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”

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 heighten 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?

Specific Comments/ Major Compulsory Revisions

1. The monthly follow up of seronegative villagers was presumably both clinical and serological (this needs clarification in the text), yet single point IgG titres were used rather than four-fold increases. 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? 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. 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. 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.

This is a very helpful comment made by Dr. Boulos. In revising our manuscript we realized that the information about the needs assessment process (questionnaires) included in our manuscript was somewhat confusing and perhaps unrelated to the main topic of our paper. Therefore, we decided to remove the information on the questionnaires used in the assessment process and retain only a more detailed description of the standardized inspection forms used in the Environmental Health Inspection program. We had used specific inspection forms for the inspection of various premises such as restaurants, swimming pools, water supply systems, cooling towers etc. Unfortunately, these standardized inspection forms were written in Greek language and it is quite a labor-intensive task to translate them in order to include them as additional information files as suggested by the reviewer. Please find attached the 19 inspection forms in Greek. If you are interested, we may translate the most important standardized inspection forms used for restaurants and swimming pools.
The terminology regarding buffers has been used in the manuscript as recommended by the reviewer.
This is a very detailed comment on the methodology used. We have added information in the manuscript regarding the algorithms used as requested by the reviewer (page X). The above sentence has been revised according to the reviewer’s suggestion. We have also included the recommended citations.
We would like to thank the reviewer for the references provided to us. We had actually meant to say that there were no previous reports on the use of GIS for the Environmental Health Surveillance of Olympic Games. We have modified our statement accordingly.
The Epi Info databases used included the registration information of all environmental health items and the corresponding electronic files of the 19 standardized inspection forms. We have clarified this information in the text as well.
We used Epi Map because Epi Info 2002 was used for the development and maintenance of the above databases (see comment 6). In addition, the same software (Epi Info 2002) was used of the statistical analysis of the data collected and the presentation of the results in electronic reports. All these functions were easily performed using the menu features of Epi Info.
We thank the reviewer for his comment. We have followed his recommendation and moved some of the literature review in the introduction section. Despite we used the GIS application for Environmental Health Inspection, we have included a paragraph comenting on the other applications of GIS as suggested by the reviewer.
We have made every effort to eliminate typos and other errors from the revised manuscript.
We would like to thank the reviewer for his instructive comments regarding our figures. We have followed his recommendation and improved our figures as suggested.

Minor Comment

The causative agent of Q fever, Coxiella burnetii, is spelt in three different ways within this manuscript! Reference for the shell vial culture technique should be provided. Q fever continues to be a public health problem beyond the Mediterranean. 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. Page 5: delete "rickettsial" as C. burnetii is phylogenetically distant from the rickettsiae. It was confusing to see "three phases" in the introduction when two phases were discussed in the abstract. The reference for phase 1 results should be included in the introduction after "reported elsewhere" (presumably this is reference 7). What samples were used for cultivation attempts (blood, bone marrow, respiratory secretions)? Were the culture positive cases also seropositive?

We would like to thank the reviewer for providing us with valuable information. We have revised the manuscript accordingly.

It is true as the reviewer indicates that there was significant variability of the inspections performed thereby necessitating the development of 19 different standardized inspection forms, each one used to inspect a specific environmental health item (restaurant, swimming pool, cooling tower etc). We have carefully reviewed our manuscript in order to clarify this issue and provide detailed description of the work undertaken.
All the inspectors were trained to use all standardized inspection forms and therefore there was no specialization of inspectors. However, as mentioned in the manuscript, the inspectors always performed the duties in pairs. However, in the assignment of inspectors into pairs, their experience in inspecting food premises or water sites was taken into consideration in order to have complementary expertise. This information was added into the manuscript.
The reviewer is right to point out that the reason for choosing scenario 6 was based on the
availability of human and other resources. It is true that we had inspected all food premises
within a 200 meter radius around tourist, archaeological and Olympic venues. We included
the inspection of 2% of all other premises outside the 200 meter radius based on a random
sample of those environmental health items. It should be noted that prior to Olympic games
the food premises were neither registered nor routinely inspected. Moreover, there was no
categorization of food premises according to public health risk (High, medium and low).
Therefore, it was impossible to undertake such a task in a relatively short period of time and
under the pressure to prepare for the Olympic Games. We thought that using a random sample
of all other premises would provide us with a representative picture of the quality of food
offered around the Olympic Cities.
We would like to thank the reviewer for giving us the opportunity to clarify this issue. The
results of the random inspections around the Olympic Cities, during the pre-Olympic period,
were publicized by the Ministry of Development, National Food Authority. As assumed by
the reviewer, during the Games, all inspections were focused on food premises within the
Olympic Village, the Olympic venues and hotels.
It is true that archaeological sites were used to target food premises around them. We are not
clear whether we need to further clarify this issue.

Done.
We have replaced miles with kilometers. Please see our response to comments 6 and 7 from
the first reviewer regarding the databases of Epi Info.
We have eliminated the word “statistical” since we have not performed any statistical
analyses with respect to the work described in this paper.

The GIS was not used to investigate any real cases since there were no human cases reported.
We have added another column describing the food premises as requested by the reviewer.
This is a very interesting comment made by the reviewer. It would be valuable to link
inspection results with information about human cases for different diseases. We were not
able to do such analyses because the human surveillance data collected by another
organization were not geocoded or displayed on a GIS map. A comment has been added in
the discussion regarding this issue.
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.

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 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.
- In the material and method, statistical analysis method is not appropriated as no comparison have been reported in the text nor in the table.
- 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 on Q fever are somewhat old there is more recent and up to date references on Q fever than Aiken (1987)

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categorization of food premises according to public health risk (High, medium and low). Therefore, it was impossible to undertake such a task in a relatively short period of time and under the pressure to prepare for the Olympic Games. We thought that using a random sample of all other premises would provide us with a representative picture of the quality of food offered around the Olympic Cities.

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**Minor Essential Revisions**

- There is errors in spelling "burnettii" sould 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....)

It is true that archaeological sites were used to target food premises around them. We are not clear whether we need to further clarify this issue.