**QUEEN'S
UNIVERSITY
BELFAST**

Helminths, polyparasitism, and the gut microbiome in the Philippines

Gordon, C. A., Krause, L., McManus, D. P., Morrison, M., Weerakoon, K. G., Connor, M. C., Olveda, R. M., Ross, A. G., & Gobert, G. N. (2020). Helminths, polyparasitism, and the gut microbiome in the Philippines. *International Journal for Parasitology*, 50(3), 217-225. <https://doi.org/10.1016/j.ijpara.2019.12.008>

Published in:

International Journal for Parasitology

Document Version:

Peer reviewed version

Queen's University Belfast - Research Portal:

[Link to publication record in Queen's University Belfast Research Portal](#)

General rights

Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

1 **Helminths, polyparasitism, and the gut microbiome in the Philippines**

2 Catherine A. Gordon ^a, Lutz Krause ^b, Donald P. McManus ^a, Mark Morrison ^b, Kosala G. Weerakoon
3 ^{a,c,d}, Mairead C. Connor ^e, Remigio M. Olveda ^f, Allen G. Ross ^g, Geoffrey N. Gobert ^{e,*}

4 ^a *Molecular Parasitology Laboratory, Immunology Division, QIMR Berghofer Medical Research*
5 *Institute, Brisbane, Australia*

6 ^b *University of Queensland Diamantina Institute, Faculty of Medicine, Translational Research*
7 *Institute, Brisbane, Australia*

8 ^c *School of Public Health, University of Queensland, Brisbane, Australia*

9 ^d *Department of Parasitology, Faculty of Medicine and Allied Sciences, Rajarata University of Sri*
10 *Lanka, Saliyapura, Sri Lanka*

11 ^e *School of Biological Sciences, Queen's University Belfast, Belfast, United Kingdom*

12 ^f *Department of Immunology, Research Institute of Tropical Medicine, Manilla, the Philippines*

13 ^g *Menzies Health Institute Queensland, Gold Coast, Australia*

14 *Corresponding author. Geoffrey Gobert. *E-mail address:* g.gobert@qub.ac.uk

15

16 ABSTRACT

17 Polyparasitism, involving soil-transmitted helminths (STH) and *Schistosoma* blood flukes, is
18 common in low to middle income countries. These helminths impact on the gut environment and can
19 cause changes to the gut microbiome composition. Here we examined the gut microbiome in
20 individuals with polyparasitism from two human cohorts in the Philippines utilising DNA
21 sequencing-based profiling. Multiple helminth species infections were high with 70.3% of study
22 participants harbouring at least two parasite species, and 16% harbouring at least five species.
23 Increased numbers of helminth co-infections, in particular with the gut-resident STH, were
24 significantly associated with increased bacterial diversity; however no significant parasite-gut
25 microbiome associations were evident for individuals infected only with *Schistosoma japonicum*. In
26 general, a healthy gut is associated with high bacterial diversity, which in these human cohorts may
27 be the result of helminth-mediated immune modulation, or due to changes in the gut environment
28 caused by these parasitic helminths.

29

30 *Keywords:* Soil-transmitted helminths; Hookworm; *Ancylostoma*; *Ascaris*; *Trichuris*; *Schistosoma*;
31 Gut microbiome; The Philippines

32

1. Introduction

Polyparasitism, or infection with multiple parasite species, is highly prevalent within human populations of many economically poor countries. An estimated 2 billion people are infected worldwide with soil-transmitted helminths (STH) (Bethony et al., 2006), with individual species presenting a range of host pathologies while inhabiting different host tissue/organ sites (Fig. 1). STH share similar geographic ranges and similar infection pathways, with co-infections common (Gordon et al., 2017). Hookworms (*Necator americanus* and *Ancylostoma* spp.), whipworms (*Trichuris trichiura*) and roundworms (*Ascaris lumbricoides*) comprise the major clinically important STH species. STH transmission control is largely achieved by good hygiene practices, having access to latrines, and wearing shoes (Bieri et al., 2013; Campbell et al., 2014; McManus et al., 2014).

Parasitic helminths generally cause chronic disease, particularly in children, including growth stunting, reduced cognitive ability and anaemia in the case of hookworms and *Schistosoma* spp. (King, 2010; Parija et al., 2017). Hookworms are the only gut-resident helminths that penetrate the gut wall while feeding on host blood; the other gut-resident helminths attach to the intestinal lumen and impact on the mucosal immune system and homeostasis (Leung and Loke, 2013). Adult schistosomes dwell in the blood vessels of their human hosts. Intestinal helminth parasites and gut bacteria live in such close proximity in the intestine that it is likely considerable interaction between the two groups of organisms may lead to perturbations of the gut microbiome (Reynolds et al., 2015; Zaiss and Harris, 2016). There are some reports that helminth parasites increase host gut microbiome diversity (Cantacessi et al., 2014; Lee et al., 2014), whereas other studies showed no change in infected individuals (Cantacessi et al., 2014; Kay et al., 2015). How the presence of a single or multiple parasitic helminth species in the gut impacts this habitat, and whether the composition of the microbial population is affected, is largely unknown.

South East Asia (SEA) is a hot bed for STH infections, with approximately one-third of global cases occurring in this region (Jex et al., 2011). The Philippines is no exception, being highly endemic

not only for STH but also *S. japonicum* and *Taenia* spp., often occurring as clinical co-infections (Gordon et al., 2015c). Here we report the gut microbiome of individuals with single or multiple parasitic helminth infections in a rural area of the Philippines (Gordon et al., 2015c). The study is timely as polyparasitism as a parameter has not been investigated when considering its potential impact on the gut microbiome of humans.

2. Materials and methods

2.1. Ethics

As previously described (Gordon et al., 2015b, 2015c), informed written consent was received from all participants and approval provided by the Ethics Committee of the Research Institute of Tropical Medicine (RITM), Manila, the Philippines, and the QIMR Berghofer Medical Research Institute (QIMRB) Human Research Ethics Committee, Australia (Approval Number: H0309-058 (P524)).

