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Controlled Hookworm Infection for Medication-free Maintenance in Patients with Ulcerative Colitis: A Pilot, Double-blind, Randomized Control Trial

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Background: Human hookworm has been proposed as a treatment for ulcerative colitis (UC). This pilot study assessed the feasibility of a full-scale randomized control trial examining hookworm to maintain clinical remission in patients with UC.

Methods: Twenty patients with UC in disease remission (Simple Clinical Colitis Activity Index [SCCAI] ≤4 and fecal calprotectin (fCal) <100 ug/g) and only on 5-aminosalicylate received 30 hookworm larvae or placebo. Participants stopped 5-aminosalicylate after 12 weeks. Participants were monitored for up to 52 weeks and exited the study if they had a UC flare (SCCAI ≥5 and fCal ≥200 µg/g). The primary outcome was difference in rates of clinical remission at week 52. Differences were assessed for quality of life (QoL) and feasibility aspects including recruitment, safety, effectiveness of blinding, and viability of the hookworm infection.

Results: At 52 weeks, 4 of 10 (40%) participants in the hookworm group and 5 of 10 (50%) participants in the placebo group had maintained clinical remission (odds ratio, 0.67; 95% CI, 0.11-3.92). Median time to flare in the hookworm group was 231 days (interquartile range [IQR], 98-365) and 259 days for placebo (IQR, 132-365). Blinding was quite successful in the placebo group (Bang's blinding index 0.22; 95% CI, −0.21 to 1) but less successful in the hookworm group (0.70; 95% CI, 0.37-1.0). Almost all participants in the hookworm group had detectable eggs in their faeces (90%; 95% CI, 0.60-0.98), and all participants in this group developed eosinophilia (peak eosinophilia 4.35 × 10^9/L; IQR, 2.80-6.68). Adverse events experienced were generally mild, and there was no significant difference in QoL.

Conclusions: A full-scale randomized control trial examining hookworm therapy as a maintenance treatment in patients with UC appears feasible.

Lay Summary

This pilot study has shown a full-scale RCT examining hookworm therapy as maintenance therapy in patients with ulcerative colitis is feasible, safe, and will be well-tolerated.

Key words: ulcerative colitis, inflammatory bowel disease, helminth, hookworm, clinical trial

Introduction

The pathogenesis of inflammatory bowel disease (IBD), comprising ulcerative colitis and Crohn's disease, involves complex genetic, environmental, microbial, and immune factors.[1] The incidence of IBD is increasing at an alarming rate, particularly in developing regions of the world where IBD was once a rare disease.[2] Although the cause is not fully elucidated, one contributing factor may be the elimination of certain gastrointestinal parasites, also known as helminths, from the human intestinal flora due to improved sanitation.[3] In support of this premise, preclinical and early clinical studies have shown the introduction of certain helminths regulate hyperactive immune responses in the host and reduce disease activity in IBD without immune-suppressing the host.[4–6] An example of this mutually beneficial host-parasite interaction is the human hookworm, *Necator americanus*.[7]

Controlled hookworm infections using dose-controlled *Necator americanus* have been trialled in the treatment of several allergic and autoimmune diseases including asthma, multiple sclerosis, and celiac disease.[8–11] There is also significant public interest in hookworm as a potential therapy for disease, with widespread "underground" use of human hookworm infection driving anecdotal evidence of its benefit in treating many diseases, including ulcerative colitis.[12]

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Key Messages

What is already known?

- Patients with ulcerative colitis already use medically unsupervised hookworm therapy, suggesting it may be of benefit, but the evidence base is lacking.

What is new here?

- This pilot study is the first controlled evidence in the use of hookworm as a therapy in ulcerative colitis. It has shown hookworm therapy to be well-tolerated and safe, and a full-scale RCT is feasible.

How can this study help patient care?

- Hookworm therapy could provide an alternative therapeutic option to conventional medications and improve adherence by removing the need to take daily medication. This study is a step towards proving whether it is efficacious in ulcerative colitis.

Of importance, no randomized controlled trials (RCTs) have been conducted investigating hookworm therapy in the treatment of ulcerative colitis.

The authors of the current study recently completed a longitudinal study of controlled hookworm infections in healthy volunteers and have shown that it is safe and well-tolerated and that a viable infection remains years after a single inoculation with hookworm without the needed for repeated infections.[13] That study also highlighted how the acute phase of a hookworm infection, as hookworm migrate through the lungs to the small intestine, is characterized by a pronounced systemic and local intestinal type 2 immune response (elevation in interleukin [IL]-5 and eosinophils), which dissipates as the hookworm infection enters its chronic immunoregulatory phase in the gut.[13] Previous studies examining helminths to treat IBD have focused on patients with active inflammation, but the longevity of hookworm infection and this characteristic host immune response make hookworm therapy better suited as a maintenance therapy where a rapid response to suppress active inflammation is not required.[5,14,15] Furthermore, by providing immunoregulatory benefits for years without the need to take daily medication, hookworm therapy has the potential to address the clinical challenge of poor medication adherence faced by IBD patients whilst in disease remission.[16]

To test the hypothesis that hookworm therapy is an effective maintenance treatment in ulcerative colitis first requires a pilot study to assess the feasibility of recruiting, infecting, and blinding patients with ulcerative colitis, while also assessing the safety and tolerability of a controlled hookworm infection in this patient group. A particular safety concern is the possibility that the pronounced type 2 immune response experienced in the early stages of a hookworm infection may trigger a flare of ulcerative colitis.[13,17] To address the feasibility and safety of proceeding to a full-scale RCT, this double-blinded, randomized controlled pilot study recruited patients with ulcerative colitis currently in disease remission and only being treated with 5-aminosalicyclic acid (5-ASA), and randomized them to receive 30 hookworm larvae or placebo. Their 5-ASA was stopped 12 weeks postrandomization, and participants were monitored for up to 52 weeks and exited the study if they had a UC flare (Simple Clinical Colitis Activity Index [SCCAI] ≥5 and fecal calprotectin [fCal] ≥200ug/g). The primary outcome was difference in rates of clinical remission at week 52. Differences were assessed for quality of life (QoL) and feasibility aspects including recruitment, safety, effectiveness of blinding, and viability of the hookworm infection.

Materials and Methods

Participant Selection

Participants were recruited using several methods including researchers directly contacting patients following a search of 2 local hospitals' IBD patient databases, placing advertising posters in waiting areas of local IBD centers, and a referral pathway for local IBD physicians and nurses to refer interested patients. After reviewing medical records, potentially eligible patients were sent information about the study, which was followed by a phone call by the researchers to gauge interest and further screen eligibility. An in-person screening visit was then performed for those wanting to participate.

