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Full Paper

Investigation of gut microbiota and short-chain fatty acids in *Strongyloides stercoralis*-infected patients in a rural community

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Intestinal parasitic infections can change gut microbiota and short-chain fatty acids (SCFAs). We aimed to study the interaction among *Strongyloides stercoralis*, human gut microbiota, and serum SCFAs in a community. Fifty-two subjects in Donchang sub-district, Khon Kaen Province, northeastern Thailand, were included based on specific inclusion and exclusion criteria. Characteristics of the participants were matched between those positive for *S. stercoralis* infection alone (no other intestinal parasites; Ss+, n=26) and uninfected controls (infection status confirmed by polymerase chain reaction (PCR); Ss−, n=26). Serum short-chain fatty acids were evaluated by gas chromatography-mass spectrometry. DNA was extracted from individual faecal samples and then pooled into two groups (Ss+ and Ss−) for amplification and sequencing of the V3–V4 region of the 16S gene with next-generation technology. We explored the impact of infection with *S. stercoralis* on the faecal microbiota: individuals infected with this parasite exhibited increased alpha diversity of bacteria. At the genus level, gut microbiota in Ss+ patients showed high abundances of *Escherichia-Shigella* and *Bacteroides* but low abundances of the genera *Bifidobacterium, Lactobacillus,* and *Blautia.* PCR of individual samples to identify certain species of interest gave results consistent with those from next-generation sequencing of pooled samples and showed that significantly more Ss+ samples contained *Bacteroides fragilis*. Intriguingly, a major SCFA, acetic acid, was significantly decreased in *S. stercoralis* infection. In conclusion, *S. stercoralis* infection caused an imbalance of gut microbiota and decreased acetic acid in serum. This information adds to the knowledge concerning the effect of intestinal nematode-related chronic diseases.

Key words: *Strongyloides stercoralis,* gut microbiota, bacteria, next-generation technology, short-chain fatty acids

INTRODUCTION

The parasitic adult females of *Strongyloides* spp. inhabit the gastrointestinal tracts of humans and other vertebrates [1], where they interact with the host gut microbiota, which can have a substantial impact on gut homeostasis [2]. *Strongyloides stercoralis*, the causative agent of strongyloidiasis, is a soil-transmitted helminth. Recently, it was estimated that 8.1% of the global population is infected with *S. stercoralis*, corresponding to 613.9 million people [3]. In Thailand, the prevalence of *S. stercoralis* ranges from 0.05% to 57.0% based on the diagnostic method used [4, 5], with the highest prevalence in northeastern,

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northern, and southern parts of the country. Infection with *S. stercoralis* in otherwise healthy people might not induce severe gastrointestinal symptoms. However, in immunocompromised hosts, such as HIV (human immunodeficiency virus) patients, the cycle of autoinfection can dramatically increase, resulting in potentially life-threatening conditions, including hyperinfection and disseminated strongyloidiasis [6]. In such cases, hyperinfection can induce severe gastrointestinal symptoms [7] and other complex diseases, such as kidney disease [8].

The gut microbiota is the sum of microbial communities (including bacteria, viruses, archaea, fungi, and other unicellular and multicellular microorganisms) that inhabit the gastrointestinal tract [9]. They have beneficial and harmful effects on the human host. On the one hand, most resident bacteria exert a range of useful functions, such as protection against infections, development of immunity, synthesis of vitamins, and absorption of nutrients [10]. On the other hand, an imbalance in the microbial communities results in gut dysbiosis [11], contributing to the progression and severity of many diseases, such as chronic autoimmune and allergic disorders, obesity, diabetes, and kidney injury [12].

Several factors can alter the gut microbiota [13], including the presence of intestinal parasites that can extract nutrients for their own use, damage host tissues, and release toxic waste products [14]. Changes in the gut microbiota leads to the alteration of gut-derived metabolites and short-chain fatty acids (SCFAs) [15]. SCFAs are key metabolites produced in the large intestine by bacterial fermentation of dietary fibre and resistant starch. Acetic acid, propionic acid, and butyric acid are the principal SCFAs involved, with a molar ratio of 60:20:20 [16], respectively, and this ratio changes to approximately 91:5:4 in systemic circulation [17]. Higher levels of SCFAs increase the capacity for energy harvest, anti-inflammatory status, and satiety and lead to better overall health status. Reduction of SCFAs and dysbiosis of the gut microbiota contribute to various diseases, such as inflammatory bowel disease and rheumatoid arthritis [18].

A recent study investigated how parasitic nematode infections affect the composition of the gut microbiota and production of SCFAs [2]. SCFA levels may vary according to the species of nematode involved. For instance, increased levels in the gut have been reported in chronic infections with the murine helminth *Heligmosomoides polygyrus bakeri* [19], and reduced levels were found in *Ascaris suum* infection in the pig [20]. SCFA levels were also decreased in *S. stercoralis*-infected subjects from a non-endemic area [15]; however, the effect of *S. stercoralis* infection on SCFA levels in endemic communities is unclear. In addition, ethnicity and geographical location are factors influencing the composition and diversity of the gut microbiota [21]. The present study aimed to explore the interaction of gut microbiota and host co-metabolites in *S. stercoralis* infection in an endemic area—Khon Kaen Province, Thailand.