2.2. Study area and design

Stool samples were collected from two human cohorts in 2011 (Cohort-1; $n=161$), and 2015 (Cohort-2; $n=58$) from barangays in the municipalities of Palapag and Laoang in Northern Samar Province, the Philippines, as described (Fig. 2) (Supplementary Table S1) (Gordon et al., 2015a, 2015b; Olveda et al., 2017; Weerakoon et al., 2017). Of the total of 219 stool samples, 38 were helminth-negative by real time PCR (qPCR) and droplet digital PCR (ddPCR) (Supplementary Fig. S1); all helminth-negative individuals were from cohort 2. The ages of participants ranged from 4-72 years with the average age across the two cohorts being 33 years. Barangays in this study had been subjected in the past to annual mass treatment with praziquantel (PZQ) as part of the national schistosomiasis control program, although none of the selected study participants had been treated in the year prior to stool collection. School-aged children in the two cohorts were, at the time of stool

82 collection, subjected to annual albendazole treatment for STH as part of the national control program
83 in the Philippines (DepED, 2015a, b). Stool samples were stored in 80% (v/v) ethanol for subsequent
84 DNA isolation and molecular analysis.

85 DNA from subjects in cohort-1 was previously subjected to qPCR for *S. japonicum* (Gordon
86 et al., 2015b) and multiplex qPCR for *Ancylostoma* spp., *Necator americanus*, *Ascaris lumbricoides*,
87 *Taenia* spp. and *Trichuris trichiura* (Gordon et al., 2015c). DNA from individuals in cohort-2 was
88 previously used in a ddPCR for *S. japonicum* (Weerakoon et al., 2017). In the current study cohort-2
89 was subjected to a multiplex qPCR for *Ancylostoma* spp., *Necator americanus*, *Ascaris lumbricoides*,
90 *Taenia* spp. and *Trichuris trichiura* as per cohort-1 (Gordon et al., 2015c). DNA samples from both
91 cohorts ($n=219$) were further subjected to microbiome profiling (Fig. 2).

92 DNA quality and quantity were determined using a NanoDrop 1000 (Thermo Scientific,
93 Waltham, USA); DNA samples with quantity <10 ng/ μ l and 260/280 ratio of <1.6 or more than 2.1
94 were excluded. With cohort-1, 161 DNA samples were divided into single, dual, triple, quadruple,
95 and quintuple helminth infection groups; then a set number of samples was randomly selected from
96 each of these infection groups for microbiome analysis. For cohort-2, all DNA samples were analysed
97 by multiplex qPCR after which 38 DNA samples negative for all helminth parasites and 20 helminth-
98 positive DNA samples, selected based on age and gender matching with cohort-1, were submitted to
99 microbiome analysis.

100 *2.3. DNA sequencing-based profiling of the gut microbiome*

101 The composition of the gut microbiome of all 219 cohort participants was profiled by 16S
102 rRNA amplicon sequencing (*rrs*) at the Australian Genome Research Facility (AGRF; Brisbane,
103 Australia) following their standard protocols. The *rrs* V3 and V4 hypervariable regions were
104 amplified using primers 341F and 806R; Forward sequence: 5'-CCTAYGGGRBGCASCAG-3',
105 Reverse sequence: 5'-GGACTACNNGGGTATCTAAT-3'. AmpliTaq gold 360 MasterMix (Life
106 Technologies, Carlsbad, USA) was used for the first PCR assay and cycling conditions were as

107 follows: initialisation at 95°C for 7 min followed by 30 cycles of 94°C for 45 s, 50°C for 60 s and
108 72°C for 60 s, followed by a final extension of 72°C for 7 min. A secondary PCR to index the
109 amplicons was performed using TaKaRa Taq DNA polymerase (Clontech, Mountain View, USA).
110 The resulting amplicons were measured by PicoGreen fluorometry (Invitrogen, Carlsbad, USA) and
111 normalised. The equimolar pool was measured by qPCR (KAPA, Roche, Basel, Switzerland)
112 followed by Illumina MiSeq sequencing with 2x300 bp paired-end chemistry.

113 Reads were assembled by aligning the forward and reverse reads using PEAR (V-0.9.5)
114 (Zhang et al., 2014). Primers were identified, trimmed and *rrs* amplicon sequences processed using
115 Quantitative Insights into Microbial Ecology (QIIME-1.8) (Caporaso et al., 2010). Sequences were
116 clustered into Operational Taxonomic Units (OTUs) using UCLUST on the basis of similarity to
117 known bacterial sequences available in the GreenGenes database (v13.8, 97% sequence similarity
118 cut-off) and rarefied to a sequence depth of 28,700. Rare OTUs with <5 assigned amplicon sequences
119 or <0.0001 fraction of total sequence reads were excluded and OTU reference sequences were aligned
120 using pyNST. The trimmed multiple alignment was then used to infer a phylogenetic tree (using
121 fasttree) which, in turn, was used as input for UniFrac to estimate microbial beta diversity.

122 2.4. Statistics

123 SAS software (SAS Institute) was used for descriptive statistics and calculation of 95%
124 confidence intervals (95% CIs) of the parasitology data, while Calypso (version 8.54) (Zakrzewski et
125 al., 2017) was used for mining the *rrs* microbiome dataset and for data visualisation. Rarefied and
126 filtered OTU tables were uploaded to Calypso and square root transformed. To examine differences
127 in the gut microbiota between infected and uninfected cohort participants, data were grouped as ‘case’
128 – parasite infected, and ‘control’ – negative for parasites. Data were further stratified in some
129 instances to determine the effect of multiple parasite species (1-5) on bacterial diversity, and grouped
130 as gut helminths (*T. trichiura*, hookworm, *A. lumbricoides*, *Taenia* spp.), *S. japonicum* only
131 infections, and controls (negatives). Principal coordinate analysis (PCoA using weighted UniFrac
132 distances) and Redundancy Analysis (RDA) were undertaken to assess whether variance in microbial

community composition could be attributed to any of the study groups or clinical variables, such as age and gender. Microbial species diversity was characterised using the Shannon and richness diversity indices. Correlations with continuous variables (e.g. infection number) were calculated using Spearman's rank order correlation. Individual taxa that were significantly differentially abundant across study groups were identified by ANOVA and *P* values were corrected for multiple testing by false discovery rate (FDR). The significance level alpha was set to 0.05.

2.5. Data accessibility

Raw data are available from Medley Data. This includes a code book for annotation, an annotations excel workbook, and the raw OTU results in csv format (<http://dx.doi.org/10.17632/59j46prhvf.1>)

3. Results

3.1. High prevalence of helminth parasites in the Palapag and Laoang endemic areas

The majority of infected individuals (70.32%) harboured at least two different helminth parasites with 7.31% (95% CI: 3.83-10.78) being infected with at least five species (Supplementary Table S1). The highest prevalence was observed for *S. japonicum* (71.69%; 95% CI: 65.68-77.70) followed by *A. lumbricoides* (48.86%; 95%CI: 42.19-55.53) and *T. trichiura* (47.49%; 95% CI: 40.82-54.15) (Table 1).

