Author's response to reviews

Title: Screening of post-mortem tissue donors for Coxiella burnetii infection after large outbreaks of Q fever in The Netherlands.

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Author's response to reviews: see over
Dr. Paget,

Editor at BioMed Central

Dear Dr. Paget,

Below you will find our replies to the points raised by the reviewers of our manuscript “Screening of post-mortem tissue donors for Coxiella burnetii infection after large outbreaks of Q fever in The Netherlands” (1733175573103833). The revised manuscript has been uploaded into the system.

We hope the manuscript now fulfills the requirements for publication in BioMed Central Infectious Disease.

Kind regards,

Marja van Wijk

Replies to the comments of Referee 1 (Wim van der Hoek):

Major Compulsory Revisions:

1. The main conclusion listed in the abstract is that the used assays are sufficiently specific for use on post-mortem samples. This seems difficult to reconcile with the main text under ‘Serological testing’ where serious problems with false-positivity are described.

   We have more clearly stated that the EIA and IFA used are sufficiently specific, but that one has to be aware that differences between batches may exist. The line in the abstract now reads: “The strong correlation between notifications and seroprevalence confirms that the used assays are sufficiently specific for use on post-mortem samples, although one has to be aware of differences between batches.” (Abstract page 2)

2. One would expect a statement in the abstract about the sensitivity of the assays used, as high sensitivity of the screening test would be important to avoid harm to recipients of tissue. This bears on the sensitivity of the EIA. It has been shown that sensitivity of the EIA is good for diagnosis of acute Q fever but much lower than IFA in seroprevalence surveys (Blaauw et al. 2011 in Epidemiol Infect). The authors argue in the discussion section of the paper that low sensitivity is not a major problem, which seems reasonable in the case of tissue donation, but they also claim to provide “the first estimate of the general seroprevalence of antibodies against Coxiella of 3.0% after the recent outbreaks of Q fever in The Netherlands”. In order to arrive at a reliable estimate of seroprevalence, the authors should have tested a random sample of EIA negative sera in order to be able to correct for the low sensitivity of EIA. This was the procedure used in the study that the authors refer to for comparison (Schimmer et al. 2012). In the study by Schimmer et al, the corrected prevalence was 2.4% instead of the 1.5% based on EIA only. As the 3% seroprevalence is likely to be an underestimate of the real seroprevalence, I would suggest that the authors do not list this as their major finding under ‘Conclusions’ on page 13 and mention the limitations of the EIA-based estimate.

   We agree with the referee that testing all samples with IFA would lead to a more reliable estimate of the seroprevalence. However, the EIA measurements were made briefly after the outbreaks at a time that waning of the signal from recent infections is not yet expected. The study from Schimmer was made using samples from before the outbreak. In that case the most sensitive test is required, or the seroprevalence will not be indicative of the cumulative incidence in the past. We have stated this
more clearly by adding a sentence to the discussion: “With the current study, that was undertaken at the end of/right after the outbreak period, this underestimation is expected to be limited.” (page 14). As suggested, we have changed the sequence of the conclusions in the conclusion section so that the seroprevalence data are not the first, primary conclusion. Furthermore, the sentence in the conclusion has been altered to more clearly indicate that the reported seroprevalence concerns the seroprevalence in the Dutch deceased tissue donor population and not the general population, as also suggested by the second referee. It now states: “Furthermore, this study provides a first estimate of 3.0% of the seroprevalence of antibodies against Coxiella in the Dutch deceased tissue donor population after the recent outbreaks of Q fever in The Netherlands.” (page 15)

3. The paper is not clear on the role that donor characteristics and Q fever incidence should play in a screening algorithm. The authors could expand the discussion section with their opinion on
(1) the general need for screening of tissue donors after an outbreak;
(2) how long such screening should continue;
(3) should screening be implemented in the entire country or only in the high incidence / outbreak area?
There will be no definite answers but if the authors feel that there is insufficient evidence for a complete screening algorithm, they should say so.

We have included a new paragraph at the end of the Discussion section that discusses the raised issues. The paragraph states: “After the Q fever outbreaks in The Netherlands the Health Council of the Netherlands advised to test tissue donors donating tissues with a higher risk of transmission for contamination with C. burnetii [10]. Because of the geographic spread to almost the whole country in the course of the outbreak, the screening was performed nationwide. The necessity of a nationwide screening was confirmed by the results of this current study in which more than half of the seropositive donors (17 of 31) lived outside the risk areas for Q fever. No guideline was given by the Health Council as to when testing of donors can be stopped. The interval between initial infection and when chronic Q fever becomes manifest is reported to be years [13]. Since the main concern is transmission through tissues from donors with chronic Q fever, it may be reasonable to stop testing when the number of new chronic Q fever patients in The Netherlands drops back to pre-outbreak levels and/or when, due to the gradual waning of the IgG signals after the outbreak, the seroprevalence will return to pre-outbreak levels.”

