Author's response to reviews

Title: Quantifying Serum Antibody in Bird Fanciers' Hypersensitivity Pneumonitis.

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Author's response to reviews: see over
Reply to Editors comment
Subjects signed an informed consent proforma and donated a blood sample. The Medical Ethics Committee of Stobhill Hospital approved the study (ref. 00/44). This has been entered into the Materials section of the paper

Reviewer 1.
The General Comment that the reader would wonder whether the categorization of the subjects under study is valid based upon only the questionnaire.
Response
This is an important general aspect for this type of study group. There is no easy clinical categorization for these subjects since it is a syndrome and can change with time, but symptoms [along with antibody] are still a major diagnostic index. Our approach was to be as clear as possible on describing the symptoms and to be as open as possible on their categorization, in order not to be prescriptive. Our main aim was to address the lack of standardization relating to the investigation of antibody in this disease, only then can we improve our understanding of its relevance.

Minor Essential Revisions
In the text at page 9, Figure 4 should be labeled as Figure 3.
Response
This has been done [line 201].

As the authors mentioned, chronic insidious HP patients seldom complain of acute symptoms including fever and their positive rate of specific antibody against avian antigen by conventional EIA are relatively low. Insidious type HP cases might be included in ‘unlikely’ HP group. This possibility should be discussed.
Response
This is good point and gets to the heart of a clinically important subgroup of at-risk subjects. By their nature we will not be able to identify subclinical disease using our simple questionnaire and clinical interview, although part of the symptom assessment was specifically about undue shortness of breath. The methods recommended in this paper may help to resolve this if we can better describe the situations where the association between antibody is clear in order to identify where it is less well understood for example in insidious disease. Furthermore our well-motivated subjects are likely to provide a cohort that can be re-visited in order to identify at an early stage those cases of insidious type HP. This has been added to the text [lines 236-241].

The Discretionary Revision point raised is also well founded.
Response
I would also have prefer to categorize these patients more categorically as HP, symptomatic breeders without HP, and asymptomatic breeders after further clinical evaluations including physical findings, pulmonary function tests, and image findings. This was not possible in the convention center where the subjects had gathered. A much larger study will be required to establish the true sensitivity and specificity of this method (automated fluorimetry) in the diagnosis of HP and we recommend that in the conclusion [lines 275-279].
Reviewer 2.
The General Comment that the study has been carried out on a very small number of individuals and these have only been evaluated by clinical questionnaire.

Response
We note this point in the conclusion section, stressing that further large-scale studies should be carried out before this technique is adopted as the standard diagnostic technique for measuring anti-avian antibodies [lines 269-270, and 275-279].

Major Compulsory Revisions.
I am unsure as to the relevance of the data shown in figure 1 and section antibody activity against different avian species antigen unless you are going to suggest that you could use pigeon antigens/budgie antigens to diagnose all forms of bird breeders HP. You must comment on this data in your conclusion or take it out.

Response.
We found that the information content of the assay using pigeon serum as antigen was the same as for all the other antigen sources tested, and I had referred to this in lines 36-37 and 230-231. I had not included this in the Conclusion section in the interests of brevity, but I am happy to do so [Lines 272-273].

As an aside (which does not have to be followed up in this paper) this data is interesting from the fact that as far as I am aware people who are symptomatic against one species are not necessarily symptomatic against another species. If as you suggest these individuals have cross reacting antibodies to serum antigens across the spectrum of species tested it would suggest that these anti-serum antibodies do not play a role in the pathogenesis of disease.

Response.
It is intriguing whether there is sensitivity to different species. I am not aware of any literature that resolves this.
I agree that having antibody is exquisite evidence for antigen exposure, and cross-reacting antibody suggests that in the case of avian exposure the species doesn’t seem to matter in the induction of the main antibody. I would argue also that antibody seems necessary but not sufficient for pathogenesis, but since antibody detection is important it would be done better using a standardized method.

Page 8. The section on comparison between EIA and precipitation techniques is muddled. Why was there no comparison between fluorimetry and precipitin formation as I assume that the same 50 samples were used for all three techniques. This must be added. Also from this data I am not sure how many individuals in each of your clinical groups were precipitin positive, this should also be added. When you discuss antibody titres in relation to being precipitin positive does 22 (14) relate to precipitin
positive or negative individuals from the text it seems to relate to positives. This whole section should be tidied up and made clearer.

Response.
With the benefit of this useful comment I can see that this short section needed clarification. The single purpose was to quantify the detection limit of precipitins and I have amended this section, along with transposing the titers as spotted by the reviewer. [lines 190-195].

In the conclusion there should be a comment about this being a pilot study and further studies looking at larger groups of patients and clinically defined individuals must be carried out before fluorometry is adopted as the standard diagnostic technique for pigeon fanciers hypersensitivity pneumonitis.

Response.
[lines 269-270, and 275-279].

Minor Essential Revisions

Page 3 line 1 (HP) should be added after hypersensitivity pneumonitis
Response. [line 47]
Page 4 Numbers of individuals with probable HP, Intermediate symptoms and Unlikely to be added here
Response. [lines 75,76,77]

It would be useful to add in a table with some clinical information regarding the patients e.g. number of pigeons, number of years kept birds etc with the most important section here being whether the patients smoked.

Response.
This is important - I should have made it clear that all subjects were non-smokers [line 78]
The various demographics, age, gender, and avian exposures years of keeping pigeons, numbers of pigeons kept and hours spent in contact with pigeons were unremarkable and were no different between the groups. I don’t think they merit a table but I include the statement in the results [lines 150-152].

Page 7. What food antigens in addition to hens egg antigens were these patients positive for (add a list in here).

Response.
[Line 141]

page 9. I assume that decrease in antibody titre was measured by fluorometry. Can you make this clear in the text and in the legend for figure 3. Note this figure is referred to as figure 4 in the text on page 8, please change this.

Response.
The titer for this figure was established using enzyme-immunoassay since the earlier samples in this longitudinal series were measured before fluorometry. The purpose of this was to illustrate the importance of quantifying antibody rather than the method used. I have made this clear [results lines 198-199 and legend line 384].
Figure number changed [Line 201].