3.2. Significant changes seen in the gut microbiota between helminth-positive and helminth-negative individuals

Microbial profiles were generally consistent with normal gut flora with high variability in the relative abundance of individual taxa between cohort participants (Supplementary Fig. S2). All microbiome data are provided in Supplementary Tables S2-S6.

RDA showed significant differences in the microbiome between helminth-parasitised individuals and controls ($P=0.001$), while *S. japonicum*-only infections also showed significantly

different microbiomes compared with individuals infected with intestinal helminths ($P=0.021$) (Supplementary Fig. S3). The microbiome composition was considered for each helminth infection by a separate RDA; for example, *S. japonicum* infection, irrespective of infection with other species, were compared with individuals not infected with *S. japonicum* (including helminth-negative individuals). RDA clustering for *S. japonicum*, *A. lumbricoides*, *T. trichiura*, *Taenia* spp., and *Ancylostoma* spp. were all statistically significant ($P<0.05$) (Fig. 3).

The most common bacterial phyla present in all samples analysed were Firmicutes and Bacteroidetes, followed by Actinobacteria (Supplementary Table S5). Archaea were significantly more abundant in helminth-positive individuals (cases, $n=181$; controls, $n=38$, $P\leq 0.01$, FDR=0.016) as were Firmicutes ($P\leq 0.01$, FDR=0.016) and Actinobacteria ($P\leq 0.001$, FDR=4.3E-08), while Bacteroidetes were significantly more abundant in helminth-negative individuals (controls, $n=38$, $P\leq 0.001$, FDR=4E-06).

3.3. Helminth infections are associated with increased richness and diversity of the gut microbiota

Bacterial richness was significantly higher ($P=6.96\text{e-}06$) in individuals harbouring helminth parasites compared with those who did not (Fig. 4A), and significantly higher ($pP=3.9\text{e-}07$) in individuals with intestinal helminths ($n=167$) compared with uninfected subjects ($n=38$) or in those harbouring only *S. japonicum* ($n=14$) (Fig. 4B). Bacterial richness increased significantly ($P=8.83\text{e-}05$, $R=0.28$) as the number of infecting helminth species increased (Fig. 4C). Bacterial diversity, as measured by the Shannon Index, also increased in helminth-positive individuals ($P=0.0352$, Fig. 4A) compared with helminth-negative subjects, and in *S. japonicum*-only infected individuals ($P=0.0343$; Fig. 4B).

Bacterial richness was increased ($P=0.00366$) in individuals harbouring *T. trichiura* irrespective of infection with other species, compared with individuals not infected with *T. trichiura* (Fig. 5A). Similarly, bacterial richness was significantly higher in *T. trichiura*-only infected individuals ($n=5$) compared with helminth-negative controls ($n=38$) ($P=0.0111$) (Fig. 5B).

182 3.4. *Schistosoma japonicum* infection has less impact on the gut microbiota composition than
183 intestinal helminth infection

184 We observed significant differences in the abundance of individual bacterial genera and
185 species between helminth-infected subjects and uninfected controls (Supplementary Fig. S2 and
186 Supplementary Fig. S4). When comparing *S. japonicum*-only ($n=14$) infections with helminth-
187 negative ($n=38$) and intestinal helminth infections ($n=167$), significant differences in abundance of
188 certain bacteria were evident. However, in general, *S. japonicum*-only infected individuals had a
189 similar microbiome as the intestinal helminth-infected subjects compared with those who were
190 uninfected (Supplementary Fig. S5).

191 3.5. Increased abundance of Lactobacillaceae in individuals with *T. trichiura* infection

192 Lactobacillaceae were significantly more prevalent ($P=0.0034$, FDR=0.014) in parasitised
193 individuals compared with those who were uninfected (Fig. 5). Regardless of infection with other
194 helminths, *Lactobacillus* showed a higher relative abundance in *T. trichiura*-infected individuals
195 ($n=104$), compared with *T. trichuris*-negative individuals ($n=115$) (Fig. 5). There was no change in
196 the relative abundance of the Lactobacillaceae between *T. trichiura*-only infected individuals ($n=5$)
197 compared with uninfected controls ($n=38$).

198 Bacterial richness was increased ($P=0.00366$) in individuals infected with *T. trichiura*,
199 irrespective of infection with other species, compared with individuals not infected with *T. trichiura*
200 (Fig. 5A). Similarly, bacterial richness was significantly higher in *T. trichiura*-only infected
201 individuals ($n=5$) compared with helminth-negative controls ($n=38$) ($P=0.0111$) (Fig. 5B).

202 3.6. Increased abundance of *Faecalibacterium* associated with any helminth infection

203 There was an increase in *Faecalibacterium* abundance with increasing polyparasitism
204 ($p=0.00044$, $R=0.24$) (Fig. 6A, Supplementary Fig. S6). Abundance of the genus *Faecalibacterium*
205 was increased significantly ($P=0.038$; FDR 0.093) in helminth-positive individuals compared with
206 helminth-negative subjects (Fig. 6B).

207 3.7. *No significant differences in microbiota observed between cohorts*

208 Comparisons were made between the cohort-1 helminth-positive individuals, cohort-2
209 helminth- positive individuals, and cohort-2 helminth-negative individuals to determine the impact
210 of batch effect and non-helminth infection status (Supplementary Fig. S7). In terms of richness, there
211 was a significant increase in diversity between cohort-1 and cohort-2 helminth-positive individuals
212 compared with those who were helminth-negative (Supplementary Fig. S7B). The Shannon Index
213 was not significant (0.053) but showed a trend in both cohorts towards increased diversity in
214 helminth-positive individuals compared with those who were helminth-negative.

215 4. Discussion

216 This is the first known study to investigate the effect of helminth polyparasitism, a feature
217 common in many rural communities of developing countries, on the human gut microbiome. Previous
218 reports examining the gut microbiota and parasitism did not consider individuals infected with more
219 than one helminth species, exemplified by recent studies with schistosome infections (Kay et al.,
220 2015; Schneeberger et al., 2018). Another key limiting feature of previous studies was the use of
221 insensitive microscopically-based diagnostic procedures to identify helminth infections. DNA
222 diagnosis using multiplex qPCR is considerably more sensitive (Gordon et al., 2011, 2015c) and thus
223 our data represent a more comprehensive overview of helminth parasites in this target study
224 population.

