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Ascaris suum infection was associated with a worm-independent reduction in microbial diversity and altered metabolic potential in the porcine gut microbiome ☆

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ABSTRACT

The effect of infection of pigs with *Ascaris suum* on the microbial composition in the proximal colon and fecal matter was investigated using 16S rRNA gene sequencing. The infection significantly decreased various microbial diversity indices including Chao1 richness, but the effect on Chao1 in the colon luminal contents was worm burden-independent. The abundance of 49 genera present in colon contents, such as *Prevotella* and *Faecalibacterium*, and 179 operational taxonomic units was significantly changed as a result of infection. Notably, infection was also associated with a significant shift in the metabolic potential of the proximal colon microbiome, where the relative abundance of at least 30 metabolic pathways including carbohydrate metabolism and amino acid metabolism was reduced, while the abundance of 28 pathways was increased by infection. Furthermore, the microbial co-occurrence network in infected pigs was highly modular. Two of 52 modules or subnetworks were negatively correlated with fecal butyrate concentrations ($r < -0.7$; $P < 0.05$) while one module with 18 members was negatively correlated with fecal acetate, propionate and total short-chain fatty acids. A partial Mantel test identified a strong positive correlation between node connectivity of the operational taxonomic units assigned to β-Proteobacteria (especially the family Alcaligenaceae) and fecal acetate and propionate levels ($r = 0.82$ and 0.74, respectively), while that of the family Porphyromonadaceae was positively correlated with fecal egg counts. Overall, *Ascaris* infection was associated with a profound change in the gut microbiome, especially in the proximity of the initial site of larval infection, and should facilitate our understanding of the pathophysiological consequence of gastrointestinal nematode infections.

1. Introduction

Different species of *Ascaris* roundworms primarily infect humans and pigs (Taylor et al., 2016). *Ascaris lumbricoides* is the most common soil transmitted helminth infecting humans, especially children in developing countries (Pullan et al., 2014). The public health impact of *A. lumbricoides* is estimated to be approximately 1.31 million daily adjusted life years (Pullan et al., 2014). *Ascaris suum* is ubiquitous in swine where it causes important economic losses, mostly due to reduced feed conversion and impaired liver metabolism, and has also been reported to be zoonotic (Miller et al., 2015). Recent evidence indicates that *A. suum* is closely related to *A. lumbricoides* (Peng and Criscione, 2012), supporting the use of *A. suum* as a model of *A. lumbricoides* infection in humans.

Ascaris suum infection in pigs induces a typical Th2 immune response characterized by increased expression of IL4, IL5, and IL13 genes (Dawson et al., 2005) and eosinophilia (Masure et al., 2013a). A strong immunity and/or resistance can be readily developed. For example, an expulsion mechanism termed self-cure can cause the elimination of most of the L4s from the small intestine between 14 and 21 days p.i. (Masure et al., 2013b). As a result, most pigs harbor few or no worms and a small proportion of hosts

☆ *Note:* All raw 16S sequences were deposited in the NCBI Sequence Read Archive (SRA accession no. SRP127199).

