**Reviewer’s report**

**Title:** Helicobacter-induced Gastric Inflammation Alters the Properties of Gastric Tissue Stem/Progenitor Cells

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**Reviewer:** Carrie Duckworth

**Reviewer's report:**

Shibata et al present a manuscript entitled, "Helicobacter-induced gastric inflammation alters the properties of gastric stem/progenitor cells" which attempts to characterise mechanisms of Helicobacter infection-associated gastric carcinogenesis using gastric organoid cultures. The manuscript in its current form is difficult to read due to grammatical errors in places. Whilst I note that the text has been examined by Text check (ref: 15042704), I suggest that further proof reading is carried out by a native English speaker. For instance, some connecting words such as 'the', 'a' and 'an' are missing in places and the use of pluralisation can be confusing. There are also several whole sentences that I do not fully understand.

The overall aim of the study is exciting in the attempt to study the effects of Helicobacter felis infection on the stem cell compartment of gastric epithelial cells without the compound effects of other cell systems found in vivo. However, I feel that the presented data fall short to satisfy the authors' conclusions. Please address the following major and minor concerns:

**Major**

1. Please describe in the text why gastric corpus organoids were generated from H. felis infected mice whereas antral organoids were generated from MNU treated mice when the MNU model was used as a positive control (page 7 line 3). These regions are very different in terms of histology, function and response to H. felis infection and putative stem cell markers are also known to have different abundances in these different regions. For instance, villin is thought to label a small subset of stem cells and these are predominantly observed in the gastric antrum.

2. Please include the sex of C57BL/6 mice used for H. felis infection/organoid studies. There are known and well documented sex and background strain differences in susceptibility to pathology with this model.

3. In the mouse model section, it is not clear as to whether the MNU study was first conducted in C57BL/6 mice to generate organoids prior to subcutaneous injection into NOD/SCID mice or whether tumours were generated in NOD/SCID mice. Please clarify in text.  

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line 16 suggests male mice were used and page 10 line 14 suggests female mice were used. Were organoids generated in males and grafted into females? Was this the same for H. felis studies?

4. The methods of organoid analyses have not been fully described. Please include descriptions of how organoid size was measured; eg from how many fields of view, original magnification, how many replicates, software used.

5. It is not clear from this manuscript whether organoids were passaged or used immediately following their establishment. A clear indication of the experimental design is needed. There is suggestion from the literature that parietal cell loss occurs following passage of gastric organoids unless they are co-cultured with a myofibroblast feeder layer (Schumacher et al J Physiol. 2015 Apr 15; 593(Pt 8): 1809-1827). Parietal cell atrophy is also well characterised following H. felis infection in vivo.

6. Were antibiotics used in organoid growth medium? Have the authors determined whether extracted gastric glands from H. felis infected mice remain infected during gastric organoid culture or whether the isolation and growth conditions ex vivo eliminate these bacteria? In the latter case, how are these organoids different to those generated from H. felis infected but later eradicated mice? Some indication of infection status of these organoids is needed.

7. Figure 2B shows that the number of organoids generated from H. felis infected mice is greater than that from untreated mice. Please indicate how this experiment was standardised. There is great variation in number of glands extracted from stomachs and how they are fragmented regardless of treatment. The numbers presented per stomach seem remarkably low.

8. The authors describe an increased organoid size and increased Ki67 staining (page 13, lines 8-10) however, this data is presented as 'data not shown' with no indication of quantification or justification for this statement. Please include the data and indicate in the methods how this data was generated.

9. The authors suggest that single organoids are derived from a single stem/progenitor cell (page 14 line 2) however, this depends on culture method used. The authors have not generated single cell gastric epithelial extracts but have generated organoids from individual glands, each of which contain several stem cells all of which could continue cycling. Please amend this paragraph.

10. The authors measured cytokines in organoids generated from infected mice and those post eradication however, on page 16 line 2 they suggest that this was conducted in organoids infected and post eradication which is not strictly true. Please adjust to read accurately.
11. Please add DCLK1 data presented in supplemental figure 1 to the main body of the manuscript including how many organoids were assessed and how this was analysed.

12. Fig 4D shows gastric organoid size 3 days post cytokine stimulation vs control. I find it hard to believe that these organoids are only 6-8μm presumably (clarification in methods needed) in diameter. Single epithelial cells would be roughly this size and organoids contain several 100 to several 1000 cells. Please check size (diameter?) calculations.

13. Have the authors compared the phenotypic changes identified in organoids generated from H. felis infected and H. felis eradicated mice with organoid infected with H. felis in vitro? Are similar phenotypic changes observed in both models?

Minor

1. Abstract: please use consistency when describing Helicobacter felis infection and use Helicobacter felis in full at first use.

2. Please add the concentration and volume of H. felis administered to mice (page 7 line 11)

3. Please describe the route of administration of antibiotics (page 7, line 17)

4. Please add concentration of N-acetylcysteine (page 8, line 15)

5. Fig 1B: I am not convinced by the images that there is an upregulation of Tff2 and Muc2 over the course of infection. Parietal cells look positively stained particularly in the Tff2 control tissues. Additionally, please check the magnification of 3M DCLK1 image and include scale bars on figures.

6. I am unclear as to how E-cadherin IHC allows the confirmation of a single layer of epithelial cells (page 13, line 4). Please include data not shown data.

7. Fig 2D: The authors suggest that the Muc4 expressing region is expanded in gastric corpus glands between 3 months, 6 months and 12 months of H. felis infection, it is difficult to appreciate this from the 3 month image as this largely contains lymphoid cells. Please show a region containing mainly epithelium as for 6 moths and 12 months. How was this quantified?

8. How were organoids prepared and quantified for subcutaneous grafts?

9. Why is one paragraph in the discussion underlined? - the last sentence of this paragraph is particularly not well phrased.
10. Isx is discussed in the discussion section however, the only previous mention of this gene was from the initial cRNA microarray. Have the authors conducted follow up studies by qRT-PCR as they have for other genes?

11. I am not clear as to how the top paragraph on page 21 is relevant to the data presented in the manuscript. Ie how are Sall4, Klf5, Cdx1, NFκB related to any of the genes identified in presented data?

**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?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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Needs some language corrections before being published

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