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Helminths, polyparasitism, and the gut microbiome in the Philippines

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ABSTRACT

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

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

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 world-wide 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

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

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

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

2.3. DNA sequencing-based profiling of the gut microbiome

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

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

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

2.4. Statistics

SAS software (SAS Institute) was used for descriptive statistics and calculation of 95% confidence intervals (95% CIs) of the parasitology data, while Calypso (version 8.54) (Zakrzewski et al., 2017) was used for mining the *rrs* microbiome dataset and for data visualisation. Rarefied and filtered OTU tables were uploaded to Calypso and square root transformed. To examine differences in the gut microbiota between infected and uninfected cohort participants, data were grouped as 'case' – parasite infected, and 'control' – negative for parasites. Data were further stratified in some instances to determine the effect of multiple parasite species (1-5) on bacterial diversity, and grouped as gut helminths (*T. trichiura,* hookworm, *A. lumbricoides, Taenia* spp.), *S. japonicum* only infections, and controls (negatives). Principal coordinate analysis (PCoA using weighted UniFrac 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 a 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 Medeley 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≤0.01, FDR=0.016) as were Firmicutes (P≤0.01, FDR=0.016) and Actinobacteria (P≤0.001, FDR=4.3E-08), while Bacteroidetes were significantly more abundant in helminth-negative individuals (controls, n=38, P≤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=6.96e-06) in individuals harbouring helminth parasites compared with those who did not (Fig. 4A), and significantly higher (pP=3.9e-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=8.83e-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).

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

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

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

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

Bacterial richness was increased (*P*=0.00366) in individuals infected with *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).

3.6. Increased abundance of Faecalibacterium associated with any helminth infection

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

3.7. No significant differences in microbiota observed between cohorts

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

4. Discussion

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

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

A low number of Proteobacteria is a marker of a healthy gut, and imbalances in the gut microbiota can be due to their increased abundance (Shin et al., 2015). In the current study we found no difference in the abundance of Proteobacteria in helminth-positive individuals compared with uninfected individuals. There was a slight decrease in Proteobacteria with increasing polyparasitism, although this was not significant (Supplementary Fig. S6). The most abundant phyla present were the Firmicutes, followed by the Bacteroidetes, and then the Actinobacteria. There was a significant increase in the Firmicutes and Actinobacteria, but a significant decrease in Bacteroidetes in parasitised subjects (Supplementary Table S2). Individual members of all these phyla can provide beneficial or elicit deleterious impacts on gut health; for instance a decrease in some Bacteroidetes and Firmicutes and an increase in Proteobacteria is commonly reported in Irritable Bowel Disease patients (Wexler, 2007). Members of the Firmicutes and Bacteroidetes are involved in a number of important metabolic processes within the intestine, specific examples being polysaccharide (fibre) degradation and carbohydrate fermentation (Wexler, 2007) and they can influence the host immune system, benefiting themselves over other species and pathogens (Wexler, 2007). A reduction in Bacteroidetes abundance associated with parasitic infection has previously been documented (Andersen et al., 2016), suggesting that regulation of these bacteria by parasites occurs. It is noteworthy that the shifts in microbiota profiles likely reflect alterations in gut function and microbial fermentation. We found an increase in the relative abundance of Firmicutes and Archaea with polyparasitism, most likely reflective of food digestion and anaerobic fermentation favouring the initial production of acetate, CO_2 and hydrogen; and leading to methane and butyrate production. This contrasts with Bacteroidetes which produce relatively higher amounts of succinate and propionate. It has been hypothesised that parasites cause alterations within the gut microbiome to their own advantage; consequently, the higher levels of butyrate-producing Firmicutes might be more advantageous to gut parasites because butyrate has been shown to inhibit potentially deleterious inflammatory responses within the intestine (Lepage et al., 2011; Riviere et al., 2016).

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

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

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

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

parasites may in fact be beneficial commensal organisms.