Minor Essential Revisions:

1. Page 3: Airborne transmission from INFECTED goats and sheep
   This has been corrected according to the suggestion.

   This has been corrected according to the suggestion.

3. Page 4: Give the reference to the report of the Health Council of the Netherlands
   This reference has been added as reference 10.

4. Page 7-8: What is the origin of the (1) Q fever incidence data; (2) bulk tank milk positive farms; (3) farms with abortion wave?
   The incidence data were obtained from the Dutch National Institute for Public Health and the environment. The data on bulk tank milk positive farms and farms with abortion waves were obtained from the Dutch Food and Consumer Product Safety Authority. This information has been added to the Data collection section (page 8).

5. Page 8: the authors found a “strong correlation” between notifications and seroprevalence, but no increased prevalence among donors living within a five-kilometre radius from an infected farm. One would expect a bit
more information on how ‘living within a five-kilometre radius from an infected farm’ was determined, i.e. a sentence on geographical analyses under ‘Statistics’ (maybe rephrase this to ‘Data analysis’).

The heading of the Statistics section has been altered to data analyses, as suggested, and the following sentence has been added to explain how the 5 kilometer radius was determined: “The 5 kilometer radius from infected farms to the residence of each donor was determined by measuring the distance between both postal codes.” (page 8)

6. **Page 11:** I do not understand the sentence “Risk assessments are hampered by limited knowledge about the magnitude of the outbreaks”. The Dutch outbreaks have been described extensively, including estimates of under ascertainment, underreporting etc.

   We have added a sentence after the statement that risk assessments are hampered by limited knowledge to explain more clearly that the data that are missing are population wide data. The sentence that has been added states: “Although the outbreaks have been studied extensively, most studies were limited to the outbreak areas.”

   Since only 14.3% of our donors lived in the outbreak areas, we believe that a risk assessment is hampered by the missing of nationwide prevalence data.

**Discretionary Revisions:**

7. **Page 5:** a number of exclusion criteria are mentioned but from the results section it is not clear how many were excluded. What were the occupations that were considered ‘hazardous’?

   As the entrance point for the study was donors with at least one approved tissue, we chose not to report the results of the donor selection process before entrance of donors into the study. Many donors were rejected during the donor selection process for many different reasons and many more might not result in transplantable tissues after quality assessment. We chose only to report the donor selection criteria regarding Q fever to make clear that donors with a very high risk for acute or chronic Q fever were not included in the study.

   Occupational hazardous professions were considered those marked in the Dutch LCI Q fever guideline as occupational hazardous, i.e. farmers and veterinarians. These examples have been added to the text. (page 5)

8. **Throughout the paper:** maybe better to describe the antibodies as “IgG antibodies against phase 2 of C. burnetii”

   This has been altered throughout the manuscript as suggested.

9. **Page 5:** what were the criteria for EIA positivity?

   EIA positivity was determined by determining the cutoffs according to the manufacturer’s instructions. This has been added to the text. “The cut-offs for (borderline) positivity were determined as according to the manufacturer’s instructions.“ (page 5)

10. **Page 9:** a reference to the Dutch Q Fever Consensus Group guidelines on diagnosis chronic Q fever, as described in the literature would be appropriate.

    A reference to this article has been added to the methods section (page 5, reference 13).

11. **Page 12:** “Virion/Serion EIA”. Better to use same description as under ‘Methods’.

    This has been corrected to the Serion EIA, the same as under ‘Methods’ (page 13).

12. **Table 1:** I fail to understand this table; it gives the number of donors tested, no results of testing? If all samples were negative, why not simply report this in the main text?
The table indicates for each type of tissue:
- the number of donors who donated that specific tissue
- the number of donors in whom that specific tissue was available for testing and
- the results of PCR testing on the specific tissue.
Since various tissues were available from the positive donors and not for all donors all tissues were available for testing, we decided to include this in a table. We have tried to put the same information in text, however this needs substantially more space and is more complicated. Therefore we decided to include the table. To clarify the content of the table more clearly we have adapted the text referring to the table in order to explain the contents of the table more clearly. The sentence now states “The number of tissues that were available for PCR testing and the results of PCR testing are presented for each type of tissue in table 1.” (page 10)

Replies to the comments of Referee 2 (Ted Eastlund):

Specific comment

The first sentence on the CONCLUSION needs change. It states: "This study provides the first estimate of the general seroprevalence of antibodies against Coxiella of 3.0% after the recent outbreaks of Q fever in The Netherlands. They should add that it is a seroprevalence of the deceased tissue donor population. Otherwise the reader may conclude it is a seroprevalence of the general population, including the healthy public.

Conform the suggestion of the referee we have adjusted the sentence to more clearly specify the population we investigated. It now states: "This study provides a first estimate of 3.0% of the seroprevalence of antibodies against Coxiella in the Dutch deceased donor population after the recent outbreaks of Q fever in The Netherlands.”