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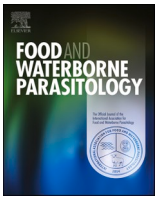

Ascaris lumbricoides harbors a distinct gut microbiota profile from its human host: Preliminary insights

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A R T I C L E I N F O

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A B S T R A C T

In indigenous populations where soil-transmitted helminths (STH) infections are endemic, STH parasites (i.e., *Ascaris lumbricoides*, *Trichuris trichiura*, hookworms) often co-exist and co-evolve with the gut microbiota of their human hosts. The association between STH infections and the gut microbiota of the colonized human hosts has been established, but few studies explored the gut microbiota of the parasites. This preliminary study aimed to characterize the microbiota of the STH parasite for further understanding the STH parasite-host relationship. The gut microbial genomic DNA from four adult *A. lumbricoides* worms recovered from a six-year-old indigenous Negrito boy living in an STH-endemic village in Perak, Peninsular Malaysia was extracted and sequenced for the V3-V4 region of the 16S rRNA. The microbiota profiles of these worms were characterized and compared with the gut microbiota of their human host, including the profiles from four STH-positive and three STH-negative individuals from the same tribe and village. The gut microbial structure of *A. lumbricoides* was found to be differed significantly from their human host. The worms contained lower gut bacterial abundance and diversity than human. This difference was evident in the beta diversity analysis which showed a clear separation between the two sample types. While both Firmicutes (52.3%) and Bacteroidetes (36.6%) are the predominant phyla followed by Proteobacteria (7.2%) in the human gut, the microbiota of *Ascaris* gut is highly dominated by Firmicutes, constituting 84.2% relative abundance (mainly from the genus *Clostridium*), followed by Proteobacteria (11.1%), Tenericutes (1.8%) and Bacteroidetes (1.5%). The parasites were also found to alter the microbial structure of the human gut following infection based on the relatively higher bacterial abundance in STH-positive versus STH-negative participants. Further studies with a greater number of *Ascaris* adults and human hosts are needed to confirm the gut microbiota profiles.

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1. Introduction

In indigenous populations, soil-transmitted helminths (STH) such as *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms often co-exist and co-evolve with the gut microbiota of their human host (Cho and Blaser, 2012). It has been established that STH infections were associated with increased microbial diversity and alteration in the composition of human gut microbiota (Lee et al., 2014; Toro-Londono et al., 2019). These alterations are presumed to be part of STH's establishment strategies, including egg hatching mechanism (Hayes et al., 2010) and modulation of the host's immune responses (Ramanan et al., 2016). The exact mechanism behind these strategies remains unclear, and one hypothesis is related to the roles of the parasite's microbiota.

Similar to mammalian hosts, parasitic worms also harbour their own bacterial endosymbionts or gut microbiota. This internal microbiota can potentially contribute to the fitness of parasites during changing events (such as in the organism's life cycle) and environmental variations. For example, the interaction between the blood filarial nematode with its endosymbiont bacteria, the *Wolbachia* (Taylor et al., 2005). *Wolbachia* play a critical role in supporting the fecundity and survivability of the parasite in infected human hosts by providing essential nutrients such as riboflavin, flavin adenine dinucleotide, and nucleotides. In return, the filarial nematode provides the amino acids required for the growth of *Wolbachia* (Taylor et al., 2005).

Meanwhile, microbiota study on the free-living soil nematode of *Caenorhabditis elegens* revealed a species-rich microbial community dominated by the Proteobacteria (*Enterobacteriaceae* and *Pseudomonadaceae* families), which was found to be significantly different from the nematode's natural soil environment from which they are isolated from (Dirksen et al., 2016; Zhang et al., 2017). The finding was interesting based on the preconceived notion that organisms could probably acquire their microbiota from their living environment. In terms of the role, specific unique microbiota such as the *Ochrobactrum* serves as a food source for the *C. elegens*, promoting its population growth under unfavourable conditions like high temperatures (Dirksen et al., 2016).

An experimental animal study on *Trichuris muris* (a parasitic helminth of mice) has revealed distinct intestinal microbial composition from its murine host. However, after establishing themselves within the host, the helminths altered the host's microbiota (White et al., 2018). Notably, the parasite's gut system contained different bacteria, mainly from the Proteobacteria, including families *Sphingomonadaceae, Oxalobacteraceae*, and *Enterobacteriaceae*. This composition was believed to be linked with the helminth's survivability and ability to establish long-term infections, particularly in chronically infected hosts (White et al., 2018).

