Author's response to reviews

Title: Lack of association between serological evidence of past Coxiella burnetii infection and incident ischaemic heart disease: nested case-control study

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Referee 1:

We agree with Professor Raoult that indirect immunofluorescence (IF) is indeed the gold standard reference method for assessing past infection with Coxiella burnetii. We had originally intended to use IF for this study. However it was made a condition of funding by the R&D office (on recommendation of the referees that reviewed our grant proposal) that we used an Elisa assay to perform this test in the 3 studies on human serology that they funded. These studies are:

1) the present study looking at cardiovascular risk (PRIME)
2) a study of 4000 individuals looking at seroprevalence in Northern Ireland
3) A study of seroprevalence in farmers, veterinarians and abattoir workers

We looked at manufacturer's data for EIA/IF comparisons for the 3 commercial IgG Elisa assays available to us and chose this assay because the data were the most convincing. For the assay we chose (Vircell) we have manufacturers data comparing this assay to IIF & with another EIA assay. This data looks fine (98% sensitivity and 100% specificity compared to the Virion Serion assay and 96.5% sensitivity compared to IgG IFA)

We hare currently doing a study on farmers, vets and abattoir workers using the Vircell assay. Of just over 300 farmers and vets tested so far in this study; 51% are seropositive using the Vircell EIA. This is a much higher seroprevalence rate than we are seeing for the PRIME study.

Using some of the PRIME sera and farmers study sera we have some limited local validation data comparing IF and EIA: Samples were selected by ELISA result; 32 positives and 49 negatives and tested blind by IF. Of a total of 81 tested by ELISA & IF:

ELISA: 32/81 POSITIVE
IF: 33/81 POSITIVE

(1 conflicting result tested Negative by ELISA and tested Positive by IF)

We intend to include the validation data in the study of farmers when we publish this however, if the referees felt it was appropriate, we could include this information in the current paper.

Ref 2
We have now amended the methods section to include fuller details of the assay including antigen source.

The assay details (to insert into the paper)

In answer to the query regarding stored samples, the samples were kept in a controlled -70C state. Sera were checked visually before testing and were clear. There is no specific data about this assay on stored samples but from first principles we feel that there is no particular reason to worry about this since the sera have been maintained correctly.