Author's response to reviews

Title: Active surveillance of Q fever in human and animal population of Cyprus

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Author's response to reviews: see over
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To: The BioMed Central Editorial Team

Re: “ACTIVE SURVEILLANCE OF Q FEVER IN HUMAN AND ANIMAL POPULATION OF CYPRUS” MS: 1798934908717002

Dear Editor

We are pleased to submit a revised manuscript for your consideration. We have made every effort to modify our manuscript according to the editors’ and reviewers’ comments.

Below please find a point-by-point response to the comments provided. In addition, we tried to format our paper in order to conform to the manuscript formatting checklist as requested.

Sincerely,

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Response to Reviewers’ Comments

Reviewer: Sally Cutler

General

The authors report the acquisition of Q fever in a cohort of both villagers and their livestock followed over one year, together with a countrywide effort to identify acute cases. The study is of value as it is of paramount importance to highlight awareness of this neglected yet significant pathogen. The findings highlight the problem of subclinical seroconversion. Whether these cases will develop later complications or merely reflect exposure and possible immunity, remain open questions. Although epidemiological data is severely lacking for Q fever in many countries, this study choose to use a very low serological cutoff limit that could flaw the interpretation of the authors findings. In general a single IgG titre of 1:200 or a four-fold increase in titre would be required for serological diagnosis. Variation in titre has been reported with the use of different antigens, but the source of the diagnostic antigen was not disclosed. Was this a local strain or reference strain such as Nine Mile? If this was a reference strain, were parallel tests done with a local strain? Other criteria were also applied for diagnosis, however, details of these were not given. How was the differential diagnosis achieved?

We would like to thank the reviewer for the useful comments. Regarding the serological cutoff, we would like to clarify that we used different cutoff for seropositivity and for serological confirmation of suspected cases. The cutoff of 1:60 or 1:120 were used for seropositivity while the cutoff 1:240 plus IgM 1:50 were used for laboratory confirmation of clinically suspected cases. A Paragraph was included in the methods section to clarify this issue (page 7). Moreover regarding the serological test (antigen etc) a paragraph was also include in page 7 together with references: Sera collected were examined for the detection of IgG, IgM, and IgA antibodies against C. burnetii phase II antigen by using indirect immunofluorescent antibody (IFA) test with a commercial kit (bioMerieux, Lyon, France). According to the test guidelines, IgG antibodies were accepted positive in case of > or = 1:60 titre, while IgM and IgA antibodies in case of > or = 1:25 titre. Finally clinical and laboratory criteria to identify acute Q fever cases are now included in the methodology section.

Specific Comments/ Major Compulsory Revisions

The monthly follow up of seronegative villagers was presumably both clinical and serological (this needs clarification in the text), Clarification was done in methods section page 7 and 8.

yet single point IgG titres were used rather than four-fold increases. The specific case definition including the laboratory confirmation was included in methods section. Indeed the four-fold increases were also used.
The authors should clarify this point further. The higher seroprevalence in humans when compared with livestock is worthy of discussion. Could this indicate another source of exposure, maybe rats or other reservoir host?

We would like to thank the reviewer for giving us the opportunity to include more information on tick infestation of animals and the possible relation to the animal and human seropositivity. In a previous study in the same region we isolated C. burnetii in 7.8% of collected ticks by using shell vial technique. We included results stating relation of tick infestation and human seropositivity in result section (page 11) and a paragraph in discussion section (page 13).

The aim of explaining the small number of acute clinical cases despite high seroprevalence is not achievable with a study design such as this. This is best deleted.

This paragraph was deleted and instead the following was written: The overall aim of this study was to assess if the small number of reported acute clinical cases of Q fever in the local population of Cyprus, despite the high seroprevalence (52.7%) identified in previous studies, is due to under diagnosis of Q fever or to under reporting of the disease.

Serological and clinical case finding criteria must be detailed. IgM results are described, but only serological tests without specification of whether these were IgG or IgM is given in the materials & methods.
The serological and clinical case finding criteria were provided (pages 7, 8)

Discussion is needed outlining the problems of serodiagnosis for Q fever, including poor standardisation, variation with different antigens, detection of only about a third of acute cases as seropositive and lack of harmonised testing approaches.
A paragraph was added in the discussion section addressing this problem together with related references.

Minor Comment

The causative agent of Q fever, Coxiella burnetii, is spelt in three different ways within this manuscript!
It was corrected throughout the manuscript

Reference for the shell vial culture technique should be provided.
Reference was included

Q fever continues to be a public health problem beyond the Mediterranean.
Corrected
A better and more up-to-date reference could be used to replace reference 1 for the significance of Q fever as a public health problem.
Three new references were included

Page 5: delete "rickettsial" as C. burnetii is phylogenetically distant from the rickettsiae.
Was deleted and replaced

It was confusing to see "three phases" in the introduction when two phases were discussed in the abstract.
Deleted and corrected

The reference for phase 1 results should be included in the introduction after "reported elsewhere" (presumably this is reference 7).
These data are under publication. We included in the references the study conducted in the same region to isolate C. burnetii from ticks.

What samples were used for cultivation attempts (blood, bone marrow, respiratory secretions)? Were the culture positive cases also seropositive?
Thank you for giving us the opportunity to clarify this issue. Blood samples were collected and it is clarified in the method section

Reviewer: Philippe Brouqui

General

This is an article that reports the features of an active epidemiological survey of the occurrence of Q fever in Cyprus in humans and animals. It is a lot of work done for a survey of 750,000 inhabitants of the island. The data are convincing and emphasize on the fact that C burnetii is prevalent in Cyprus but that most primary infections are asymptomatic and that the disease occurs rarely. The research is important and the data interesting but the paper lacks work especially statistical analysis work of the huge data collected during this survey.

We improved the statistical analysis by asking help from a biostatistician

Major Compulsory Revisions

The major comments in this article is that there is obviously a large number of data that have been collected and that their restitution can be improved. For example
- the incidence rate should be given (Nb of new cases / Total number of survey population) / Year .
The incidence rate is provided for both the “high risk” region and the whole country. Moreover incidence was calculated for children and adults and for residence of urban and rural areas.
The incidence rate can be compared within groups of peoples. Ex: incidence rate for seroconversion in humans, incidence rate for seroconversion in animals, incidence rate for Q fever in humans in each village, in Cyprus, incidence rate for Q fever in kids compare to adults (it as been reported that kids were more sensitive to Q fever.) The manuscript can be improved by a little bit more statistical work may be with the help of a biostatistic expert. Throughout the text it seems that there is enough interesting data collected to be analysed. We would like to thank the reviewer for the helpful and constructive comments Statistical analysis was improved. Comparison of incidence among different groups was conducted. Moreover attack rates were provided (cases versus seropositives-exposed). The kids were found with lower seropositivity and lower incidence rate (the incidence was not statistical significant), a finding that is supporting other researchers opinion (Maltesou and Raoult D. Q fever in Children).

- In the material and method, statistical analysis method is not appropriated as no comparison have been reported in the text nor in the table. Statistical analysis is now included in the text.

- The authors have made some isolate of C burnetii with is very good because it's not easy but the reviewer would like that a short description of the shell vial technique and the serological technique be described for readers not aware of such techniques. References are now included.

- References on Q fever are somewhat old there is more recent and up to date references on Q fever than Aiken (1987) Three new references were included.

Minor Essential Revisions

- There is errors in spelling "burnettii" should be "burnetii" Some environmental characteristics of Cyprus would be helpful to readers to understand why Q fever is so likely in this country (breeding index, livestock,...). The spelling errors were corrected and environmental characteristics of Cyprus are given in the methods section (livestock).