Eligible patients were 18 to 70 years old with an endoscopic and histological diagnosis of ulcerative colitis for >3 months, were currently in disease remission, and receiving maintenance medication of oral and/or rectal 5-ASA only. Disease remission was defined as being on a stable dose of 5-ASA only for the previous 3 months and a screening SCCAI ≤4 and fCal<100 μg/g.[18]

Patients were ineligible to participate if their stool contained enteric pathogens, *Clostridium difficile* toxin, or parasite ova; had been treated with antibiotics, antiparasite medication, or nonsteroidal anti-inflammatories in the last 2 weeks; were currently receiving immunosuppressive medication (other than 5-ASA); had severe anemia (Hb <100 g/L) or a white cell count <4 or >20 × 10^9/L; had asthma requiring treatment within the last 5 years; had active human immunodeficiency, hepatitis B, or hepatitis C virus; had a history of cancer (excluding squamous cell carcinoma [SCC] or basal cell carcinoma [BCC] of the skin) within the last 5 years; or had other clinically significant diseases that could interfere with the study protocol. Women needed a negative pregnancy test and be willing to practice birth control.

Study Design

This study was a single-center, double-blinded, randomized pilot study assessing the feasibility of hookworm therapy in maintaining medication-free remission in patients with ulcerative colitis conducted at the Malaghan Institute of Medical Research, Wellington, New Zealand. The recruitment target for this study was 20 eligible patients. This study was completed in accordance with the World Medical Association's Declaration of Helsinki, approved by the Health and Disability Ethics Committee of New Zealand (HDEC 20/CEN/119), and registered with the Australian New Zealand Clinical Trial Registry (ACTRN12620000956909).

After giving informed consent, participants were screened for eligibility. Eligible participants were randomized in a double-blind fashion to receive either 30 hookworm larvae in the infective L3 lifecycle phase (L3) or a placebo consisting of Capsaicin cream, applied directly to the skin of the forearm. All patients ceased 5-ASA therapy 12 weeks after randomization. During the study, participants had scheduled study visits at baseline, 2, 4, 6, 8, 12, 16, 24, 36, and 52 weeks postrandomization,

with unscheduled visits as required. At each of these visits, the following data were obtained: current symptoms including adverse events, physical examination, disease activity assessments (SCCAI and fCal), quality of life assessment, hookworm symptom assessment, and safety bloods (complete blood count, C-reactive protein, and iron and liver profile). Analysis of stool to quantify hookworm eggs was performed at week 12 and at week 52 or upon exiting the study.

Participants discontinued the study if they developed a flare of ulcerative colitis (defined SCCAI score ≥5 and fCal >200 μg/g); recurrent mild adverse events (AEs) or a serious adverse event (SAE) that in the investigators' opinion would impact on the participant's ability to continue the study; pregnancy; or request of the participant to withdraw. Those exiting the study completed a termination visit, became un-blinded, were treated with anti-helminth therapy (100 mg of mebendazole twice a day for 3 days) if in the interventional arm, and then their IBD managed as per standard care.[19] A fecal egg count and blood eosinophils were checked 1 month following treatment to ensure successful eradication. Participants who had exited the study were not required to attend any further follow-up appointments.

Participants remaining in the study at 52 weeks after randomization were un-blinded. Participants in the interventional arm were given the opportunity to undertake a continuation phase where they were monitored every 4 to 12 weeks. Participants in the interventional arm not undertaking the continuation phase were treated with mebendazole and had their IBD managed as per standard care. Participants in the placebo arm had their IBD managed as per standard care.[19]

Study Agent Preparation and Interventions

Larvae were developed from eggs isolated from stool samples provided by human donors infected for this purpose. The larvae were repeatedly washed in an iodine solution before testing for morphological integrity and viability/motility by an experienced technician using dissecting microscopy. Aliquots of 30 L3 were stored in 200 μL of deionized water contained in small glass microtubes and kept at approximately 25°C and protected from light for up to 1 week prior to inoculation. Capsaicin 0.075% strength cream, which gives a similar sensation as hookworm larvae when applied to the skin, was used as the placebo.

Eligible participants were randomly assigned into the intervention or placebo group in a 1:1 ratio. Randomization codes were placed in sealed envelopes and chosen at random by the unblinded researcher administering the intervention. The randomization code was protected and limited to the unblinded researcher administering the intervention and another independent researcher. All other investigators, site trial staff, and the participant were blinded as to the participant's status. Unblinding of the participants occurred upon completion of the study, participant withdrawal, or a medical emergency.

Administration of the intervention/placebo was performed by an unblinded trained researcher who was not otherwise involved in the study. The gauze containing hookworm larvae or placebo were prepared in a separate room to the participant. Hookworm larvae contained in a 200-μL aqueous solution were extracted using a pipette with a single use glass pipette tip and placed on a 5 to 7-cm commercial dressing. The carrier tube was rinsed with 200 μL of deionized water with the rinse waste collected and applied to the gauze. Capsaicin cream was also placed on the gauze so that the gauzes applied to both groups appeared identical. The dressing was then applied to the ventral surface of the forearm and left in place for 24 hours. The placebo was presented in the same way with the gauze containing capsaicin cream and 200 μL of water (without hookworm larvae). On administration, participants were advised that there may be local skin irritation and itch at the site of inoculation, like what would be experienced if chili were rubbed on the skin.

Outcome Measures

Baseline characteristics

Baseline characteristics assessed included gender, age, smoking, body mass index (BMI), disease characteristics, current dose of 5-ASA, past use of immunomodulators, biologics or steroids, baseline laboratory measures, and quality of life and symptom-based scores.