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

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Figure legends

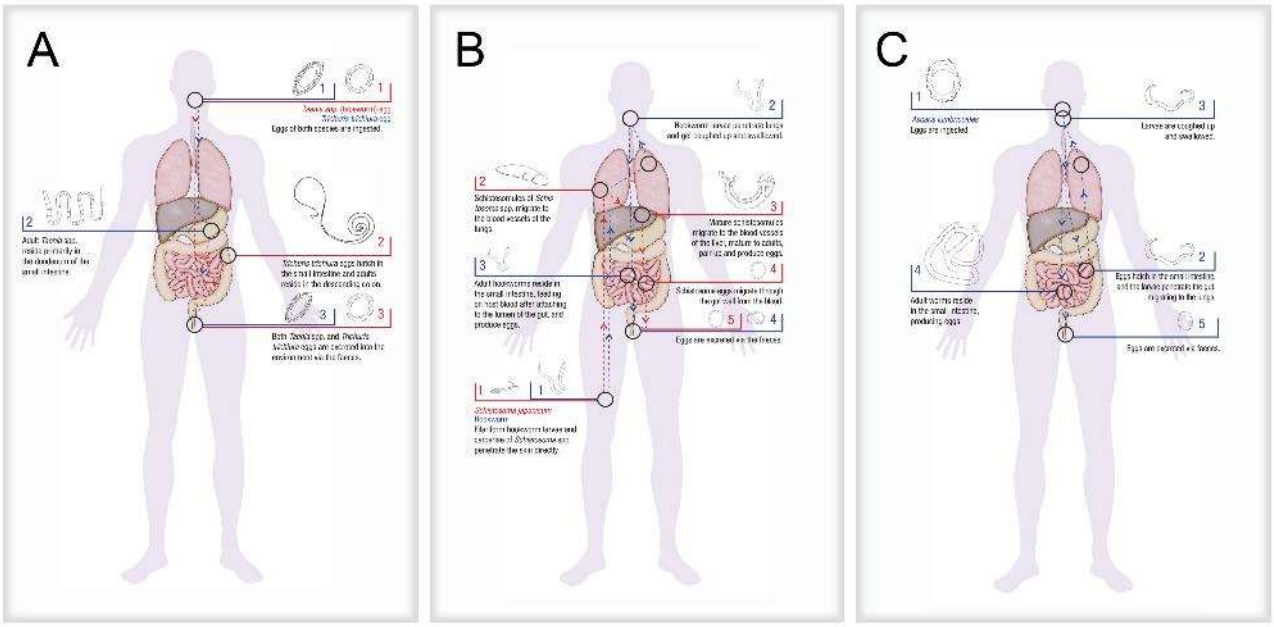


Fig. 1. Parasite lifecycles in the human host. (A) Lifecycle of *Trichuris trichiura* (blue; 1,2,3 on left) and *Taenia* spp. (red; 1,2,3 on right). In both species eggs are ingested (1) and eggs hatch in the gut. The adults of *Taenia* spp. live in the small intestine, particularly the duodenum (2) while *T. trichiura* adults reside in the large intestine, primarily the descending colon (2). Adults of both species produce eggs which are excreted in the faeces (3). (B) Lifecycle of *Schistosoma japonicum* (red; 1,2,3,5 on left, 4 on right) and Hookworm (blue; 1,2,4 on right, 3 on left). Larval forms of both species penetrate the skin directly (1) and migrate to the lungs. Hookworm larvae penetrate into the alveolar space and undergoing tracheal migration whereby the larvae are coughed up and swallowed (2). The hookworm larvae mature into adults in the gut, attaching to the lumen of the gut and feeding on host blood (3), and producing eggs which are excreted via the faeces (4). Schistosome larvae do not leave the blood vessels surrounding the lungs and are swept to the vessels around the liver where they mature and pair up (3), producing eggs which traverse the gut wall (4) to be excreted in the faeces (5). (C) Lifecycle of *Ascaris lumbricoides* (blue). Mature eggs are ingested (1) and hatch in the gut. The larvae penetrate the gut wall (2) and migrate to the lungs where they penetrate into the alveolar spaces and undergo tracheal migration whereby they are coughed up and swallowed (3). Now mature, the adult worms reside in the small intestine (4) and produce eggs which are excreted in faeces (5).

