Date: 07/02/2021

Company Editor-in-Chief
World Journal of Gastroenterology

Dear Dr. Ma,

Thank you for your response, conditional acceptance and an opportunity to revise our manuscript entitled "Characterization of gut microbiome and metabolome in *Helicobacter pylori* patients in an underprivileged community in the United States" (manuscript No: 66632). We appreciate the comments given by the reviewers. The manuscript has now been revised answering each of the queries raised by all the reviewers. Item-by-item answers to the reviewers' comments are enclosed with this letter. We hope that the modified manuscript addresses all the questions raised. We look forward to hearing from you soon.

Sincerely,

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Reviewer 1

We are grateful to Reviewer 1 for his/her positive evaluation of the manuscript and thoughtful suggestions for its revision, which helped us to improve the manuscript.

Response to Specific comments

1. Reviewer: *In this study the Authors analyzed stool samples from H. Pylori-positive patients and negative controls. Alpha and beta diversity analyses were performed, as well as measurements of stool fatty acids content. Lower alpha diversity in elderly patients and beta diversity differences were observed in H. pylori positive patients, with significant disruption. Although the attempt put in place by the Authors might be commendable, there are some major flaws in this study that need either correction or explanation. My main perplexity raises from the difficult task to be performed to directly correlate H. pylori, a bacterium present almost exclusively in the stomach, to gut dysbiosis, a concept which is difficult to accept on the basis of biological plausibility.*

Response: We agree that it is indeed intriguing that the gastrotropic *H. pylori* is associated with alterations in distal gut microbiota. Nevertheless, these associations have been demonstrated in both animal and human studies. Heimesaat *et al*. showed that after 14 months of gastric infection with *H. pylori*, the cecum and colon of Mongolian gerbils harbored significantly higher loads of *E. coli, Enterococcus, Bacteroides*, and *Prevotella* spp.

compared to controls[1] (References are included at the end of the item-by-item by responses). The authors ventured that secreted mediators altered by gastric *H. pylori* infection (hydrochloric acid, gastrin, somatostatin, cytokines) could exert downstream effects on the lower GI tract that influence the resident bacterial species. Kienesberger *et al*. demonstrated that infection of mice with *H. pylori* significantly influenced microbial community structure (beta-diversity) in the distal gut (fecal, cecal, and ileal samples) compared to controls [2]. Again, the authors noted that the observed changes in expression of certain immunological genes (T-reg and Th17 cell response-related genes) and hormone (ghrelin) regulation in response to *H. pylori* may cause systemic effects at distant tissues. Two recent studies have also found significant differences in stool microbiota of patients with *H. pylori* infection compared to controls. Frost *et al*. found significant changes in fecal microbiome diversity and composition as measured by alpha and beta diversity, respectively, in *H. pylori* positive individuals compared to controls and the latter association was strongly correlated with *H. pylori* stool antigen load [3]. Although *H. pylori* infection was generally associated with higher fecal microbiome alpha diversity, high *H. pylori* fecal antigen load was associated with reductions in putatively beneficial genera (*Bacteroides, Barnesiella, Fusicatenibacter,* and *Alistipes*). The authors speculated that reduced gastric acid secretion associated with *H. pylori* may permit the passage of acid-sensitive bacteria that alters distal gut microbiome composition. They also cited the immunoregulatory features of *H. pylori* shown in murine models of allergic asthma as evidence of a potential immune-mediated mechanism by which the organism alters distal gut flora. Dash *et al*. similarly observed increased alpha diversity among stool samples from *H. pylori*-positive patients [4]. We have now revised our Introduction (lines 189-195) to reflect these studies and provide the reader with precedence for the association of gastric *H. pylori* infection with distal gut dysbiosis.

2. **Reviewer:** *Background, lines 3-5: see above. Moreover, it is unclear how this study, as stated in the background, could help improve H. pylori treatment which is based on antibiotics and not on gut microbiota modulation.*

Response: We are grateful to Reviewer 1 for this important question. His/her comment refers to the statement in the abstract that: "To combat the increasing antibiotic resistance of *H. pylori*, the need for new therapeutic strategies has become more pressing." We have now revised the statement as: "As the burden of antibiotic resistance increases, the need for new adjunct therapies designed to facilitate *H. pylori* eradication and reduce negative distal outcomes associated with infection has become more pressing." (lines 64-67)

The conclusions of this study, which enable a better understanding of *H. pylori*-associated gut dysbiosis, may hold value in multiple contexts. First, while a number of probiotics have been shown to reduce the incidence of antibiotic-associated adverse effects in patients undergoing treatment for *H. pylori*, some have also shown efficacy in improving eradication rates when used adjunctively [5-7].

