Worm expulsion is independent of alterations in composition of the colonic bacteria that occur during experimental *Hymenolepis diminuta*-infection in mice

Adam Shute\(^a,b\), Arthur Wang\(^b\), Timothy S. Jayme\(^a,b\), Marc Strous\(^c\), Kathy D. McCoy\(^b\), Andre G. Buret\(^a,b,d\), and Derek M. McKay\(^a,b\)

\(^a\)Host-Parasite Interactions Program, University of Calgary, Calgary, Alberta, Canada; \(^b\)Gastrointestinal Research Group and Inflammation Research Network, Department of Physiology and Pharmacology, Calvin, Joan and Phoebe Snyder Institute for Chronic Diseases, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada; \(^c\)Department of Geoscience, University of Calgary, Calgary, Alberta, Canada; \(^d\)Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada

**ABSTRACT**

The tapeworm *Hymenolepis diminuta* fails to establish in mice. Given the potential for helminth-bacteria interaction in the gut and the influence that commensal bacteria exert on host immunity, we tested if worm expulsion was related to alterations in the gut microbiota. Specific pathogen-free (SPF) mice, treated with broad-spectrum antibiotics, or germ-free wild-type mice were infected with *H. diminuta*, gut bacterial composition assessed by 16S rRNA gene sequencing, and worm counts, blood eosinophilia, goblet cells, splenic IL-4, -5 and -10, and colonic cytokines/chemokines mRNA were assessed. Effects of a PBS-soluble extract of adult *H. diminuta* on bacterial growth in vitro was tested.

*H. diminuta*-infected mice displayed increased \(\alpha\) and \(\beta\) diversity in colonic mucosa-associated and fecal bacterial communities, characterized by increased Lachnospiraceae and clostridium cluster XIVa. In vitro analysis revealed that the worm extract promoted the growth of anaerobic bacteria on M2GSC agar. *H. diminuta*-infection was accompanied by increased Th2 immune responses, and colon from infected mice had increased levels of IL-10, IL-25, Muc2, trefoil factor 3, and \(\beta\)2-defensin mRNA. SPF-mice treated with antibiotics, or germ-free mice, expelled *H. diminuta* with kinetics similar to control SPF mice. In both settings, measurements of Th2-immune responses were not significantly different across the groups. Thus, while infection with *H. diminuta* results in subtle but distinct changes to the colonic microbiota, we have no evidence to support an essential role for gut bacteria in the expulsion of the worm from the mouse host.

**Introduction**

The mammalian intestine houses an immense and complex bacterial community, is the body’s largest repository of immune cells, and can be infected, acutely or chronically, with helminth parasites. This trans-kingdom – bacteria, helminth, host – interaction has co-evolved for millennia and the relationship can be critical in gut homeostasis and overall well-being of the host.\(^1\)

It is intuitive to accept that helminths residing in the intestine would affect bacteria in this niche; a postulate reinforced by culture-independent sequencing of the small-subunit ribosomal RNA gene (16S) revealing order/family level shifts in bacterial abundance and diversity in helminth-infected humans, rodents and other mammals.\(^2\)

Acknowledging parasite-host specificity, infection with helminth parasites often results in contraction of Bacteroidetes and expansion of Firmicutes in the hosts’ colon.\(^3\) While reports using 16S rRNA sequence analysis describe alterations in the gut microbiome of helminth-infected individuals, the functional consequences of such shifts on the helminth-bacteria relationship and enteric homeostasis remain incompletely understood. Ova hatching and establishment of the whipworm *Trichuris muris* in the mouse require the presence of commensal bacteria.\(^4\) Similarly, it appears that gut bacteria are essential for the nematode *Heligmosomoides polygyrus* to thrive.\(^5\) The protection against airway inflammation evoked by *H. polygyrus* was attributed to the short-chain fatty acid (SCFA) acetate, produced by the gut microbiota.\(^6\)
The cestode, *Hymenolepis diminuta*, unlike many nematodes and trematodes, does not migrate through host tissues, and lacking hooks or abrasive structures it does no obvious damage to the small intestine. Lacking an alimentary track, *H. diminuta* worm absorbs nutrients across its tegument. *H. diminuta* successfully establishes in the permissive rat host but is expelled from mice via signal-transducer and activator of transcription (STAT)-6-mediated immunity. In rats, *H. diminuta* reduces the abundance of aerobic bacteria, and metagenomic analyses of rat cecal contents or feces revealed subtle changes in the microbiome with a putative bloom of *Clostridiales*. Infection with *H. diminuta* in wild mouse lemurs is associated with a reduction in specific orders of gut bacteria. Therefore, the current study addresses two questions. First, does infection with *H. diminuta* on the mouse gut microbiota are lacking. Addressing this knowledge gap will advance understanding of the helminth–host interaction and putative mechanisms by which this helminth, or others, can affect mucosal immunity and susceptibility to disease.

