Author’s response to reviews

Title: Onchocerca-infected cattle produce strong antibody responses to excretory-secretory proteins released from adult male Onchocerca ochengi worms.

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Letter to the editor

Manuscript: Strong antibody responses of cattle infected by Onchocerca filariae to excretory-secretory proteins released from adult male Onchocerca ochengi worms.

Dear Editor
On behalf of all co-authors, I am submitting herewith our revised manuscript entitled: „Strong antibody responses of cattle infected by Onchocerca filariae to excretory-secretory proteins released from adult male Onchocerca ochengi worms” for consideration for publication in BMC Infectious Diseases.

We greatly appreciate the comments and suggestions put forward by the peer-reviewers. We have strived to address each and every comment when revising the manuscript. Below, we provide a short response to each point, the corrections and changes in the manuscript has been marked in yellow.

My coauthors and I feel that this topic should be of interest to those who work on the elimination of Onchocerca infection in cattle and humans in endemic countries. We are confident that the manuscript has substantially improved in its clarity and relevance, in particular for the filaria community and the public health community, and look forward to your final disposition.

My co-authors and I would be grateful for the opportunity to publish our findings in BMC Infect Dis.

With kind regards,

Alexander Kwarteng (Reviewer 1):

While I agree that a lot of effort has gone into this work, I think its important the authors address these minor comments.

1. I would like to know the source of O. volvulus antigen used (I failed to see that if its there).
   
   Response: The source of O. volvulus antigen is from Ghanaian patients and were provided by N. Brattig from the Bernhard Nocht Institute (page 7 under Preparation of adult worm somatic extracts)

2. I am not sure whether endotoxin test (assays) was performed on the filarial extracts before used given the presence of endobacterial-Wolbachia in these parasites. Am thinking how the presence of the Wolbachia will influence the immune response in general.
   
   Response: Endotoxin, relevant for cellular studies, has been tested in our O. volvulus extracts (Brattig et al., 2004, PMID: 15210803)

3. Please check the spelling of Kruskal Wallis in the abstract under methods.
   
   Response: The error has been corrected in abstract.
Ben Makepeace (Reviewer 2): This manuscript on the immune response of cattle and humans to Onchocerca ochengi excretory-secretory products contains some new information of limited interest, namely that antibody responses to male worm antigens appear to be stronger than those to the female worm. Unfortunately, it makes no attempt to identify the male worm antigens in question.

Response: Currently sequenced excretory-secretory proteins will be published separately.

There are numerous problems with the manuscript as it stands, not least in the description of the methods and with some of the figures.

Major issues:

1. Figure 2 is impossible to interpret. Presumably, it refers to the proportion of ES batches in which bands of certain molecular weights were visible by SDS-PAGE. However, for the somatic extracts where "n = 5", individual bands are present in 10% or less of the extracts, which appears to be mathematically impossible (since one batch of SX would represent 20% of the total examined). For the ES products, the number examined is much larger, but even the most prevalent bands have a frequency of only 25%. Either the figure has been prepared incorrectly, or most ES batches contained no visible protein. If the latter is true, then only those batches with visible protein should be included.

Response: Figure 2 has been deleted accordingly.

2. It is not clear how many ES batches were used to coat ELISA plates and what quality control was in place. Were several batches pooled for each worm sex? If so, how many, and was a common pool used for all ELISAs (if not, the variation in antigen profile might render the serological data meaningless)? For the female worms, were these from non-gravid only (no attempt seems to have been made to remove Mf from culture medium prior to protein extraction).

Response: For the present study one batch comprising filariae from five onchocercomas from Ghanaian onchocerciasis patients was applied and five batches of O. ochengi nodules from exposed cattle for the isolation of non-gravid female and male worms. Microfilariae were excluded. For the gel electrophoretic analysis one batch of O. ochengi female and male worms was applied. Concerning the serological analysis, ELISA tests were performed in parallel when transition the batch to another and the comparability of the ELISA verified. (page 8-9). O. volvulus somatic extract was used as control for O. ochengi antigens.

