Reviewer’s report

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

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Reviewer: Ben Makepeace

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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.

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.

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).

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]?

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.

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.

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.

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.

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".

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

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.).
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.

5. The sentence, "Specifically, in Cameroon, this drug was reported to induce severe
inflammatory reactions in people co-infected with loasis 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.

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".

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!

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.

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.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
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