Primary outcome measure

The maintenance of clinical remission, defined as fCal <200 μg/g and SCCAI ≤4 at week 52 postrandomization, was the primary measure of efficacy. The SCCAI is a validated symptom-based score that includes 5 clinical variables (day and night stool frequency, urgency of defecation, blood in the stool, general well-being, and extracolonic features). An SCCAI ≤4 indicates symptom remission and ≥5 active symptoms.[18,20,21] Fecal calprotectin is a validated objective biomarker of disease activity. For this study, thresholds to define disease remission at baseline and active disease were <100 μg/g and ≥200 μg/g, respectively.[22]

Secondary outcome measures

The difference in time remaining in clinical remission postrandomization, adverse events, and quality of life between hookworm and placebo groups were secondary end points. Quality of life was assessed using the short inflammatory bowel disease questionnaire (SIBDQ), a validated instrument to assess quality of life in patients with inflammatory bowel disease. It comprises 10 questions scored using a 7-point Likert scale, with lower scores indicating a poorer quality of life. Use of the SIBDQ, authored by Irvine et al, was made under license from McMaster University, Hamilton, Canada.[23] Possible symptoms attributable to a hookworm infection were assessed using a modified version of the Talley gut symptom questionnaire.[24] Symptoms were graded as mild (nagging or annoying), moderate (strong negative influence on daily living), and severe (disabling) and were self-reported by each participant. Adverse events were assessed using Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0.[25]

Assessment of blinding

The success of blinding was assessed at week 12 postrandomization. Participants were asked to guess their treatment assignment, with 3 guessing options, "hookworm group," "placebo group," or "do not know."[26]

Assessment of infection viability

The viability of the hookworm infection was assessed by quantifying hookworm eggs by blinded trained researchers within the stool from participants at week 12 postrandomization, at week 52 postrandomization, or upon

study withdrawal, using light microscopy. Blood eosinophils were also measured at each study visit.

Assessment of fecal markers of inflammation

Fecal calprotectin levels have been shown to become mildly elevated during the acute stages of a hookworm infection in some healthy participants. The source of fCal appears to be from eosinophils that have accumulated in the gastrointestinal tract in response to the hookworm infection rather than neutrophilic inflammation typical of active ulcerative colitis.[13] To help confirm that hookworm-related elevations in fCal were not misdiagnosed as an ulcerative colitis flare, post hoc eosinophilic (eosinophilic derived neurotoxin [EDN]) and neutrophilic (human neutrophil lipocalin [HNL]) specific fecal markers were performed using enzyme-linked immunosorbent assay (ELISA; Supplementary Methods).

Statistical Analysis

Statistical analyses were performed according to intention-to-treat principles. Recruitment rates and reasons behind patients declining participation and ineligibility or failing screening were analyzed using descriptive statistics. Differences in continuous and categorical variables between the 2 groups were assessed using the Mann-Whitney *U* test and Pearson χ^2 test, respectively. The success of blinding was analyzed using the Bang blinding index, presented with one-sided 95% confidence intervals. The Bang index ranges from −1 to 1, in which 1 indicates complete lack of blinding, 0 perfect blinding, and −1 indicates opposite guessing.[26,27] The primary efficacy measure and time remaining in clinical remission were compared between the intervention and placebo groups using the Fisher exact test and the Mann-Whitney *U* test, respectively. The χ^2 tests were used to compare adverse events between the 2 groups. Quality of life data was analyzed using a linear mixed model, adjusted for baseline QoL scores.[28] Due to an early termination of follow-up for some participants, this analysis was restricted to QoL outcomes measured up to 12 weeks. Results are presented as overall hypothesis tests regarding differences in outcomes across follow-up times and estimates for mean treatment effects at each follow-up time, presented with their 95% confidence intervals; *P* values less than 0.05 were considered statistically significant. All statistical analyses were performed using GraphPad Prism 9 package (GraphPad Software, Inc., San Diego, CA) or R 4.1 (R Institute, Vienna).

Since this was a feasibility study, there was no formal sample size analysis for clinical outcomes. To adequately assess the feasibility aims of the study, a sample size of 10 participants in each arm was considered suitable.[29]

Questionnaires were completed electronically or using paper copies. Data were stored using the Research Electronic Data Capture software (REDCap, Vanderbilt, USA).[30]

Results

Recruitment

Participants were recruited between July 2020 and July 2021. The initial search of the local hospital IBD databases generated a list of 1208 patients with inflammatory bowel disease. From that list, 1072 (89%) were excluded when assessed against inclusion and exclusion criteria after physician screening of medical records or a phone call with the patient. The main reasons for exclusion were due to not having ulcerative colitis, being on immunosuppression other than 5-ASA, or no longer taking 5-ASA. Of the patients approached to participate in the study, 74 of 136 (54%) declined to participate, 33 of 136 (24%) did not respond or did not give a final answer, and 29 of 136 (21%) signed consent. Reasons given for declining participation included being unable to commit their time to the study, feeling content with the current management of their ulcerative colitis, feeling concerned about the increased risk of having a disease flare, being away during the study period, or being disinclined to undergo infection with hookworm (Figure 1).

Of the 29 patients who signed consent, 9 (31%) patients did not pass screening. Reasons included screening fCal outside the eligibility criteria (78%), subsequent change in diagnosis to Crohn's disease (11%), and withdrawing consent due to developing new health issues (11%). Of the 20 remaining participants, 10 were randomized to receive hookworm and 10 placebo, as planned. Compliance with the study protocol was excellent with all participants attending every study visit and completing all questionnaires and biological samples. No patients were lost to follow-up, and no participant withdrew consent prior to completing the study (Figure 1).

Baseline Characteristics

Baseline characteristics were similar with respect to age, gender, BMI, smoking history, time since diagnosis, previous immunosuppression use, disease severity at worst, and baseline fCal, SCCAI, CRP, SIBDQ, eosinophil count, and ferritin. Imbalance was seen on a few variables: participants in the intervention group had a higher mean baseline hemoglobin than in the placebo group (148.5 g/L vs 138.5 g/L), and the extent of disease tended to higher for the placebo group (eg, E3: $n = 6$ for control vs $n = 1$ in hookworm group; Table 1).

Maintenance of Clinical Remission

At 1-year postrandomization, 4 of 10 (40%) participants who received hookworm remained in clinical remission (fCal <200 µg/g and SCCAI <5) compared with 5 of 10 (50%) participants who received placebo (odds ratio, 0.67; 95% CI, 0.11-3.92), showing no strong evidence for a difference in remission for either group. The median time to flare in the hookworm group was 231 days (IQR, 98-365) and 259 days in the placebo group (IQR, 132-365). All participants were observed until the time of flare or end of the follow-up period (Figure 2).

Assessment of Blinding

Two participants (1 participant in each study arm) had a flare of ulcerative colitis prior to 12 weeks postrandomization, so they had exited the study prior to when blinding was assessed. Of the 18 participants remaining in the study at 12 weeks postrandomization, 7 (78%) participants in the intervention group believed they had received hookworm, and 4 (44%) participants in the placebo group believed they had received placebo (Table 2). The Bang blinding index in the hookworm arm was 0.70 (95% CI, 0.37-1.0), indicating strong evidence for unblinding, and in the placebo arm was 0.22 (95% CI, −0.21 to 1), in line with relatively successful blinding.