MATERIALS AND METHODS

Study design and ethics statement

This study was conducted using samples collected for a previously reported survey [22] from January 2017 to May 2018 in Donchang sub-district, Muang District, Khon Kaen Province, northeastern Thailand, under the Chronic Kidney Disease Northeastern Thailand (CKDNET) program. The study design is shown in Fig. 1. The participants were recruited in accordance with the criteria used by a previous study [22]. Intestinal parasitic infections were diagnosed from stool samples using the modified agar plate culture (mAPC) method and the formalin-ether concentration technique (FECT) as previously reported [22].

In the present study, the inclusion criteria were infection with *S. stercoralis* alone (no other intestinal parasites) and no current use of antibiotics. Samples from individuals with chronic diseases, such as kidney disease and autoimmune disease, were excluded. Samples from uninfected individuals were used as controls. The uninfected individuals were matched with the infected individuals

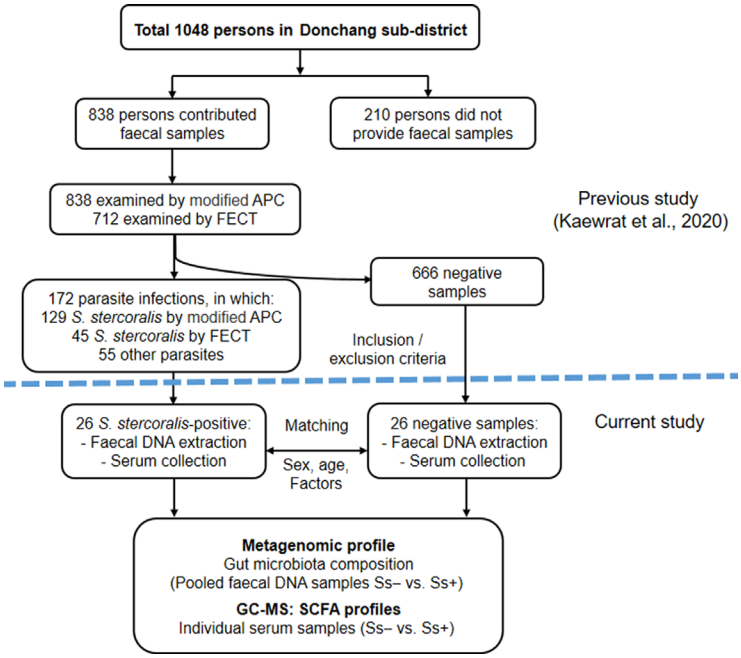

Fig. 1. Flow diagram for selection of subjects and samples analysed.

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in terms of characteristics, including gender, age, and biochemical factors. The datasets were obtained from the medical records of CKDNET and from a recent study [22]. Samples from 52 participants, who met the above criteria, were separated into two groups: uninfected with *S. stercoralis* (Ss−, n=26) and infected with *S. stercoralis* (Ss+, n=26). Serum and faecal samples of these 52 participants were made available to us by the CKDNET biobank, where they had been kept at −20°C. Twenty-six faecal DNA samples from the control group (Ss−) were confirmed as free of *S. stercoralis* infection by using conventional polymerase chain reaction (PCR) tests (Supplementary Fig. 1). To identify and compare the gut microbiota between the two groups, DNA extracted from stool samples was pooled by group, and a portion of the bacterial 16S rRNA gene was sequenced using next-generation sequencing (NGS). To determine the association between gut microbiota and metabolites, the serum SCFAs were analysed using gas chromatography-mass spectrometry (GC-MS). The Human Ethical Review Committee of Khon Kaen University approved the protocol of this study (HE631200).

Faecal DNA extraction and 16S rRNA gene sequencing

DNA was extracted from 52 stool samples using a commercial kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Each extraction yielded about 50–100 ng/mL of DNA, which was stored at −20°C until required. The concentration of the extracted DNA was measured using a Nanodrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). DNA quality was checked by electrophoresis through a 1.5% agarose gel. In addition, DNA from each sample was individually subjected to PCR amplification of the V3–V4 region of the 16S bacterial rRNA gene [23] before pooling the samples. The PCR product was electrophoresed in a 1.5% agarose gel to confirm its size, which was expected to be about 450–500 bp. An equal amount of DNA from each faecal sample was contributed to pooled samples (Ss+ and Ss−) for next-generation sequencing.

Sequencing libraries were generated using a NEBNext® Ultra DNA Library Prep Kit for Illumina (Illumina, San Diego, CA, USA), following the manufacturer's recommendations, and index codes were added. The library quality was assessed using a Qubit® 2.0 Fluorometer (Thermo Scientific) and Agilent 2100 Bioanalyzer system. Libraries were sequenced on an Illumina platform, and 250 bp paired-end reads were generated.

Sequencing results were assembled and quality checked. Reads were sorted into different groups (Ss+ and Ss−) based on their unique barcodes. They were truncated by cutting off the barcode and primer sequences. The reads were then merged using FLASH software to generate raw tags. Quality filtering on the raw tags was performed using the QIIME software. Tags were then compared with the reference database using the UCHIME software, and chimeric sequences were removed. Operational taxonomic units (OTUs) were clustered based on ≥97% similarity of sequences using the Uparse software, and taxa were annotated using the GreenGene Database. Information on the abundance of each OTU was normalised relative to the sample with the fewest sequences. Subsequent analyses of alpha diversity were all performed based on this normalised data. Alpha diversity refers to the species diversity for a sample as determined with six different indices (observed species, Chao1, Shannon, Simpson, ACE, Good's coverage). All these indices were calculated with QIIME and MOTHUR. The abundance distribution of the 35 most abundant genera in samples were displayed in the species abundance heatmap, which showed the similarities and differences of samples. The heatmap was created based on relative abundance profiles using the R-heatmap library. Raw data are available from Mendeley Data (DOI: 10.17632/fbr8k496vw.1).