A better understanding of *H. pylori* associated gut dysbiosis is potentially most valuable in addressing the negative distal outcomes associated with *H pylori*. Associations of *H. pylori* with colorectal cancer and metabolic disease, in particular, have been observed in other studies, and authors have theorized that these may be mediated by gut dysbiosis [8, 9]. We have now revised our Abstract-Background (lines 64-67) and Introduction (lines 196-209) to clarify the potential value in the knowledge gained from this study.

Although the next entire discussion is not included in the manuscript, we would like to further elaborate here on this interesting aspect raised by the reviewer**.** Our interest in understanding the gut microbiome in individuals with *H. pylori* relative to healthy controls is based in part on preclinical studies showing that psychological stress increases colonization and proliferation of *H. pylori*, and this effect is prevented by administration of RU486 (a type II glucocorticoid (GC) receptor antagonist)[10] . Furthermore, we have shown in preclinical studies that chronic psychosocial stress increases the relative abundance of *Helicobacter* spp. in the gut microbiome, in association with increases in spontaneous colitis, exaggerated release of proinflammatory cytokines, interferon gamma and interleukin 6, from freshly isolated mesenteric lymph nodes stimulated with anti-CD3 antibody *ex vivo*, exaggerated dextran sulfate sodium (DSS)-induced colitis in a model of inflammatory bowel disease (IBD) and increased in anxiety-related defensive behavioral responses[11]. Importantly, these effects of chronic psychosocial stress were absent in mice that were *Helicobacter*-free, and could be reinstated by infecting with *Helicobacter* sp. [12, 13]. Together, these studies demonstrate that *Helicobacter* infection is an important determinant of physiological and behavioral responses to stress. Further, microbiome-based interventions can prevent these stress-induced outcomes, presumably by preventing inflammatory responses associated with *Helicobacter* infection, even without affecting the relative abundance of *Helicobacter* in the gut microbiome. These studies suggest that microbiome-based interventions may be useful as an adjunct therapy to antibiotic treatment, for reduction of antibiotic-induced side-effects, or for preventing distal outcomes of *H. pylori* infection, including stress-induced inflammation, as well as symptoms of anxiety and depression, which can be elevated in individuals with *H. pylori* infection [14]. Together, these findings suggest that an improved understanding of the relationship between *H. pylori* infection and gut microbiome diversity and community structure may inform strategies for development of microbiome based interventions designed as an adjunct therapy to standard antibiotic therapy, with the aim of improving both proximal (i.e., *H. pylori* eradication) and distal (*e.g.*, inflammation and mental health) outcomes.

3. Reviewer: *The general feeling is that the Authors are facing a number of observations that are possibly indirectly associated to H. pylori (e.g. previous treatments, lifestyle, diet).*

Response: Information was collected about each participant regarding (i) if they had b een treated for *H. pylori* infection in the past, (ii) if so, the date infection was diagn osed, (iii) treatments given, and (iv) outcomes: eradication testing with dates. Only on e patient had *H. pylori* infection 10 years ago and had successfully completed treatme nt at that time. History about antibiotic treatments for any infection and PPI use for both *H. pylori* patients and control subjects in the past five years was also documente d and these data values were included in the statistical analyses. Our statistical analys es showed that the differences in microbiome composition and diversity seen in *H. py lori* patients were not due to the previous treatments. We have now included these de tails (lines 381-388).

We do agree with the reviewer that previous treatments, lifestyle, and diet are importa nt confounders for the diversity and composition of the gut microbiome. We had men tioned in our manuscript that: “Conversely, the data may reflect a preceding decrease in gut microbial diversity that facilitates *H. pylori* infection, possibly mediated by oth er factors or exposures that accumulate with age. In other words, patients who experi ence decreased gut microbial diversity as they age, potentially mediated by diet or ch ronic inflammation, may be more prone to *H. pylori* infection, particularly by mid-adu

lthood." However, given the challenge in controlling for hard-to-measure variables suc h as lifestyle and diet, we do not believe their presence precludes drawing conclusion s from statistically significant observations associated with *H. pylori* infection. As desc ribed above, increased understanding of the diversity and community composition of t he gut microbiome may be useful for development of microbiome based interventions designed as an adjunct therapy to standard antibiotic therapy, with the aim of impro ving both proximal (*i.e*., *H. pylori* eradication) and associated distal (*e.g***.** colorectal ca ncer, metabolic disease) outcomes. We have now acknowledged these potential confou nders in the text (lines 624-628).

