Reviewer's report

Title: Q fever infection and pregnancy outcome during the 2007-2008 outbreaks in the Netherlands

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Reviewer: ANNA PSAROULAKI

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This is a well written article with importance in its field. The authors clearly state the limitations of the study acknowledging in part previous studies which are building on. The authors grasp the opportunity to assess the possible relationship between Q fever exposure and pregnancy outcome using a significantly large number of samples during the early stages of the Q fever outbreak in the Netherlands. However, there are some major compulsory revisions that need to be addressed in order to decide on acceptance or rejection of this work.

A) Major compulsory revisions

1)-Title: “Q fever infection…”.

Solely antibody titre values against C. burnetii of #1/64 are not sufficient to diagnose a Q fever infection. In addition to clinical data, laboratory diagnosis of Q fever is based on seroconversion or a fourfold increase in titer which will indicate acute infection. The combination of elevated levels of IgG (>1/200) and IgM (>1/25) to phase II antigens could also indicate a recent infection. On the other hand, high titers of IgG (1/800) and/or IgA (>1/50) to phase I antigen are found in chronic infections. I strongly suggest therefore that the title should be changed to C. burnetii exposure…… or C. burnetii seroprevalence……..

2)-Methods, second paragraph:

Again, recent Q fever infection cannot be defined only by the presence of anti-phase II IgG +IgM antibodies particularly during an outbreak such as the one in Netherlands where there is increased probability of exposure to the bacterium. Since a second sample serum to observe a possible seroconversion was not available, elevated levels of IgG (>1/200 instead 1/64) combined with IgM (>1/64) to phase II antigens could be a more realistic approach to indicate a recent infection.

3)-Results, Seroprevalence:

The laboratory confirmation of a recent or a past infection which the authors present is weak.

“These were combined with 16 sera with an IgM II #1:64 in the group of ‘possible recent infection’

Solely low IgM titres cannot indicate recent infection by C. burnetii. The specific
antibody titre values could possibly come as a result of cross reactivity taking therefore into consideration falsely positive samples. The sera therefore that fall into the group of possible recent infection should not be combined with sera with both IgM and IgG antibody titre values falsely increasing the number of seropositive samples.

4)-Discussion, fifth paragraph:
“A positive IgM against phase II has been considered useful for the diagnosis of acute Q fever for a long time [Hunt et al, 1983].

This sentence is misleading. Indeed, Hunt and his co-workers stated that it is possible in virtually all acute cases, to diagnose Q fever by testing a single convalescent serum. However this should be collected between 2 and 8 weeks after the onset of symptoms. Furthermore, they added that in practice, the best approach, during 1983 I may add, is to test for Q fever complement-fixing antibodies and if that test was positive to then test for phase 2-specific IgM. If that was also positive, "a current (or recent) infection" could be reported. To my experience there is no laboratory today that relies solely in a positive IgM titre, particularly using a cut-off of # 1/64, to diagnose a Q fever positive sample.

Even though the authors admit at the contradictory sentence following the one cited above that “an isolated positive IgM against phase II could be the result of an aspecific reaction”, still they combine the samples with solely IgM II # 1/64 with those with IgM II and IgG II #1/64 in the group of possible recent infection increasing their number of positive samples.

In the article by Tissot Dupont the authors observed that the antibody titre cut-off values suggested by the manufacturer resulted in very low specificity of the test producing large number of falsely positive results. In order to effectively increase the specificity of the test the cut-off values had to be increased based on the local baseline to IgM II # 1/256 and IgG II # 1/1024. It has to be further noted that Denmark is considered a low prevalence country concerning Q fever. Thus, in the case of an outbreak such as the one in Netherlands the cut-off values should probably be further increased in order to achieve an acceptable specificity of the test. It is my opinion therefore that the cut-off values used in this study are far too low to be indicative of a positive Q fever sample serum, particularly during an outbreak of such an extent where the probability of exposure to the bacterium is highly increased. Thus, too many falsely positive samples could be included in the study. It would be interesting to assess the change in the number of the positive samples after increasing the cut-off values of the test to the ones used in the study in Denmark. In any case, since prevalence of Q fever varies greatly worldwide the establishment of cut-off values using healthy volunteers and/or pregnant women of a non-endemic area is of the utmost importance in order to tackle the problem of cross-reactivity and falsely Q fever positive samples.

B) Minor essential revisions
1) - Abstract, Conclusion
It is not advisable to compare the results of this study with the 4 studies cited in the article since 3 of them (Carcopino et al 2007, Denman et al 2009 and Diaz et al 2001) are supported on confirmed Q fever cases based on seroconversion and PCR.

2) - Methods, first paragraph:
“Exposure (infection with C. burnetii during pregnancy)...”
Exposure to the bacterium does not necessarily mean or lead to infection.

Storage conditions of the sample sera should be mentioned in order to assess the quality of the samples. It is known, for example, that storage of sera at 4°C for prolonged periods of time will negatively affect the nature of the possibly present antibodies. This phenomenon can lead to the presentation of different antibody titre values from the same sample over time. In this case, where just a cut-off of #1/64 is used for determining exposure to C. burnetii even a single dilution change could affect the result.

3) - Methods, Sample size calculation:
The authors assume that the prevalence of antibodies against C. burnetii among pregnant women in the high-risk area would be around 10%. Further information is needed to demonstrate how this assumption came up.

C) Discretionary Revisions
1) Results, Pregnancy outcome, paragraph 2
The authors state that the mean birth weight of the babies was not significantly associated with positive serology. It would be interesting to check whether this parameter, among other adverse pregnancy outcomes, is affected when taking into consideration sample sera only from 2008 which is actually the first year of the outbreak. An additional appealing study would be the comparison of the data collected concerning pregnancy outcomes in the Q fever endemic region with a non endemic one in the Netherlands.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
'I declare that I have no competing interests’