PCR

To confirm the absence of *S. stercoralis* infection in the control group (n=26), conventional PCR was performed on DNA extracted from stool samples, as previously described [24] with a slightly modified annealing temperature (60°C).

To confirm the presence or absence of certain bacteria (*Ligilactobacillus agilis*, *Limosilactobacillus fermentum*, *Bacteroides*, and *Escherichia-Shigella*) in individual samples, PCR was performed, and PCR products were sequenced using BTSeq™ Barcode-Tagged Sequencing and sent to BLAST for identification. Primers (Table 1) were designed using Primer 3 version 4.1.0 based on 16S rRNA gene sequences from the EzBiocloud Database (https://www.ezbiocloud.net). Each primer pair was tested in BLAST to determine whether false priming sites might be present in the relevant genomes (Supplementary Table 1).

PCR amplification was performed in a final volume of 20.1 µL containing a 5.1 µL volume of master mix (2 µL of 10× buffer

Table 1. Primer pairs used to amplify portions of the 16S rRNA gene of *Bacteroides fragilis*, *Escheric*hia coli, *Ligilactobacillus agilis,* and *Limosilactobacillus fermentum*

Type species	Primer sequence (5′-3′)	Tm	PCR product length (bp)	Reference number	Percentage of identification* (%)
Bacteroides fragilis	F: TTGCTTTCTTTGCTGGCGAC	58.4	591	NCTC 9343	100
	R: TTCCGCCCACCTCTACTGTA	60.5			
Escherichia coli	F: CTTGCTGCTTTGCTGACGAG	60.5	465	ATCC 11775	100
	R: TTGCACCCTCCGTATTACCG	60.5			
Ligilactobacillus agilis	F: GGAGGCAGCAGTAGGGAATC	62.5	426	DSM 20509	100
	R: ACACTTTCGAACCTCAGCGT	58.4			
Limosilactobacillus fermentum	F: AACTCCATGTGTAGCGGTGG	60.5	529	DSM 20438	98.96
	R: GATGATCTGACGTCGTCCCC	62.5			

PCR: polymerase chain reaction; BLAST: The Basic Local Alignment Search Tool.
*Percentage of identification of PCR products to corresponding species as determined by BTSeq™ Barcode-Tagged Sequencing and BLAST alignment.

$MgCl_2$, 0.4 µL of 50 mM $MgCl_2$, 0.6 µL of 10 mM dNTPs, 1 µL of each primer, and 0.1 µL *Taq* DNA polymerase), 1 µL of 9–155 ng/µL of DNA, and 14 µL of PCR-grade water. The DNA amplification was performed with a pre-denaturation step of 94°C for 5 min; then 25 cycles of 94°C for 30 sec, annealing for 25 sec (at the mean Tm of each primer pair in Table 1), and 72°C for 2 min; followed by a final amplification at 72°C for 10 min. The amplification products were confirmed by electrophoresis in a 1.5% agarose gel, and the product bands were visualised under a UV transilluminator (Gel Doc™ XR+ Molecular Imager, Bio-Rad).

Identification of serum short-chain fatty acids

Short-chain fatty acids were investigated following methods reported previously [25], with slight modification in that we used GC-MS. Briefly, 100 µL of serum sample was extracted with 20 mg of NaCl, 10 mg of citric acid, 20 µL of 1 M HCl, and 100 µL of butanol. The mixture was vortexed for 2 min and centrifuged at 18,000 × g for 15 min. The supernatant was transferred to microtubes, and 2 µL was injected into the gas chromatograph. The SCFAs were analysed with a GC-MS (Agilent 7890A GC-7000 Mass Triple Quad) equipped with a capillary column (DB-WAX, 60 m × 0.25 mm × 0.25 µm, J&W Scientific, Folsom, CA, USA) and a quadrupole mass detector. The volatile free fatty acid mix standard (acetic acid, butyric acid, formic acid, heptanoic acid, hexanoic acid, isobutyric acid, isovaleric acid, 4-methylvaleric acid, propionic acid, and valeric acid) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Two microliters of liquid sample was injected into the injector. Helium gas was used as the carrier gas with a constant flow rate of 1.0 mL/min. The GC oven temperature was initially 40°C for 10 min, increased to 160°C in 3°C/min increments, then increased to 240°C in 10°C/min increments (hold 10 min). The mass spectrometer was used in electron ionisation mode with the ion source temperature set at 250°C and the ionisation energy set at 70 eV. Scan mode was used in the range of 30 to 500 m/z. The Agilent MassHunter Qualitative Analysis B.04.00 software was used for data analysis. Identification of SCFAs was performed by comparing mass spectra with NIST mass spectral libraries (National Institute of Standards, 2011 version).