4. Reviewer: *The discussion is too long, rambling, and needs to be focused.*

Response: Please note that some aspects also got added to the discussion in response to comments made by reviewers. The discussion is long; however it will be useful for the readers to have comprehensive information about various aspects of the study. As per the reviewer's suggestion, we have tried to make the discussion concise by removing the paragraph comparing our findings to those of other studies evaluating changes to distal gut microbiota in association with *H. pylori* (now referenced briefly in the Introduction) and also modified the paragraph about effect of *H. pylori* with respect to age.

5. Reviewer: *Introduction, ref 8-12: these are reviews, not experimental studies, and the associations merely hypothetical; ref 15 describes Hp-associated consequences in the upper gut***.**

Response: We have revised the Introduction to acknowledge associations as theoretical or speculative where appropriate and emphasize associations observed in primary literature and causal effects seen in experimental studies. Indeed previous reference 15 was not the most appropriate to cite when discussing the potential consequences of *H. pylori*-associated distal gut dysbiosis. We have now deleted this reference. We have now also revised this statement and included a reference to Butt J, *et al*., who found in a large case-control study that seropositivity for the *H. pylori* virulence factor VacA was associated with increased odds of colorectal cancer (lines 200-202) [8].

6. Reviewer: *Methods, patients and controls: relevant information are missing. What was the diagnosis in Hp+ patients? When, how, how many times had they been treated? It may well be possible that observed differences might be the effect of previous treatments, not Hp.* How was Hp+ diagnosed in controls?

Response: *H. pylori* patients are identified in our electronic medical record system (EPIC) by ICD-9 codes of 041.86, 531.90, 535.60, 795.79, 008.47, 531.00, V12.08. Histological examination of the biopsied samples showed positive outcome indicating *H. pylori* infection. As mentioned in response 3 above, information was collected about each participant regarding (i) if they had been treated for *H. pylori* infection in the past, (ii) if so, the date infection was diagnosed, (iii) treatments given, and (iv) outcomes: eradication testing with dates. Only one patient had *H. pylori* infection 10 years ago and had successfully completed treatment at that time. History about antibiotic treatments for any infection and PPI use for both *H. pylori* patients and control subjects in the past five years was also documented and these data values were included in the statistical analyses. Our statistical analyses showed that the microbiome differences seen in *H. pylori* patients were not due to the previous treatments.

The control subjects were screened for lack of previous history and current documentation for *H. pylori* infection as well as lack of any GI-related symptoms before recruitment. Their stools were tested by RT-PCR based biprobe assay [15] for presence of *H. pylori* DNA. None of the stool samples from the control subjects showed presence of *H. pylori* DNA. We have now included these details in the revised manuscript (lines 248-256).

7. Reviewer: *statistics: By and large, the sample size is very small and raises concerns as far as the conclusions can be supported. Was a sample size calculation made? Was the statistical power sufficient? Can these small numbers support age-based differences? Convince me and the general reader.*

Response: As little is known about the microbiome of *H. pylori* patients in an underprivileged community, this study is exploratory. A sample size calculation for microbiome analysis was not thus performed prior to collecting samples. Casals-Pascual *et al*. outline that in human microbiome studies knowledge of the baseline microbiome diversity and composition, along with knowledge of the magnitude of changes that confer clinically relevant outcomes are important for determining sample size [16]. However, this could not easily be predicted in our sample demographic due to a lack of previous research. Though the effects of *H. pylori* and low socioeconomic status (SES) on the gut microbiome have been investigated separately in previous studies, the two states (*H. pylori* infection and low SES) could potentially have conflicting relationships with diversity and composition, making it difficult to determine the baseline microbiome diversity and composition without large assumptions. Thus, much of this study provides the baseline to characterize the microbiome of *H. pylori* patients in an underserved population, which was previously understudied to a degree by which sample size calculations would have required a large number of assumptions. Though the power is likely weak in this study, especially for post-hoc analyses such as alpha diversity in *H. pylori* patients over 40 years of age *versus* control participants over 40 years of age, multiple lines of evidence, including both the Kruskal-Wallis test with age group and the Spearman correlation with actual age support the relationship in our sample. These results can provide a basis for future study design and power calculations. We have now modified the text to include this rationale (lines 475-495)**.**