### Results

**Infection with *H. diminuta* increases diversity of mouse colonic bacteria**

Analysis of the V3-V4 region of 16S rRNA gene amplicon sequencing results revealed no difference in the Shannon α-diversity index (i.e. within an individual sample) between fecal samples and colonic tissue of BALB/c mice (Suppl. Figure 1A). A statistically significant difference in overall taxonomic composition between the two sampling sites from uninfected BALB/c mice was revealed by PERMANOVA analysis (Suppl. Figure 1B); however, significant shifts in specific OTUs were not identified using differential abundance testing (DeSeq2). Thus, we opted to examine mucosa-associated bacteria in *H. diminuta*-infected mice. The Shannon index revealed a significant increase in α-diversity in samples from mice 8 d post-infection (dpi.) (Figure 1a) compared to non-infected mice. As expected, treatment with broad-spectrum antibiotics resulted in substantial loss of bacteria as assessed by aerobic culture on blood agar, total 16S rRNA as determined by qPCR, shifted β-diversity (Suppl. Figure 2), and reduced Shannon index (Figure 1a). Analysis of β-diversity using weighted-UniFrac distance and PERMANOVA analysis (Figure 1b) (and also with centered log-ratio transformation (CLR) (Suppl. Figure 3)) confirmed a statistically significant (*p* < .05) effect of *H. diminuta*-infection with samples from 5, 8 and 11 dpi clustering together and away from controls.

Sequence variant analyses indicated family-level changes in mucosa-associated bacteria in mice infected with *H. diminuta* for 8 d. Lactobacillaceae and members of the *Clostridiales* order (Figure 1c) increased in abundance following infection with *H. diminuta*. DESeq2 analysis revealed that the relative sequence abundance of bacteria affiliated with *Clostridium* cluster XIVa, *Lachnospiraceae*, and *Ruminococcaceae* were increased by ~150-, ~130- and 65-fold, respectively (with adjusted *p* values = .0002, 0.0004, 0.0003, respectively), at 8 dpi (Figure 1d). Increased *Lachnospiraceae* following infection with *H. diminuta* was supported by CFU growth on selective M2GSC medium under anaerobic conditions (Figure 1e). *H. diminuta*-evoked changes in the gut microbiota were not observed in *H. diminuta*-antibiotic-treated mice, likely being masked by the major impact of the antibiotics (Suppl. Figure 2C).

Despite the subtle difference between mucosal and fecal samples, the latter have the advantage of being able to show changes in a single animal over time. Adopting this approach, we confirmed the increase in gut microbiota α and β diversity in *H. diminuta*-infected mice compared to time-matched, non-infected controls by comparing feces from the same mouse over time (Suppl. Figure 4).
Figure 1. Sequence analysis of the V3-V4 region of the bacterial 16S rRNA gene reveals subtle shifts in the composition of colonic mucosa-associated bacteria following infection with *H. diminuta*. Male BALB/c mice were infected with 5 *H. diminuta* and assessed 5, 8 and 11 days post-infection (dpi). (A) Shannon diversity index (α-diversity) (horizontal line, median; black diamond, mean; box plots, 25–75% quartiles; vertical line, minimum and maximum value; * and **, *p* < .05 and *p* < .01 compared to control (uninfected). (B) Weighted-Unifrac distance metric for β-diversity, followed by a PERMANOVA test for significance, demonstrates that infection with *H. diminuta* changes the taxonomic composition of the colonic microbiota. (C) Relative family abundance based on amplicon sequence variants assessment. (D) Differential abundance analysis using DESeq2 comparing control and *H. diminuta*-infected mice (n = 4-5; * indicated *Clostridium* cluster XIVa across the time-line). (E) Bacterial colony-forming units (CFU) after 24 h of 37°C anaerobic culture of feces from control and 8-d-infected mice on M2GSC medium agar (n = 7–8 mice from two experiments; *, *p* < .05; o, order; f, family; g, genus).
Hymenolepis diminuta extract (HdE) promotes growth of anaerobic bacteria

Exposure to HdE in vitro did not affect the growth of fecal bacteria grown under aerobic conditions on LB or blood agar (Figure 2a). In contrast, HdE caused a significant bloom of anaerobic bacteria cultured on LB or M2GSC agar (but not blood agar), resulting in a ~3-fold increase in CFU upon treatment with 500 μg/mL of HdE (Figure 2b).