Statistical analysis

Demographic, socioeconomic, and biochemical characteristics from the two groups (Ss+ and Ss−) were matched. Descriptive statistics of gender are presented as frequencies, and continuous datasets (age, BMI, blood pressure, HbA1c, estimated glomerular filtration rate (eGFR), glucose, LDL, and eosinophils) are presented as means ± standard deviations (SDs). Comparisons of these parameters between the Ss+ and Ss− groups were done using χ^2 tests for the categorical variable and the independent-samples t-test and/or Mann–Whitney U test for the continuous data. The correlation of serum SCFA values between the two groups was analysed using Pearson's correlation coefficient. The level of statistical significance was considered to be $p<0.05$ with a 95% confidence interval (95% CI). All analyses were performed using IBM SPSS statistics version 20. GraphPad Prism 8 was used to draw the figures.

RESULTS

Study population characteristics and biochemical parameters

The baseline demographic characteristics and biochemical parameters are shown in Table 2. There were no differences in age, gender, or body mass index between the Ss+ and Ss− groups because the participants were chosen such that they were matched between the groups for these parameters. For the same reason, biochemical parameters, including blood pressure, eGFR, HbA1c, glucose, and LDL cholesterol levels, did not differ between the two groups.

Gut microbiota changed by S. stercoralis *infection*

Gut microbial alpha diversity

Alpha diversity indices are commonly used to describe the ecological diversity within microbial community samples. Table 3 indicates the difference in alpha diversity between the two groups. The alpha diversity indexes according to the number of reads, number of OTUs, ACE estimator, Chao1 estimator, PD whole tree, and Shannon index (but not Good's coverage) were higher in the *S. stercoralis*-infected group than in the uninfected control group.

Table 2. Characteristics of pooled samples

Category	Ss− (n=26)	Ss+ (n=26)	p value
Gender			
Male	16	16	
Female	10	10	
Age	64.5 ± 9.5	64.4 ± 9.3	0.96
BMI (kg/m^2)	24.8 ± 3.0	23.1 ± 4.0	0.08
Blood pressure systolic (mmhg)	127.4 ± 15.3	130.3 ± 14.7	0.49
Blood pressure diastolic (mmhg)	79.9 ± 9.5	79.9 ± 8.9	0.98
Eosinophils (%)	5.8 ± 5.1	12.7 ± 6.9	0.0001
Hemoglobin A1c (%)	6.2 ± 1.8	6.5 ± 2.3	0.52
eGFR (mL/min/1.73 m^2)	84.8 ± 14.6	86.8 ± 13.0	0.59
LDL cholesterol (mg/dL)	121.8 ± 31.6	128.0 ± 38.1	0.64
Glucose (mg/dL)	101.8 ± 36.9	106.9 ± 46.7	0.95

BMI: body mass index; eGFR: estimated glomerular filtration rate; LDL cholesterol: low-density lipoprotein cholesterol; Ss: *S. stercoralis* infection groups (Ss+ and Ss−).

Operational taxonomic unit analysis

The bacterial 16S rRNA gene sequences from the 52 faecal samples were assigned to 536 OTUs overall, encompassing 13 bacterial phyla, 26 classes, 36 orders, 57 families, and 160 genera. To compare the relative abundances of bacterial taxa between groups (Ss− and Ss+) more intuitively, stacked histograms of the ten most abundant taxa at the levels of the phylum, class, order, family, and genus are shown in Fig. 2. At the phylum level, Firmicutes dominated (Ss− 77.97% vs. Ss+ 65.42%), followed by Bacteroidetes (8.6% vs. 21.05%), Proteobacteria (5.65% vs. 10.9%), Actinobacteria (5.53% vs. 1.16%), and Fusobacteria (2.1% vs. 1.06%). Participants infected with *S. stercoralis* (Ss+) had higher numbers of sequence reads from Proteobacteria and Bacteroidetes and fewer reads from Firmicutes and Actinobacteria than the Ss− group (Fig. 2A). This was consistent across the class, order, family, and genus levels. Particularly striking differences were noted between the two groups (Ss− vs. Ss+) in the abundances of the genera *Bacteroides* (5.64% vs. 11.68%), *Escherichia-Shigella* (1.27% vs. 6.36%), *Blautia* (9.93% vs. 4.28%), *Bifidobacterium* (4.72% vs. 0.22%), and *Lactobacillus* (10.57% vs. 1.86%; Fig. 2E). Interestingly, the presence of the genus *Bacteroides* was confirmed by PCR in individual samples, and the results showed that its abundance was significantly elevated in the Ss+ group.

A heatmap of the top 35 genera overall is shown in Fig. 3. The two groups (Ss−, Ss+) differed. In the Ss− group, *Lactobacillus*, *Blautia*, *Clostridium_sensu_stricto*_1, *Bifidobacterium*, *Peptoclostridium*, *Erysipelotrichaceae*_UCG−003, *Fusobacterium*, [*Eubacterium*]_*hallii*_group, and *[Ruminococcus]_torques*_group were highly abundant, as shown by the red- to light green-coloured cells. In contrast, in the Ss+ group, *Bacteroides*, *Escherichia-Shigella*, *Faecalibacterium*, *Ruminococcaceae*_UCG-002, *Prevotella*_9, *Holdemanella*, *[Eubacterium]_coprostanoligenes*_group, *Roseburia*, and *Ruminococcus*_2 were more abundant.