Given these intriguing findings, it is of great interest to investigate the microbial communities of the human parasite, *Ascaris lumbricoides*. To date, there has been only one study (in Thailand) which has explored the gut microbiota of *A. lumbricoides* (Klomkliew et al., 2022). In addition, there was a recent study which investigated the gut microbiota of *Ascaris suum* and their pig host (Midha et al., 2022). Hence, we aimed to characterize the gut microbiota of *A. lumbricoides* in comparison to the gut microbiota of their human host in Malaysia. Considering very limited available studies, we believe that our preliminary findings will be essential in understanding the strategies and defence mechanisms employed by *Ascaris* for long-term establishment in humans (parasite-host relationship).

2. Materials and methods

2.1. Ethics approval

This study was approved by Ethics Committee of the Universiti Teknologi MARA [reference no: 600-IRMI (5/1/6)] and National Medical Research Register (NMRR), Ministry of Health, Malaysia [NMRR-17-3055-37,252 (IIR)]. Additionally, the study obtained permission from the Department of Orang Asli Development (JAKOA) under reference no: JAKOA/pp.30.052J1d9 (29). As this study involved participants under 18, written consent forms were obtained, either signed or with a thumbprint, from their parents or legal guardian.

2.2. Samples characteristics

This study was part of the STH epidemiology and anthelminthic research among the indigenous Orang Asli Negrito, as previously reported (Muslim et al., 2019; Muslim and Lim, 2022). Samples (stool and adult worm of *A. lumbricoides*) were collected from a Negrito village located at the forest fringe (5° 55′ 82" North, 101° 41′ 46″ East) area in Gerik, Perak. With a population size of 60 to 80 people (Jahai sub-tribe), this village reported a high STH prevalence rate of >80%. The villagers are relatively homogenous in terms of diet (mainly consisting of rice and vegetables particularly spinach and tapioca) and lifestyle.

In brief, stool samples were collected and examined for the presence of STH ova using direct smear, Kato-Katz, formalin-ether concentration techniques and staining (Trichrome and modified Ziehl-Neelsen) before mass-albendazole administration (single dosage, 400 mg) was conducted in the same village three weeks after. Those included had no history of antibiotic/anthelminthic consumption for the three months before sample collection, were afebrile, and asymptomatic during collection. Details of STH examination and albendazole administration were as previously described (Muslim and Lim, 2022). Four adult worms (two males, two females) of the *A. lumbricoides* were recovered from a 6-year-old boy (expelled from the anus) three days after albendazole administration. The child was initially found to be positive with a moderate infection of mix *A. lumbricoides* and *T. trichiura* but with no clinical symptoms such as diarrhoea or abdominal pain. All worms were washed and rinsed multiple times with sterile Phosphate-Buffered Saline (PBS) on site (until the solution became clear), were placed in a sterile Schott glass bottle (200 ml) and transported to the Faculty of Medicine, Universiti Teknologi MARA (Sungai Buloh Campus), in dry ice, within 6 h.

To compare with the gut microbiota profiles of adult *Ascaris*, we selected eight human stool samples that were collected at baseline: i) the host ($n = 1$), ii) STH-positive participants who had similar STH profiles to the host (moderate-severe *Ascaris* & *T. trichiura*) ($n = 4$)

and, (iii) STH-negative participants at the time of collection ($n = 3$). All *Ascaris* worms were processed immediately upon arrival at the laboratory. The worms were surface sterilized again by washing and rinsing more than ten times using sterile PBS. They were dissected longitudinally, and the gut of the *Ascaris* was extracted using a sterilized surgical set under an aseptic environment inside the laminar flow cabinet.