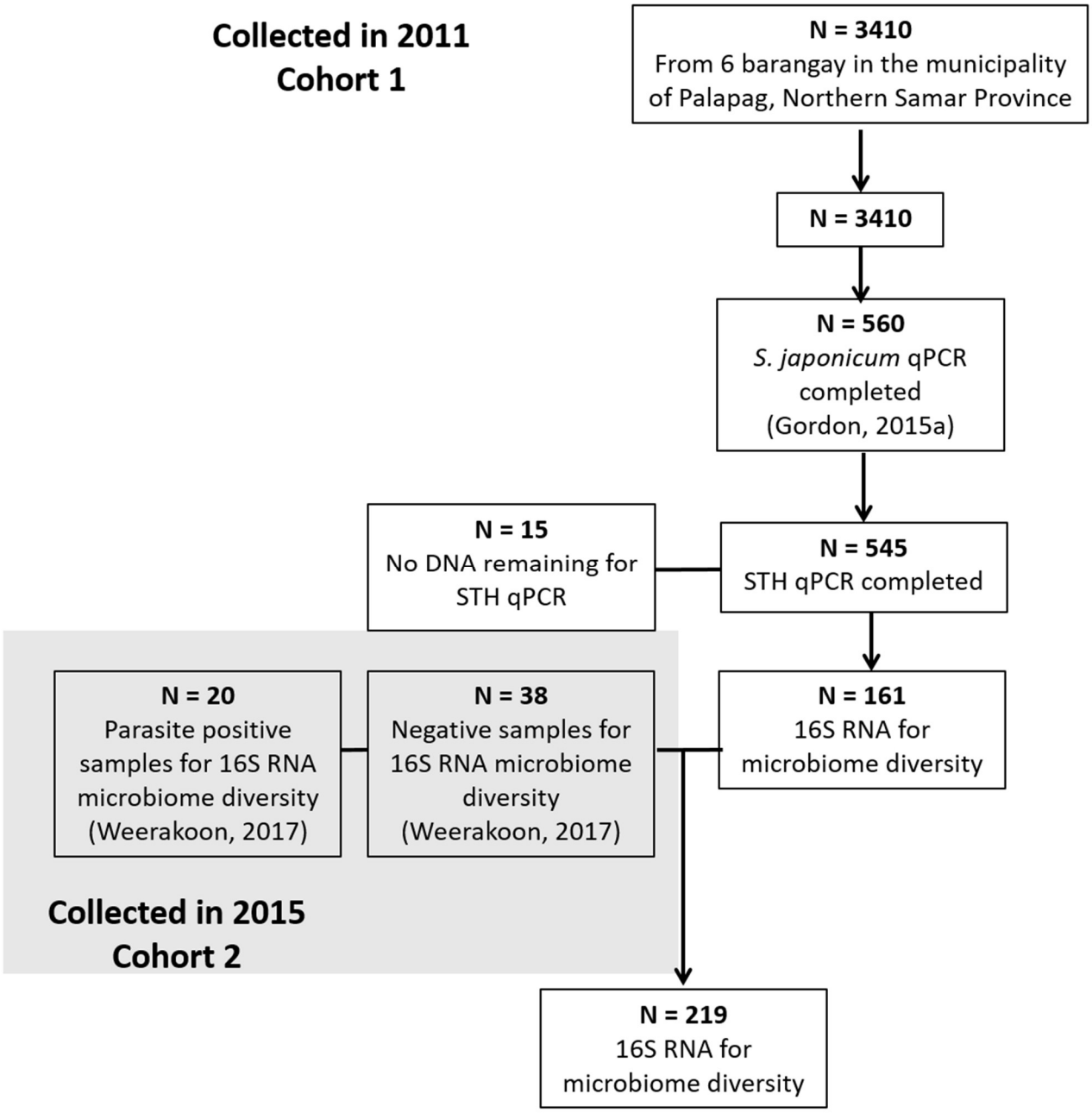


Fig. 2. Flow diagram showing initial cohort sample collection and real-time PCR (qPCR) analysis through to the 16S RNA sequencing (Gordon et al., 2012; Gordon et al., 2015c; Weerakoon et al., 2017). STH, soil transmitted helminth.

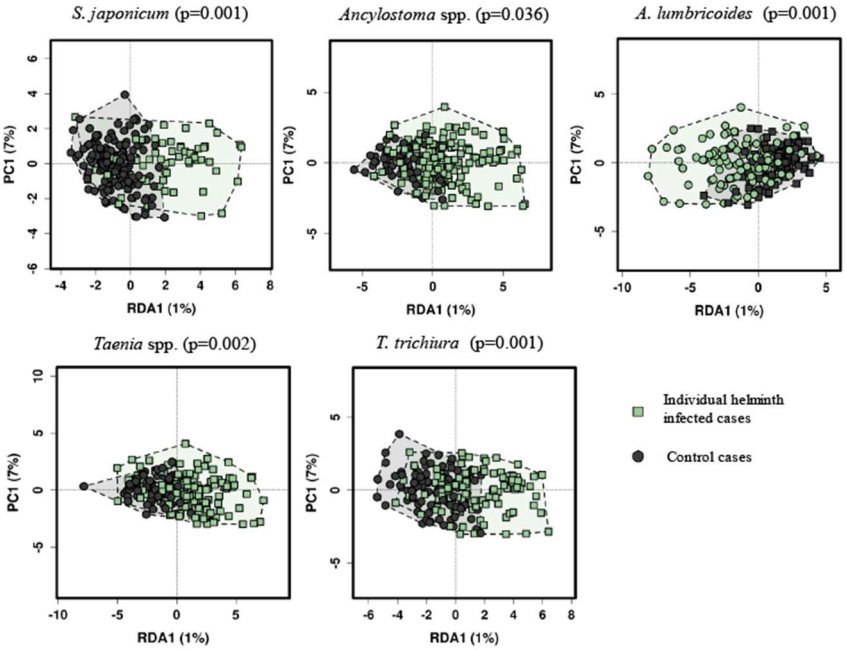


Fig. 3. Redundancy Analysis **(**RDA) of helminth-infected individuals in green and (remaining individuals including negatives) in black. RDA was run on relative operational taxonomic unit (OTU) abundance of infected cohort subjects with specific helminth species irrespective of infection with other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