8. Reviewer: *Discussion, lines 6-11. There is no evidence of this: the study is not designed to answer this question.*

Response: This reviewer comment refers to the statement: "Collectively, these results could suggest an *H. pylori*-mediated transformation of the fecal microbiome that progresses with age and becomes apparent by mid-adulthood. Central to this transformation is a decline in fecal microbial diversity that may be the result of long-standing infection in which *H. pylori* alters the gastrointestinal environment at the expense of other taxa. This conclusion is consistent with those of other studies that attributed decreased diversity of gastric microbiota to the ability of *H. pylori* to outcompete other species (references are given)."

We agree our study is not designed to answer this question; we have now revised our statement to clarify this (lines 526-530).

9. Reviewer: *Discussion, 2nd para, lines 3-... H. pylori infection is usually achieved during infancy, and carried on through adulthood. This para needs revision.*

Response: We have revised this paragraph as per the reviewer's suggestion (lines 534-549).

10. Reviewer: *Discussion page 18. The hypothesis of a gut mucosal disruption induced by H. pylori is not supported by the data.*

Response: We now have revised the statement to reflect that it is a speculation and not conclusion from our data (lines 582-584).

Reviewer 2

We thank the reviewer for his/her positive evaluation of our manuscript and his/her th oughtful suggestions, which helped us to improve our manuscript.

The reviewer said that "*In my opinion, this manuscript is provided the new knowled ge and novelty.*"

Response: We thank the reviewer for this comment.

1. Reviewer*: The biopsies between June 1 st, 2017 and December 31st, 2020 were el igible for inclusion. Stool samples from 19 H. pylori patients and 16 control subjects were analyzed. Why does take a long time (3+Yrs.) to collect only 35 patients?*

Response: Patients diagnosed between June 1, 2017 and December 31st, 2019, were e ligible for inclusion. We apologize for the typographical error that it is 2019 and not 2020. We have now corrected this in the Materials and Methods section (line 245). N o particular preference was given to demographics or any other patient characteristics while recruiting. We recruited all those who were willing to participate. Our sample s ize is modest due to the stringent limitations posed by the socioeconomic attributes of our patient population. According to the US census data, Camden County has the lo west median income and highest poverty and unemployment rates in southern New Je rsey. It also has the lowest educational attainment and the greatest socioeconomic disp arities among ethnic minority groups. More than one third of the population in Camd en City, where our hospital is located, lives below the national poverty line, and the median household income is USD 26,105. Our local community is thus considered on e of the poorest and most economically distressed communities in the United States. A significant fraction of adults over 25 years old (almost one quarter) have not comp leted high school. It is possible that this influences patient's appreciation of the releva nce of the study. Please note that in the present study, 13 out of 19 participating *H. pylori*-infected patients had an education level of high school or less. A large number of potential participants were less inclined to perform stool sample collection and del ivery. Patients also lacked readily available means of transportation for transport of sa mples to the clinic, especially as the window for collecting stool samples was short a s samples need to be collected after the infection was confirmed, but before the patie nt started antibiotics. We have now included this in the Discussion section and have also given a reference (lines 497-515).

2. Reviewer*: Patients were asked to provide a stool sample before initiating eradication therapy. The characterization of gut microbiome and metabolome in before and after eradication of Helicobacter pylori is more interested. Do you have the data?*

Response: The goal of the present study was to have a greater, more individualized understanding of gut dysbiosis among patients experiencing *H. pylori* colonization, esp ecially in our underserved population, which will in turn help to create opportunities f or targeted therapy, as well as a potential for creating predictive models for antibiotic selection, and even exploring restorative probiotic therapy to help lessen the adverse impact of eradication therapy. We thus focused on gut microbiome and metabolome a nalyses before the antibiotic treatment. We do agree with the reviewer that it will be interesting to carry out these analyses after antibiotic treatments. Although that is bey ond scope of our present study, we made note of it as an important objective for fut ure studies.