225 The majority of published research on the gut microbiome has been undertaken in developed
226 countries where the impact of helminth parasites, a major consideration here, is generally not a feature
227 that is relevant or is considered. Our two study cohorts examined were from a rural, low
228 socioeconomic area in the Philippines, and thus the microbiome profile might be expected to be quite
229 different from that found in urban dwellers and residents from more economically developed
230 countries (Mah et al., 2008; Tyakht et al., 2014; De Filippo et al., 2017). Accordingly, we found a
231 large number of unclassified bacteria at the species level and due to the limitations in classifications

232 of “rural microbiota species”, examination of genus and family was used. We found a relatively high
233 abundance of *Prevotella copri*, a hallmark of rural infections and, in the Philippines, this species is
234 positively associated with a rural non-westernised diet (Nakayama et al., 2017). In addition, *P. copri*
235 was significantly associated with parasitic helminth infection ($P=0.0072$) as reported previously for
236 *S. haematobium* infection and the microbiome (Kay et al., 2015) (Supplementary Table S2).

237 We found increasing numbers of helminth species co-infections correlated significantly with
238 increased bacterial richness and diversity (Fig. 4C). Bacterial richness in *S. japonicum*-only infections
239 ($n=14$) was reduced and comparable to that found in uninfected individuals (Fig. 4B), suggesting *S.*
240 *japonicum* might have less of an effect on the gut microbiome than the STH. This could also be a
241 reflection of decreased richness due to a lower burden of helminths. We saw in this study an increase
242 in bacterial richness and diversity with increasing numbers of helminths as well. Intestinal
243 schistosomes impact physically on the host intestine only when their eggs traverse the gut lumen to
244 be excreted in faeces, although some become trapped in intestinal tissues, inducing mucosal
245 granulomatous inflammation (McManus et al., 2018). Previous studies examining the gut microbiota
246 and schistosome infection found significant differences between infected and uninfected individuals,
247 including differences in the microbiota composition in different age groups (Yatsunenko et al., 2012;
248 Kay et al., 2015). In contrast, we found no significant differences in our study by either age or gender
249 (data not shown). Ages in our study cohorts ranged from four to seventy-six, while differences had
250 previously been identified for children <1 year of age (Yatsunenko et al., 2012).

251 The increased bacterial diversity we report in STH-infected individuals compared with those
252 who were uninfected, supports similar studies on the gut microbiota in rural Malaysians ($n=51$) (Lee
253 et al., 2014) and Sri Lankans ($n=76$) (Jenkins et al., 2017), and highlights the impact of
254 gastrointestinal helminths on the microbiota diversity in parasitised individuals in both locations. The
255 Malaysian study also demonstrated that bacterial populations varied significantly between Malaysian
256 and New York City residents ($p=0.01$) (Lee et al., 2014).

257 A low number of Proteobacteria is a marker of a healthy gut, and imbalances in the gut
258 microbiota can be due to their increased abundance (Shin et al., 2015). In the current study we found
259 no difference in the abundance of Proteobacteria in helminth-positive individuals compared with
260 uninfected individuals. There was a slight decrease in Proteobacteria with increasing polyparasitism,
261 although this was not significant (Supplementary Fig. S6). The most abundant phyla present were the
262 Firmicutes, followed by the Bacteroidetes, and then the Actinobacteria. There was a significant
263 increase in the Firmicutes and Actinobacteria, but a significant decrease in Bacteroidetes in
264 parasitised subjects (Supplementary Table S2). Individual members of all these phyla can provide
265 beneficial or elicit deleterious impacts on gut health; for instance a decrease in some Bacteroidetes
266 and Firmicutes and an increase in Proteobacteria is commonly reported in Irritable Bowel Disease
267 patients (Wexler, 2007). Members of the Firmicutes and Bacteroidetes are involved in a number of
268 important metabolic processes within the intestine, specific examples being polysaccharide (fibre)
269 degradation and carbohydrate fermentation (Wexler, 2007) and they can influence the host immune
270 system, benefiting themselves over other species and pathogens (Wexler, 2007). A reduction in
271 Bacteroidetes abundance associated with parasitic infection has previously been documented
272 (Andersen et al., 2016), suggesting that regulation of these bacteria by parasites occurs. It is
273 noteworthy that the shifts in microbiota profiles likely reflect alterations in gut function and microbial
274 fermentation. We found an increase in the relative abundance of Firmicutes and Archaea with
275 polyparasitism, most likely reflective of food digestion and anaerobic fermentation favouring the
276 initial production of acetate, CO₂ and hydrogen; and leading to methane and butyrate production.
277 This contrasts with Bacteroidetes which produce relatively higher amounts of succinate and
278 propionate. It has been hypothesised that parasites cause alterations within the gut microbiome to
279 their own advantage; consequently, the higher levels of butyrate-producing Firmicutes might be more
280 advantageous to gut parasites because butyrate has been shown to inhibit potentially deleterious
281 inflammatory responses within the intestine (Lepage et al., 2011; Riviere et al., 2016).

282 In this respect, we observed a significant increase in *Faecalibacterium* in parasitised

283 individuals compared with uninfected controls, and an increase in abundance with increasing parasite
284 diversity (Fig. 6 and Supplementary Fig. S6). *Faecalibacterium* is typically associated with a healthy
285 microbiome in Western populations, and reduced numbers of these bacteria have been associated
286 with Crohn's disease and other inflammatory conditions in the gut (Galecka et al., 2013; Cao et al.,
287 2014).

288 Knowledge of Archaea, such as *Euryarchaeota*, within the gut is rudimentary, particularly in
289 regards to their association with human disease (Koskinen et al., 2017). Certain Archaeal species are
290 suggested to relate to Irritable Bowel Syndrome and colorectal cancer due to their role in the
291 production of methane in the gut, with higher levels of methane linked to constipation-related
292 disorders (Gaci et al., 2014). We found *Euryarchaeota* abundance significantly increased with
293 increasing polyparasitism (Supplementary Fig. S6); this is to our knowledge the first time this
294 observation has been made and may help explain further interactions within the gut. In contrast a
295 study by Li et al. (2016) of goats infected with the intestinal nematode *Haemonchus contortus*
296 displayed a lower abundance of *Euryarchaeota* in comparison to uninfected animals, suggesting this
297 relationship is complex and may vary according to the host species infected and the infecting parasite
298 species (Galecka et al., 2013).