3. No information is given on how the somatic extracts were prepared. The Results section suggests that these were from female worms only; is this correct? What was the geographic origin of the O. volvulus worms [since they vary genetically between regions - see Choi et al. (2016) DOI: 10.1038/NMICROBIOL.2016.207]?
Response: The preparation has been written on page 7 under Preparation of adult worm somatic extracts. The O. volvulus worms originated from Ghana. In Fig. 2 extracts of female O. volvulus and O. ochengi have been applied (page 11 under Immune recognition of SX and ES proteins from female and male of O. ochengi and of SX of O. volvulus female filariae by IgG in sera from bovine and human hosts).

4. Very little information is given about the origin of the cattle sera, which were presumably newly collected for this study. The sentence "The sera originated from 28 bovines exposed from birth to natural transmission of O. ochengi by S. damnosum s.l. at the age of 4 and 24 bovines at 36 months" is unhelpful. I assume it should read, "Sera originated from cattle exposed from birth to O. ochengi for 4 months (n = 28) or 36 months (n = 24)"? I also assume that the field site was on the banks of the River Vina du Sud (make this explicit), but what was the infection status of the dams that produced these calves? What was the sex ratio of the animals? Were they Ngaoundéré Gudali breed? At least for the animals from 36 months, some basic information on the prevalence and intensity of nodules and Mf should be provided.

Response: The information has been written as proposed. All dams are infected, cattle are Gudali Zulu in a herd of 1/1.2 (female/male) sex ratio (page 8 under Collection of sera).

5. In Fig. 1, the gel slices seem to be poorly aligned in some cases. For the male worm ES at 24 h, the gels from cultures containing 21 or 23 worms are well-aligned, but these are offset by approx. 5 kDa relative to the culture from 20 male worms.

Response: The lanes has been rearranged as well as possible

6. The quality of the English is very poor in places, especially in the Introduction and Discussion. The Discussion opens with the almost unintelligible sentences, "A clear difference base on differences of molecular weight is observable in figure 2. That variation is observable with a variation of time of culture of number of culture worms." The Discussion then proceeds to hop between various seemingly unconnected points before terminating abruptly. It needs to be completely rewritten, clearly considering the limitations of the study and the context in the light of previous works. For instance, it completely neglects the prior work of Cho-Ngwa and Titanji at the University of Buea using O. ochengi antigens for measuring serological responses in human onchocerciasis patients. This group also analysed O. ochengi ES products and actually went on to identify some of the proteins (see Cho-Ngwa et al., 2011 doi:10.1016/j.meegid.2010.08.004). The Discussion should consider the implications of measuring immune responses to adult antigens in calves that had only been exposed for 4 months.

Response: The English has been improved and the discussion edited and in highlighted in yellow (pages 15-17).
7. The final paragraph of the Results reports various longwinded comparisons between serological responses to SX and ES in individual animals. It would be more informative to run a Spearman Rank correlation and report whether any of this is actually significant.

Response: The final paragraph has been deleted (page 15).

Minor issues

1. The title seems to state the conclusions of the study the wrong way round! It should be something like "Cattle show stronger antibody responses to excretory-secretory products from adult male Onchocerca ochengi than to adult female products".

Response: The title has been improved as followed: Strong antibody responses of cattle infected by Onchocerca filariae to excretory-secretory proteins released from adult male Onchocerca ochengi worms.

2. The abstract misspells Kruskal-Wallis as "Chruskall-Wallis".

Response: This error has been corrected.

3. In the Introduction, it is stated that "36 million people are still infected (Zouré et al. 2014)" with onchocerciasis. The latest figures from the Global Burden of Disease Study 2015 is 15.5 million infected [with 1.1 million years lived with disability] (see doi: 10.1016/S0140-6736(16)31678-6.).

Response: This paragraph has been deleted.

