Author's response to reviews

Title: Immune markers characteristic for asymptotically infected and diseased Entamoeba histolytica individuals and their relation to sex

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Author's response to reviews: see over
Dear Editor,

thank you very much for editing the manuscript MS 1992030071346497 - Immune markers characteristic for asymptomatically infected and diseased *Entamoeba histolytica* individuals and their relation to sex.

We hopefully fulfilled and discussed all remarks made by Reviewer 1 carefully. However, the major remarks of Reviewer 2 might be dissolved by the following comments:

We included data from a previous study showing that the positive serology to *E. histolytica* in the *E. dispar* carrier used in the present study did not differ from endemic controls that were stool PCR-negative for *E. histolytica* and *E. dispar* (Blessmann et al., *J Clin Microbiol* 2002, 40(12):4413-4417). In addition, a respective citation was included for the in house ELISA (Lotter et al., 1995, *Trop Med Parasitol* 46(3): 180:2) used in this study.

The remarks are answered in detail in the Point-by-Point response.

We would be grateful if our response in the following meets the editor’s intention.

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Point-by-point Response

Reviewer 1#: William Petri
Thank you very much for your assessment of the manuscript.

Critiques:

1) We have modified the sentence into “In this study we characterize for the first time an asymptomatic carrier stage in amebiasis that is associated with a significant immune reaction and provide immunological markers that might give first hints towards an understanding of immune mechanisms underlying the control or development of invasive amebiasis” to avoid an overstatement in the conclusion.

2) First: we agree that including colitis patients from the same endemic area would be substantially helpful to differentiate the three relevant infection stages of amebiasis: asymptomatic, colitis and ALA and would open the opportunity to identify more markers.

However, we believe that the immune mechanisms underlying ALA development might not be transferable to the development of colitis, i) since the immune regulatory mechanisms of the liver differ from those found in other organs, and ii) colitis does not necessarily occurs prior to ALA, in other words, both diseases occur independently.

With this study we want to show that the asymptomatic carrier stage exists and in contrast to infections with *E. dispar*, an interaction with the immune response possibly due to subclinical lesions in the gut occurs, even in healthy individuals. In addition, due to the knowledge derived from our mouse model, we find certain correlation to the markers we identified possibly supporting ALA in human. However, it would be
necessary to identify markers responsible for the development of colitis to fully answer the question of the fate of an asymptomatic colonization.

3) The reviewer is right, it is conceivable that not only ALA but also colitis is associated with the development of specific serum IgG. To our regret, respective sera from colitis patients were not available for this study and we only could cite respective investigations (Line 344), however, there are no reports on sex differences in amebic colitis, but indeed this does not mean that the IgG levels does not differ between male and female colitis patients. A respective study, including sera from children, would be very interesting and we are open to investigate serum collectives of such collectives in the future.

4) As far as we know, there is no sex difference in colitis and as far as we know from the literature, there is no difference in the CCL2 serum levels between healthy women and men, but disease dependent, differences in the CCL2 serum levels occur. But indeed, this should be analyzed in a study on colitis patients.

5) We have checked the Figure legend 5, but we have included *E. dispers* samples in the Fig 5 graph.

6) We have excluded a previous Figure from this manuscript and now Fig. 2 shows titers.

7) The reviewers remark is correct, as we wrote in the discussion (Line 384) we assume that once ALA occurs, either in male or in female patients, the immune pathological processes are the same and do not differ between the sexes. We really want to point out and suggest, that the level for a control or susceptibility to disease lies in the asymptomatic stage.
Reviewer 2# Carol Gilchrist

Major revision:

1) We thought that the *E. dispar* carrier would be the far most best “control” group as these were from the same endemic area, healthy, definitely not infected with *E. histolytica*. This cohort (*E. dispar* stool PCR positive) has already been analysed in a previous study and seropositivity to *E. histolytica* has been compared with a cohort of samples from *E. histolytica* - *E. dispar* stool PCR negative individuals. We found no differences in the seropositivity to *E. histolytica* between these two groups, indicating that *E. dispar* infection does not lead to an increase in *E. histolytica* specific antibodies and that we detect here the background titer of the amebiasis endemic area (Blessmann et al., 2002, J Clin Microbiol 4413-4417; Table 1. *E. dispar* stool PCR positive:  20.8% seropositivity for *E. histolytica*; *E. histolytica* - *E. dispar* stool PCR negative: 18.3% seropositivity for *E. histolytica* vs. *E. histolytica* PCR positive: 82.6% seropositivity for *E. histolytica*). However, to take the remark into account, we have included a respective commentary as well as the citation in the text (Methods: Line 162; Discussion: Line 349). The in-house ELISA we used in the present study is longtime used in our diagnostic laboratory, and a description of the sensitivity and specificity is published 1995 by Lotter et al…Evaluation of three serological tests…Trop Med Parasitol 46(3): 180:2. A respective citation is included in the Methods: Line 175.

Minor essential revisions:

2) we corrected cohorte into cohort
3) we corrected IgGA to IgA in Figure 1,
4) we deleted “about” from the text