2.3. 16S rRNA-based metagenomic analysis

The *Ascaris* gut and human stool samples were subjected to genomic DNA isolation using the NucleoSpin® soil (Macherey-Nagel GmbH & Co. KG, Germany) kit (suitable for the stool samples as well due to the similar complexity) based on the manufacturer's protocol. PCR amplification of the V3-V4 regions of the 16S rRNA was later conducted using 16S Amplicon primers 341F (5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG) and 805R (5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG) containing Illumina overhang adapter sequences as previously described (Takahashi et al., 2014). Sample libraries were constructed and pooled in equimolar concentration, followed by paired-end sequencing (2 × 250 bp) on an Illumina Miseq platform at Majorbio Bio Tech Co. Ltd. (Shanghai, China). Poor quality bases of the paired-end sequence data (FASTQ) were trimmed using Trimmomatic v0.39 (Bolger et al., 2014) and were merged using USEARCH v11 (Edgar, 2010). The sequences were then imported into the Quantitative Insights into Microbial Ecology 2 (QIIME2) v2019.7 (Caporaso et al., 2010; Bolyen et al., 2019). Denoising and identification of Amplicon Sequence Variants (ASV) tables were carried out using DADA2 (Callahan et al., 2016). Mitochondrial and chloroplast ASVs were filtered out from the feature table and representative sequences. Taxonomic classification involved training a 16S V3–V4 Naive-Bayes classifier on V3–V4-trimmed 16S sequences from the SILVA v132 database (Quast et al., 2012) with 99% ASVs reference sequences using the q2-classifier plugin in QIIME 2 (Bokulich et al., 2018). A total of 710,442 high-quality merged sequence reads (median 56,377) were generated. The read counts were rarefied to 32,964 (minimum read count among the samples).

Alpha-diversity analysis was performed based on the observed ASV and Shannon matrices (Chao and Shen, 2003). Kruskal-Wallis's pair-wise statistic was used to compare between groups (significant value of $P < 0.05$). Part of the visualization and analysis was done using R 4.1.3 (R Core Team, 2021). The rooted tree, ASV table, metadata, as well as the taxonomy file were imported from qiime2 to R via the qiime2R package (Bisanz, 2018). Jaccard and Bray-Curtis dissimilarity matrices was calculated from the Hellinger standardized ASV data using the Vegan package (Oksanen et al., 2019). Principal coordinates analysis (PCoA) plots were generated using the ggplot2 package (Wickham et al., 2016) to visualize the differences in beta diversity between parameters. Permutational multivariate analysis of variance (PERMANOVA) of the data was calculated using the ADONIS function.

Comparative metagenomics between humans and *Ascaris*, helminth infection status, and sexes of *Ascaris* worms were further performed using METAGENassist (Arndt et al., 2012). Significant differences in the abundance of each phylum and genera between parameters were tested using univariate statistics including fold change (FC) analysis (threshold set at 2.0), independent *t*-test, and ANOVA with Post hoc Tukey test with a significant $P < 0.05$. Multivariate statistics were used to determine any discriminant pattern in the beta-diversity of gut microbiota between parameters using Partial Least Square Discriminant Analysis (PLSDA). The performance of the PLSDA model was evaluated based on R^2 values (<0.33, weak; 0.33–0.67, moderate; 0.67 and above is a substantial model) (Peng and Lai, 2012). The leave-one-out cross-validation (LOOCV) with a 1000 permutation test was used to determine the significance value of the PLSDA ($P < 0.05$). The importance of each phylum and genus was derived from loading plots of PLSDA and variable importance in projection (VIP). Key phyla and genera with a score over 1.0 were considered important contributors to the clustering of microbiota structure (Akarachantachote et al., 2014).

3. Results

A total of 12 samples (4 *Ascaris*; 8 human stools) were included in this communication. Characteristics of the samples are described

Table 1
General characteristics of the samples.

Group ($N = 12$)	Sample ID	Sex	Age	Description
Worm gut $n = 4$	Worm1	Female worm	NA	The adult of *A. lumbricoides* worms were recovered from Pe27 subject.
	Worm2	Female worm	NA	
	Worm3	Male worm	NA	
	Worm4	Male worm	NA	
Human gut $n = 8$	*Pe27	Male	6	Participants positive with moderate-severe *A. lumbricoides* & *T. trichiura* infections during fecal collection at pre-treatment (labelled as Post_feces)
	Pe04	Female	7	
	Pe20	Male	4	
	Pe100	Male	7	
	Pe21	Female	3	
	Pe12	Male	11	Participants negative for parasites during stool collection at pre-treatment (labelled as Neg_feces)
	Pe18	Female	5	
	Pe48	Female	9	

NA = Not applicable; *host.

