Author’s response to reviews

Title: Safety and Tolerability of Experimental Hookworm Infection in Humans with Metabolic Disease: Study Protocol for a Phase 1b Randomised Controlled Clinical Trial

Authors:
Doris Renate Pierce (doris.pierce@my.jcu.edu.au)
Lea Merone (lea.merone@my.jcu.edu.au)
Chris Lewis (chris.lewis2@sa.gov.au)
Tony Rahman (tony.rahman@health.qld.gov.au)
John Croese (jcroese@bigpond.com)
Alex Loukas (alex.loukas@jcu.edu.au)
Malcolm McDonald (malcolm.mcdonald@jcu.edu.au)
Paul Giacomin (paul.giacomin@jcu.edu.au)
Robyn McDermott (robyn.mcdermott@jcu.edu.au)

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Responses to reviewers

We thank the Editor and reviewers for their positive feedback that has strengthened the presentation of our protocol. Please find a point-by-point response to each of the reviewer’s comments below.

Reviewer 1

Reviewer’s comment
First in the design justification for not doing a cross over design must be considered.

Response
We thank the reviewer for this suggestion. A cross-over design would indeed strengthen the study’s findings; however, such a design is not feasible for this particular trial. First, the length of the trial (a minimum of 104 weeks) requires considerable commitment from the participants during this time, and it is unreasonable to expect each participant to complete the 3 arms of the trial, which would mean a
minimum commitment of 6 years. Second, and more important, it is currently not confirmed at which
point in time after deworming (or to what degree this happens at all) hookworm-induced changes in
metabolic and immune responses and the microbiome have abated and prior status is re-established,
which would have to occur before the participants could start the next arm. This uncertainty could
result in a carry-over effect of the previous treatment and would also make it extremely difficult to plan
a timeline for the trial and commit the required funding and resources.

Reviewer’s comment
Since this study is based on worm therapy and associated immunological aspect, therefore it is
imperative that the history of parasitic infection of the study participants should be a major recruitment
criterion. However that was not mentioned.
Also it is important to have idea of the place of residence of these subjects which pertains to endemic
normal status of the individual, if they belong to any area where the parasite is naturally endemic.

Response
We thank the reviewer for raising this important point. History of parasitic infection is recorded as part
of the medical history during the screening visit (Section 3.1). Importantly, the faecal sample collected
at Baseline will be available for retrospective analysis of the presence of some of the most common
gastrointestinal nematode parasites (Necator, Ascaris and Ancylostoma) using a multiplex qPCR. Hence,
while we do not undertake a rigorous investigation of the parasite status of individuals prior to
enrolment, mostly due to the unlikeliness of this occurring in people from metropolitan Australia, we
do have the ability to take previous or current parasitic infection into consideration into account during
our final analyses.

The human hookworm Necator americanus is not endemic to the recruitment area (see Section 1.3 for
description of the study’s setting), and Section 1.3 has been modified to clarify this point (Lines 175-
176).

Reviewer’s comment
Also whether during the course of the study if any pregnancy occurs, how it will be dealt with, must be
mentioned, not only screening at the onset of the study will suffice.

Response
A sentence has been added to Section 3.3.2 to address this potential issue (Lines 284-285).

Reviewer’s comment
It was mentioned that the mandatory requirement for the study participants to 'purchase' the
anthelminthic medication, which seems objectionable as it is the moral /ethical responsibility of the
investigator to provide rather than asking to 'purchase'. It's also quite contradictory to mention in the
next sentence that 'however, it is not mandatory for participants to comply with this direction'! If it is a
mandatory requirement then how it is not mandatory for the participants to follow that direction?

Response
We thank the reviewer for identifying this contradictory point. We can confirm that the medication will
indeed be provided to the participants free of charge. It is a mandatory requirement to provide the
medication, but not mandatory for the participants to consume the medication should they prefer to
keep their worms. Please see amendments to Paragraph 3.3. (Lines 243-245).
Reviewer’s comment
Moreover, it is also very difficult to accept that ‘Participants electing to keep their helminths will be advised that their future medical care is their own responsibility.’ Since the study is still continuing (even it is optional for the participants) after 2 years, then the benefit of this follow-up is added onto the study results and will enrich the data, therefore it is unethical to discontinue the provision for medical care, leaving that as their responsibility.

Response
Only participants electing to take the anthelmintic treatment will be able to opt into the 52-week extension to assess the impact of deworming (see Section 3.3, Line 246-248). These participants will still be provided with medical care during the extension period.

Participants choosing to forego the anthelmintic treatment, that is to keep their worms, at completion of 104 weeks will not be able to opt into this extension period, as this period specifically assesses the impact of deworming. These participants will, therefore, be considered as having completed the trial. Medical advice will be provided to the participants leaving the trial at 104 weeks and electing to keep their worms, and future medical care will be passed on to their general practitioner (Line 246-248).

Reviewer’s comment
The study variables has not been mentioned very clearly and a broad outline only is provided, which makes the protocol weak.

Response
We have been careful to outline all of the relevant study measures throughout the protocol, however some of these are displayed across multiple sections. For example, Primary outcome: Section 3.4 describes evaluation of the primary outcome (Safety of infection) by number of reported AEs and SAEs, assessment of general health, and successful completion of the 24-month trial (Line 312-314). Following on from this, section 3.3.2 and Section 3.1 provide further details on the assessment of AEs/SAEs and general health, respectively. Additional File 3 (CRF for Adverse Events) has now been added to specify the recording of AEs and SAEs.