Two opposing abundance trends for some bacteria in S. stercoralis *infection*

Opposing trends in the abundances of some bacteria between the two groups are shown in Fig. 4. The Ss− pool had a high abundance of reads from the genera *Bifidobacterium*, *Blautia*, and *Lactobacillus*. In contrast, higher abundances of *Escherichia-Shigella*, *Desulfovibrio*, and *Proteus* were found in the Ss+ pool. At the species level, the abundances of *Bifidobacterium*

Table 3. Alpha diversity of the gut microbiota in the Ss− and Ss+ groups, calculated according to several indices

Group	No. of reads	No. of OTUs	Good's (%)	ACE	Chao 1	PD whole tree	Shannon	Simpson
Ss−	63699	380	0.999	381.535	374.221	27.801	6.258	0.971
Ss+	80023.5	432	0.998	449.960	449.987	36.511	6.781	0.982

OTU: Operational taxonomic units; ACE: Abundance-based coverage estimator; PD: Phylogenetic diversity.

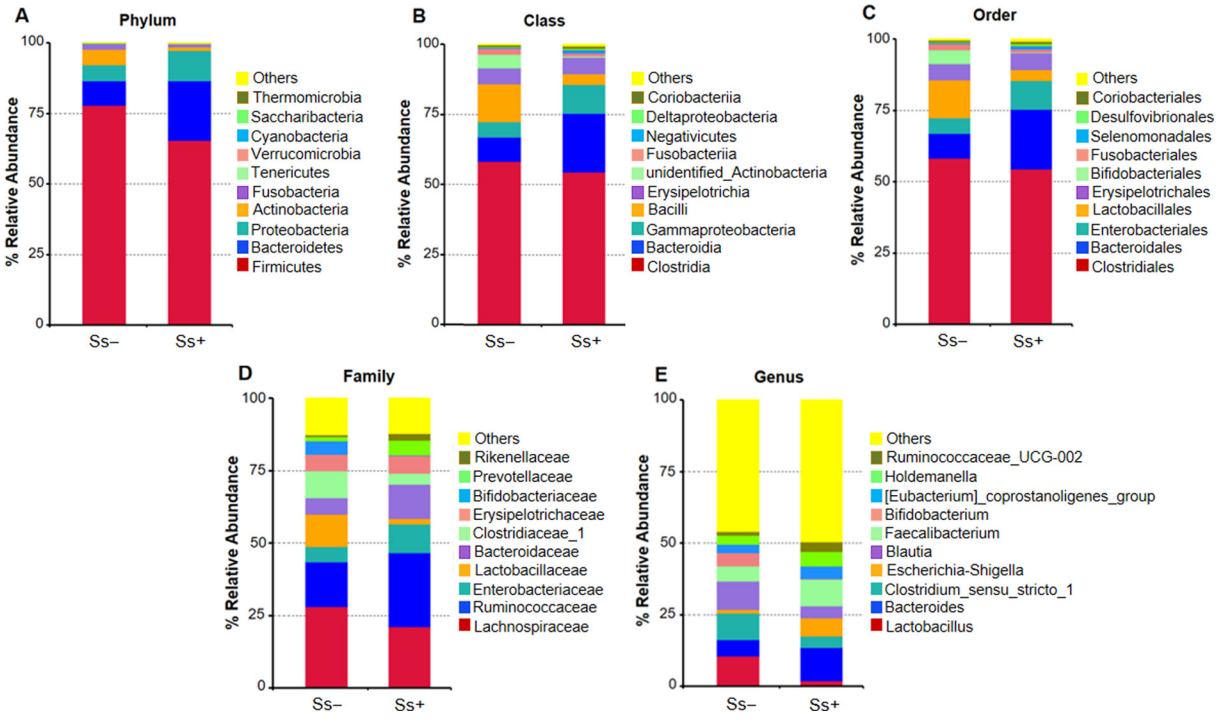

Fig. 2. The gut microbiota composition at the level of the (A) phylum, (B) class, (C) order, (D) family, and (E) genus. Control group (Ss−, n=26) compared with *S. stercoralis* infection group (Ss+, n=26). The V3–V4 region of the 16S gene was sequenced from pooled samples of the Ss+ and Ss− groups using next-generation technology.

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pseudocatenulatum, *L. agilis*, *L. fermentum*, *Ligilactobacillus salivarius*, and *Latilactobacillus sakei* were much lower in the Ss+ subjects. In contrast, *Bacteroides fragilis*, *Desulfovibrio piger*, and *Proteus vulgaris* were increased in this group.

Since bacteria in the genera *Bacteroides* and *Escherichia* were more common and those in the genus *Lactobacillus* were less common in the Ss+ pool, we therefore investigated the presence of them in individual samples. It is known that inflammatory bowel disease can be associated with the presence of *Escherichia coli* and *B. fragilis* [26] and that *L. agilis* and *L. fermentum* are antagonistic to *E. coli* [27]. Presence or absence of these bacteria in each sample was determined by conventional PCR using specific primers, a band of the right size on a gel being taken as evidence of presence. PCR products from two individual samples in each group were sequenced using BTSeq™ Barcode-Tagged Sequencing to confirm the identities of the bacterial species. Sequenced PCR products were >98% similar to corresponding species (Table 1 and Supplementary Table 1). We detected *L. agilis* and *L. fermentum* in all samples in the Ss− group and most samples in the Ss+ group (Table 4 and Supplementary Fig. 2). However, the difference between groups was not significant (odds ratio <1 and p>0.05). *Bacteroides fragilis* was significantly more frequently found among samples in the Ss+ group than in the Ss− group (OR=6.35, p=0.02).