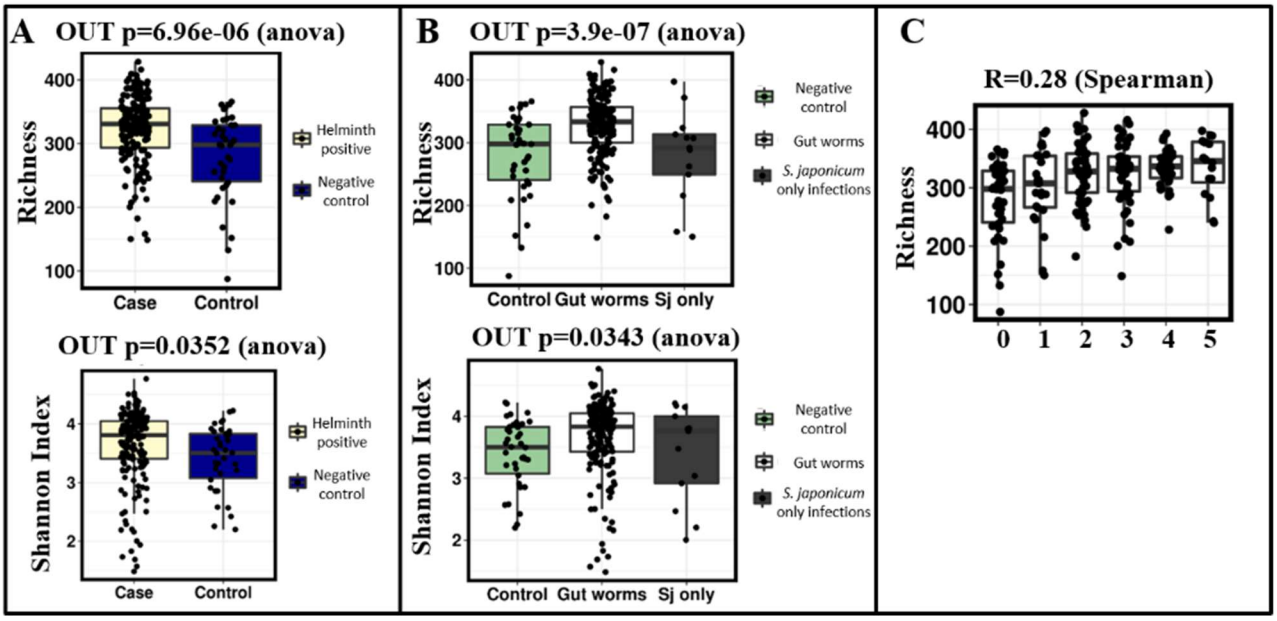


Fig. 4. Association between helminth infection status and microbial richness. **(**A) Comparison of helminth-positive individuals and helminth-negative controls (uninfected individuals) considering bacterial richness and diversity via the Shannon Index. **(**B) Comparison of helminth negative individuals (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

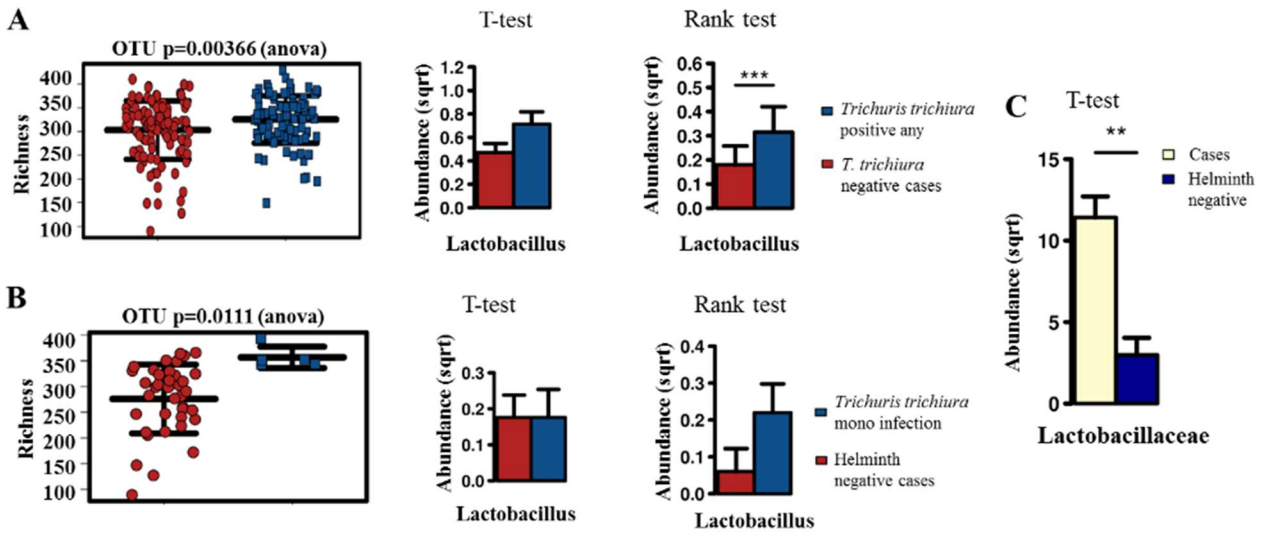


Fig. 5. Bacterial richness (total) and abundance of *Lactobacillus* when considering: (A) *Trichuris trichiura* infection (*n*=103), regardless of other infections, (blue, right) compared with *T. trichiura* negatives (*n*=116) (red, left) and (B) *T. trichiura*-only infections (*n*=5) (blue, right) compared with helminth negatives (controls) (*n*=38) (red, left). (C) The abundance of Lactobacillaceae in all parasitised individuals (cases) (*n*=181) versus controls (*n*=38). *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