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harbor large numbers of worms. This overdispersion distribution may have relevance in developing effective parasite control strategies. It is well known that the host microbiome profoundly shapes immunity (Hooper et al., 2012) and a minimal disruption in the microbiota by parasite reinfection contributes to the restoration of gastric function in animals which are partially immune to parasites (Li et al., 2011). The complex interactions among the host, host gut microbiome and parasites have important functional implications. For example, *Trichuris muris*-induced alterations in the mouse gut microbiome control parasite numbers in the host gut via inhibiting subsequent infections, representing a novel parasite survival mechanism (White et al., 2018). Furthermore, *A. suum* worms release antimicrobial factors, which directly interfere with bacterial growth and biofilm formation in the host, suggesting that parasites play a direct role in shaping the microbiome (Midha et al., 2018). Recent studies show that intestinal worms significantly impact the general homeostasis of the host; and that the interaction between worms and the intestinal microbiome alters host immunity and inflammation. Other studies have shown that intestinal helminths markedly change gut microbial composition and related aspects of host metabolism (Walk et al., 2010; Broadhurst et al., 2012; Li et al., 2012; Wu et al., 2012; Rausch et al., 2013; Osborne et al., 2014). Moreover, it is documented that the extent of host immunity to *Ascaris* is independent of the adult worm counts (Urban et al., 1988). It would be intriguing to see if *A. suum*-induced changes in the gut microbiome are worm burden-independent. Partially driven by such curiosity, we attempted to understand porcine proximal colon and fecal microbial communities in response to an *A. suum* experimental infection using 16S rRNA gene sequencing and bioinformatic tools. We were specifically interested in identifying microbial co-occurrence patterns and gut microbiome features that may be strongly correlated with parasitological and physiological traits as potential targets for future explorations in microbial ecology engineering.

2. Materials and methods

2.1. Animals and parasitology

Animal experiments were conducted as approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (Protocol# EC2013/109), which strictly followed The Institutional Animal Care and Use Committee (IACUC) guidelines and European Animal Welfare Directives. Infection protocols and sampling were the same as previously described (Zaiss et al., 2015). Briefly, 20 Rattlerow Seghers hybrid piglets (10 weeks old) with free access to food and water were inoculated with infective *A. suum* eggs, and four other parasite-naïve piglets of the same age were orally dosed with PBS and served as uninfected controls. The 20 piglets in the infected group received three doses of 300 infective eggs on three consecutive days via oral inoculation. Parasite eggs per gram of feces (EPG) were monitored every 2 days from day 47 post inoculation (pi) onward. At 54 days pi, both infected and uninfected pigs were sacrificed. Intestinal contents were collected at ~30 cm distal from the junction of the caecum and proximal colon together with feces and snap frozen in liquid nitrogen prior to storage at −80 °C until total DNA was extracted. Short chain fatty acid (SCFA) data were measured previously in a large study that included the animals used in this study (Zaiss et al., 2015). The number of *A. suum* worms present in the small intestine was counted visually. Of the 20 inoculated pigs, 10 were selected for this study based on their worm burdens. These pigs were divided into two groups, those with a relatively high worm count (worm burden high, WBH; $n = 5$) and those with low or no worm count (worm burden low, WBL; $n = 5$). The mean number of worms recovered from WBL and WBH was 0.60 ± 0.89 and 128.40 ± 41.73, respectively (±S.D., P value = 1.32×10^{-4}). Similarly, EPG for WBL and WBH groups were 30.00 ± 44.72 and 2810.00 ± 2421.88, respectively ($P = 3.33 \times 10^{-2}$). No significant changes in growth rate or feed intake between WBL and WBH groups were detected.

2.2. 16S rRNA gene sequencing and sequence analysis

The 16S rRNA gene sequencing was performed as previously described (Li et al., 2016). Total DNA was extracted from proximal colon contents and fecal samples using a QIAamp DNA stool kit (Qiagen, Valenica, CA, USA) with some modifications, including the addition of a bead-beating step and increasing the lysis temperature to 95 °C for 5 min. The V3-V4 regions of the bacterial 16S rRNA gene were amplified and sequenced using the primers (forward primer_341/357F: CCTACGGGNGGCWGCAG, reverse primer_805/785R: GACTACHVGGGTATCTAATCC) on an Illumina MiSeq sequencer (Li et al., 2016).