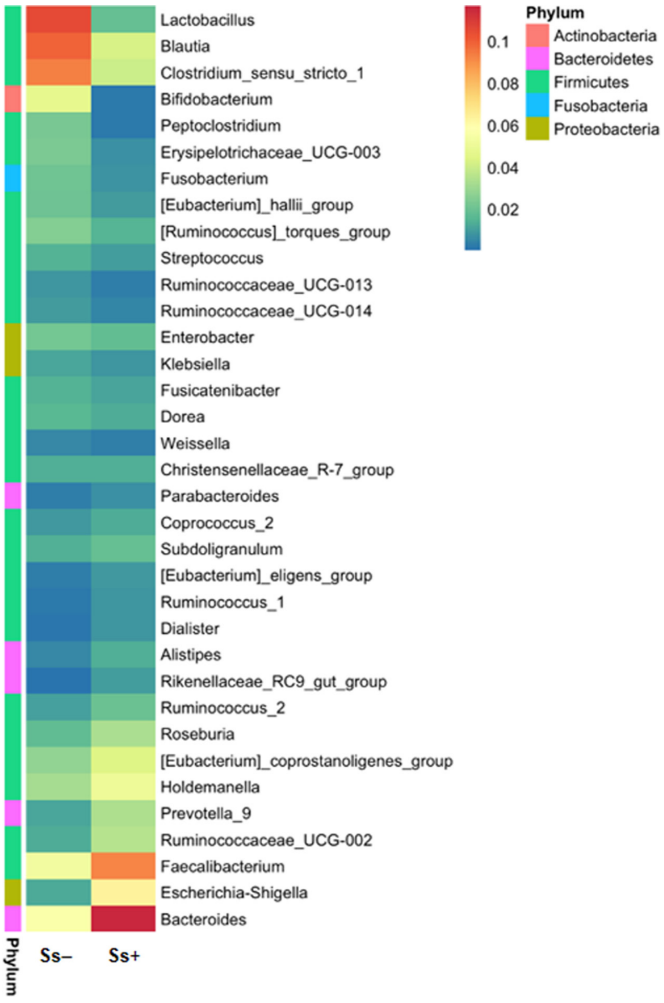

Fig. 3. Heatmap of the top 35 bacterial taxa at the genus level. The X-axis and Y-axis represent the sample name and genus, respectively. The relative values in the heatmap are depicted by colours, with increasing abundance from dark green to red. Control group (Ss−, n=26) compared with *S. stercoralis* infection group (Ss+, n=26). The V3–V4 region of the 16S gene was sequenced from pooled sample of the Ss+ and Ss− groups using next-generation technology.

Serum SCFA concentration reduced in S. stercoralis *infection*

Concentrations of SCFAs were determined by GC-MS in individual serum samples. The results showed that acetic acid was significantly lower in Ss+ individuals compared with Ss− individuals (p<0.05; Fig. 5A). A significant decrease in acetic acid concentration was observed in Ss+ female individuals compared with both Ss+ and Ss− males (Fig. 5B). The difference in acetic acid levels was particularly marked between males in the Ss− group and females in the Ss+ group (p<0.01). Among Ss+ individuals older than 65 years, the concentrations of acetic acid were significantly lower than among Ss− individuals in the same age group (Fig. 5C). There was a positive correlation (Pearson's correlation) between the concentrations of acetic acid and eGFR (r=0.35, p=0.027). There was a negative correlation between the concentration of acetic acid and age (r=−0.55, p=0.0001).

DISCUSSION

Here, we demonstrated the influence of *S. stercoralis* infection on gut microbiota and gut-derived metabolites in rural communities in Donchang sub-district, Khon Kaen, Thailand. High-throughput sequencing of the faecal microbiota revealed that *S. stercoralis* infection leads to an increase in the number of OTUs as well as greater community richness (ACE and Chao1 estimators) and community diversity (Shannon and Simpson indexes). This is in line with a previous study in elderly Italians [15]. However, compared with the Italian study, we found greater numbers of reads from the phyla Proteobacteria and Bacteroidetes in Ss+ individuals but fewer reads from the phyla Firmicutes and Actinobacteria than the Italian control group [15]. This discrepancy may be attributable to a range of differences, such as life style, endemic area, diet, and socioeconomic development [21]. Although high alpha diversity has generally been associated with better health [28], our data revealed that members of *Escherichia-Shigella*, *Desulfovibrio*, *Proteus*, and *Bacteroides* (including *B. fragilis*) were enriched in Ss+ individuals, while representatives of *Lactobacillus, Bifidobacterium*, and *Blautia* were diminished (Fig. 4).

Members of the genus *Escherichia-Shigella* were more abundant in infected individuals. This taxon is commonly associated with diarrhoeal disease and bacillary dysentery [29] and is possibly also associated with Parkinson's disease [30], bladder cancer [31], and coinfection with parasites such as *Schistosoma mansoni* [32]. In addition, a rise in the proportion of *Escherichia-Shigella* is positively correlated with levels of metabolic toxins such as trimethylamine-N-oxide and indoxyl sulfate [33].