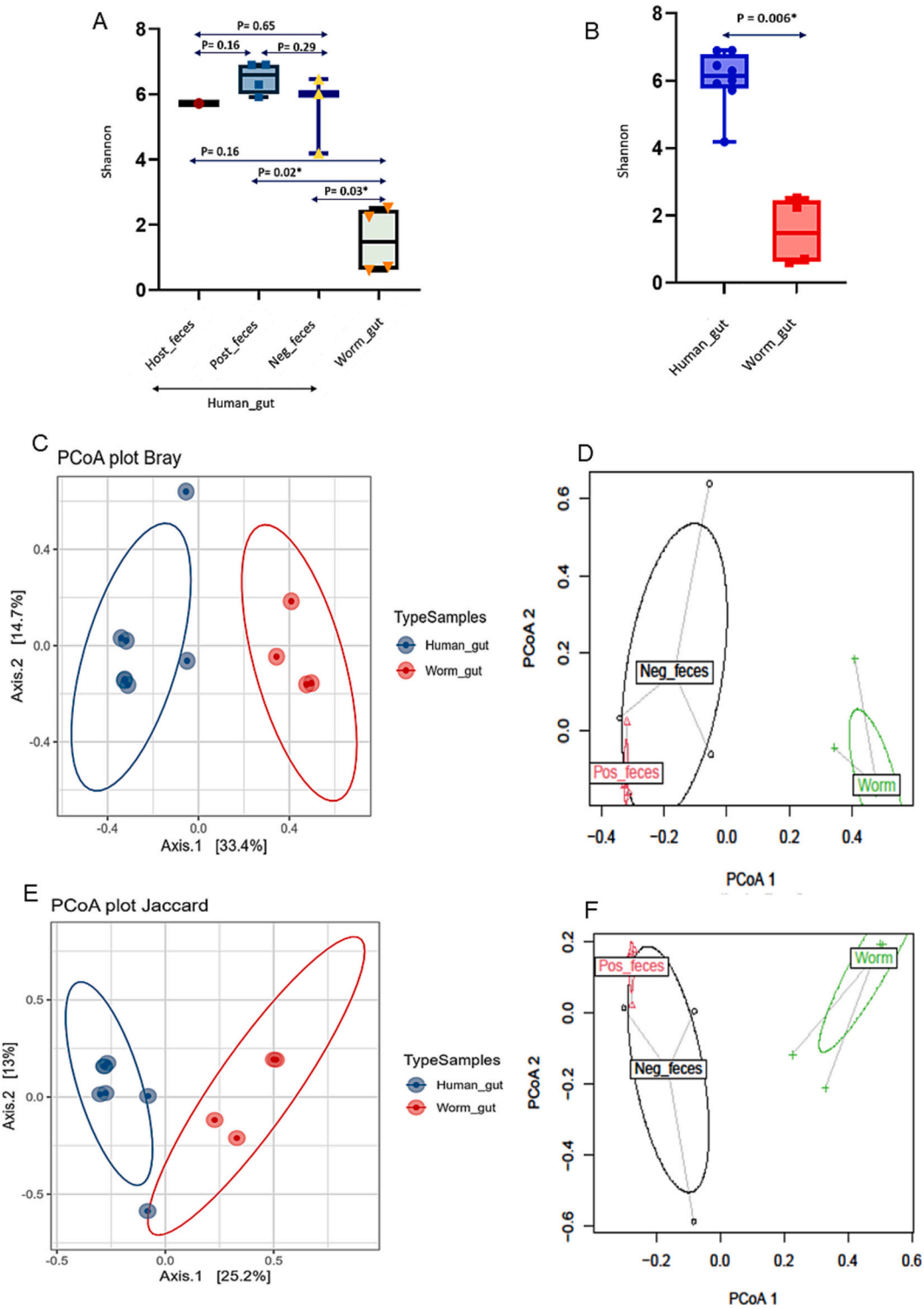
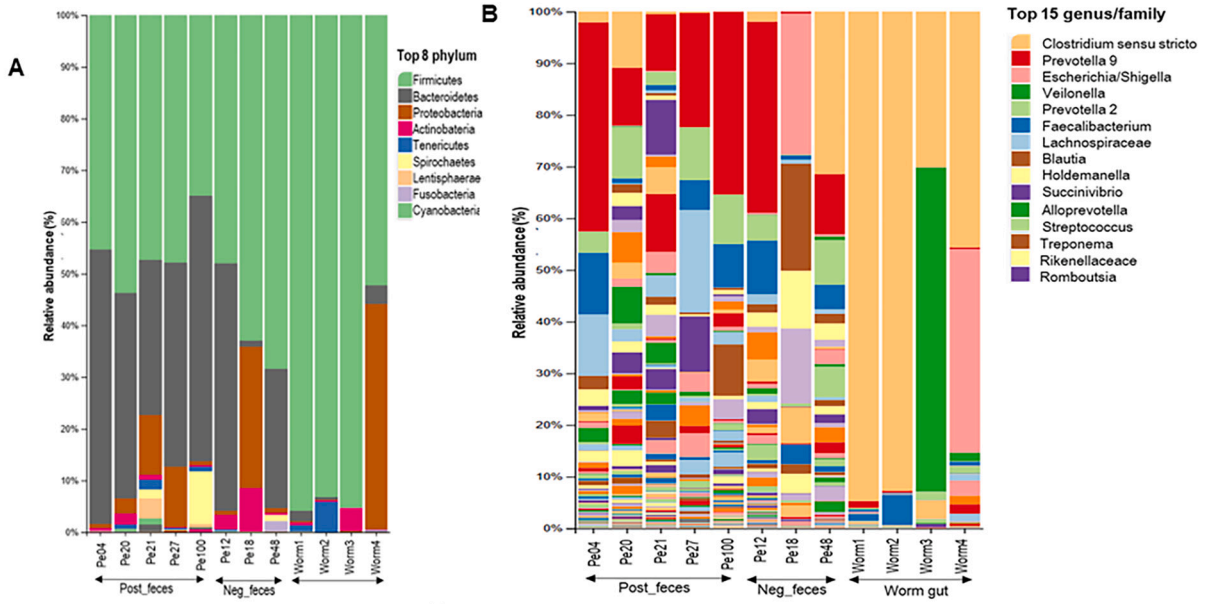