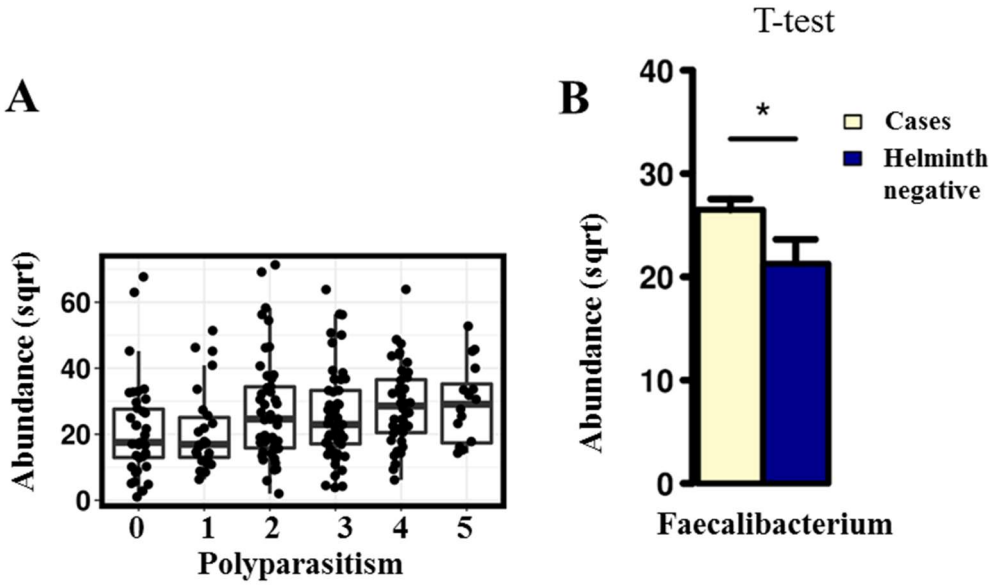
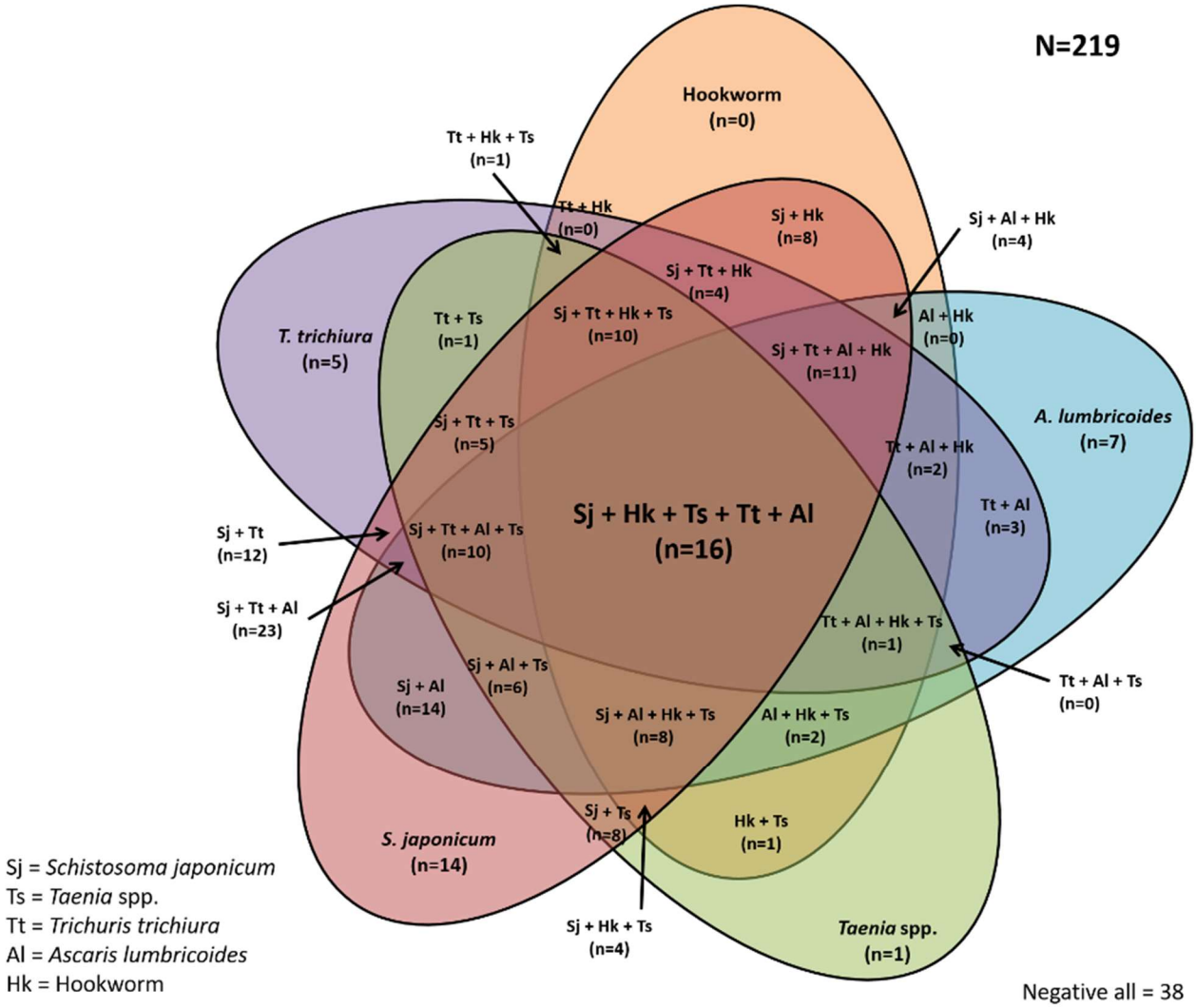
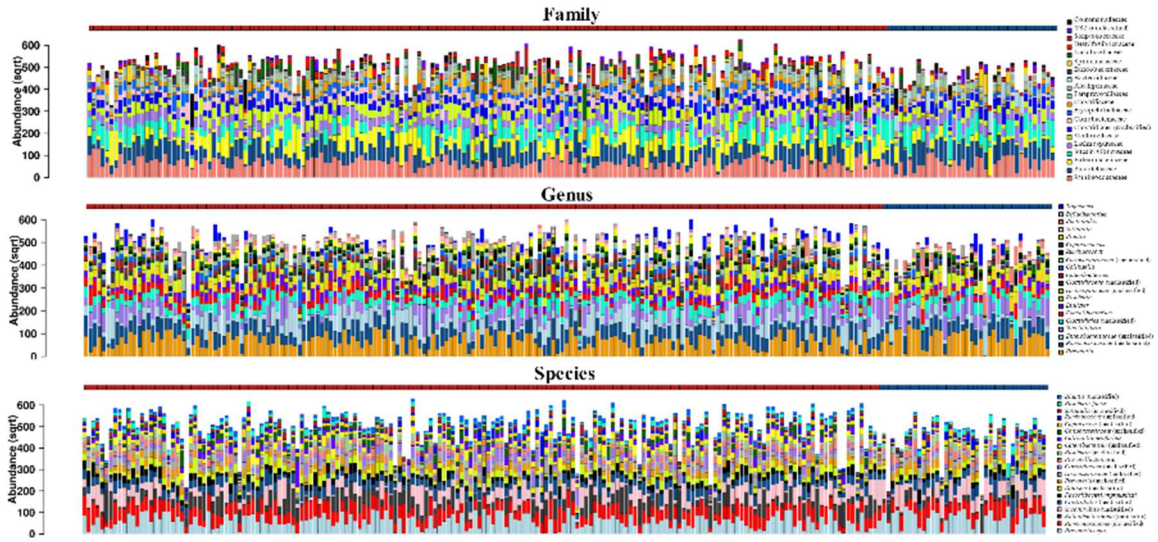


