Reviewer's report

Title: Chlamydia trachomatis antigens in enteroendocrine cells of the small bowel in patients with irritable bowel syndrome

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Reviewer: Mark Pimentel

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In this manuscript by Dluogs, et al entitled, “Chlamydia trachomatis antigens in enteroendocrine cells of the small bowel in patients with irritable bowel syndrome”, the authors present data to suggest that subjects with IBS have positive staining for certain components of C. trachomatis that is significantly different from controls. This could be a very important discovery in IBS. However, there are some concerns about various components of the manuscript.

There is some anxiety of the part of this reviewer about the methods. How did the authors get full thickness biopsies on so many IBS patients. Besides the ethical risks of such a procedure, who would agree to such a study. Agreeing to such invasive testing must have affecting the type of subject seen. Were these severe subjects. I would argue that they must have been severe since 2 had deficient actin staining which is sometimes attributed to visceral myopathy (not IBS). This may result in pseudoobstruction. Another suggestion that they were severe is the high rate of small bowel abnormalities consistent with enteric dysmotility. Can the authors be more specific? We need to know a lot more about these patients. Also, their sexual behaviors. Why would they have Chlamydia trachomatis in their GI tract?

Obviously these were not consecutive IBS patients given the proposed procedure of laparoscopy. How many patients were approached to get this number to consent? It might suggest how representative their population is. This is really important because this may not be applicable to IBS as a whole but only patients severe enough to do an invasive study like this.

I am very familiar with the previous work by this group but it is puzzling how they even came across the idea of looking for this. It might be helpful to know more specifically why they chose this since the rationale is very subtle in the introduction.

Back the results. The controls are odd also. Why so many obese subjects. Was the full thickness biopsy part of a bariatric procedure? How obese were they? I think since they brought it up, it might be good to see some demographic comparisons between IBS and controls such as BMI, age, etc.

I am confused about how the authors decided that the staining was positive. I know the readers were blinded (although I did not find out till the results that this was the case- this needs to be in the methods), but was one EEC with LPS
considered positive or did they need more than one. I suspect any detectable staining was considered positive. This should be stated explicitly.

With regards to the 4 subjects that were previously positive and some time later re-biopsied, the authors state they were negative. In the discussion they suggest a possibility that over time the Chlamydia infection or affect may wane. This does not make sense. In the methods, the authors already state that the average duration of IBS was 6.5 years. If C. trachomatis is important in IBS, why would it clear in 1 year and not clear in 6.5 years? This makes no sense at all.

Did the authors do PCR on all the original specimens or only on the 4 subjects stated in the last paragraph. This needs to be done in a larger group.

Maybe the LPS they are detecting is from other bacteria. Perhaps their assay is cross-reacting with the many intestinal bacteria that produce various forms of LPS. Other LPS antibodies should be tested before declaring C. trachomatis the main organism.

Were there differences between subgroups of IBS? It seems you might have enough numbers to look at D-IBS vs C-IBS, etc.

While this could be a very important study, this study warrants much careful scrutiny.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I declare that I have no competing interests