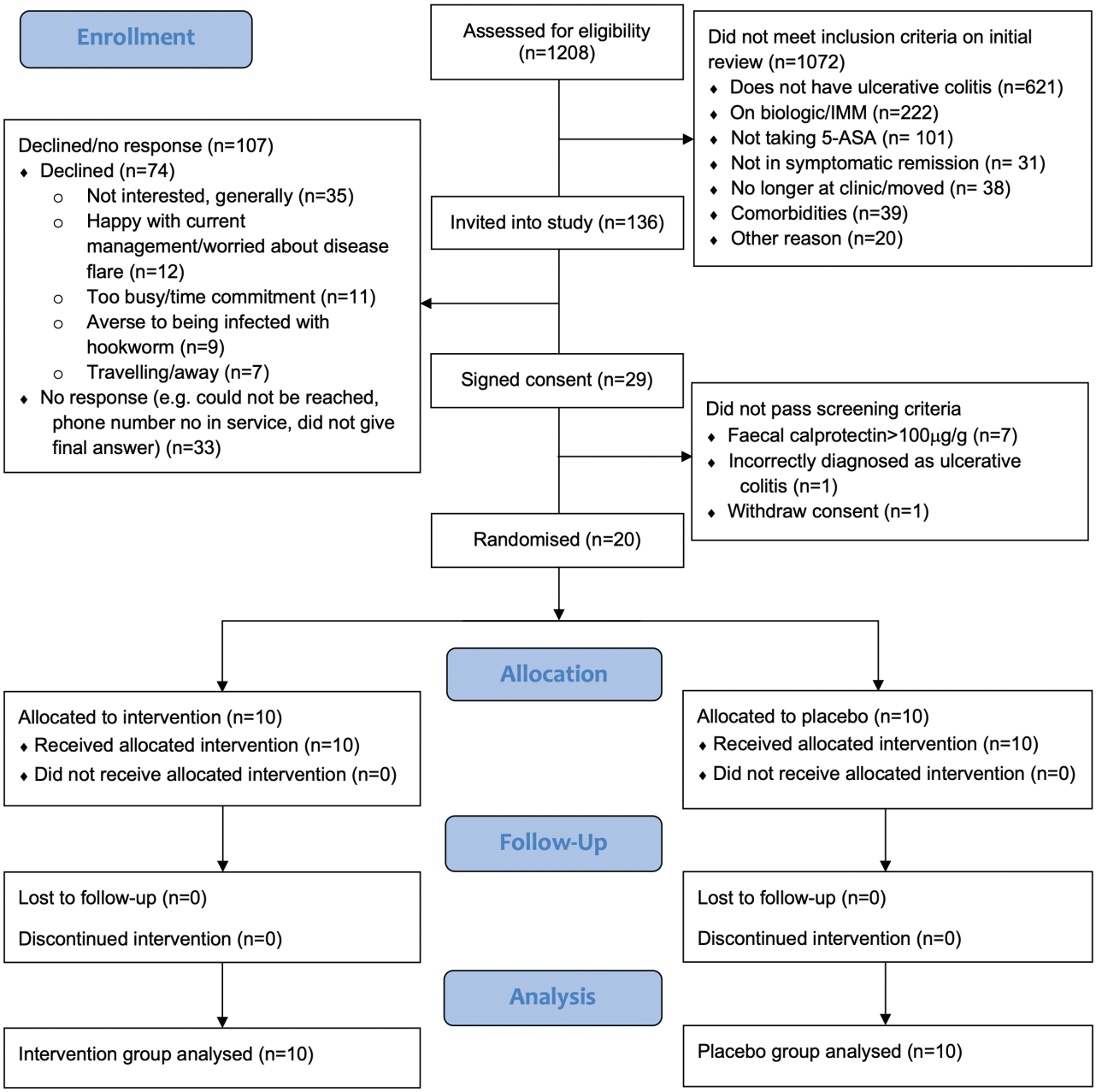

Figure 1. Consort flow diagram showing recruitment and participant progress. Abbreviations: IMM, immunomodulator; 5-ASA, 5-aminosalicylic acid.

Confirmation of Hookworm Infection

Nine of 10 (90%; 95% CI, 0.60-0.98) participants who received hookworm had detectable eggs in their feces during the study. Of these, 8 participants had detectable eggs at week 12 postrandomization (median 50 eggs/g; IQR, 37.5-262.5), and 9 had detectable eggs at completion of the study (either week 52 or upon exiting due to a disease flare; median 225 eggs/g; IQR, 50-612.5). The single participant in the intervention group without detectable eggs during the study had a flare of their ulcerative colitis and received anti-helminth treatment at week 13 postrandomization. All participants who received hookworm experienced peripheral blood eosinophilia, with the peak in median eosinophil count seen at week 6 postrandomization (4.35×10^9/L; IQR, 2.80-6.68). No participants in the placebo group had detectable eggs or experienced an eosinophilia (Figure 3).

Adverse Events

Compared with the placebo group, participants in the hookworm group experienced more rashes at the site of application (8 of 10, 80%; 95% CI, 49%-96%) vs 0 of 10 (0%; 95% CI, 0%-28%; $P < .001$) and more nausea (6 of 10, 60%; 95% CI, 31%-83%) vs 1 of 10 (10%; 95% CI, 0.5%-40%; $P < .05$). There was no significant difference in other adverse events ($P > .05$; Supplementary Table 1). All adverse effects were Common Terminology Criteria for Adverse Events (CTCAE) grade 1 or 2 (mild or moderate), with no serious adverse events. There was no significant change in hemoglobin (Supplementary Figure 1), ferritin, creatinine, alanine transaminase, or albumin from baseline at any of the measured time points for the hookworm or placebo groups ($P > .05$ for all comparisons).

Table 1. Baseline demographic, disease and laboratory characteristics. Disease extent based on Montreal classification for ulcerative colitis.