299 An interesting finding in mouse models of *Trichuris muris* is the skewing of the immune
300 response towards inflammation (Holm et al., 2015), corresponding with a decrease in bacterial
301 diversity and an increase in *Lactobacillus* abundance. We found a similar trend in increasing
302 abundance of *Lactobacillus* in subjects infected with *T. trichiura* (Fig. 5C). As there was marked
303 polyparasitism in the study area, this could have been due to the effect of other helminth species and,
304 indeed, there was a significant decrease in *Lactobacillaceae* in parasitised individuals compared with
305 uninfected controls; *Lactobacillus* abundance was significantly associated with parasitised
306 individuals (Supplementary Table S2). Thus we also examined individuals infected with only *T.*
307 *trichiura* ($n=5$) and compared them with parasite-negative controls. We observed a similar increased

308 trend by the rank test, although there was no significant difference by T-test between the two groups
309 in *Lactobacillus* abundance, likely due to the small number of *T. trichiura*-only infected individuals
310 analysed. Mouse models have also shown a corresponding decrease in bacterial richness (Holm et al.,
311 2015) which we did not observe (Fig. 5B).

312 As a result of shared geographical endemic zones and infection pathways for the different
313 species, polyparasitism, due to STH and *S. japonicum*, is widespread in the rural Philippines with
314 single species infection being relatively rare (Gordon et al., 2015c). Accordingly, we examined stool
315 samples from two cohorts from the same Palapag area, collected at different times, as few helminth-
316 negative individuals were available in cohort-1 to act as controls. Utilising PCoA and RDA we found
317 a minimal batch effect between the two cohorts, and any differences reported here were not the result
318 of the different stool collection times; for example, significant findings of increased richness in
319 parasitised individuals were evident regardless of when the stool samples were collected
320 (Supplementary Fig. S7).

321 The interactions between bacteria, helminth parasites and their hosts are incredibly complex.
322 Helminth infection generally leads to phases of both acute and chronic disease, and the entry routes
323 of parasitic helminths into the host can lead to bacterial translocation which, in turn, can result in
324 sepsis. By contrast helminth parasites are known to down-regulate inflammation and, as such, show
325 potential for the treatment of inflammatory diseases. The current study focused on analysis of
326 collected stool samples, which may not be fully representative of the entire gastrointestinal tract (GIT)
327 (Lavelle et al., 2015), as bacteria resident in the upper GIT were potentially missed. However,
328 collecting such samples involves invasive procedures that are difficult to undertake in the field on a
329 large scale. Nevertheless, we clearly demonstrated that human infection with helminth parasites led
330 to an increase in bacterial richness and diversity, a feature often associated with a healthy gut. The
331 increased numbers of so called ‘beneficial’ bacteria, such as *Faecalibacterium*, we report in the
332 helminth-infected individuals points to a positive association leading to the concept that helminth

333 parasites may in fact be beneficial commensal organisms.

334 Decreased bacterial richness and diversity is associated with dysbiosis and a range of diseases
335 associated with poor gut health. Our findings of increased richness and diversity in helminth
336 parasitized individuals may suggest a role for helminths, which are known to decrease inflammatory
337 responses, in good gut health.

338

339 **Acknowledgements**

340 We would like to acknowledge the excellent work of Tal Bavli (QIMRB, Australia) for
341 designing Fig. 1. The authors would like to acknowledge funding from UBS Optimus Foundation,
342 Switzerland and the Australian National Health and Medical Research Council for the cohort
343 sample collection in the Philippines and the Australian Infectious Diseases (AID) Research Centre
344 for seed funding for the microbiome 16S RNA analysis at the Australian Genome Research Facility
345 (AGRF).

346

347

348

349 **References**

- 350 Andersen, L.O., Karim, A.B., Roager, H.M., Vigns, L.K., Krogfelt, K.A., Licht, T.R., Stensvold,
351 C.R., 2016. Associations between common intestinal parasites and bacteria in humans as
352 revealed by qPCR. *Eur J Clin Microbiol Infect Dis* 35, (9) 1427-1431.
- 353 Bethony, J., Brooker, S., Alboico, M., Geirger, S.M., Loukas, A., Diemart, D., Hotez, P.J., 2006.
354 Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *The Lancet*
355 367, 1521-1532.
- 356 Bieri, F.A.M., Gray, D.J.P., Williams, G.M.P., Raso, G.P., Li, Y.-S.P., Yuan, L.P., He, Y.M.P.H.,
357 Li, R.S.B., Guo, F.-Y.B.A., Li, S.-M.B.A., McManus, D.P.D., 2013. Health-education
358 package to prevent worm infections in Chinese schoolchildren. *New Eng J Med* 368, (17)
359 1603-1612.
- 360 Campbell, S.J., Savage, G.B., Gray, D.J., Atkinson, J.M., Magalhães, R.J.S., Nery, S.V., McCarthy,
361 J.S., Velleman, Y., Wicken, J.H., Traub, R.J., Williams, G.M., Andrews, R.M., Clements,
362 A.C.A., 2014. Water, sanitation, and hygiene (WASH): A critical component for sustainable
363 soil-transmitted helminth and schistosomiasis control. *PLoS Negl Trop Dis* 8, (4) e2651.
- 364 Cantacessi, C., Giacomini, P., Croese, J., Zakrzewski, M., Sotillo, J., McCann, L., Nolan, M.J.,
365 Mitreva, M., Krause, L., Loukas, A., 2014. Impact of experimental hookworm infection on
366 the human gut microbiota. *J Infect Dis* 210, (9) 1431-1434.
- 367 Cao, Y., Shen, J., Ran, Z.H., 2014. Association between *Faecalibacterium prausnitzii* Reduction and
368 Inflammatory Bowel Disease: A Meta-Analysis and Systematic Review of the Literature.
369 *Gastroentero Res Pract* 2014, 872725.
- 370 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer,
371 N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D.,
372 Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M.,
373 Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T.,
374 Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community
375 sequencing data. *Nat Methods* 7, (5) 335-336.
- 376 De Filippo, C., Di Paola, M., Ramazzotti, M., Albanese, D., Pieraccini, G., Banci, E., Miglietta, F.,
377 Cavalieri, D., Lionetti, P., 2017. Diet, Environments, and Gut Microbiota. A Preliminary
378 Investigation in Children Living in Rural and Urban Burkina Faso and Italy. *Front*
379 *Microbiol* 8, 1979.
- 380 DepED, 2015. Implementation of the national school deworming day, in: Education (Ed.), Division
381 memorandum. DepED, Bohol, the Philippines, pp. 1-20.
- 382 DepED, 2015. Second Round of National School Deworming Day for School Year 2015-2016, in:
383 Education (Ed.), Division memorandum. DepED, The Philippines, pp. 1-2.
- 384 Gaci, N., Borrel, G., Tottey, W., O'Toole, P.W., Brugere, J.F., 2014. Archaea and the human gut:
385 New beginning of an old story. *World J Gastroenterol* 20, (43) 16062-16078.
- 386 Galecka, M., Szachta, P., Bartnicka, A., Lykowska-Szuber, L., Eder, P., Schwiertz, A., 2013.
387 *Faecalibacterium prausnitzii* and Crohn's disease - is there any connection? *Pol J Microbiol*
388 62, (1) 91-95.
- 389 Gordon, C.A., Gray, D.J., Gobert, G.N., McManus, D.P., 2011. DNA amplification approaches for
390 the diagnosis of key parasitic helminth infections of humans. *Mol Cell Probes* 25, (4) 143-
391 152.
- 392 Gordon, C.A., Acosta, L.P., Gray, D.J., Olveda, R., Jarilla, B., Gobert, G.N., Ross, A.G., McManus,
393 D.P., 2012. High prevalence of *Schistosoma japonicum* infection in carabao from Samar
394 province, the Philippines: implications for transmission and control. *PLoS Negl Trop Dis* 6,
395 (9) e1778.
- 396 Gordon, C.A., Acosta, L.P., Gobert, G.N., Jiz, M., Olveda, R.M., Ross, A.G., Gray, D.J., Williams,
397 G.M., Harn, D., Yuesheng, L., McManus, D.P., 2015a. High prevalence of *Schistosoma*