H. diminuta-evoked changes in gut immunity

Comparison of segments of mid-colon from non-infected control BALB/c mice with those from mice infected 8 d previously with H. diminuta
revealed significant changes in the expression of mRNA for adaptive and innate immune factors. While *Il4* and *Il22* expression levels were unchanged, transcription of *Il10* and *Il25* were increased (Figure 3). Expression of mRNAs of the goblet cell factors, mucin 2 (Muc2) and trefoil factor 3 (TFF3) were increased, as was that of β-defensin. In contrast, transcription of the antimicrobial C-type lectins, regenerating islet-derived (Reg)IIIβ, and RegIIIγ were unaffected by infection with *H. diminuta* (Figure 3).

**Gut bacteria are not required for expulsion of *H. diminuta***

BALB/c and C57BL/6 mice mobilize Th2 cytokines and Th2-driven immune effector mechanisms following infection with *H. diminuta*, and expel a five-worm burden within 9–12 dpi.13 The kinetics of these events was not appreciably different in BALB/c and C57BL/6 mice, with only a marginally quicker response from the male C57BL/6 mice (Suppl. Table 1). Co-treatment of BALB/c mice with broad-spectrum antibiotics did not affect the kinetics of expulsion of *H. diminuta*, blood eosinophilia, jejunal goblet cell hyperplasia (Figure 4a–c) or IL-4, IL-5 or IL-10 from stimulated splenocytes at 5 or 8 dpi (Figure 4d–f). Interestingly, splenocytes from infected mice that also received antibiotics had reduced IL-5 output in response to conA stimulation at 11 dpi (Figure 4e, f). Given the lack of effect of antibiotics on worm expulsion, we did not pursue the mechanism(s) or putative significance of the reduced IL-5 at 11 dpi, although it hints at helminth-bacteria cross-talk in the control of systemic immune responses.

In order to assess whether intestinal microbes are required for successful expulsion of the parasite, GF mice were infected with *H. diminuta*. To ensure that bacteria were not transferred to the GF mice, cysticercoids were first treated with antibiotics, which had no effect on the ability of the helminth to excyst in vitro (Suppl. Figure 5A), or their ability to infect mice, as demonstrated by successful infection in *Il4rα−/−* mice that do not expel the worm (Suppl. Figure 5B). 16S rRNA analysis of freshly isolated cysticercoids, antibiotic-treated cysticercoids, and cecal slurry from germ-free mice administered antibiotic-treated cysticercoids confirmed a lack of bacterial contamination of the cysticercoids (Suppl. Figure 5C). Comparison of GF and SPF BALB/c mice revealed similar baseline Th2 parameters (Suppl. Table 2). Similar to SPF and antibiotic-treated SPF mice, *H. diminuta* failed to establish in GF BALB/c (inset Figure 5a) or GF C57BL/6 mice (Figure 5a) with the majority of GF mice expelling the parasite by 8–11 dpi, although a single mouse bore a stunted worm in the small intestine at 14 dpi. *H. diminuta*-infected GF C57BL/6 mice displayed blood eosinophilia and jejunal goblet cell hyperplasia.:25

![Figure 2. Extract of *H. diminuta* promotes the growth of murine fecal bacteria. Feces (100 mg) collected from male BALB/c mice (10 weeks old) was suspended in sterile PBS and cultured with a PBS-soluble whole worm extract of *H. diminuta* (HdE) for 6 h at 37°C, prior to a 24 h (37°C) culture under (A) aerobic or (B) anaerobic conditions on blood, Luteni broth (LB) or M2GSC (specific for anaerobes only) agars. Colony-forming units (CFU) were counted (data are mean± SEM; n = 4–8 mice from 1 to 2 experiments; *, p < .05 compared to 0 HdE).](image-url)
hyperplasia (Figure 5b, c), and increased IL-4, IL-5 and IL-10 production by mitogen-stimulated spleenocytes (Figure 5d–f).