3. Reviewer*: In Table 1. Patient demographics and clinical characteristics. There are many confounding factors that interfering gut microbiome. Please discuss.*

Response: We analyzed the effect of each of the factors included in Table 1 on alph a and beta diversity. Age was the only factor that showed statically significant differe nces (*p*-value of ≤ 0.05) in the *H. pylori* patients compared to the control subjects. We have now included this statement in the results section, as follows (lines 374-379): Age was the only factor that showed statically significant differences (*p*-value of ≤ 0.05) in alpha diversity in the *H. pylori* patients compared to the control participants, with *H. pylori* patients over 40 years of age having lower richness than control parti cipants over 40 years of age. Significant differences in community composition and gr oup dispersion were seen between *H. pylori* patients and control participants."

4. Reviewer*: The typing error in symbol of ⎡g/g and δ-8-desaturase.*

Response: We apologize for the typographical errors. The symbols in the μg/g and de lta 6 and 8 desaturases have now been included in the correct format.

Reviewer 3

We greatly appreciate the reviewer's very positive reception of our manuscript and rec ommendation for acceptance with high priority. We thank this reviewer for his/her tho ughtful suggestions, which helped us to improve our manuscript.

The reviewer said that "*The original article entitled "Characterization of gut microbi ome and metabolome in Helicobacter pylori patients in an underprivileged community in the United States" is of high quality, both in terms of language and the results p resented. The discussion of the results is also done exhaustively. Therefore, I percept t he manuscript very positively and have only a few small suggestions /corrections, whi ch I consider appropriate to introduce.*"

Response: We thank the reviewer for this comment.

1. Reviewer*:* Corrections: - "the impact of H. pylori infection on distal gut, i.e. fecal, microbiota, and observed" -> the impact of H. pylori infection on distal gut, i.e., fec al microbiota, and observed - μg/mL -> please correct in several parts of the manuscr ipt (the unit is invisible).

Response: We have now corrected the symbols throughout the manuscript. We have a lso placed a comma after 'i.e.'

2. Reviewer: "*The increased abundance of Gemellaceae in H. pylori patients is consi stent with the observation of increased organisms from the Gemella genus in patients with current H. pylori infection reported by Gao et al." -> as this is one of the few microorganisms that has been shown to be increased in the abundance during H. py lori infections, please add an extra sentence or two concerning an association of Ge mellaceae with human infections or physiology. For example: https://www.ncbi.nlm.nih. gov/pmc/articles/PMC4059512/ and https://pubmed.ncbi.nlm.nih.gov/25003194/* ."

Response: We thank the reviewer for bringing these two articles to our attention. We have now included these two references in our manuscript and have added a sentenc e in the discussion as suggested by the reviewer, as follows:

"Increased relative abundance of Gemellaceae in the Crohn's ileum has been reported by other groups (lines 574-575; references are included)"

3. Reviewer: "*persistent infection with H. pylori may create a niche favorable for tax a that are found in increased abundance in gastric cancer, including Lactobacillus an d Lachnospiraceae" -> I believe that after this sentence one or two sentences explain ing this phenomenon should be added: the increased amount of these microorganisms seems to be associated with the presence of high concentrations of lactic acid produc ed by both cancer cells and bacteria and can be used by both a fuel source. For ex ample: https://pubmed.ncbi.nlm.nih.gov/31394110/.*"

Response: We thank the reviewer for bringing this article to our notice. We have no w referenced this in our text and have added in the discussion: "It has been suggeste d that lactic acid bacteria can influence gastric cancer by a number of mechanisms su ch as (i) supply of exogenous lactate, that acts as a fuel source for cancer cells, (ii) production of reactive oxygen species and N-nitroso compounds, and (iii) by allowing colonization of carcinogenic non-*H. pylori* bacteria [lines 616-620; references are giv en]".

4. Reviewer: "*Figure 4: in one place H. pylori is written without italics - Figure 7: the image on the right is too dark -> in the electronic version it can read easily, bu t not after printing; please change to a lighter version.*"

Response: We have italicized the words '*H. pylori*' in the legend of Figure 4. We ha ve changed the font color to white in the dark circles in Figure 7, so it will be print ed appropriately.

Reviewer 4

We thank this reviewer for his/her thoughtful suggestions, which helped us to improve our manuscript.

