Author's response to reviews

Title: Development of functional gastrointestinal disorders after Giardia lamblia infection

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Author's response to reviews: see over
Responses to 1\textsuperscript{st} reviewer comments:

Specific Comments
1. Introduction: The first report of post-infective IBS was actually published more than twenty years ago by Chaudhary & Truelove in 1962.  
\textbf{Answer}: We thank the reviews for this correction and changed the introduction to acknowledge this first report, which also mentions amoebic gastroenteritis inducing IBS in some of the patients they describe.

2. Introduction: Post-infective IBS, and for that matter functional dyspepsia, have in fact been reported after other types of infection for example viruses and even protozoan parasites and this has not been mentioned. These observations obviously suggest that any form of gastrointestinal infection may result in post-infectious IBS and that problems following giardiasis might be anticipated.  
\textbf{Answer}: We left out viruses in the manuscript as it seems they give much less risk for PI-IBS and also of such short duration that one may argue if it can be called PI-IBS. However, in the general context we describe this in the introduction, we agree viruses as well as amoeba should also be mentioned.

3. Methods: We are told functional dyspepsia was sought after but not given any detail of how this was identified.  
\textbf{Answer}: We put in the Rome II criteria questions in the standardised questionnaire. This has now been changed and clarified in the revised manuscript.

4. Methods: We are told some patients were re-biopsied one year later, but not informed whether the whole group was followed up. It would be very important to assess whether symptoms diminished or resolved over time and as they have recorded symptom scores should be possible to ascertain. This point is important because people with non-specific diarrhoea can develop persisting symptoms for a surprisingly long time after the initial event.  
\textbf{Answer}: We see that the text is unclear about this and have rewritten it to clarify the timeline of an initial workup not described in detail here but where initial duodenal biopsies were taken, and the later follow-up at which the data from this study were obtained and at which time new biopsies were taken from a few patients. Later follow-up was beyond the scope of this manuscript but has been performed by questionnaires and these data are currently being analysed.
5. Methods: it would have been nice to have a control group to see how many people spontaneously developed symptoms over a similar period of observation. **Answer:** Regrettably for this study we did not have a control group, and we therefore did not try to estimate the percentage for PI-IBS occurring after giardiasis in this publication. However, by other study designs we are presently trying to answer the question posed by the reviewer.

6. Results: We are told most people denied previous abdominal illness before their infection. It would be important to know how this was established especially as it seems that many may not have consulted a doctor. Many people deny abdominal symptoms unless specifically asked about problems such as difficulties with bowel function or bloating which they may not have consulted about. This issue does not necessarily mean that the findings reported here are irrelevant as gastrointestinal infections also make established IBS worse. **Answer:** In the text we have now made it clearer that this was established in the structured interview, and we do agree with the reviewer that memory of previous abdominal complaints are often suffering due to recall bias. A passage about this have been added in the discussion.

7. Results: They report that many patients claimed to be intolerant of milk. Lactase deficiency can follow gastrointestinal infections – did they undertake any lactose tolerance testing. **Answer:** We did not test this in patients before data collection in this study was finished, as the method was not available to us before that. However we have tested duodenal lactase activity in biopsies from 10 newly referred patients with post-giardiasis FGID with no correlation to reported milk intolerance. We state this in the revised manuscript and also refer to a study of PI-IBS after bacterial gastroenteritis where lactose intolerance was not found to be implicated.

8. Discussion: There is a lot of interest in the role of low grade inflammation in IBS especially in relation to the post-infective variety of this condition. **Answer:** We agree very much that this is an exiting field and hope to look further into the biopsy material for inflammation markers.

9. Discussion: Antibiotics can lead to IBS and there is a literature on this which probably ought to be quoted. Presumably these patients were treated with antibiotics and this issue needs discussing. **Answer:** We agree that this is an important point and have added a passage about it in the discussion and quoted the core publication in this discourse.

10. Discussion: The authors dismiss female gender as a risk factor although I think (but I am not certain) that female gender has been reported as a risk factor. **Answer:** Female gender have in several early articles on PI-IBS been found to be a risk factor (about 2-3 x higher risk), but in later multivariate analyses not an independent risk factor when adjusting for psychological factors. We chose not to go into this discussion
in this manuscript as we do not have psychometric data and it would make the introduction lengthy. The point is well discussed in the references given (2 & 5).

11. Discussion: I have previously referred to a range of organisms being responsible for post-infective IBS, if the authors decide not to mention it in the Introduction it should certainly be covered in the discussion.
Answer: See point 2

12. Discussion: The role of stress is dealt with very superficially. It would be worth discussing this in more detail in the light of previous data suggesting psychological factors predict the development of IBS following infection.
Answer: We agree that stress is dealt with lightly, and the issue is of great interest. Unfortunately, we do not have more data to elaborate on this issue other than a single question. We view this as an exploratory question that warrants further study, and we do not feel comfortable discussing this in more detail without more detailed data of better quality than we planned for in this study.
Responses to 2nd reviewer comments

The method used to identify successful eradication has some limitations since microscopy of fecal samples has a sensitivity around 85%. This should be acknowledged in the manuscript.

**Answer:** We agree this should be acknowledged and have added a passage about this into the discussion of the revised manuscript. We state the minimum number of samples analysed for all patients, but most patients had 3 samples analysed on 2-5 occasions, thus increasing the sensitivity.

Although this is a prospective study the presence/absence of symptoms before the infection was assessed retrospectively. This is a limitation since it appears that many patients were asked about their previous symptoms a long time after the diagnosis of Giardia infection. This may generate a recall bias.

**Answer:** We agree that recall bias is a limitation regarding previous abdominal complaints. We have in the revised manuscript mentioned this in the discussion.

The study is uncontrolled and this may overestimate the prevalence of symptoms. What is the prevalence of FGID in age, gender and region matched controls?

**Answer:** The study in uncontrolled and we avoid the attempt to make a calculation of the prevalence of PI-IBS after giardiasis as the design is not suitable for that. However, we have added in the revised manuscript the IBS-prevalence in a region- and age matched population from a recent study in a Norwegian reference population (ref 9) as the referee requests. (Around 10% in the relevant age group).

Could be the authors more precise on the tests they did on the patients to exclude other diseases? Did they exclude celiac disease or major food allergies?

**Answer:** In the revised manuscript we are addressing this comment in the method section. Celiac disease was excluded by evaluating serum anti-endomysial and anti-tissue transglutaminase antibodies and duodenal biopsies. In patients reporting worsening after specific foods a more specific panel of blood and skin allergy tests were performed. This was done on a clinical basis and not as part of this study, but without identifying IgE mediated food allergy fitting the reported subjective food allergy.