Discussion

Enteric helminths and bacteria that can cause mild to severe disease have been the focus of extensive research to define host immune responses to combat these pathogens and identify mechanisms of immunopathology. Thus far, research has typically focused on helminths or bacteria, but seldom both simultaneously. This is a simplified approach to a complex ecosystem. It is germane to ask if infection with a helminth affects the composition of the gut bacteria and vice-versa, and the consequences, if any, of helminth-bacteria interaction for host immunity and susceptibility to disease.

Sequencing the V3-V4 region of the bacterial 16S rRNA gene revealed increased complexity of the gut microbiota of *H. diminuta*-infected male BALB/c mice. These statistically significant changes were characterized by increased Clostridiales (i.e. Firmicutes), specifically Ruminococcaceae and Lachnospiraceae (e.g. Clostridium XIVa). It is difficult to compare between the mouse and rat host with respect to changes in gut bacteria following infection with *H. diminuta* as the models, kinetics of infection and samples examined (gut, feces, cecal contents) all differ. The data available for the rat suggest that infection with *H. diminuta* leads to a reduced α-diversity and that there may be an outgrowth of Ruminococcaceae. Mice infected with the cestode *Echinococcus granulosus* for 4 months had a largely normal gut microbiota with enrichment of *Eisenbergiella* and *Parabacteroides* genera. Extending these studies, a burgeoning literature demonstrates shifts in the composition of bacterial communities in the gut of mice, humans and other mammals infected with helminths, primarily nematodes, but also flukes, such as *Schistosoma mansoni*, *Clonorchis sinensis*, and *Opisthorchis felineus* that do not inhabit the gut.

Two scenarios can explain the *H. diminuta*-evoked changes in the colonic microbiota. First, it could be a direct effect of products released from the worm. Second, it could be a consequence of helminth-evoked changes in host immunity (the possibility of *H. diminuta* cysticercoids having bacteria are disputed by the absence of 16S rRNA gene detection in the cysticercoids or cecal contents of infected GF mice). Remarkably, we found that a crude extract of *H. diminuta* directly stimulated the growth of feces-derived anaerobes cultured on LB- or M2GSC-agar. This is a notable finding and contrasts with the antibiotic effect of *H. polygyrus*- and *Ascaris suum*-derived molecules. Corroborating the direct effect of HdE on bacterial growth, more CFUs grew on M2GSC-agar when feces from *H. diminuta*-infected mice were cultured anaerobically.

![Figure 3](image-url). Infection with *H. diminuta* increases colonic expression of immune genes. Male BALB/c mice were infected with 5 *H. diminuta* and 8 days post-infection (dpi) segments of mid-colon were excised, processed and qPCR performed for interleukin (IL)-4, 10, 22 and 25, mucin-2 (Muc2), trefoil factor 3 (TFF3) and the antimicrobial peptides, β2-defensin (β2Def), regenerating islet-derived protein 3 (RegIII) β and γ (data are mean ± SEM; n = 6 mice from 1 experiment; ∗, p < .05 compared to uninfected control mice).
Figure 4. Broad-spectrum antibiotic treatment does not affect the expulsion of *H. diminuta*. Male BALB/c mice were given antibiotic-supplemented drinking water 5 days prior to infection with 5 *H. diminuta* and maintained on antibiotics until necropsy when (A) worms were counted in small intestinal washings, (B) blood eosinophils counted, (C) jejunal periodic-acid Schiff positive goblet cells counted, and (D-E) concanavalin A (2 μg/mL for 48 h) stimulated cytokine production from spleen cells (5 × 10⁶) measured by ELISA (data are mean ± SEM; n = 4–8 mice from two experiments; dpi, days post-infection; abx, antibiotics; * and **, p < .05 and p < .01 compared to control (con) uninfected mice, respectively; #, p < .05 compared to no abx group).
determine unequivocally how helminth-derived molecules affect bacterial growth will require concerted efforts with multiple media/agars.