Characteristic	Intervention (n = 10)	Placebo (n = 10)
Women, *n* (%)	4 (40%)	5 (50%)
Age, mean (SD)	53.5 (9.3)	48.0 (12.9)
Age category, *n* (%)		
18-30	0 (0)	1 (10)
30-50	3 (30)	5 (50)
50-70	7 (70)	4 (40)
Smoking, *n* (%)		
Current	1 (10)	1 (10)
Past	5 (50)	3 (30)
BMI (mean, SD)	19.6 (7.0)	26.4 (6.2)
Time since diagnosis, years (median, IQR)	16 (2.8-20)	16 (4.5-23.0)
Disease extent, *n* (%)		
E1	1 (10)	1 (10)
E2	8 (80)	3 (30)
E3	1 (10)	6 (60)
Disease severity at worst, *n* (%)		
Mild	1 (10)	1 (10)
Mod	9 (90)	7 (70)
Severe	0 (0)	2 (20)
Previous IMM, *n* (%)	2 (20)	3 (30)
Previous biologic, *n* (%)	0 (0)	0 (0)
Steroid use in last year, *n* (%)	1 (10)	2 (20)
Previous steroid use, *n* (%)	8 (80)	6 (60)
Severity of inflammation at last endoscopy, *n* (%)		
Nil	2 (20)	3 (30)
Mild	2 (20)	5 (50)
Mod	6 (60)	2 (20)
Severe	0 (0)	0 (0)
Baseline SCCAI score (median, IQR)	2.0 (2.0-2.0)	2.0 (2.0-2.0)
Baseline SIBDQ (median, IQR)	59.0 (52.8-64.5)	59.50 (55.8-64.0)
Baseline fCal, ug/g (median, IQR)	32.0 (29.0-38.8)	33.0 (28.8-64.8)
Baseline CRP, mg/L (median, IQR)	1.0 (1.0-8.0)	1.0 (1.0-1.5)
Baseline Hb, g/L (median, IQR)	148.5 (143.3-154.8)	138.5 (128.5-144.0)
Baseline ferritin, ug/L (median, IQR)	109.0 (38.0-157.0)	83.50 (57.8-99.5)
Baseline eosinophil count, x109/L (median, IQR)	0.2 (0.1-0.4)	0.2 (0.1-0.4)

Abbreviations: BMI, body mass index; IMM, immunomodulator; SCCAI, simple clinical colitis activity index; SIBDQ, short inflammatory bowel disease questionnaire; fCal, fecal calprotectin; CRP, C-reactive protein; Hb, haemoglobin

Self-described Symptoms

Participants that received hookworm reported more rashes, nausea, abdominal pain, and diarrhea compared with those that received placebo. The rash at the site of infection was generally experienced 2 weeks after inoculation and lasted 2 to 3 weeks, whereas the gastrointestinal symptoms (nausea, diarrhea, abdominal pain, cramping, and bloating) were experienced from approximately week 4 to week 10 after inoculation before returning to baseline (Figure 4).

Quality of Life

After adjusting for baseline scores, there was no significant difference in mean SIBDQ scores between participants in the hookworm group and placebo group across follow-up (main effect for group differences at any follow-up time, P = .38; interaction term test for differences by group across follow-up, P = .26). Differences were broadly similar postrandomization between groups at 2 weeks (mean difference 2.75; 95% CI, −3.87 to 9.36), 4 weeks (1.22; 95% CI, −5.53 to 7.96), 6 weeks (−3.17; 95% CI, −9.92 to 3.58), 8 weeks (−2.60; 95% CI, −9.35 to 4.14), and 12 weeks (1.63; 95% CI, −5.25 to 8.50; Figure 5).

Re-establishing Disease Remission Post-flare

Of the 11 (55%) participants who had a flare of ulcerative colitis, 7 of 11 (64%) reestablished disease remission (SCCAI <5 and FC <200 µg/g) by restarting oral +/- rectally administered 5-ASA. The remaining 4 of 11 (36%) participants required oral steroids in addition to 5-ASA due to an inadequate initial response to 5-ASA alone. After treatment of the disease flare, 10 of 11 (91%) patients returned to their baseline 5-ASA dose, and 1 patient (9%) required the addition of an immunomodulator to maintain clinical remission.

Continuation Phase

Following unblinding, the 4 participants in the hookworm group that remained in remission at 1-year postrandomization chose to continue with the hookworm infection and remain off 5-ASA. To date (mean follow-up time postrandomization of 28 months), these 4 participants remain in clinical remission and off 5-ASA. Two participants have requested fecal egg-counts, which both remain positive at 19 and 25 months postrandomization (900 eggs/g and 100 eggs/g, respectively).

Of the 6 participants in the hookworm group who had a flare of their ulcerative colitis, 5 (83%) chose to eradicate hookworm, and 1 (17%) chose to maintain their hookworm infection while also restarting 5-ASA. Successful eradication of hookworm was confirmed in all 5 (100%) participants by the absence of eggs on stool microscopy and normalization of blood eosinophils.

Fecal Calprotectin, Eosinophil- and Neutrophil-specific Fecal Markers

One participant in the hookworm group had an elevation in fCal to >200 µg/g (fCal, 340 µg/g) without meeting the study's criteria for a UC flare. This coincided with an elevation in fecal EDN (300 µg/g) but not fecal HNL (below limit of detection), suggesting the origin of fCal was eosinophilic. All participants in the hookworm group had an elevation in fecal EDN levels during the acute stages of the hookworm infection (median baseline EDN 34.8 vs 8 weeks postinfection

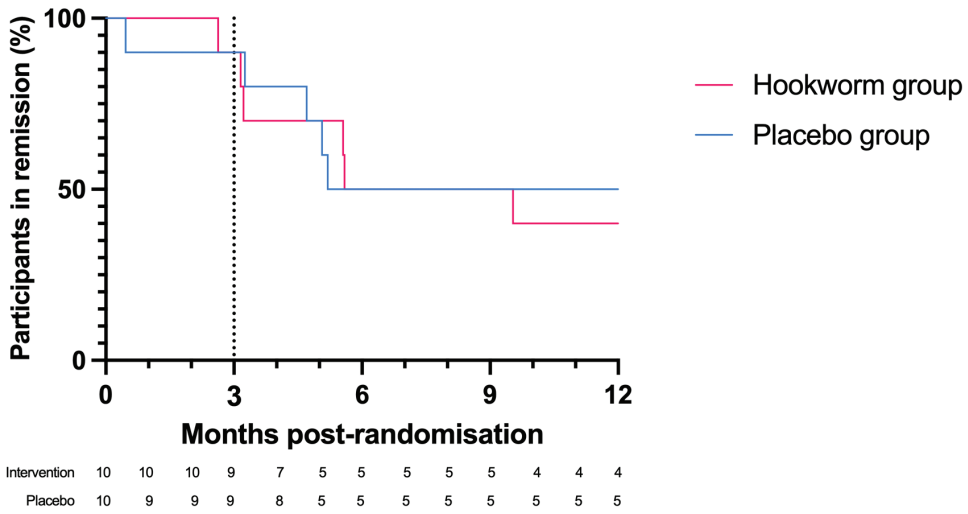

Figure 2. Proportion of participants remaining in remission (Simple Clinical Colitis Activity Index ≤5 and fecal calprotectin <200 μg/g) in the hookworm and placebo groups. Dotted line indicates when the participants ceased 5-aminosalicylic acid.

