Author's response to reviews

Title: Immuno-epidemiology of human Schistosoma haematobium infection: preferential IgG3 antibody responsiveness to a recombinant antigen dependent on age and parasite burden.

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Author's response to reviews: see over
RE: submission of revised manuscript

We are pleased to submit our revised manuscript entitled ‘Immuno-epidemiology of human Schistosoma haematobium infection: preferential IgG3 antibody responsiveness to a recombinant antigen dependent on age and parasite burden’.

We have addressed all the comments made by the reviewers and made all the changes suggested. The two main comments from reviewer 1 regarded the importance of vaccination studies and effect of dying worms on the immune response. To address the first point, we mention a preliminary vaccination study conducted in hamsters in the discussion and to address the second point we have highlighted the possible importance of dead worms also in the discussion.

The major comment from reviewer 2 was related to the methodology, i.e. use of 1ug/ml of SWAP antigen. To address this, we repeated the ELISA assay on 41 random samples using 20ug/ml of antigen mentioned by the reviewer to measure levels of IgG1, IgG2, IgG3 and IgG4 and the results from this assay were very similar to those from our original assays in the manuscript. We have therefore used the original results in the manuscript.

We made all the other suggested discretionary revisions. Detailed responses to the all the comments from the reviewers are given below. We now hope that our manuscript is suitable for publication in BMC Infectious Diseases.

Yours sincerely
Francisca Mutapi
Revisions in response to reviewer’s comments

Title: Immuno-epidemiology of human *Schistosoma haematobium* infection:
preferential IgG3 antibody responsiveness to a recombinant antigen dependent on age
and parasite burden.

Reviewer 1: Michael J Doenhoff:

*Major compulsory revisions*

We have taken on board the effect of dying worms stimulating the Sh13 response as
well as the Sh13 response being a marker for the changing immune responses as
suggested by the reviewer and included the following statements in our discussion
‘…then the anti-Sh13 response is being elicited by adult worms (both living and
dying) and is directed against adult worms’ and ‘……but we have yet to establish that
the anti-Sh13 response contributes to, rather than simply, reflects the development
protective immunity against schistosome infections.’.

We agree with the reviewer that correlation analyses alone do not give direct evidence
that an immune response is protective and that vaccination studies will be more
informative. We point this out in our discussion. We also have some preliminary data
from a vaccination study of 10 hamsters which we have referred to in the discussion
as follows ‘…..We have however obtained encouraging results from our preliminary
vaccination studies in 10 hamsters which have shown that Sh13 stimulates an
antibody response in hamsters and that vaccinated hamsters have little or no infection
compared to unvaccinated controls which had heavy infection and liver pathology
(data not shown)…’ in order to address the reviewer’s comment.

*Discretionary revisions*

We have made all the 5 changes as suggested.
Reviewer 2: Daniel Colley

Major compulsory revisions

We have re-run a subset of the samples (41 random samples) using 20ug/ml of SWAP to determine if the range of OD we obtained for the 4 IgG subclasses in the original assays using 1ug/ml is similar to the 20ug/ml assays (in our titration assays we had run the assays at 1, 5, and 10ug/ml and decided on 1ug/ml). The range of ODs remained the same, for example, 0-1.40 in original data and 0-1.50 for the repeat IgG1 and 0.-0.09 and 0-0.09 respectively for IgG2. In addition, to determine if the data for each individual assay were similar in the two assays, we conducted a correlation assay between the two data sets and the correlation coefficients ranged from 0.891-0.912 for the 4 IgG subclasses. We have therefore left the original figure in the manuscript and indicated in the text that we did run a subset of the samples at 20ug/ml SWAP as follows ‘……a random subset of 41 samples run using 20ug/ml SWAP together with negative and positive controls showed that similar results were obtained using 1ug/ml and 20ug/ml of SWAP..’. The only difference we obtained between the two assays was that the reaction with the substrate occurred faster. We appreciate that there will be differences in isotype reactivity between different populations, and that some of those differences may be methodological. We hope that we have addressed the reviewer’s concerns.

Discretionary revisions

We have made all the 5 changes as suggested by the reviewer.