The fact that non-gut helminths elicited changes in the colonic microbiota of their hosts points to an indirect effect via the host. In accordance with this, the local changes in the transcription of immune genes after infection with *H. diminuta* (i.e. colon cytokines, muc2, TFF3, β2-defensin), could potentially affect the composition and function of the gut microbiota. Additional studies with knockout mice are needed to determine a functional link between these *H. diminuta*-evoked immune changes in the colon and the

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**Figure 5.** Germ-free mice successfully expel *H. diminuta*. Male C57BL/6 germ-free mice were infected with 6–8 *H. diminuta* and on necropsy at 5, 8, 11 and 14 days post-infection (dpi) (A) worms were counted in small intestinal washings (inset: infectivity of germ-free BALB/c mice in the same experimental paradigm), (B) blood eosinophils counted, (C) jejunal periodic-acid Schiff positive goblet cells counted, and (D-E) concanavalin A (2 μg/mL for 48 h) stimulated cytokine production from spleen cells (5 × 10^⁶) measured by ELISA (data are mean ± SEM; n = 3 (day 5 and 14) or 8, 1–2 experiments; *, p < .05 compared to control uninfected mice).
changes in the composition of gut bacteria. These data underscore how a small-intestine dwelling helminth can affect the colon and align with reports of mice infected with *H. diminuta* being protected from colitis.\(^{13}\)

As data continue to accumulate on helminth-evoked changes in the composition of their hosts’ enteric microbiota, the functional significance of these changes needs to be determined. The apparent increase in Lachnospiraceae in *H. diminuta*-infected mice is noteworthy, because members of this family produce short-chain fatty acids from dietary fiber that affect many aspects of host physiology, including immunity.\(^{27}\) Similarly, increases in the probiotic bacteria *Lactobacillus spp.* and *Bifidobacter spp.* were reported in *C. sinesis* and *Enterobius vermicularis*-infected mice, respectively.\(^{23,28}\) Thus, one can hypothesize that helminth-evoked changes in the composition of the gut bacteria affect the helminth–host relationship or concomitant immunity. Neither scenario is unprecedented. For example, hatching of *T. muris* eggs required a gut microbiota,\(^{4}\) suppression of airways inflammation or improved insulin sensitivity in mice infected with nematodes involved the gut microbiota,\(^{29}\) and, contrarily, *H. polygyrus*-evoked exaggeration of *Citrobacter rodentium*-induced colitis occurred, at least in part, via the microbiota.\(^{30}\)

We assessed a role for the gut bacteria in the expulsion of *H. diminuta* from mice. Treating mice with broad-spectrum antibiotics did not affect the kinetics of expulsion of *H. diminuta*, mobilization of local effector cells (e.g. goblet cells) or the development of systemic Th2 responses. In accordance with these findings, antibiotic treatment of *S. mansoni*-infected C57BL/6 mice did not affect worm burden, eggs per female, or cytokine levels in the spleen; however, serum IgE and mitogen evoked synthesis of IFNγ and IL-10 by mesenteric lymph node cells were reduced.\(^{31}\) Notably the antibiotic-\(^{+}\)*S. mansoni*-treated mice had fewer granulomas in the liver and reduced intestinal pathology, suggesting that part of *S. mansoni*-evoked disease was indirect via bacteria.

The use of antibiotics has two caveats: antibiotics can have non-antibiotic effects (e.g. induction of neutrophil apoptosis or increased peristalsis\(^{32,33}\)) and some bacteria remain after treatment. Consequently, we utilized germ-free mice, noting that the immune system in these mice differs from SPF mice, and they may have a propensity to enhanced Th2 immunity.\(^{34,35}\) Increased Th2 immunity in GF mice would predict expedited loss of *H. diminuta*, although if a microbiota is critical to overall immune development, expulsion could be delayed. While there was variability between the individual GF mice, there was no obvious defect (or enhancement) in their local or systemic immune responses following infection with *H. diminuta* (compare Figures 4 and 5). This translated into effective expulsion of the worm, with the majority of the mice expelling their entire worm burden by 8 dpi, marginally quicker than SPF mice. Thus, we have no data to support enteric bacteria significantly affecting the mobilization of Th2 responses following infection or affecting the expulsion of the parasite.

Data in this area are scant. In accordance with the findings with *H. diminuta*, the immunological response of GF mice to infection with *H. polygyrus* was similar to that in SPF mice: worm burdens were not different at 2-weeks post-infection but worms were slightly smaller, and egg output from the infected GF mice was slightly reduced.\(^{26}\) However, 4–6 weeks post-infection, GF mice harbored fewer *H. polygyrus* (previously designated *Nematospioides dubius*) than conventional mice and mono-colonization with *Lactobacillus* favored survival and development of the helminth.\(^{36}\) Thus, there is host-parasite specificity in the role of gut bacteria in modifying the outcome of the host-parasite interaction, which may be influenced by the amount of tissue damage caused by the helminth that in turn allows bacteria greater access to immune cells in the mucosa.

