   | Neg      |
| 39 | Vascular wall                          | None          | 2.048         | Pos   | 34.00         | Postoperative   | Pos      |

Abbreviations: IgG, immunoglobulin G; PCR, polymerase chain reaction; CT, cycle threshold; FISH, fluorescence *in situ* hybridization; pos, positive; neg, negative; TMP/SMX, trimethoprim/sulfamethoxazole; NA, not applicable; doxy, doxycycline; HCQ, hydroxychloroquine; rifa, rifampicin; moxi, moxifloxacin; cipro, ciprofloxacin; inconcl, inconclusive; inhib, inhibited.

gene (DNA) of *C. burnetii* were selected using the ARB software (<http://www.arb-home.de>). All samples were examined by an experienced researcher on FISH for *C. burnetii* in veterinary practice (TKJ), and 55% of the samples were also examined by a second researcher (SBB). More information on the FISH and PCR procedure can be found in the [supplementary text and supplementary Fig. 1](#).

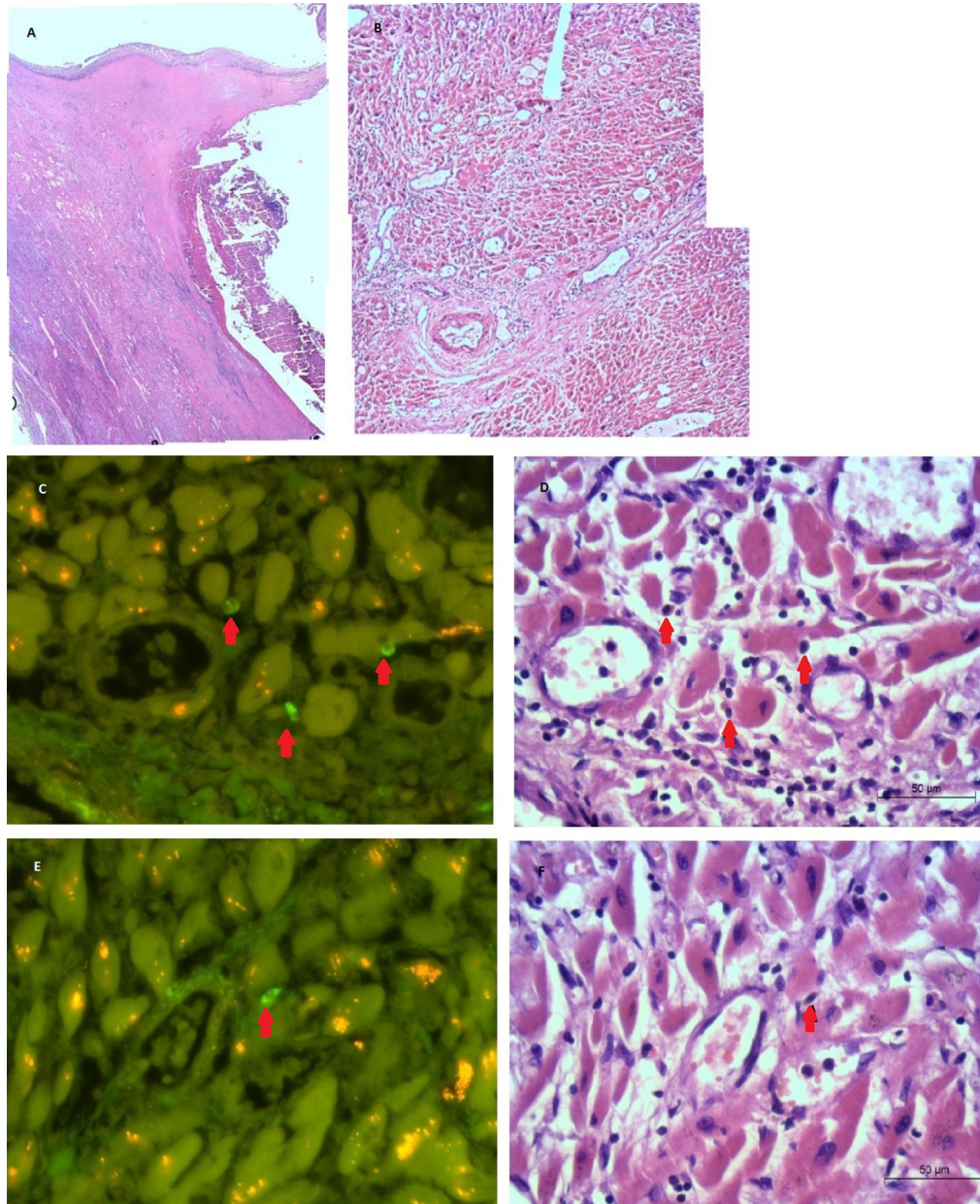
#### Statistical analysis

Analyses were performed with SPSS Statistics, version 26.0. Categorical variables were tested with chi square test or Fisher's exact test as appropriate. Continuous variables were tested with

student's *t* test for normally distributed variables or Mann Whitney U for non-normally distributed variables. Sensitivity, specificity, and overall diagnostic accuracy were calculated through a 2x2 contingency table with "exact" Clopper-Pearson confidence intervals [11].

#### Ethical statement

The local Medical Ethics Committee stated that this study needed no specific review. The Internal Review Board of the local participating hospitals approved the use of remnant clinical samples and waived the need for informed consent from patients.