Fig. 6. Comparison of *Faecalibacterium* abundance in control versus parasitised individuals. (A) Comparison of abundance of *Faecalibacterium* with increasing numbers of infecting helminth species ranging from no infections up to five co-infections (P=0.00044, Spearman correlation). (B). Relative abundance of *Faecalibacterium* in helminth-negative controls (n=38) versus infected individuals (cases; n=181) (P=0.038; FDR, false discovery rate =0.093). *$P \leq 0.05$.

Supplementary figure legends



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



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

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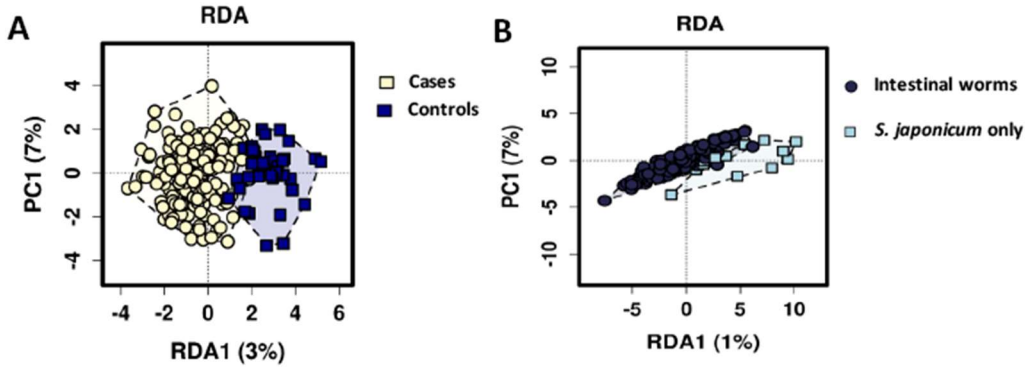

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569 **Supplementary Fig. S3.** Supervised Redundancy Analysis (RDA) of: (A) helminth-positive cases
570 (cream) versus negative controls (blue) (*P*=0.001); and (B) intestinal helminths (dark blue) versus
571 *Schistosoma japonicum*-only (light blue)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

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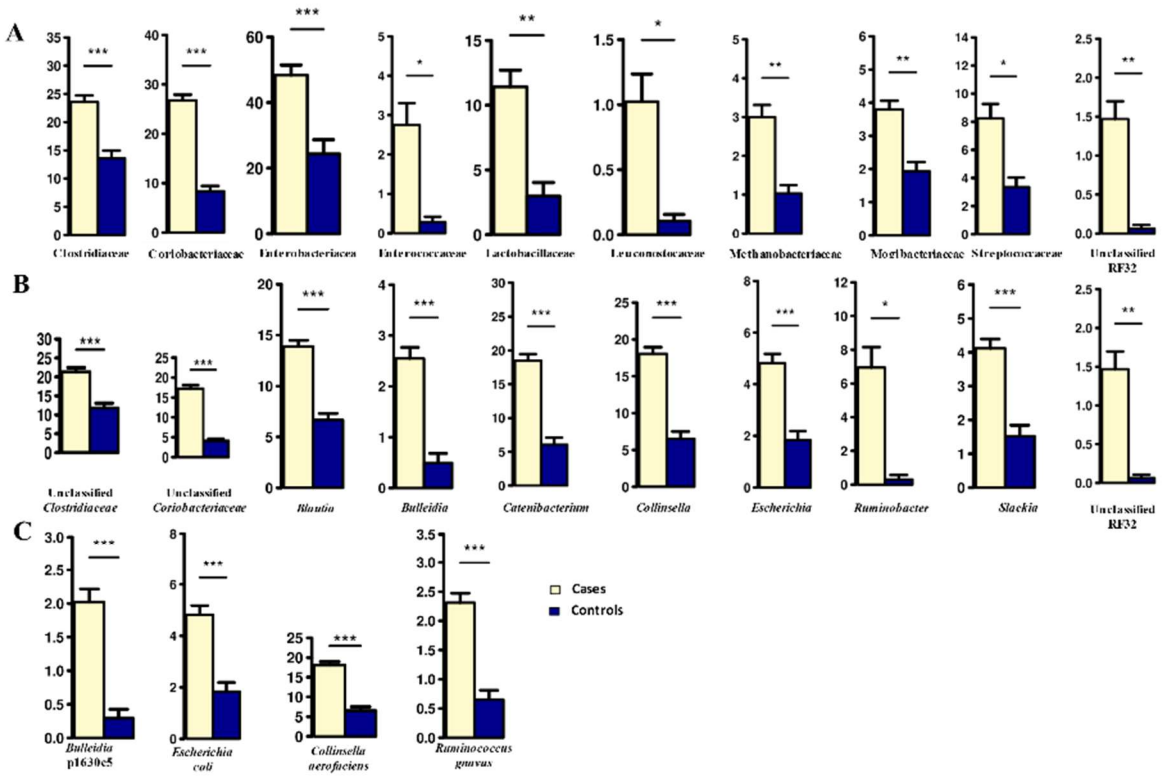