1. Reviewer: "*The number of cases involved in this study is small, and the compositi*

on of the experimental group and the control group is not consistent in people over 40 years old and under 40 years old. The results of this study cannot represent the u nderprivileged community."

Response: As mentioned above, we recruited all those who were willing to participat e and no particular preference was given to demographics or any other patient charact eristics while recruiting. Alpha diversity was shown to be significantly lower in *H. py lori* patients over 40 years of age compared to control participants over 40 years of a ge through a Kruksal-Wallis test in our analysis, which takes into account the numb er of test and control subjects and is appropriate for comparing sample groups of une qual sizes. Moreover, the Kruskal-Wallis test is a rank-based approach that is typicall y more conservative than other tests that could have been used, such as an ANOVA or t-test. Additionally, the Fisher transformation on Spearman rho values for the correl ation with alpha diversity with age provides another line of evidence supporting this i n our sample group. Recruitment of both cohorts was not carried out based on any p articular subject characteristics in order to avoid biases. Our sample size is modest du e to the stringent limitations posed by the socioeconomic attributes of our patient pop ulation. According to the US census data (this reference is now included in the revise d manuscript), Camden County has the lowest median income and highest poverty an d unemployment rates in southern New Jersey. It also has the lowest educational attai nment and the greatest socioeconomic disparities among ethnic minority groups. More than one third of the population in Camden City, where our hospital is located, lives below the national poverty line, and the median household income is USD 26,105. Our local community is thus considered one of the poorest and most economically dis tressed communities in the United States. A significant fraction of adults over 25 year s old (almost one quarter) have not completed high school. It is possible that this inf luences patient's appreciation of the relevance of the study. Please note that in the pr esent study, 13 out of 19 participating *H. pylori*-infected patients had an education lev el of high school or less. A large number of potential participants were less inclined to perform stool sample collection and delivery. Patients also lacked readily available means of transportation for transport of samples to the clinic, especially as the windo w for collecting stool samples was short as samples need to be collected after the inf ection was confirmed, but before the patient started antibiotics. We have now included this in the Discussion section and have also given a reference for the community ch aracteristics as mentioned above (lines 475-515).

2. Reviewer*:* "*The results of this study for people over 40 years of age were also ob served in other reported studies of older or antibiotic users.*"

Response: Thank you for the comment. To our knowledge, this is the first study spe cifically describing an age-dependent decrease in fecal microbial diversity associated w ith *H. pylori* infection. Other studies have reported decreased gastric microbial diversit y associated with *H. pylori* infection, and these are now referenced in the Discussion as: "While other authors have attributed decreased diversity of gastric microbiota to th e ability of *H. pylori* to outcompete other species, secretion of antibacterial peptides, and local pH alterations, the mechanisms by which *H. pylori* affect distal gut microbi ota remain unclear (references are given). Although our study was not designed to un cover these mechanisms, multiple factors hypothesized by other authors, including alter ations in hydrochloric acid secretion, gastrointestinal hormones, and immune regulation,

may be involved in the mediation of distal gut dysbiosis (lines 523-529)." We have added a recent reference highlighting that *H. pylori* eradication *via* antibiotic therapy was indeed associated with restoration of the gastric microbial diversity to levels seen in uninfected patients- "The impact of *H. pylori* on fecal microbial diversity observed in our study may be reversible, just as a recent study showed that *H. pylori* eradication could restore gastric microbial diversity to levels seen in uninfected controls (lines 530-532; reference is included)." Interestingly, other studies have shown increased fecal microbiome diversity in association with *H. pylori* infection, and these contrasting findings are referenced as well in the Discussion- "In contrast to our study, Dash *et al*. observed increased alpha diversity among stool samples from *H. pylori*-positive individuals, although they found no effect on beta diversity[4]. Finally, Frost *et al*. found significant changes in fecal microbiome diversity and composition as measured by alpha and beta diversity, respectively, in *H. pylori*-positive individuals compared to controls, and the latter association was strongly correlated with *H. pylori* stool antigen load[3]."

We have revised the manuscript addressing each of the suggestions/comments given by all the reviewers as described above. We thank the reviewers again for their suggestions, which we believe resulted in significant improvements in the manuscript.

