Reviewer's report

Title: Antibodies against Coxiella burnetii and pregnancy outcome during the 2007-2008 Q fever outbreaks in the Netherlands

Version: 2 Date: 5 January 2011

Reviewer: ANNA PSAROULAKI

Reviewer's report:

In the initial manuscript the authors correlated Q fever infection and pregnancy outcome. My original criticisms concerning the major compulsory revisions 1-4, were to emphasize that the data presented in the manuscript are obviously not possible to support a confirmed C. burnetii infection. One cannot talk about confirmed infection based on a SINGLE serum sample (moreover- since no titration was performed -based only on a single antibody titre). In fact, this is clearly stated in the package insert of the commercial IFAT kit used by the authors for detection of antibodies against C. burnetii “A 4-fold IgM antibody endpoint titer increase is considered supportive evidence of current or recent acute infection”.

However, if the option of testing acute and convalescent sample sera, particularly with no patients’ clinical data, does not exist, then the diagnostic cut-off should be increased in order to avoid misdiagnosed infections.

In their answer the authors challenge the sentence of my comments that “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 Q fever” by citing two articles that used similar cut-off points. The first article by Field et al. uses a different IFAT kit (in-house built) and the second by Frangoulidis et al. is based also on patients’ clinical symptoms.

To further support my comments I attach the laboratory criteria required by CDC for diagnosis of acute Q fever and confirmation of C. burnetii infection (CDC, 2009 Case Definition)

Laboratory confirmed case:

-Serological evidence of a fourfold change in immunoglobulin G (IgG)-specific antibody titer to C. burnetii phase II antigen by indirect immunofluorescence assay (IFA) between paired serum samples, (CDC suggests one taken during the first week of illness and a second 3-6 weeks later, antibody titers to phase I
antigen may be elevated or rise as well), or
-Detection of C. burnetii DNA in a clinical specimen via amplification of a specific
target by polymerase chain reaction (PCR) assay, or
-Demonstration of C. burnetii in a clinical specimen by immunohistochemical
methods (IHC), or
-Isolation of C. burnetii from a clinical specimen by culture.
Laboratory supportive case:
-Has a single supportive IFA IgG titer of #1:128 to phase II antigen (phase I titers
may be elevated as well).
-Has serologic evidence of elevated phase II IgG or IgM antibody reactive with C.
burnetii antigen by enzyme-linked immunosorbent assay (ELISA), dot-ELISA, or
latex agglutination. Note: For acute testing, CDC uses in-house IFA IgG testing
(cutoff of #1:128), preferring simultaneous testing of paired specimens, and does
not use IgM results for routine diagnostic testing.
According to CDC cases classification are: Probable acute Q fever: A clinically
compatible case of acute illness (meets clinical evidence criteria for acute Q
fever illness) that has laboratory supportive results for past or present acute
disease (antibody to Phase II antigen) but is not laboratory confirmed. Confirmed
acute Q fever: A laboratory confirmed case that either meets clinical case criteria
or is epidemiologically linked to a lab confirmed case.

Comment 1

Major Compulsory Revisions
Since the data presented in the manuscript is obviously not possible to support a
C. burnetii infection, the authors can only talk for #gG and IgM antibodies
seropositivity against C. burnetii and not for infection. I believe that in the initial
manuscript there was confusion relating the aim of the study. To my opinion, the
aim of the study is to determine the prevalence of anti- C. burnetii antibodies in a
large but specific target group (pregnant women) and to correlate seropositivity
with pregnancy outcome. If the authors of this article agree that this is the object
of the study, they have to make revisions throughout the manuscript under this
scenario.
Indeed in the revised manuscript the authors acknowledged in part this criticism
changing the article’s title, and they have made some changes. However,
throughout the manuscript including the tables, results and discussion- there
should be changes made to show the correlation of pregnancy outcome and seropositivity and not infection. Thus, terms such as “recent infection”, “possible recent infection” and “past infection” should be replaced by the terms “presence of anti-phase II IgM and anti-phase II IgG antibodies with a titre of #1:64”, “presence of a solitary IgM II #1:64”, “presence of both anti-phase I and II IgG antibodies with a titre of #1:64 without IgM being present”, respectively. The remaining samples were scored as seronegative for C. burnetii antibodies.

Comment 2

Major Compulsory Revisions

Page 13 Discussion

Paragraph 3: “There is no consensus...... between different studies”

Concerning other studies of seroprevalence determination in population groups, the authors of this article stand true mentioning that there are no definite rules. Indeed an IgG #1/64 cut off is usually satisfactory in sero-epidemiological studies aimed to estimate the seroprevalence of anti-C. burnetii antibodies for different groups of general population (refer the article “Epidemiological study of Q fever in humans, ruminant animals, and ticks in Cyprus using a geographical information system” by Psaroulaki A. et al Eur J Clin Microbiol Infect Dis. 2006 Sep;25(9):576-86) and please see Table 4 of the above article)

The authors do need to refer to seroepidemiological studies performed in general populations (this is compulsory).

The article we have mentioned is only indicative and is not compulsory to be used in the specific manuscript.

Comment 3

Major Compulsory Revisions

Page 14 Discussion

“In our study........... screening test”

The text should be reorganized based on the suggestions of comment 1.

An additional comment related to authors’ answer for comments A 1-4

Using non-rigorous (‘relaxed’) parameters, the majority of people with a disease will be classified correctly as having the disease but there is a relatively high probability that people without the disease (healthy) to be classified as they had it.

In general, in disease prevalence determination studies within a population, the
process may involve a significant bias which depends on the prevalence rate (low or high) and the sensitivity and specificity of the methods used. The overestimation could be relatively large for small values of prevalence. Particularly when it comes to screening, test results of false positives and false negatives should be seriously taken into account, since these test results aim to find people with the disease at an early enough stage where not even medical care is yet sought, with the hope that early treatment will be of greater benefit. Individuals for whom screening tests give a positive result should be further examined to determine the probable diagnosis and early treatment. This is determined by the positive screening rate of true positive samples (predictive value).
If the prevalence is low the predictive value would also be low, even for high values of sensitivity and specificity. These facts should be taken into account when assessing the usefulness of a screening test.

**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