**Fig. 1.** Myocardial tissue surrounding an infected valvular prosthesis of a chronic Q fever patient. a. HE-stained section, overview of infected myocardial tissue, x2.5; b. HE-stained section, overview of infected myocardial tissue, x10; c. FISH section, endocardium with green stained intracellular *Coxiella burnetii*, x40; d. HE-stained slide of same area as Fig. 2c, x40. Black triangle point to macrophages with FISH positive intracellular *C. burnetii* seen in Fig. 2c. e. FISH section, endocardium with green stained intracellular *C. burnetii*, x40; f. HE-stained slide of same area as Fig. 2e, x40. Black triangle points to macrophage with FISH positive intracellular *C. burnetii* seen in Fig. 2e.



## Results

### Chronic Q fever patients

Tissue samples from 39 chronic Q fever patients were analysed. Mean age at chronic Q fever diagnosis was 65.2 years (SD, 14.9 years) and 79.5% were male. Most patients had proven chronic Q fever (92.3%), two patients (5.1%) probable chronic Q fever due to being on immunosuppressive therapy or because of pregnancy, and one patient (2.6%) possible chronic Q fever without a focus. The most common focus of infection was vascular (58.9%), i.e. an infected arterial aneurysm or vascular prosthesis.

### Tissue samples

From the 39 chronic Q fever patients, 49 tissue samples were analysed (Table 1). Of 34 *C. burnetii* PCR positive tissue samples, 14 (41.2%) were also positive with FISH (Table 1). The other 20 *C. burnetii* PCR positive tissue samples were either FISH negative (17/34, 50.0%) or inconclusive (3/34, 8.8%). Of 14 *C. burnetii* PCR negative tissue samples, 11 (78.6%) were also negative with FISH. Two (14.3%) *C. burnetii* PCR negative placentas were FISH positive and one (7.1%) PCR negative tissue had an inconclusive FISH result (Table 1). One PCR result was inconclusive due to inhibited amplification. Four (4/49, 8.2%) samples could not be assessed because of autofluorescence and these FISH results were deemed inconclusive. Fig. 1 demonstrates several FISH and HE-stained slides.

In PCR positive tissues, FISH positive samples did not differ from FISH negative ones with regards to PCR cycle threshold ( $C_T$ ) value (mean, 26.8 vs. 25.9,  $p = 0.77$ ), time to fixation (<1h, 64.3% vs. 64.7%,  $p = 0.98$ ), *C. burnetii* PCR serum positivity (PCR positive, 35.3% vs. 64.7%,  $p = 0.22$ ), therapy at time of tissue retrieval (21.4% vs. 47.1%,  $p = 0.26$ ), or level of phase I Immunoglobulin G (IgG) titres at time of tissue retrieval (median, 4096 vs. 4,096,  $p = 0.43$ ).

### Diagnostic accuracy of FISH

The sensitivity and specificity of FISH compared to PCR for detecting *C. burnetii* in tissue from chronic Q fever patients is 45.2% (95% CI, 27.3% – 64.0%) and 84.6% (95% CI, 54.6% – 98.1%), respectively. The overall diagnostic accuracy is 56.8% (95% CI, 41.0% – 71.7%).

## Discussion

Currently, diagnosis of chronic Q fever is based on serology, PCR, clinical, and radiologic factors [9]. In this study we show that FISH is not a good alternative for PCR. Only 41.2% of PCR positive tissues were also FISH positive. In contrast, two PCR negative cases were clearly FISH positive. The PCR results on these tissues might be falsely negative, or the *C. burnetii* might be latently present in tissue and often not detected with PCR as described in earlier research [12]. FISH had a sensitivity of 45.2% (95% CI, 27.3% – 64.0%) and a specificity of 84.6% (95% CI, 54.6% – 98.1%), resulting in an overall diagnostic accuracy of 56.8% (95% CI, 41.0% – 71.7%).

Analysis of possible explanations for discordant results, including prior treatment or time to sample fixation, failed to show statistical differences [13]. The same holds for factors associated with chronic Q fever-related mortality, as *C. burnetii* PCR serum positivity and phase I IgG titres at time of tissue retrieval [14]. Another explanation might be sampling error: FISH might have been performed on a section of the tissue with less inflammation.

Lastly, PCR was performed in routine care upon retrieval of samples from 2010 to 2018, while FISH was performed during this study on the same samples, after being stored as FFPE blocks for 3 to 9 years at room temperature. However, if tissue is stored appropriately, RNA has been detected for at least 40 years after fixation [15].

Although we tried to maximize the number of samples by collecting them from the three hospitals that treat the most chronic Q fever patients in the Netherlands, sample size was still small. Therefore, a difference between FISH positive and negative samples with regards to the above stated possible explanatory factors might be missed due to a lack of power. Our collection of samples is the most varied studied thus far, ranging from vascular walls to lung tissue and placentas, and extensive data on patient characteristics and course of the disease was available for all cases. In addition, all samples were hybridized with four *C. burnetii* probes.

We conclude that FISH has limited value in the routine diagnostics of chronic Q fever. With this study, we have shed some light on the value of FISH for detecting *C. burnetii* in tissue of chronic Q fever patients.

## Transparency declaration

The authors declare no competing interests.

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## Contributions

JMW and SBB contributed equally to this paper. JMW and SBB contributed to conceptualization, data curation, formal analysis, methodology, project administration, visualization, writing - original draft, and review & editing. TKJ and MB contributed to data curation, methodology, visualization, and review & editing. MMMA and PTGAN contributed to formal analysis, methodology, writing - review & editing. CPB-R and AIMH, contributed to conceptualization, funding acquisition, formal analysis, methodology, writing - review & editing, and supervision. PCW and JJO contributed to conceptualization, funding acquisition, formal analysis, methodology, project administration, writing - review & editing, and supervision.

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The results of this study were presented during the ECCMID 2022.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2022.06.015>.

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