We thank the Science Editor and the Company Editor-in-Chief for conditionally accepting our manuscript. We have provided all the forms as asked by the Science Editor. We have also addressed all of the queries raised by the Science Editor below:

Science editor comments that asked for our action:

Comment: The questions raised by the reviewers should be answered:

Response: We have revised the manuscript addressing each of the suggestions/comments given by all the reviewers. Item-by-item answers to the reviewers' comments are given above.

Comment: The signed Conflict-of-Interest Disclosure Form and Copyright License Agreement, and Informed consent or waiver form.

Response: We have now attached these three forms. Please note that we had uploaded IRB approval in our first submission. We have now also attached to it the informed consent form that was used for the study. However, as per our IRB rules, we are not permitted to include the consent forms obtained from participants as that will violate the code of patient confidentiality. We had sent an inquiry to the journal before (helpdesk ticket ID 10408) and were informed that our process was acceptable.

Comment: I found the similar article by the Google search.

Response: We uploaded a copy of the manuscript to a pre-print server as that is allowed per the journal guidelines (as a decision was not made by the journal at that time). As gut microbiome and *Helicobacter pylori* infection are currently hot fields of study, we wanted to make sure that our results are documented before another research group carries out similar research and publishes. Pre-printing manuscripts is a common practice at our research institutes.

Comment: The authors did not provide the approved grant application form(s). Please upload the form.

Response: We have now included grant form. Please note that this is an internal grant awarded by our institution.

Comment: The authors did not provide original pictures.

Response: This is not applicable as we do not have any pictures in the manuscript.

Comment: Please provide the original figure files. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor

Response: We have now provided all figures in power point format that can be reprocessed by the editor.

Comment: The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text (and directly before the References).

Response: We have now added the "Article Highlights" section as suggested by the Science Editor.

Check list of Step 7: Revision Files to be uploaded

(1) 66632-Answering Reviewers
(2) 66632-Audio Core Tip
(3) 66632-Biostatistics Review Certificate
(4) 66632-Conflict-of-Interest Disclosure Form
(5) 66632-Copyright License Agreement
(6) 66632-Approved Grant Application Form(s) or Funding Agency Copy of any Approval Document(s)
(7) 66632-Institutional Animal Care and Use Committee Approval Form or Document
(8) 66632-Institutional Review Board Approval Form or Document
(9) 66632-Non-Native Speakers of English Editing Certificate
(10) 66632-Video- Not applicable
(11) 66632-Image File
(12) 66632-Table File
(13) 66632-The ARRIVE Guidelines
(14) 66632-Supplementary Material

References used in the item-by-item responses:

1. Heimesaat MM, Fischer A, Plickert R, Wiedemann T, Loddenkemper C, Göbel UB, Bereswill S, Rieder G. *Helicobacter pylori* induced gastric immunopathology is assoc iated with distinct microbiota changes in the large intestines of long-term infected Mo ngolian gerbils. *PLoS One* 2014; **9**(6): e100362 [PMID: 24941045 PMCID: PMC4062 524 Heimesaat and Stefan Bereswill are PLOS ONE Editorial Board members. This d

oes not alter the authors' adherence to all the PLOS ONE policies on sharing data an d materials. DOI: 10.1371/journal.pone.0100362]

2. Kienesberger S, Cox LM, Livanos A, Zhang XS, Chung J, Perez-Perez GI, Gorkie wicz G, Zechner EL, Blaser MJ. Gastric *Helicobacter pylori* Infection Affects Local a nd Distant Microbial Populations and Host Responses. *Cell Rep* 2016; **14**(6): 1395-14 07 [PMID: 26854236 PMCID: PMC4758874 DOI: 10.1016/j.celrep.2016.01.017]

3. Frost F, Kacprowski T, Rühlemann M, Bang C, Franke A, Zimmermann K, Nauck M, Völker U, Völzke H, Biffar R, Schulz C, Mayerle J, Weiss FU, Homuth G, Lerc h MM. *Helicobacter pylori* infection associates with fecal microbiota composition and diversity. *Sci Rep* 2019; **9**(1): 20100 [PMID: 31882864 PMCID: PMC6934578 DOI: 10.1038/s41598-019-56631-4]

4. Dash NR, Khoder G, Nada AM, Al Bataineh MT. Exploring the impact of *Helicob acter pylori* on gut microbiome composition. *PLoS One* 2019; **14**(6): e0218274 [PMID: 31211818 PMCID: PMC6581275 DOI: 10.1371/journal.pone.0218274]

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