In summary, having found distinct changes in the composition of the colonic bacteria following infection of mice with the tapeworm *H. diminuta* (likely a combination of direct effects of molecules released from the worm and indirectly via host immunity), mechanistic studies revealed that gut bacteria were not required for the successful eradication of the helminth. However, this does not dismiss the possibility that the perturbation of the gut microbiota following infection with *H. diminuta* has other physiological/immunological consequences for the host.\(^{37}\) With the increasing characterization of tri-kingdom interactions, meeting the challenge of defining the functional ramifications for each player – helminth, bacteria,
and host – will significantly advance understanding of host–parasite interactions.

**Material and methods**

**Mice and H. diminuta infection**

Male BALB/c or C57BL/6 (7–9 weeks old, Charles River Laboratories, Quebec, Canada) and BALB/c Il4rα−/− (colony at Univ. Calgary) mice were housed in HEPA filtered micro-isolator cages with free access to food and water in a temperature-controlled facility with a 12 h:12 h light:dark cycle. Germ-free BALB/c and C57BL/6 mice were bred and maintained in flexible film isolators at the IMC, University of Calgary, Canada. Germ-free status was routinely monitored by culture-dependent and culture-independent methods and all mouse colonies were independently confirmed to be pathogen-free. All experimental procedures were approved from the University of Calgary, Canada, Animal Care Committee and followed the Canadian Council on Animal Care guidelines for the use of animals in biomedical research, under protocol AC17-0115.

Mice under mild manual restraint were infected with five *H. diminuta* cysticercoids via oral gavage in 200 μL of 0.9% NaCl. On necropsy, the small intestine was removed, flushed with PBS, and the intestine opened longitudinally and rinsed in PBS, and the number of worms counted under a dissection microscope. GF mice received 6–8 cysticercoids that had been treated with antibiotics for 2 h (kanamycin 400 mg/L, gentamicin 35 mg/L, colistin 42 mg/L, and metronidazole 215 mg/L). Viability of antibiotic-treated cysticercoids was tested *in vitro* by induced excystment (exposure to pepsin (1%), trypsin/sodium tauroglycalate (1%) in Hanks solution) and the ability to infect Il4rα−/− mice that do not expel the worm.

**Antibiotic (abx) treatment**

Mice were administered abx 5 d prior to infection in drinking water, which was maintained throughout the experiment: kanamycin (40 mg/L), gentamicin (3.5 mg/L), colistin (4.2 mg/L), and metronidazole (21.5 mg/L). Abx-water was renewed every 48 h. In addition, mice received an intraperitoneal injection of vancomycin (4.5 mg/kg) 2 d prior to and 3 d after infection with *H. diminuta*.39

**Blood eosinophilia and enteric goblet cells**

Blood smears, collected on coded slides, were stained with Wright-Giemsa and, in a blinded fashion, 200 immune cells were counted and percentage eosinophilia determined. A ~ 1 cm segment of mid-ileum was immersion-fixed in 10% formalin, and following dehydration in graded ethanol and clearing in xylene, samples were embedded in paraffin, and 5 μm sections collected onto coded microscope slides. Sections were stained using periodic-acid Schiff’s (PAS) reagent and goblet cells counted in 15 villus-crypt units (VCU)/section.15

**Cytokines**

Spleen and mesenteric lymph nodes (MLN) were excised aseptically, dispersed into a single cell suspension and red blood cells were lysed in ACK (ammonium-chloride-potassium) lysing buffer. Subsequently, cells (5 × 10⁶/ml in RPMI 1640 medium with 10% FBS and antibiotics (Gibco, USA)) were treated with concanavalin A (2 μg/mL) for 48 h at 37°C.38 Interleukins (IL)-4, −5 and −10 were measured in duplicate samples of the cell-free culture medium by ELISA using paired antibodies following manufacturer's instructions (R&D Systems Inc.).