Table 2. Assessment of participant blinding at 12 weeks postrandomization.

	Patients answer, n (%)			
Allocated group	Hookworm	Placebo	Do not know	Total
Hookworm	7 (78)	1 (11)	1 (11)	9
Placebo	2 (22)	4 (44)	3 (33)	9
Total	**10** (56)	**5** (28)	**4** (22)	**18**

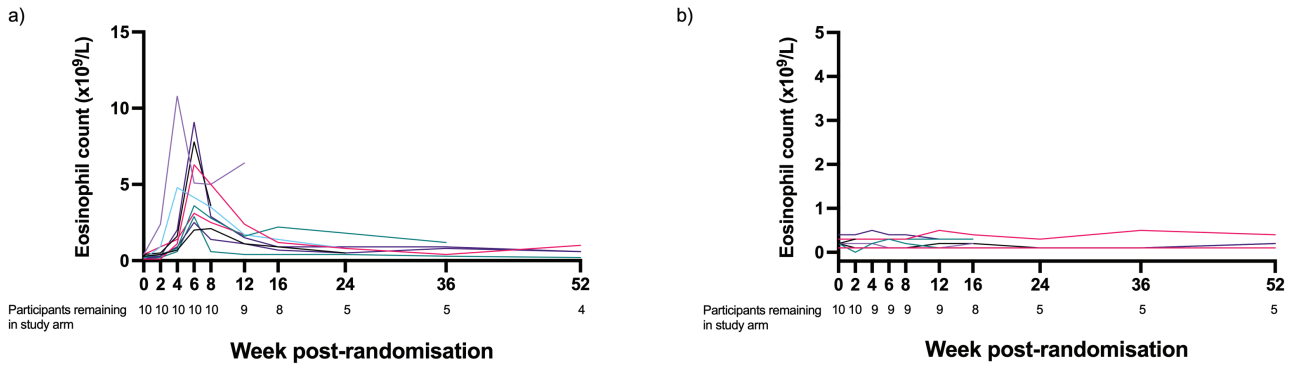

Figure 3. Peripheral blood eosinophils in participants randomized to (A) hookworm and (B) placebo groups.

EDN 573.1 μg/g, $P < .05$), but no increase was seen in the placebo group other than during a flare of ulcerative colitis. Fecal HNL levels did not significantly change in either group other than during a flare of ulcerative colitis. Overall, this suggests that a hookworm infection induces eosinophilic, but not neutrophilic, intestinal inflammation (Figure 6).

Discussion

The rationale for using helminths, such as hookworm, as a therapeutic in IBD initially emerged from epidemiological data demonstrating an inverse relationship between the incidence of helminth infections and IBD.[31] This forms part of the "hygiene hypothesis" and more specifically "the old friends hypothesis," which are plausible explanations for the increasing incidence of autoimmune, inflammatory, and allergic diseases worldwide.[3] In addition, preclinical models of IBD have shown that certain helminths can supress intestinal inflammation and have provided insight into potential mechanisms of action, including an increased production of regulatory immune cells and cytokines, and favorable changes in the microbiome resulting in increased short-chain fatty acid production.[4,31–33]

This pilot study examining a controlled hookworm infection as maintenance therapy in patients with ulcerative colitis was performed to test the feasibility and safety in preparation for a full-scale RCT. Feasibility issues were clearly resolved: patients can be successfully recruited to a study of

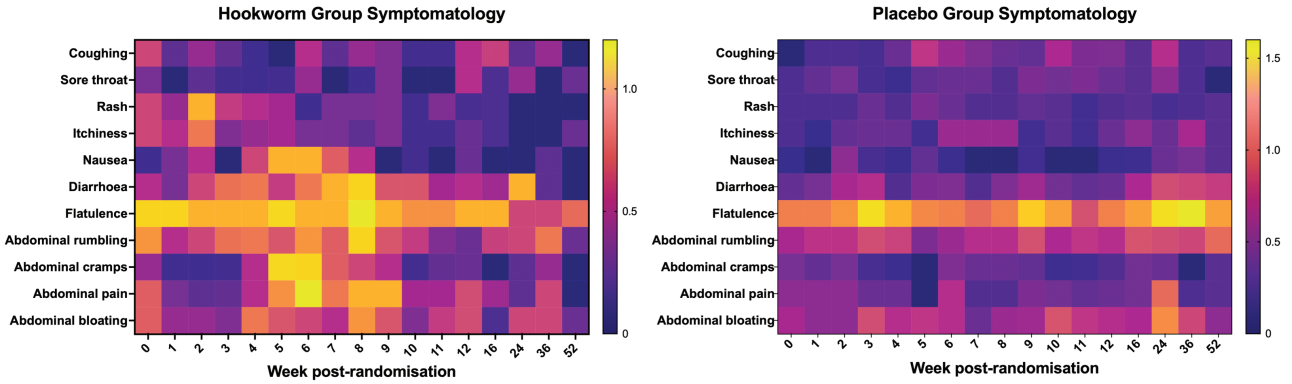

Figure 4. Symptoms as shown by heatmapping in participants that received (A) hookworm and (B) placebo. Color of each cell indicates the average score of all participants in each group at a particular week postrandomization as shown in legend. Symptoms were assessed using a modified version of the Talley gut symptom questionnaire.[24] Symptoms were self-graded by the participant as 0 = absent, 1 = mild (nagging or annoying), 2 = moderate (strong negative influence on daily living), and 3 = severe (disabling).