The sequence data were preprocessed using MiSeq Control Software (MCS) v2.4.1. Raw sequences were first analyzed using FastQC v. 0.11.2 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) to check some basic statistics such as GC contents and per base quality score distribution. Raw reads were then filtered and primers were trimmed (Li et al., 2016; Liu et al., 2017). The processed pair-end reads were then merged using PandaSeq v2.8 (https://github.com/neufeld/pandaseq) to generate representative complete nucleotide sequences (contigs) using default parameters. The overlapping regions of the paired-end read were first aligned and scored, and reads with low score alignments and high rates of mismatches (≥3 mismatches) were discarded. Retained quality sequences were analyzed using the Quantitative Insights into Microbial Ecology pipeline (QIIME v1.9.1, http://qiime.org/), as previously described (Li et al., 2016; Liu et al., 2017). An "open reference" protocol in the QIIME pipeline was used for operational taxonomic unit (OTU) picking. The default QIIME parameters were used, except that the quality filtering based on the OTU abundance threshold was lowered to 0.0001%. The GreenGene database (v13.8) was used for taxonomic assignment (greengenes.lbl.gov). PyNAST (v1.2.2, https://github.com/biocore/pynast) was used for sequence alignment. OTU relative abundance values were then analyzed using the linear discriminant analysis (LDA) effect size algorithm (LEfSe) (Segata et al., 2011) to identify taxa and Kyoto Encyclopedia of Genes and Genomes (KEGG) gene families and pathways that display significant differences between two biological conditions. Furthermore, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt v1.0.0, http://picrust.github.io/picrust/)) was used with default parameters to predict gene contents and metagenomic functional information based on the OTU table generated using the open reference protocol in QIIME. Briefly, the OTU table was first normalized by dividing each OTU by the known/predicted 16S copy number by using the PICRUSt work flow: *normalize_by_copy_number.py*. The gene contents or the abundance of KEGG Orthology (KO) were predicted from the normalized OTU table using the work flow: *predict_metagenomes.py*. The predicted metagenomic function was further analyzed by collapsing thousands of KEGG orthologs into higher functional categories (pathways) (*categorize_by_function.py*). In addition, specific OTU contribution to a given function or pathway was identified by using the work flow: *metagenome_contributions.py*, as described previously (Li et al., 2016; Liu et al., 2017). Approximately 570, 693 ± 304, 089 (mean ± S.D.) raw sequence reads per sample were generated for this study ($n = 28$). Raw 16S sequences were deposited in the NCBI Sequence Read Archive (SRA accession no. SRP127199).

2.3. Network construction and visualization

The global microbial co-occurrence network was constructed for the proximal colon microbial community of the infected pigs using a Random-Matrix theory (RMT) based pipeline (http://ieg4.rccc.ou.edu/mena/) (Deng et al., 2012; Zhou et al., 2011). The OTUs detected in ≤50% of all samples were excluded due to a drastic effect of OTU sparsity on the precision and sensitivity of network inference (Weiss et al., 2016). A similarity matrix, which measures the degree of concordance between the abundance profiles of individual OTUs across different samples (Zhou et al., 2011), was then obtained by using Pearson correlation analysis of the abundance data. A threshold cut-off value (0.88) was automatically determined by calculating the transition from the Gaussian orthogonal ensemble to the Poisson distribution of the nearest-neighbor spacing distribution of eigenvalues in the pipeline and then applied to generate an adjacent matrix for network inference (Deng et al., 2012). The fast-greedy modularity optimization procedure was used for module separation. The within-module degree (Z) and among-module connectivity (P) values were then calculated and plotted to generate a scatter plot for the network to gain insights into the topological roles of individual nodes in the network according to the Olesen classification (Olesen et al., 2011). A Mantel test was performed to measure the relationship of the network topology and physiological traits by calculating OTU significance and node connectivity (Zhou et al., 2011). The network structure was visualized using Cytoscape v3.6.1 (https://cytoscape.org/)