398 *japonicum* and *Fasciola gigantica* in bovines from Northern Samar, the Philippines. PLoS
399 Negl Trop Dis 9, (2) e0003108.

400 Gordon, C.A., Acosta, L.P., Gobert, G.N., Olveda, D.M., Ross, A.G., Williams, G.M., Gray, D.J.,
401 Harn, D., Yuesheng, L., McManus, D.P., 2015b. Real-time PCR demonstrates high human
402 prevalence of *Schistosoma japonicum* in the Philippines: implications for surveillance and
403 control. PLoS Negl Trop Dis 9, (1) e0003483.

404 Gordon, C.A., McManus, D.P., Acosta, L.P., Olveda, R., Williams, M., Ross, A.G., Gray, D.J.,
405 Gobert, G.N., 2015c. Multiplex real-time PCR monitoring of intestinal helminths in humans
406 reveals widespread polyparasitism in Northern Samar, the Philippines. Int J Parasitol 45, (7)
407 477-483.

408 Gordon, C.A., Kurscheid, J., Jones, M.K., Gray, D.J., McManus, D.P., 2017. Soil-transmitted
409 helminths in tropical Australia and Asia. Tropical Medicine & Infectious Disease 2, (4).

410 Holm, J.B., Sorobetea, D., Kiilerich, P., Ramayo-Caldas, Y., Estelle, J., Ma, T., Madsen, L.,
411 Kristiansen, K., Svensson-Frej, M., 2015. Chronic *Trichuris muris* Infection Decreases
412 Diversity of the Intestinal Microbiota and Concomitantly Increases the Abundance of
413 *Lactobacilli*. PLoS One 10, (5) e0125495.

414 Jenkins, T.P., Rathnayaka, Y., Perera, P.K., Peachey, L.E., Nolan, M.J., Krause, L., Rajakaruna,
415 R.S., Cantacessi, C., 2017. Infections by human gastrointestinal helminths are associated
416 with changes in faecal microbiota diversity and composition. PLoS One 12, (9) e0184719.

417 Jex, A.R., Lim, Y.A.L., M. Bethony, J., Hotez, P.J., Young, N.D., Gasser, R.B., 2011. Soil-
418 transmitted helminths of humans in Southeast Asia-towards integrated control. Adv
419 Parasitol 74, 231-265.

420 Kay, G.L., Millard, A., Sergeant, M.J., Midzi, N., Gwisai, R., Mduluzi, T., Ivens, A., Nausch, N.,
421 Mutapi, F., Pallen, M., 2015. Differences in the Faecal Microbiome in *Schistosoma*
422 *haematobium* Infected Children vs. Uninfected Children. PLoS Negl Trop Dis 9, (6)
423 e0003861.

424 King, C.H., 2010. Parasites and poverty: the case of schistosomiasis. Acta Trop 113, (2) 95-104.

425 Koskinen, K., Pausan, M.R., Perras, A.K., Beck, M., Bang, C., Mora, M., Schilabel, A., Schmitz,
426 R., Moissl-Eichinger, C., 2017. First Insights into the Diverse Human Archaeome: Specific
427 Detection of Archaea in the Gastrointestinal Tract, Lung, and Nose and on Skin. Mbio 8,
428 (6).

429 Lavelle, A., Lennon, G., O'Sullivan, O., Docherty, N., Balfe, A., Maguire, A., Mulcahy, H.E.,
430 Doherty, G., O'Donoghue, D., Hyland, J., Ross, R.P., Coffey, J.C., Sheahan, K., Cotter,
431 P.D., Shanahan, F., Winter, D.C., O'Connell, P.R., 2015. Spatial variation of the colonic
432 microbiota in patients with ulcerative colitis and control volunteers. Gut 64, (10) 1553-1561.

433 Lee, S.C., Tang, M.S., Lim, Y.A.L., Choy, S.H., Kurtz, Z.D., Cox, L.M., Gundra, U.M., Cho, I.,
434 Bonneau, R., Blaser, M.J., Chua, K.H., Loke, P., 2014. Helminth colonization is associated
435 with increased diversity of the gut microbiota. PLoS Negl Trop Dis 8, (5) e2880.