Fig. 1. Alpha and beta diversity analyses between gut microbiota of *Ascaris* and gut microbiota of human hosts. Overall microbial diversity between Host, STH-positive participants, STH-negative participants and *Ascaris* worms. **(A)** and between *Ascaris* and human samples **(B)** (Kruskal-Wallis). Bray Curtis beta diversity (ADONIS: R2 = 0.310, $P = 0.003$; ANOSIM, permutations 999: $R = 0.854$; $P = 0.002$) between sample type **(C)** and Beta dispersion across all groups (Bray Curtis) **(D).** Jaccard beta diversity (ADONIS: R2 = 0.235, $P = 0.003$; ANOSIM, permutations 999; R = 0.854, $P = 0.004$) **(E)** and Beta dispersion across all groups (Jaccard) **(F).** Footnote: Host_feces = *Ascaris*'s human host; Pos_feces = STH-positive participants; Neg_feces = STH-negative participants.



C

Phyla	Human	Worm
Firmicutes	↓51.6	↑84.2
Bacteroidetes	↑36.6	↓1.5
Proteobacteria	↓7.2	↑11.1
Actinobacteria	↑1.9	↓1.3
Spirochaetes	↑1.7	↓0.0
Tenericutes	↓0.6	↑1.8
Cyanobacteria	↑0.3	↓0.0
Elusimicrobia	↑0.1	↓0.0
Total	100.0	100.0

D

Genus	Human	Worm
Faecalibacterium	↑20.5	↓0.4
Clostridium	↓15	↑76.2
Veillonella	↓0.1	↑16.5
Succinivibrio	↑12.2	↓0.08
Blautia	↑9.6	↓0.03
Catenibacterium	↑6.7	↓0.04
Subdoligranulum	↑3.6	↓0.03
Roseburia	↑3.4	↓0.1
Treponema	↑3.4	↓0.1
Streptococcus	↑2.6	↓1
Bifidobacterium	↑2.4	↓0.2
Phascolarctobacterium	↑2.4	↓0.5
Prevotella	↑18	↓1.2
Others	0.1	3.62
Total	100	100