Our results also indicated that the genus *Bacteroides*, particularly *B. fragilis*, was more abundant in *S. stercoralis* infection (Fig. 3). *B. fragilis* is a commensal organism that can become an opportunistic pathogen through the production of proteolytic enterotoxin, (*B. fragilis* toxin or fragilysin). This toxin can cause secretory diarrhoea and colonic epithelial

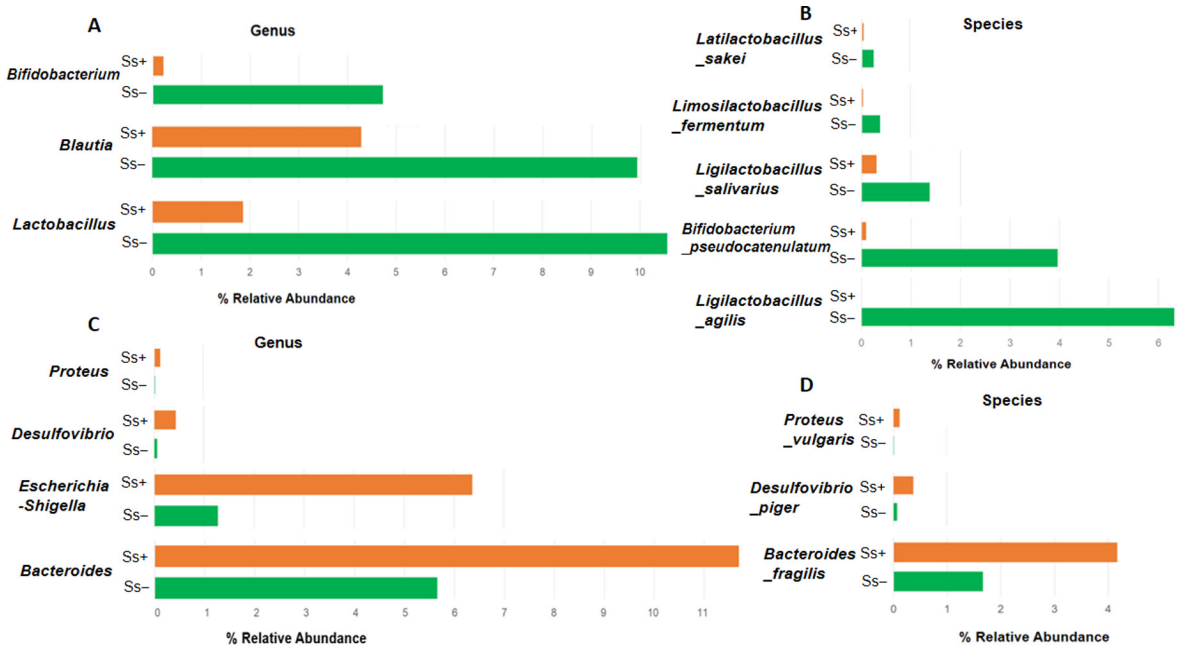

Fig. 4. Comparisons of the abundances (numbers of sequence reads) of some bacteria between Ss− and Ss+ samples. (A) Genus and (B) species of *Bifidobacterium*, *Blautia*, and *Lactobacillus*; (C) genus and (D) species of *Proteus*, *Desulfovibrio*, *Escherichia-Shigella,* and *Bacteroides*. Ss−, control group; Ss+, *S. stercoralis* infection group.

Table 4. Frequency of occurrence of four bacterial species among samples

Name	Ss− group (n=26)	Ss+ group (n=26)	Odds ratio OR (95% Cl)	p value
Bacteroides fragilis	17 (65.4%)	24 (92.3%)	6.35 (1.2–33.1)	0.02
Escherichia coli	25 (96.2%)	26 (100%)	3.11 (0.12–80.1)	0.49
Ligilactobacillus agilis	26 (100%)	25 (96.2%)	0.32 (0.01–8.2)	0.49
Limosilactobacillus fermentum	26 (100%)	25 (96.2%)	0.32 (0.01–8.2)	0.49

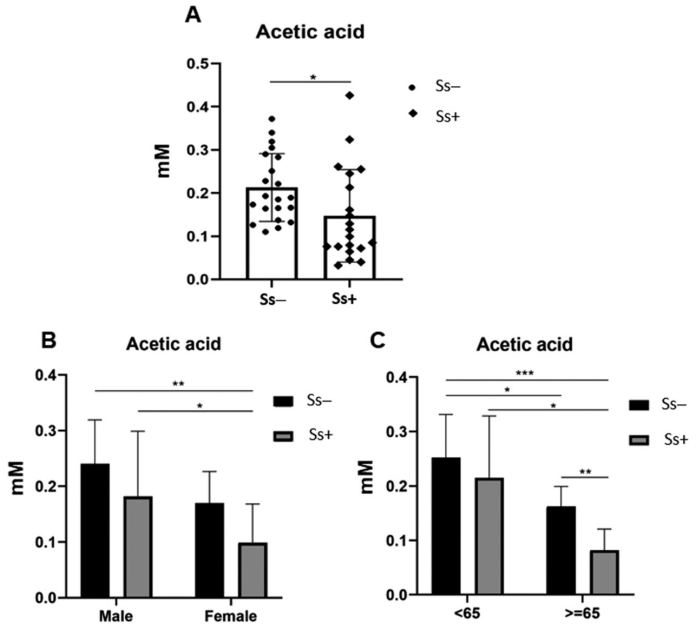

Fig. 5. (A) The concentrations of acetic acid in the *S. stercoralis* infection group (Ss+, n=20) and control group (Ss−, n=21). (B) The influence of gender and (C) age on the level of acetic acid in the Ss− vs. Ss+ group. *p<0.05, **p<0.01, ***p<0.001. Analysis of the differences in amounts of acetic acid between the two groups (based on t-test) and among four sub-groups according to gender and age (based on one-way ANOVA).