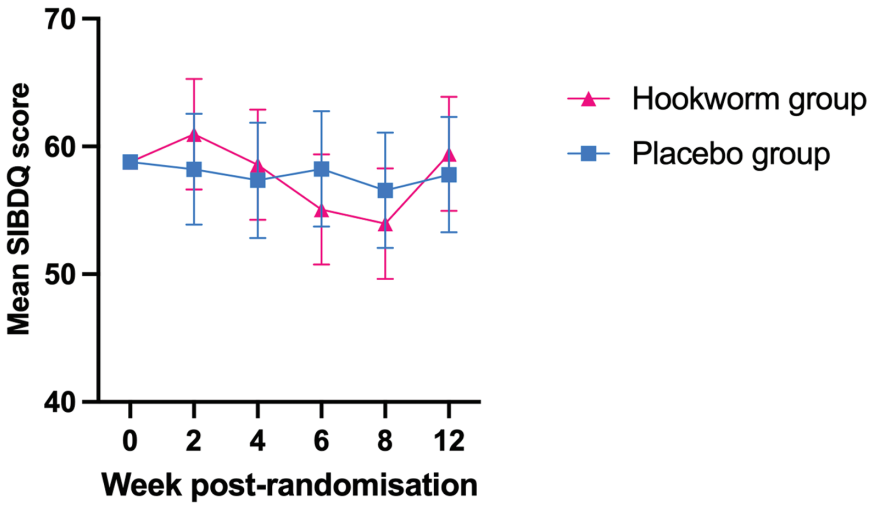

Figure 5. Comparison of short inflammatory bowel disease questionnaire (SIBDQ) scores for the hookworm group and placebo group after adjusting for baseline scores. Mean, 95% CI.

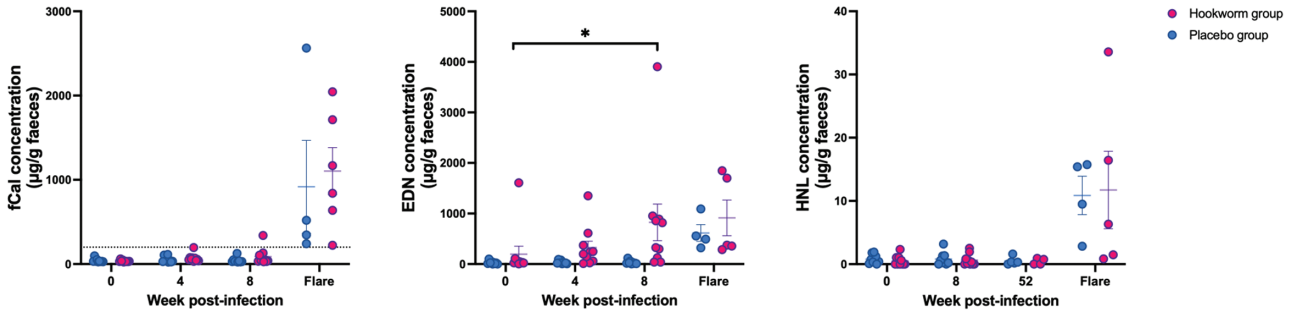

Figure 6. Fecal calprotectin (fCal), fecal eosinophil derived neurotoxin (EDN), and fecal human neutrophil lipocalin (HNL) levels at baseline, weeks 4 and 8 postrandomization, and at time of ulcerative colitis flare measured by enzyme-linked immunosorbent assay (ELISA). Mean, 95% CI. Line, fCal = 200 µg/g. *$P < .05$.

this design, a viable hookworm infection can be established in most participants from a single inoculation and can remain viable for years, and controlled hookworm infections appear well-tolerated. Of importance, there was no evidence that the acute type 2 immune response experienced by participants in the early phase of a hookworm infection triggers a disease flare in patients with ulcerative colitis, which was a key safety consideration. Additionally, in spite of some mild gastrointestinal effects in the early phase of infection, there was no negative impact on quality of life. Given the small size of the study,

there was limited statistical precision to consider differences in clinical remission by study group (50% in remission at end point for the hookworm group vs 40% for the control group).

Several clinical trials examining the benefit of experimental helminth infections in patients with IBD have now been conducted, with some showing benefit. However, the majority of these have been with the whipworm *Trichuris suis*, rather than the human hookworm *Necator americanus*.[5,14] Several characteristics of human hookworm make it a more attractive therapeutic option when compared with whipworm. Humans are the natural host for *Necator americanus*, and they have co-evolved over millions of years, whereas the natural hosts for *T. suis* are pigs. Also, whipworm has to readministered fortnightly, whereas a hookworm infection is still present years after a single inoculation, meaning regular re-infection is not required.[5,13,34] These characteristics also suggest that human hookworm induces greater immunoregulatory effects in humans, as they have successfully evolved to evade expulsion by the host's immune system.[7]

Previous clinical trials examining human hookworm as a therapeutic in IBD have included only patients with Crohn's disease and those with active disease.[6,15] Human hookworm takes approximately 6 weeks to reach the intestine following inoculation and possibly even longer to start exerting its immunoregulatory properties, suggesting that patients with active disease, where a more rapid response is generally required, are not the ideal target population for human hookworm therapy. Also, the early stages of an infection (approximately the first 12 weeks post inoculation) is characterized by a pronounced type 2 immune response, which could potentially worsen rather than improve intestinal inflammation.[13,17] This current study's novel approach was to trial hookworm as a maintenance therapy and keep patients on their usual maintenance therapy for the first 12 weeks after inoculation to help prevent an ulcerative colitis flare until hookworm enters its immunoregulatory phase of infection.

This study included patients only on 5-ASA to control their ulcerative colitis for several reasons. These patients generally have a mild disease phenotype which would increase the likelihood of restoring disease remission in the event of a flare without the need for additional immunosuppressive therapy.[19] Also, given the effect of systemic immunosuppression on the lifecycle and immune effects of a hookworm infection are not known, the inclusion of patients only on 5-ASA, which does not induce systemic immunosuppression, was considered the safest patient group. Finally, adherence to 5-ASA as maintenance therapy is relatively poor in patients with ulcerative colitis, so this patient group is likely to gain the most benefit from an intervention that potentially removes the need to adhere to daily medication.[16]

When designing this feasibility study, several potential barriers to patient recruitment were identified. Few clinical trial designs investigating new IBD therapies have included a study arm which involves the participants receiving placebo also discontinuing their usual IBD therapy, leaving them on no treatment and at an increased risk of a disease flare. Also, it was unknown whether patients with ulcerative colitis would be willing to be infected with hookworm or find it an acceptable long-term therapy. Overall, recruitment was completed within 12 months of trial registration. Predictable barriers to recruitment included a general lack of interest in participating in a clinical trial and not being able to commit their time to participate. Identified barriers that were more specifically related to the current study design included patients reluctance to participate due to the increased risk of having an ulcerative colitis flare or not wanting to participate because they found the idea of being infected with hookworm off-putting or unpleasant. These results provide valuable information on the number of study sites and length of study required to successfully complete recruitment in a full-scale RCT.