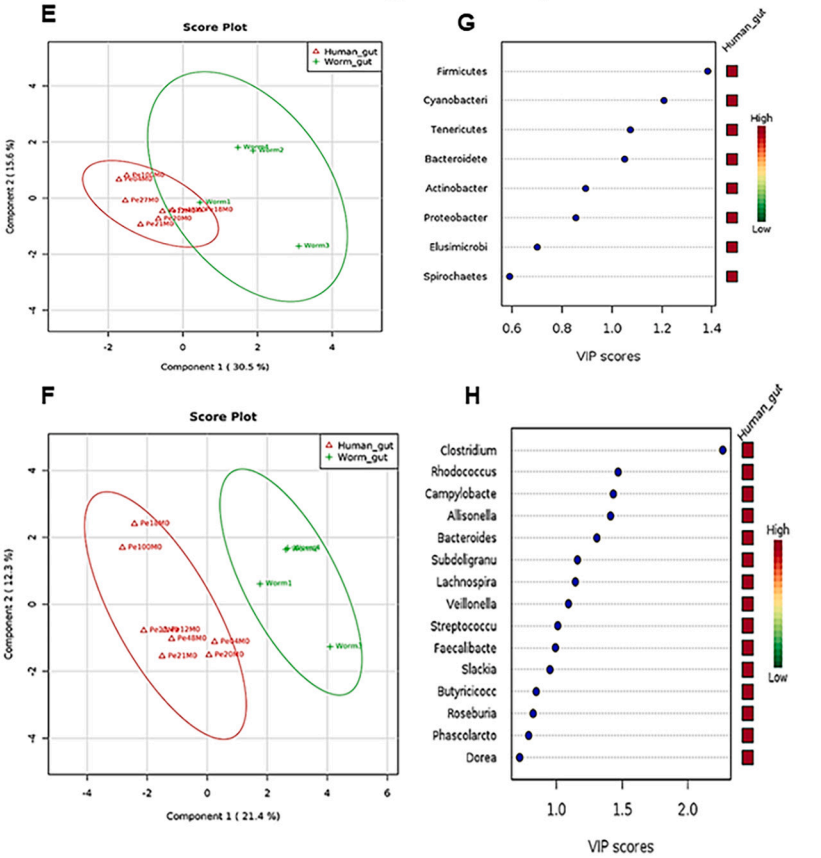


Fig. 2. Taxonomic differences in gut microbiota structure between humans and *Ascaris* worms. Overall relative abundance (%) of bacteria phyla (**A**) and genus/family (**B**) for each human and *Ascaris* samples ($N = 12$). Average relative abundance of bacteria phyla (**C**) and genus (**D**) levels between human and *Ascaris* worms.; only >0.01% of dataare shown. Partial Least Squares-Discriminant Analysis (PLSDA) score plot shows a discriminant community structure at the phylum (**E**) and genus level (**F**) between humans (red colour) and worms (green colour). The line ellipses on the PLSDA score plots indicate the 95% confidence interval. ϵ Four key discriminant phyla between humans and worms with VIP scores of >1.0 were identified (**G**). Eleven key genera with the highest VIP scores were identified (**H**). The colour key indicates the relative abundance of the identified phyla/genus in relation to the VIP score value, red: highest, green: lowest. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in Table 1. The age range for human participants was from 3 to 11 years old (four males and four females).

In total, 2337 ASVs were generated, averaging 228 ASVs per human sample and 81 ASVs per worm gut sample. Pre-analysis based on the Shannon index showed no significant difference in gut microbial diversity based on gender ($P = 0.423$) and age ($P = 0.164$) of the human subjects. We then compared the overall gut bacterial diversity and richness between *Ascaris* and human samples. We found that gut microbiota of *Ascaris* showed significantly lower bacterial diversity compared to the human host's gut microbiota ($P = 0.006$) (Fig. 1A and Fig. 1B). Similarly, a lower abundance of bacteria richness was discovered in gut *Ascaris* (median (IQR): 81.0 (55.5, 107.0) versus human samples (median (IQR): 258.5 (205.0–307.0) based on the number of ASV features ($P = 0.017$). Beta diversity analysis indicated a clear separation of clustering profiles based on the Bray Curtis ($P = 0.002$) and Jaccard ($P = 0.004$) indices, indicating a distinct microbial composition between gut microbiota of *Ascaris* and their human host's gut microbiota (Fig. 1C - Fig. 1F).

A total of 17 phylum and 223 genera were detected in all samples. The main phyla observed in both *Ascaris* gut and human gut were Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes, and Tenericutes (Fig. 2A). While both Firmicutes (52.3%) and Bacteroidetes (36.6%) are the predominant phyla followed by Proteobacteria (7.2%) in the human gut, the microbiota of *Ascaris* gut is highly dominated by Firmicutes, constituting 84.2% relative abundance (mainly from the genus *Clostridium* sensu stricto *1*), followed by Proteobacteria (11.1%), Tenericutes (1.8%) and small number of Bacteroidetes (1.5%) (Fig. 2A-D). The absence of Cyanobacteria and Elusimicrobia in the Ascaris gut was also noted as compared to the human gut (0.3%, 0.1%, respectively) Fold change analysis revealed that the *Ascaris* had higher abundances of Tenericutes (3.8 fold), Firmicutes (3.2 fold), and Proteobacteria (3.1 fold) compared to humans. There was no significant difference in the overall phylum composition except for Firmicutes, which were significantly higher in *Ascaris* compared to humans ($P = 0.03$). In contrast, human gut microbiota had higher abundances of Cyanobacteria (4.3 fold), Spirochaetes (4.2 fold), Elusimicrobia (3.4 fold), and Bacteroidetes (2.7 fold) compared to worms.