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575 **Supplementary Fig. S4.** Abundance of significantly associated bacteria in helminth infection cases

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

577 controls (blue) (Supplementary Table S2). **P*≤0.05; ***P*≤0.01; ****P*≤0.001.

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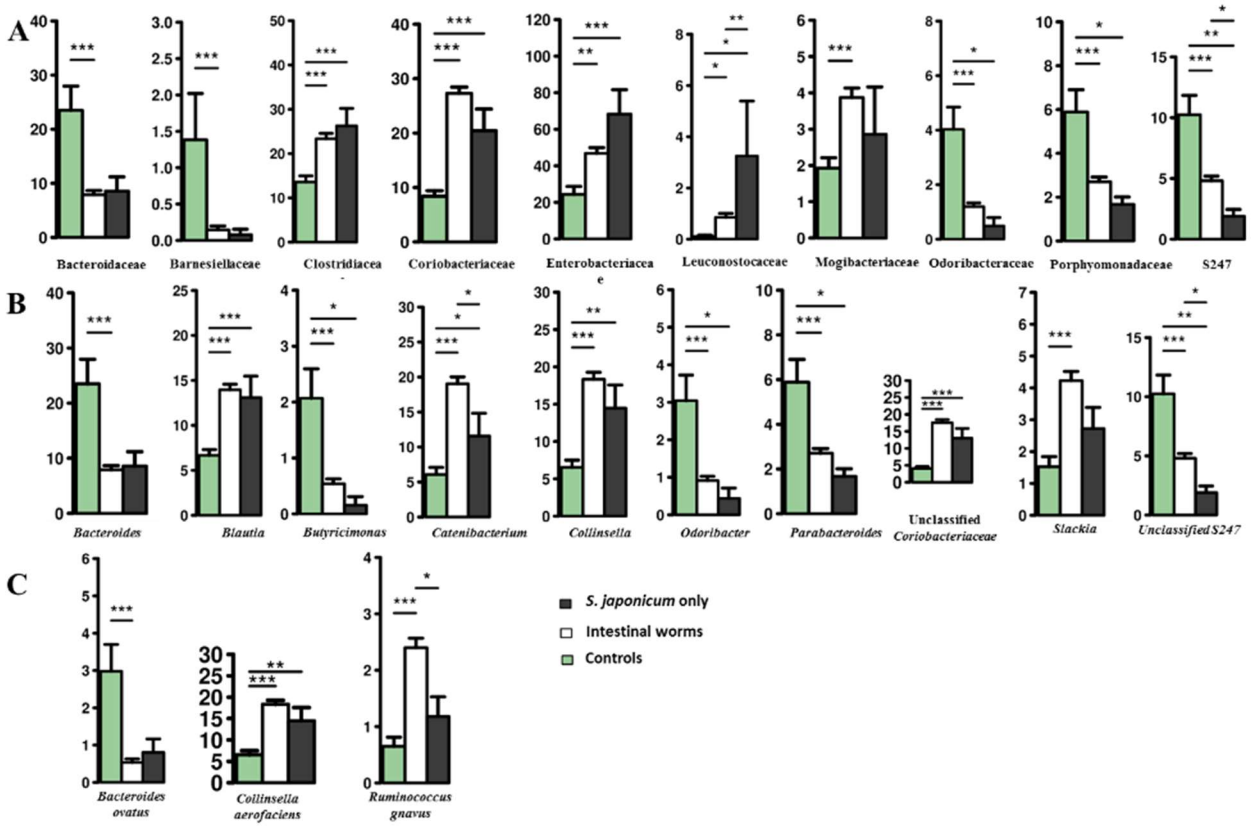

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581 **Supplementary Fig. S5.** Abundance of significantly associated bacteria in helminth infection cases:
582 (A) family; (B) **g**enus; (C) **s**pecies. Legend shows colour coding of *Schistosoma japonicum* only
583 infections (grey), intestinal helminths irrespective of *S. japonicum* infection (white), and negative
584 controls (green) (Supplementary Tables S2-S6). **P*≤0.05; ***P*≤0.01; ****P*≤0.001.