4. In the Introduction, "Control has been based on the elimination of vectors and a chemotherapeutic approach" is not really accurate. Vector elimination has only been achieved in a small number of foci (e.g., Bioko island and the Itwara focus in Uganda). Prior vector control in the OCP didn't eliminate the vector, it just suppressed it for long enough for O. volvulus adult worms to die out in the human population.

Response: This paragraph has been deleted.

5. The sentence, "Specifically, in Cameroon, this drug was reported to induce severe inflammatory reactions in people co-infected with loaisis and onchocerciasis (Tanya et al., 2013)" is a bit misleading. The problem of severe adverse advents in people with heavy loads of Loa loa Mf is not limited to Cameroon! Also, an original reference to this rather than the CAS report is required.
6. In the Introduction, doramectin and moxidectin are called avermectins. Whilst doramectin is an avermectin, moxidectin is a milbemycin. Collectively, all of these compounds are classified as macrocyclic lactones.

Response: This paragraph has been deleted.

7. This sentence in the Introduction is particularly unclear and needs rewriting: "In addition, for the adult stage an evidence of high sensitivity was given by Cho-Ngwa et al. (2003) showing that the proteins in the SX extract are as sensitive and specific as the used O. volvulus antigens for diagnosis of human onchocerciasis".

Response: The sentence has been reviewed in page 5 as followed: In addition, antigens in the somatic extract from adult female O. ochengi worms were found as sensitive and specific as O. volvulus female antigens used for diagnosis of human onchocerciasis (Cho-Ngwa et al., 2003).

8. In the Methods, it is stated that "Dead adult worms were kept in RNA stabilization solution (RNA later, Qiagen, Hilden, Germany) and stored at -70°C before transfer to the Bernhard Nocht Institute in Hamburg, Germany". This seems a very odd thing to do, but in any case, it is irrelevant to the current study!

Response: The sentence was deleted.

9. It would be helpful if Fig. 3 was redrawn in a similar way to Figs. 5 and 6, so that individual serum samples tested against both parasites are linked by a line. Then it would be clear if high responders to one tend to be high responders to the other, and vice-versa.

Response: Response: We thank the referee for the suggestion. Accordingly, we checked the data of Figs. 2 and 3 linking the indices of individual serum samples:

ad Fig. 2: Non-infected humans (naive) had comparable IgG indices for O. volvulus extract (median = 153; IQR: 111–1085) and O. ochengi extract (median = 219; IQR: 189–1039). As indicated by interconnected dots within the plot (Fig. 2a), in 7 healthy humans (64%), compared to the extract of O. volvulus, a higher titre for O. ochengi was observed without statistical relavance.

Similarly, comparable IgG indices were observed for sera from onchocerciasis patients for extracts from O. volvulus (median = 2209; IQR: 1958–2291) and O. ochengi (median = 2345; IQR: 2089–2612). As indicated by the interconnected dots within the plot (Fig. 2b), in 14 samples (78%), a higher O. ochengi titre was measured compared to O. volvulus, without statistical relavance.
ad Fig. 3: All 8 sera from uninfected humans (naive) had an increased IgG titre against ES products from male filariae (median = 983; IQR: 682–1184) compared to female ESP (median = 317; IQR: 79–837) (Fig. 3a) without statistical relevance. In onchocerciasis patients, comparable IgG titres against parasite male (median = 1834; IQR: 1756–2042) and female ES products were observed (median = 1584; IQR: 1441–1780) (Fig. 3b). As indicated by the interconnected dots within the plot, in 19 (83%) cows lower IgG titres against female parasites were measured without statistical significant (see Fig. 3a and 3b)

10. Since there is prior evidence from the same research group that male and female cattle respond differently to infection (Achukwi et al. 2004 DOI: 10.1016/j.vetpar.2004.02.015), using different symbols on the figures for male and female calves and perhaps analysing for a sex effect (if the sample size is sufficient) would be interesting.

Response: As indicated by the interconnected dots within the plots (Fig. 4-5) no statistical difference in IgG titre expression for both ES producta and extracts in male and female cows were observed (Wilcoxon rank-sum test) and thus sera from female and male cattle have not been indicated.