436 Lepage, P., Hasler, R., Spehlmann, M.E., Rehman, A., Zvirbliene, A., Begun, A., Ott, S.,
437 Kupcinkas, L., Dore, J., Raedler, A., Schreiber, S., 2011. Twin Study Indicates Loss of
438 Interaction Between Microbiota and Mucosa of Patients With Ulcerative Colitis.
439 Gastroenterology 141, (1) 227-236.

440 Leung, J.M., Loke, P., 2013. A role for IL-22 in the relationship between intestinal helminths, gut
441 microbiota and mucosal immunity. Int J Parasitol 43, (3-4) 253-257.

442 Li, R.W., Li, W., Sun, J., Yu, P., Baldwin, R.L., Urban, J.F., 2016. The effect of helminth infection
443 on the microbial composition and structure of the caprine abomasal microbiome. Sci Rep 6,
444 20606.

445 Mah, K.W., Sangsupawanich, P., Tunyapanit, W., van Bever, H., Shek, L.P., Chua, K.Y., Lee,
446 B.W., 2008. Gut microbiota of children living in rural south Thailand and urban Singapore.
447 Allergol Int 57, (1) 65-71.

448 McManus, D.P., Bieri, F.A., Li, Y.S., Williams, G.M., Yuan, L.P., Henglin, Y., Du, Z.W.,
449 Clements, A.C., Steinmann, P., Raso, G., Yap, P., Magalhaes, R.J., Stewart, D., Ross, A.G.,

- Halton, K., Zhou, X.N., Olveda, R.M., Tallo, V., Gray, D.J., 2014. Health education and the control of intestinal worm infections in China: a new vision. *Parasit Vectors* 7, (1) 344.
- McManus, D.P., Dunne, D.W., Sacko, M., Utzinger, J., Vennervald, B.J., Zhou, X.N., 2018. Schistosomiasis. *Nat Rev Dis Primers* 4, (1) 13.
- Nakayama, J., Yamamoto, A., Palermo-Conde, L.A., Higashi, K., Sonomoto, K., Tan, J., Lee, Y.-K., 2017. Impact of Westernized Diet on Gut Microbiota in Children on Leyte Island. *Front Microbiol* 8, (197).
- Olveda, D.U., Inobaya, M., Olveda, R.M., Vinluan, M.L., Ng, S.-K., Weerakoon, K., McManus, D.P., Ramm, G.A., Harn, D.A., Li, Y., Lam, A.K., Guevarra, J.R., Ross, A.G., 2017. Diagnosing schistosomiasis-induced liver morbidity: implications for global control. *Int J Infect Dis* 54, 138-144.
- Parija, S.C., Chidambaram, M., Mandal, J., 2017. Epidemiology and clinical features of soil-transmitted helminths. *Trop Parasitol* 7, (2) 81-85.
- Reynolds, L.A., Finlay, B.B., Maizels, R.M., 2015. Cohabitation in the Intestine: Interactions among Helminth Parasites, Bacterial Microbiota, and Host Immunity. *J Immunol* 195, (9) 4059-4066.
- Riviere, A., Selak, M., Lantin, D., Leroy, F., De Vuyst, L., 2016. Bifidobacteria and Butyrate-Producing Colon Bacteria: Importance and Strategies for Their Stimulation in the Human Gut. *Front Microbiol* 7.
- Schneeberger, P.H.H., Coulibaly, J.T., Panic, G., Daubenberger, C., Gueuning, M., Frey, J.E., Keiser, J., 2018. Investigations on the interplays between *Schistosoma mansoni*, praziquantel and the gut microbiome. *Parasit Vectors* 11, (1) 168.
- Shin, N.R., Whon, T.W., Bae, J.W., 2015. Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol* 33, (9) 496-503.
- Tyakht, A.V., Alexeev, D.G., Popenko, A.S., Kostryukova, E.S., Govorun, V.M., 2014. Rural and urban microbiota: To be or not to be? *Gut Microbes* 5, (3) 351-356.
- Weerakoon, K.G., Gordon, C.A., Williams, G.M., Cai, P., Gobert, G.N., Olveda, R.M., Ross, A.G., Olveda, D.U., McManus, D.P., 2017. Droplet digital PCR diagnosis of human schistosomiasis: parasite cell-free DNA detection in diverse clinical samples. *J Infect Dis* 216, 1611-1622.
- Wexler, H.M., 2007. Bacteroides: the Good, the Bad, and the Nitty-Gritty. *Clin Microbiol Rev* 20, (4) 593-621.
- Yatsunencko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R.N., Anokhin, A.P., Heath, A.C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J.G., Lozupone, C.A., Lauber, C., Clemente, J.C., Knights, D., Knight, R., Gordon, J.I., 2012. Human gut microbiome viewed across age and geography. *Nature* 486, (7402) 222-227.
- Zaiss, M.M., Harris, N.L., 2016. Interactions between the intestinal microbiome and helminth parasites. *Parasite Immunol* 38, (1) 5-11.
- Zakrzewski, M., Proietti, C., Ellis, J.J., Hasan, S., Brion, M.J., Berger, B., Krause, L., 2017. Calypso: a user-friendly web-server for mining and visualizing microbiome-environment interactions. *Bioinformatics* 33, (5) 782-783.
- Zhang, J., Kobert, K., Flouri, T., Stamatakis, A., 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* 30, (5) 614-620.

497

498 **Fig. 1.** Parasite lifecycles in the human host. (A) Lifecycle of *Trichuris trichiura* (blue; 1,2,3 on left)

499 and *Taenia* spp. (red; 1,2,3 on right). In both species eggs are ingested (1) and eggs hatch in the gut.

500 The adults of *Taenia* spp. live in the small intestine, particularly the duodenum (2) while *T. trichiura*

501 adults reside in the large intestine, primarily the descending colon (2). Adults of both species produce

502 eggs which are excreted in the faeces (3). (B) Lifecycle of *Schistosoma japonicum* (red; 1,2,3,5 on

503 left, 4 on right) and Hookworm (blue; 1,2,4 on right, 3 on left). Larval forms of both species penetrate

504 the skin directly (1) and migrate to the lungs. Hookworm larvae penetrate into the alveolar space and

505 undergoing tracheal migration whereby the larvae are coughed up and swallowed (2). The hookworm

506 larvae mature into adults in the gut, attaching to the lumen of the gut and feeding on host blood (3),

507 and producing eggs which are excreted via the faeces (4). Schistosome larvae do not leave the blood

508 vessels surrounding the lungs and are swept to the vessels around the liver where they mature and

509 pair up (3), producing eggs which traverse the gut wall (4) to be excreted in the faeces (5). (C)

510 Lifecycle of *Ascaris lumbricoides* (blue). Mature eggs are ingested (1) and hatch in the gut. The larvae

511 penetrate the gut wall (2) and migrate to the lungs where they penetrate into the alveolar spaces and

512 undergo tracheal migration whereby they are coughed up and swallowed (3). Now mature, the adult

513 worms reside in the small intestine (4) and produce eggs which are excreted in faeces (5).