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damage, leading to chronic colitis and colorectal cancer [34, 35]. However, many studies have also demonstrated a beneficial effect of *Bacteroides* on immune imbalance, mental disorders, and inflammatory diseases [36, 37]. Further studies need to explore the complex interactions involved.

As shown in the heatmap of the top 35 genera (Fig. 3), the abundances of *Lactobacillus* and *Bifidobacterium* species declined in the *S. stercoralis* infection group. We identified the presence of important *Lactobacillus* species in individual samples (Table 4). Based on odds ratios, *L. agilis* and *L. fermentum* occurred in fewer members (OR <1) of the Ss+ group relative to the Ss− group. Reduction or absence of these species might have an effect on human health, although whether for good or ill is somewhat controversial [38]. Several probiotic strains of *Lactobacillus* and *Bifidobacterium* have beneficial functions, such as the inhibition of intestinal colonisation by pathogenic microorganisms and anti-carcinogenic, anti-diarrhoeal, and immunostimulatory properties [39], as well as the maintenance of tight junctions in intestinal epithelia [40]. In contrast, *Lactobacillus* has been positively correlated with parasite colonisation in animal models, such as *Toxocara cati*-infected cats [41], *Nippostrongylus brasiliensis*-infected mice [42], *Trichuris muris*-infected mice [43], and *Heligmosomoides polygyrus*-infected mice [44]. Moreover, this genus may trigger serious infections, especially in immunocompromised individuals with prolonged hospitalization [45], suggesting that the response of gut bacterial flora to parasites is specific to each host/parasite combination.

In this study, we found some bacteria that produce SCFAs, such as members of the family Lachnospiraceae, the genera *Blautia* and *Bifidobacterium*, and the *Eubacterium. hallii* group, were in low abundance in Ss+ individuals (Fig. 3). The concentration of acetic acid was significantly reduced in the Ss+ group (Fig. 5), consistent with the finding of low levels of SCFAs in Ss+ subjects from a non-endemic area [15]. In contrast, high abundances of Lachnospiraceae and increased SCFA production were observed in *Ascaris suum*-infected pigs and *Necator americanus*-infected humans [19]. The levels of SCFAs may also be influenced by other factors, such as type of parasite, gender, and age of host [46]. Moreover, *S. stercoralis* infection in humans may also change the gut pH, causing the loss of some bacteria, such as *Bifidobacterium* and *Lactobacillus*, and elevated levels of the genus *Escherichia-Shigella*. Increased abundances of the families Enterobacteriaceae, Clostridiaceae, and Veillonellaceae are associated with increased faecal pH. This applies especially to the genus *Bifidobacterium* [47]. The pH of a microbial ecosystem is heavily influenced by the metabolites produced by the inhabitants of the ecosystem. Lactic acid bacteria (such as *Bifidobacterium* and *Lactobacillus*) produce acetic and lactic acids as major metabolic end products, which in turn significantly lower the luminal pH [48]. Our preliminary results also support the idea that *S. stercoralis* infection causes changes to the microbiota, which in turn modulates the pH of the gut environment (data not shown).

Our study has some limitations. First, we pooled the samples for next-generation sequencing, making it impossible to apply statistical analysis to individual samples. Second, we did not control for diet in our study population, a factor that could directly affect the gut microbiome. However, we assume that people in the same community, family, and culture have similar diets. A strength of the study is that we reduced confounding factors by matching gender, age, and biochemical profile factors of participants for analyses. The pooling of microbiome sample DNAs before amplification is cost-effective and has been used previously to estimate community level diversity [49–51]. However, analysis of sequence data from individual samples for gut microbiota and controlling for the diets of participants would be valuable in future studies.

In conclusion, this study presents the first human gut microbiota profiles in *S. stercoralis* infection in Northeast Thailand. Next-generation sequencing of the faecal microbiota revealed that *S. stercoralis* infection leads to an increased number of OTUs and greater community richness and community diversity. Infection with *S. stercoralis* can alter the gut microbiome by reducing the abundances of the genera *Bifidobacterium*, *Lactobacillus*, and *Blautia* (SCFA-producing bacteria) and increasing the abundances of *Escherichia-Shigella* and *Bacteroides*. The levels of serum SCFAs were significantly lower in Ss+ individuals. Taken together, these findings provide evidence indicating that the production of gut microbiota-derived SCFAs and serum acetic acid was reduced in *S. stercoralis* infection. Our data may help to establish a link between parasite colonisation and certain host phenotypical characteristics and gut microbial profiles in Khon Kaen Province, Northeastern Thailand. The fundamental question remains as to whether *S. stercoralis* infection is merely an indicator of the gut microbial ecosystem or an active manipulator of the gut microbiota structure and function [2].

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