At the genus level, the gut microbiota of *Ascaris* was dominated by *Clostridium* sensu stricto (>70%) and *Veillonella* (>13%) (Fig. 2B and D). Besides, fold change analysis indicated higher abundances of *Veillonella* (10.7 fold), *Clostridium* (6.0 fold), *Campylobacter* (4.6 fold), *Enterococcus* (2.9 fold), *Streptococcus* (2.4 fold) and *Rhodococcus* (2.3 fold) in *Ascaris* compared to humans. However, the only genus with statistically significant abundance differences between worms and humans was *Clostridium* ($P = 0.001$). In contrast, human gut microbiota had a higher abundance of *Faecalibacterium* (20.5%), Prevotella (18%), *Succinivibrio* (12.2%), and *Blautia* (9.6%). From the fold change analysis, 13 genera have an abundance two folds higher in humans, such as *Blautia* (6.5 fold), *Catenibacterium* (5.7 fold), *Anaerostipes* (4.9 fold), *Dorea* (4.7 fold), *Roseburia* (5.7 fold), *Bacteroides* (3.9 fold) and *Bifidobacterium* (2.3 fold). The PLSDA analysis reported a substantial model for discriminant patterns between gut microbiota at the phyla level ($R^2 = 0.7$) (Fig. 2E) and genus level ($R^2 = 0.8$) (Fig. 2F) between humans and worms, respectively. A further inspection of the models using VIP analysis revealed that four phylum and 11 genera had VIP scores of more than one was responsible for the discriminant pattern of gut microbiome between humans and worms (Fig. 2G and Fig. 2H).

Although not significant, the overall diversity and abundance of gut microbiota in STH-positive were relatively higher than in STH-negative individuals (Fig. 1A). STH-positive group had a higher relative abundance of Bacteroidetes (42.9%) and Spirochaetes (2.4%) (Fig. **S1: A-B**). In contrast, STH-negative group had a higher relative abundance of Firmicutes (59.7%), Proteobacteria (9.7%), and Actinobacteria (3.8%). Three phyla were elevated at least two folds in STH-positive participants; Cyanobacteria (2.6 fold), Tenericutes (2.5 fold), and Spirochaetes (2.5 fold). In STH-negative participants, Fusobacteria and Actinobacteria were elevated 5.5 fold and 2.2 fold, respectively. However, none of these were statistically significant. At the genus level, *Faecalibacterium* (22.2%) dominated the gut microbiota of STH-positive participants, while *Clostridium* was observed predominantly in STH-negative (21.9%) participants (10.9%). Three genera, *Succinivibrio*, *Treponema,* and *Phascolarctobacterium* were found mainly in STH-positive participants. In contrast, *Bifidobacterium* was detected mainly in the gut microbiota of STH-negative participants**.** A total of six genera were elevated more than two folds in STH-positive participants; *Succinivibrio* (8.6 fold), *Phascolarctobacterium* (4.9 fold), *Anaerostipes* (4.6 fold), *Parabacteroides* (3.9 fold), *Campylobacter* (2.7 fold) and *Enterorhabdus* (2.1 fold). Whereas four genera were found elevated more than two folds in STH-negative participants; B*ifidobacterium* (8.2 fold), *Cetobacterium* (5.2 fold), *Rhodococcus* (4.1 fold), and *Enterococcus* (3.1 fold). However, the differences have not been supported with statistical significance.

4. Discussion

Despite numerous studies exploring the interaction between STH parasites and the microbiota of colonized individuals, little attention has been given to investigating the gut microbiota of the parasites themselves (Dheilly et al., 2019), resulting in poor understanding of their roles. This preliminary study aimed to characterize for the first time in Malaysia, the gut microbiota of *A. lumbricoides*, a parasite that commonly affects the Orang Asli indigenous population in Malaysia. Our findings revealed that *Ascaris* harbors its own distinct set of gut microbiota, which differs from its human hosts, regardless of whether they are infected or non-infected participants in the STH-endemic community. Moreover, the gut microbiota of *Ascaris* was found to be less diverse and have lower bacterial abundance compared to the human hosts.