514

515

516

517 **Fig. 2.** Flow diagram showing initial cohort sample collection and real-time PCR (qPCR) analysis
518 through to the 16S RNA sequencing (Gordon et al., 2012; Gordon et al., 2015c; Weerakoon et al.,
519 2017). STH, soil transmitted helminth.

520

521

522

523

524

525 **Fig. 3.** Redundancy Analysis (RDA) of helminth-infected individuals in green and (remaining
526 individuals including negatives) in black. RDA was run on relative operational taxonomic unit (OTU)
527 abundance of infected cohort subjects with specific helminth species irrespective of infection with
528 other helminth species.

529

Fig. 4. Association between helminth infection status and microbial richness. (A) Comparison of helminth-positive individuals and helminth-negative controls (uninfected individuals) considering bacterial richness and diversity via the Shannon Index. (B) Comparison of helminth negative individuals (negative controls) ($n=38$) and individuals with intestinal helminths ($n=162$), and those infected with *Schistosoma japonicum* only ($n=14$) considering bacterial richness and bacterial diversity via the Shannon Index. (C) Comparison of bacterial richness with increasing number of infections ranging from helminth-negative individuals ($n=38$) to subjects with up to five co-infections. OUT, operational taxonomic unit

541

542

543 **Fig. 5.** Bacterial richness (total) and abundance of *Lactobacillus* when considering: (A) *Trichuris*
544 *trichiura* infection ($n=103$), regardless of other infections, (blue, right) compared with *T. trichiura*
545 negatives ($n=116$) (red, left) and (B) *T. trichiura*-only infections ($n=5$) (blue, right) compared with
546 helminth negatives (controls) ($n=38$) (red, left). (C) The abundance of Lactobacillaceae in all
547 parasitised individuals (cases) ($n=181$) versus controls ($n=38$). * $P\leq0.05$; ** $P\leq0.01$; *** $P\leq0.001$.

548

549

Fig. 6.

550
 551 Comparison of *Faecalibacterium* abundance in control versus parasitised individuals. (A)
 552 Comparison of abundance of *Faecalibacterium* with increasing numbers of infecting helminth
 553 species ranging from no infections up to five co-infections ($P=0.00044$, Spearman correlation). (B).
 554 Relative abundance of *Faecalibacterium* in helminth-negative controls ($n=38$) versus infected
 555 individuals (cases; $n=181$) ($P=0.038$; FDR, false discovery rate =0.093). $*P \leq 0.05$.

557

558

559 **Supplementary Fig. S1.** Venn diagram showing polyparasitism interactions for infected cohort
560 individuals.

561

562

563

564

565

566

567

Supplementary Fig. S2. Abundance plots showing bacterial classifications of family, genus, and species. The top line of each graph shows cases (red) and negative controls (blue). Figure legends show colour coding for various bacterial families, genera, and species.

568

569

570

571

572

573

Supplementary Fig. S3. Supervised Redundancy Analysis (RDA) of: (A) helminth-positive cases (cream) versus negative controls (blue) ($P=0.001$); and (B) intestinal helminths (dark blue) versus *Schistosoma japonicum*-only (light blue) infections. RDA was run on relative OTU, operational taxonomic unit abundances.

574

575 **Supplementary Fig. S4.** Abundance of significantly associated bacteria in helminth infection cases

576 by: (A) family; (B) genus; (C) species. Legend shows colour coding of cases (cream) versus negative

577 controls (blue) (Supplementary Table S2). * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

578

579

Supplementary Fig. S5. Abundance of significantly associated bacteria in helminth infection cases: (A) family; (B) genus; (C) species. Legend shows colour coding of *Schistosoma japonicum* only infections (grey), intestinal helminths irrespective of *S. japonicum* infection (white), and negative controls (green) (Supplementary Tables S2-S6). * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

587

588

589 **Supplementary Fig. S6.** Significant associations of bacteria with increased complexity of helminth
590 species infection, showing phyla (A), family (B), genus (C), and species (D).

591

592 **Supplementary Fig. S7.** Exploring possible batch effect between the two cohorts. Cohort-1 faecal
593 samples were collected in 2011, and cohort-2 in 2015 from the same area in the municipality of
594 Palapag, Northern Samar Province, the Philippines. (A) Principal coordinate analysis (PCoA) of
595 unweighted UniFrac distances comparing parasite-positive individuals in the cohort-1 collection

596 (Batch 1 case, red) and in the cohort-2 collection (Batch 2 case, grey), compared with uninfected
 597 (parasite-negative) individuals (negative control, blue). (B) Richness and Shannon index comparing
 598 parasite-positive individuals in the cohort-1 collection (Batch 1, pink) and in the cohort-2 collection
 599 (Batch 2, blue), compared with uninfected (parasite-negative) individuals (Batch 2, green). (C)
 600 Overview of bacterial genus composition from the Municipality of Palapag, Northern Samar
 601 Province, the Philippines study area. The bottom column shows collection times as per the key with
 602 the cohort-1 collection (Batch 1, blue) and the cohort-2 collection (Batch 2, red), compared with
 603 uninfected (parasite-negative) individuals (Batch 2, yellow). OTU, operational taxonomic unit
 604
 605
 606

Table 1. Infection prevalence of helminth parasites in the two cohorts

Species	No. Positive	Prevalence (%)	95% CI
<i>Schistosoma japonicum</i>	157	71.69	65.68-77.70
<i>Trichuris trichiura</i>	104	47.49	40.82-54.15
<i>Ascaris lumbricoides</i>	107	48.86	42.19-55.53
Hookworm	72	32.88	26.61-39.15
<i>Taenia</i> spp.	74	33.79	27.48-40.10
Negative	38	17.35	12.30-22.41

607 95% CI, 95% confidence interval.
 608