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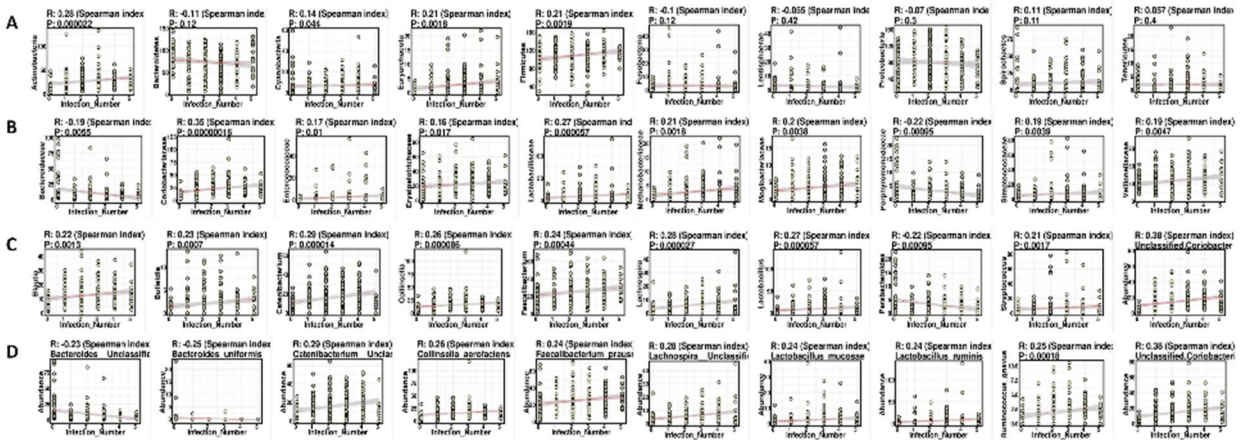
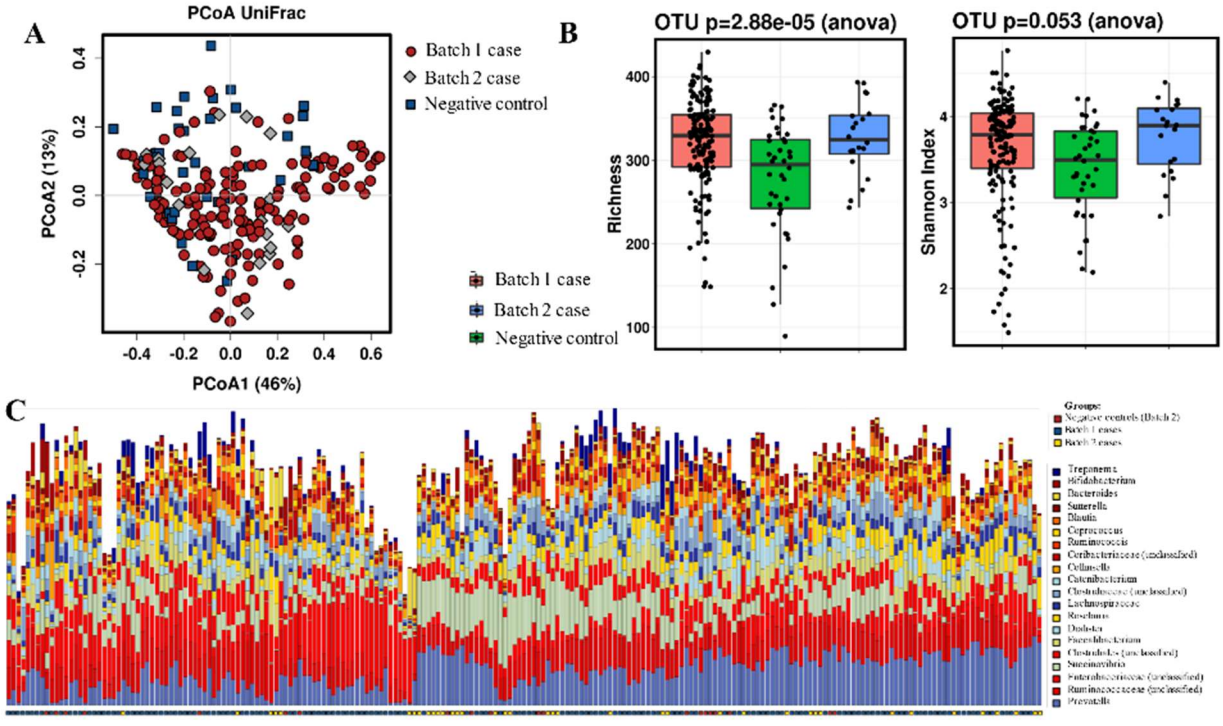


Supplementary Fig. S6. Significant associations of bacteria with increased complexity of helminth species infection, showing phyla (A), family (B), genus (C), and species (D).



Supplementary Fig. S7. Exploring possible batch effect between the two cohorts. Cohort-1 faecal samples were collected in 2011, and cohort-2 in 2015 from the same area in the municipality of Palapag, Northern Samar Province, the Philippines. (A) Principal coordinate analysis (PCoA) of 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

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

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

607 95% CI, 95% confidence interval.

608