The successful blinding of participants in hookworm clinical trials has been challenging. This current study used a novel placebo, Capsaicin cream, because of the authors' experience that this induced a sensation more similar to hookworm larvae being applied to the skin than previously used placebo agents, usually Tabasco sauce or histamine solution.[8,9,11] Although blinding was successful in participants receiving placebo, the majority of participants in the hookworm group guessed correctly that they had received hookworm. Inoculation with hookworm causes the hallmark features of a transient papular rash at the site of inoculation and mild-moderate gastrointestinal symptoms, both of which were unable to be replicated by the placebo. Another blinding method that was considered was to inoculate every participant with hookworm and then treat the control group with antihelminth therapy prior to stopping 5-ASA. Although this would likely improve blinding, it would have meant the key safety question of whether the initial type 2 immune response induced by hookworm triggers a disease flare could not be answered. Also, potentially important immunological changes may have already occurred prior to de-worming, such as alterations in the microbiome, meaning efficacy could not be reliably assessed. Careful consideration of any potential improvements to blinding of participants will be required for future hookworm clinical trials.

This study has demonstrated that hookworm therapy appears to be generally well-tolerated and safe in the studied population. A similar spectrum of adverse effects experienced by participants in previous hookworm clinical trials including a rash at the site of inoculation, nausea, abdominal discomfort, and increase in bowel frequency.[8–11] Also consistent with previous studies, adverse effects were experienced during the acute phase of the infection (first 12 weeks) before complete resolution. These adverse effects were generally described as mild or moderate by the participant and did not negatively impact their quality of life.

Traditionally, the pathogenesis of ulcerative colitis has been thought to involve a nonclassical type 2 immune response. Although this paradigm has largely been overtaken with more recent advances in the understanding of its pathogenesis, a key safety aim of this pilot study was to determine if the pronounced type 2 immune response experienced in the early phase of the hookworm lifecycle could trigger a flare of ulcerative colitis.[35] In this study, 1 patient in each study arm flared during the first 12 weeks after randomization, which suggests against this. Another important safety aspect of this study was the ability to return patients to disease remission in the event of a flare. The majority (64%) of patients that flared reestablished remission after restarting 5-ASA alone; however, 4 (36%) patients also required a course of oral steroids. Of significance, 1 (9%) participant also required the addition of an immunomodulator to their maintenance regimen. These findings are important when consenting patients to any future study with a similar design.

Previous clinical trials investigating hookworm therapy have failed to successfully establish a viable infection in a sizeable proportion of patients inoculated with hookworm. Proposed reasons for this have included an inadequate dose of hookworm being given or the effect of a long transit from the laboratory where hookworm larvae are prepared to the study site on the viability of hookworm larvae.[8,11] The authors' recent longitudinal study of controlled hookworm therapy in healthy volunteers showed that a dose of 30 hookworm larvae established a viable infection in all infected participants and is generally well-tolerated, and so the same dose was used in the current study.[13] Of the 10 participants infected with hookworm, 9 had positive fecal egg counts at week 12 postinfection. The 1 participant that did not have fecal eggs present at week 12 had a flare of their ulcerative colitis at week 13 postrandomization. Subsequent review of this participant's results showed they had developed a peripheral blood eosinophilia following inoculation, suggesting the reason for not detecting eggs in their stool was either their hookworm had not fully matured when they exited the study or they were infected with nonfecund hookworm. In the current study, participants were infected at the same study site as where the hookworm were matured, so the effect of a long transit on their viability was not assessed. Before proceeding to a multicenter study, it will be crucial to determine the optimal transport conditions to ensure the hookworm larvae remain viable.

Although the lack of endoscopic outcomes was a study limitation, the use of fCal as an objective marker of intestinal inflammation allowed regular noninvasive monitoring of disease activity, which was more suited to our primary outcome. Of note, the authors' recent study of a controlled hookworm infection in healthy volunteers showed that a hookworm infection can cause a low-level elevation in fCal (<150 μg/g) during its acute phase of infection.[13] That prior study also showed that the elevation in fCal was predominately caused by an eosinophilic rather than neutrophilic enteritis as evidenced by no increase in a neutrophil specific fecal marker in the same patients, a finding that was also confirmed in the current study. To help avoid this mild elevation in fCal being misdiagnosed as a flare of ulcerative colitis, the current study used a cutoff fCal level of >200 μg/g and included a symptom-based score which is validated in patients with UC (SCCAI to ≥5) to define a flare.

Although it is postulated that hookworm would improve ulcerative colitis through its immunomodulatory effects, this current study did not address possible mechanisms of action. Previous research examining helminth therapy in humans has not established a well-defined mechanism of action; however, it is clear that the immune response is complex and heterogeneous.[13] Given this and the heterogeneity in the pathogenesis of UC between patients, a larger study with a focus on the immunological changes at a systemic and tissue level, including its effects on the microbiome, would help to determine if certain patients respond more favorably to hookworm therapy, which may allow for a more targeted approach.[35]

In conclusion, this pilot study has shown that a further definitive trial examining hookworm therapy as a maintenance treatment in patients with ulcerative colitis is feasible and appears to be well-tolerated and safe. A full-scale RCT will require multiple study sites, which will bring the added complexity of ensuring hookworm larvae remain viable after transport to other centers, and would benefit from incorporating endoscopic assessments to better assess disease activity and examination of potential mechanisms by which hookworm may provide its beneficial effect. Given the potential for hookworm therapy to be an alternative therapeutic option to conventional medication that may be favored by patients, proceeding to a larger study is warranted.

Supplementary Data

Supplementary data is available at *Inflammatory Bowel Diseases* online.

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Ethical Considerations

This study was approved by the Health and Disability Ethics Committee of New Zealand (HDEC 20/CEN/119).

Author Contribution

All authors contributed to the study design. T.C.M., B.L., K.M., F.V., S.L.N., and M.C. acquired the study data; B.L., K.M., F.V., S.L.N, B.Y., T.T.K., and M.C. performed the laboratory analyses. T.C.M and J.S. performed the statistical analyses, and all authors assisted in the interpretation of the study results. T.C.M., B.L., and S.L.N. prepared the initial article draft. All authors contributed to the critical revision of this manuscript and approved the final submitted version.

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Conflicts of Interest

No financial disclosures or conflicts of interest.

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