**qPCR of gut immune factors**

Colonic RNA was isolated using the Aurum Total RNA Mini Kit (Bio-Rad Laboratories, Hercules, Ca) as per the manufacturer’s protocol, quantified with the Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE), andler μg of RNA was converted to cDNA using an iScript kit (Bio-Rad Laboratories Canada, Mississauga, ON, Canada). Quantitative real-time polymerase chain reaction (qPCR) of murine colonic tissue was performed as previously described in40 using primer sequences shown in Suppl. Table 3.
**Bacterial composition**

One gram of proximal colon (or 100 mg feces) was homogenized using 0.2 g of 2.8 mm ceramic beads (Mo Bio Laboratories, #13114-50) in a Bullet Blender (Next Advance [http://www.nextadvance.com]). Samples were homogenized for 3 min (speed setting 10), followed by a 45 sec stop period and then second centrifugation. Next, 0.2 g of 0.1 mm glass beads was added to the sample and blended for 3 min (speed setting 10). DNA isolation was performed using a previously described protocol. 41

Bacterial community profiling was performed through 16S rRNA sequencing. First, forward and reverse barcoded primers of the V3-V4 region (341F-785R) of the bacterial 16S rRNA gene were selected for amplification (Platinum Taq Polymerase, Invitrogen Life Tech) 42 and purification prior to undergoing Illumina MiSeq analysis. Adaptor sequences were removed using “cutadapt” ([cutadapt.readthedocs.io/en/stable/guide.html](http://cutadapt.readthedocs.io/en/stable/guide.html)). Resulting fastq files were truncated to 250nt in length and reads with a quality score less than 2 were eliminated using the R phyloseq package DADA2 ([joey711.github.io/phyloseq/](https://joey711.github.io/phyloseq/)). Taxonomy was assigned to an amplicon sequence variant (ASV) table using the SILVA SSU Ref v.128 classifier.

Prior to analyzing the generated microbiome count data, prevalence filtering was performed. In addition, and due to the compositional nature of the data, in another analysis, the centered log-ratio transformation was performed as a follow-up measure to ensure correct assumptions on the original data. 43 Following a previously published workflow ([f1000research.com/articles/5-1492/v1](http://f1000research.com/articles/5-1492/v1)) diversity indices (Shannon for α-diversity), and distance metrics were generated (weighted unifrac distance and PERMANOVA for β-diversity). Differential abundance analysis was performed using the R program, “DESeq2” ([bioconductor.org/packages/release/bioc/html/DESeq2.html](http://bioconductor.org/packages/release/bioc/html/DESeq2.html)) to test for differentially expressed variants between experimental groups.

PCR for the V3-V4 region of the 16S rRNA gene was applied to DNA (as above) extracted from H. diminuta cysticercoids freshly retrieved from beetles, abx-treated cysticercoids and extracts of cecal slurry from germ-free mice that had been infected with abx-treated cysticercoids. Extracts of feces from SPF-mice served as a positive control. Polyacrylamide gel electrophoresis was performed and the PCR product visualized using ethidium bromide.

**Preparation of H. diminuta extract (HdE)**

H. diminuta antigen was isolated as previously. 44 Fifteen live adult H. diminuta worms (removed from small intestines of rats) were rinsed in sterile PBS, treated with gentamicin (1 h, 37°C), rinsed twice in sterile PBS and then homogenized in PBS. Homogenates were centrifuged and the PBS-soluble supernatant was collected. Bradford assay was used to determine protein concentrations.

**In vitro culture of bacteria**

Fecal samples (100 mg) were collected from naïve mice and placed in 1 mL of sterile PBS. Samples were vortexed for 2 min and centrifuged for 5 min at 8000 x g. Under both aerobic and anaerobic conditions, supernatant (200 μL in replicates of three per sample) was transferred to the first row of a 96 well plate. The three replicates were treated with H. diminuta antigen (100 or 500 μg/mL) and incubated for 6 h at 37°C, plated onto Lutendi broth (LB) media, blood and M2GSC media (anaerobic only) agars for 24 h at 37°C in aerobic or anaerobic conditions, after which colony-forming units (CFU) were counted.

**Data presentation and analysis**

Results are expressed as the mean ± SE (unless stated otherwise) and n is the number of mice. Data are analyzed using Graph Pad Prism 5.0 in which statistical comparisons for parametric data were performed via one-way ANOVA with Tukey’s posttest and the Kruskal–Wallis test with Dunn’s posttest was used for non-parametric data. P< .05 was set as the level of acceptable statistical difference.

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ORCID

Timothy S. Jayme http://orcid.org/0000-0001-9655-374X
Kathy D. McCoy http://orcid.org/0000-0002-3900-9227
Derek M. McKay http://orcid.org/0000-0002-0557-8210

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