These findings were aligned with previous research on *Ascaris* gut microbiota in Thailand (Klomkliew et al., 2022), the gut microbiota of *Ascaris suum* in pigs in Germany (Midha et al., 2022), as well as an experimental study of *T. muris* gut microbiota in mice (White et al., 2018). In line with the latter experimental study, it has been hypothesized that the specific gut microbiota composition is essential for maintaining the parasite's fitness within its colonized hosts (White et al., 2018). They also suggested that once established in the host, the parasite may manipulate the host's gut microbiota, which is essential to prevent further infections and control the number of worms, as a low worm count could lead them to progress to the chronic stage of infection (White et al., 2018). This theory may explain the differences in microbiota profiles seen between STH-positive and STH-negative participants in this study. Similar observations have been reported in various field studies exploring the relationship between STH infections and human microbiota (Lee

et al., 2014; Huwe et al., 2019).

In this study, the *Ascaris* was obtained 3-days post- albendazole treatment. There is concern that albendazole might impact the microbiota of the *Ascaris*, potentially leading to misleading findings. Prior studies on albendazole's impact on human gut microbiota has shown inconsistencies. For example, Martin et al. (2018) reported no significant effect on microbiome composition, while Easton et al. (2019) observed a significant increase in *Clostridiales* and a reduction of *Enterobacteriales* in human gut microbiota following treatment. However, it is worth noting that the analyses in previous studies were conducted mainly after 14 days, 21 days or even 21 months post- treatment. Thus, the effect of albendazole administration on *Ascaris* gut microbiota within 3 days of treatment is unclear. Such investigation may not be feasible in field studies. However, we speculate that although some initial shifts might occur within three days, a complete alteration in gut microbial composition might require more time due to various factors perhaps severity or individual's variation. Important to note as well, the complete expulsion of *Ascaris* usually take up to 10 days post-treatment.

In this study, the *Ascaris* gut microbiota is primarily dominated by members from the phyla Firmicutes followed by Proteobacteria (11.1%), Tenericutes (1.8%) and Bacteroidetes (1.5%). The finding is almost comparable to a gut microbiota study of *Ascaris* in Thailand (Klomkliew et al., 2022). However, we observed a different genus profile, mainly comprising *Clostridium* and *Veilonella*, as opposed to *Lactococcus* and *Streptococcus* found previously in the latter. We hypothesize that the differences could be related to various factors including variations in host characteristics such as geographical locations, lifestyles, dietary habits, environmental factors and STH infection status (in our study the STH-positive participants were infected with a mix of *T. trichiura* and *A. lumbricoides* infections). Nevertheless, a study on the gut microbiota of *Ascaris suum* also demonstrated the presence of *Clostridium* in their gut profile (Midha et al., 2022). This spore-forming bacteria species live in anerobic environment but can withstand aerobic environment by forming spores. It is commonly found in the gut of humans and other vertebrates, animal, insects, soil and even the intestinal tract of earth worms (Koubová et al., 2015). Indeed, further studies with larger sample sizes and a subsequent systematic review are imperative to validate the bacterial signatures in the parasite's gut.

Despite the findings, some limitations in this study need consideration. The limited number of adult *Ascaris lumbricoides* and their hosts restricted the scope of our study, allowing only preliminary insights and descriptive results to be drawn. Further comparisons are challenging due to the scarcity of existing studies on this subject, emphasizing the need for more comprehensive research to advance our knowledge on the parasite microbiome. To address this, we are now advancing our investigation, replicating the same objectives but with more samples of *Ascaris* adults (over 30 worms are required for gut bacteriome analysis to achieve 80% statistical power) and human hosts. One question we are also exploring is the origin of the gut bacteria in *Ascaris* or where they are derived from. While some suggest that the microbiota may originate from the site of infection or egg hatching, particularly the jejunum (Midha et al., 2022), we also speculate that it could be related to the soil microbiome in which the eggs become embryonated and infectious before being transmitted to humans, an interesting aspect we are currently investigating. Another limitation pertains to our reliance solely on microscopic examinations during the baseline STH investigation. Although a variety of microscopy detection methods were employed and each were conducted independently by three laboratory staff members, we did not incorporate molecular detection methods for determining the parasitic infection status.

In conclusion, our preliminary analysis sheds light on the composition and potential functions of the gut microbiota in *Ascaris lumbricoides*. However, more extensive research with larger sample sizes and refined approaches are needed to validate and expand upon these findings, ultimately advancing our knowledge of the parasite's microbiome and its significance in host-parasite interactions.

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Declaration of competing interest

The authors declare that they have no competing interests.

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