The study has a long list of secondary outcomes that are detailed throughout – for example changes in metabolic markers are detailed in Section 3.4, while immunological parameters are described in 4.2.3 and microbiome parameters in 4.2.4.

Reviewer’s comment
How the meta-genomic profile of microbiome will be linked to the parasitic effect on Metabolic syndrome has to be explained with justification. Specific immunological markers also need to be spelt out.

Response
Section 4.2.4 has been amended to include a detailed description of analysis of the parasite-mediated effect on the microbiome and its correlation with immune and metabolic markers. Following quality filtering, the QIIME pipeline will be used for operational taxonomic unit (OTU) analysis and Beta diversity will be analysed using UniFrac. Longitudinal comparisons will be made between baseline, 12
and 24 months looking at diversity and any changes in OTUs between treatment and placebo groups. These data, along with clinical and immunological data, will be analysed subsequently in an integrative fashion to more comprehensively define the biological impact of experimental Na infection on individuals with metabolic disease. (Lines 391-399).

Specific immunological markers (serum cytokines and flow cytometric analyses) have now been further detailed in Section 4.2.3 (Lines 380-382, 384-388).

Reviewer 2

Reviewer’s comment
Page 4, ref [4]: Please replace by relevant recent literature reviewing the concept of obesity-induced chronic low-grade inflammation (or meta-inflammation), e.g. PMID: 21233852, 26553134, 28179656.

Response
Has been replaced with PMID 28179656.

Reviewer’s comment
Page 5: A reference to the recent study reporting that helminth infection promote colonization resistance via type 2 immunity might be added to illustrate the possible immune-mediated modulation of gut microbiota by the parasite (PMID:27080105)

Response
The suggested reference has been integrated (Lines 125-128).

Reviewer’s comment
Page 6, line 20: "obese young". Taken into that you'll include subjects between 18 and 50 year-old, I'm not sure that you should keep the term "young" here…(although debatable when being close to reach this canonical age!)

Response
The term “young” has been replaced with primary age window for progression to T2DM (Lines 184-185).

Reviewer’s comment
Page 8: the criteria for defining dyslipidemia (see for example ESC/EAS Guidelines for the Management of Dyslipidaemias, Eur Heart J. 2016) and abnormal liver function (ALAT/ASAT levels ?) should be better specified. Of note, blood glucose level 2-hour post glucose challenge (>140 mg/dL and <199 mg/dL being considered as pre-diabetics) is usually a more stable criteria for MetS than HOMA-IR (quite high day-to-day intra-individual variations, increasing the risk of having a significant number of pre-screened eligible subjects not confirming this criteria at the day of baseline visit).

Response
We thank the reviewer for this suggestion. Further information regarding individual measures to identify dyslipidaemia and abnormal liver function has been added on page 8 (Lines 189-192).
Reviewer’s comment
Page 10: It is unclear how the safety will be checked during the first 6 months, the period during which the most significant side effects might occur. Do the participants only contact the investigators when sometimes unexpected happen or is there any pro-active communication between them during the first weeks? What would be the frequency for filling the structured questionnaires during the first 6 month (page 12)? It would definitely be important to carefully monitor the adverse effects/quality of life during the first weeks/months post-infection

Response
We thank the reviewer for raising this important point. As the reviewer correctly points out the most significant side effects are likely to occur during the first few weeks after inoculation with the most likely timing for the onset of gastrointestinal symptoms being around 4-6 weeks following inoculation as the worms anchor themselves to the intestinal mucosa to facilitate feeding and avoid ejection by gut peristalsis (PMID 27929101). A previous trial (PMID 21408161) using controlled hookworm infection in individuals with celiac disease reported abdominal pain, increased flatulence, nausea and bloating as a common side effects during this initial colonisation, which resolved completely by week 16. Itching at the inoculation site can last up to week 4. Participants in the current trial will receive 2 inoculations 8 weeks apart with the second inoculation providing an ideal opportunity to identify/monitor any unexpected side effects. Additionally, an extensive information sheet provided to all participants before giving informed consent includes details about potential side effects of the treatment, and this information is reiterated during the first inoculation. Also, participants are encouraged during the first inoculation to contact the trial doctor should they notice any unusual signs and symptoms. If deemed necessary by the trial doctor, participants will be called in for an additional examination, during which they can elect to discontinue with the trial. Should the symptoms be consistent with potential side effects of hookworm infection, the participant may become unblinded and offered anthelmintic medication. Section 3.3.2 (paragraph 2) has been expanded to include this information (Lines 289-301).

Reviewer’s comment
Page 11: The information relative to the source and L3 preparation are rather sparse. Is there any quality control step before inoculation?

Response
The infective larvae are cultured freshly on site when required for inoculation following an established protocol that has been used for similar previous studies. Larvae are decontaminated using iodine disinfectant and individually selected by an experienced researcher based on morphological integrity and active motility, so we can be certain that larval viability is optimal. A paragraph detailing this information has been added to Section 3.3.1 (Lines 262-265).

Reviewer’s comment
Page 11: What happens if unexpected pregnancy occurs during the trial?

Response
A sentence has been added to Section 3.3.2 to address this potential issue (Lines 284-285).

Reviewer’s comment
Page 17: "systemic immune system"; please rephrase
Response
The word “systemic” has been removed.