3. Results

3.1. Ascaris infection was associated with a significant reduction in gut microbial diversity

Rarefaction curve analysis suggested that the sequencing depth in this study was adequate (Supplementary materials; doi: 10.17632/zwr6x3f4vy.2). Common α microbial diversity indices such as Chao1, Phylogenetic Diversity (PD whole tree), Shannon, and Simpson as well as Pielou's evenness, were evaluated (Table 1). Our results suggested that *A. suum* induced a significant decrease in various microbial diversity indices, especially in the luminal contents of the proximal colon. For example, Shannon (mean ± S.D. = 5.96 ± 0.28 for controls, 5.06 ± 0.18 for infected pigs) and Simpson indices were significantly reduced in the proximal colon contents by infection ($P < 0.05$). Similarly, the Chao1 index was decreased from 1158.00 ± 88.13 (mean ± S.D.) in the control uninfected pigs to 791.40 ± 66.00 in the infected pigs ($P < 0.05$). Moreover, the reduction in Chao1 was independent of worm burden. Compared with the control group, Chao1 was significantly decreased in the WBH and WBL groups (Table 1). β-diversity indices were also significantly impacted by infection (data not shown).

3.2. Ascaris infection was linked to a significant change in microbial composition of the porcine gut microbiome

The difference in relative abundances of various taxa between the uninfected control and infected pigs as well as between WBL and WBH segregated groups was analyzed using LEfSe (Segata et al., 2011). The infection was associated with a significantly greater change in the composition of the proximal colon microbial community (Fig. 1A) than the fecal microbial community at various taxonomic levels (Fig. 1B) compared with uninfected controls. Among the 16 phyla identified from the dataset, the abundances of two phyla, Spirochaetes and Planctomycetes, were significantly reduced compared with the uninfected controls ($P < 0.05$; Supplementary materials doi: 10.17632/zwr6x3f4vy.2). Of note, the abundance of the phylum Verrucomicrobia was significantly reduced only in the WBH group, while that of Spirochaetes was reduced only in the WBL group.

Approximately 49 detected genera were associated with infection in the proximal colon. The abundance of 12 genera was significantly altered in both the WBL and WBH groups. Among them, the abundance of four genera was significantly increased as a result of infection. For example, the abundance of *Megasphaera* was significantly increased regardless of worm burden (Fig. 2A–C) while the abundance of *Bacillus* was repressed in both WBL and WBH groups (LDA > 2.0; Supplementary Materials (doi:10.17632/zwr6x3f4vy.2)). At least six genera had significant changes in the abundance only in the WBH group compared with uninfected controls. For example, *Catenibacterium*, *Faecalibacterium* (Fig. 2B) and *Lactobacillus* had a significantly increased abundance while abundance of *Phascolarctobacterium* decreased. Among 14 genera significantly changed by infection in the WBL group only (Supplementary Materials (doi:10.17632/zwr6x3f4vy.2)), 11 had a significantly higher abundance in uninfected controls while three had a significantly higher abundance in the WBL group. For example, the infection-induced increase in the relative abundance of *Prevotella* (Fig. 2D), the most abundant genus, and *Megamonas*, was evident only in the WBL group. Notably, *Acidaminococcus* was the only genus with its abundance significantly increased regardless of the worm burden or sampling site (both in the proximal colon and fecal microbial communities) compared with uninfected controls (Fig. 2A; Supplementary Materials (doi:10.17632/zwr6x3f4vy.2)).

The relative abundance of 179 OTUs was significantly changed in the infected groups (LDA > 2.0) with 125 OTUs repressed and 54 OTUs elevated. For example, the abundance of 25 of the 29 OTUs assigned to the family *Ruminococcaceae* was significantly repressed by infection. In contrast, the majority of the 11 OTUs assigned to the family Veillonellaceae were significantly increased. The change in abundance of OTUs assigned to the genus *Prevotella* was somewhat bidirectional; 21 OTUs were significantly repressed while 22 OTUs increased. In addition, the abundance of an OTU (GreenGene ID# 529940) belonging to *Faecalibacterium prausnitzii* was elevated 4.3 fold by infection (Table 2). The relative abundance of 69 of the 179 OTUs was significantly changed regardless of the worm burden (Table 2). However, the abundance of 32 OTUs was significantly changed only in the WBH group (Supplementary Materials (doi:10.17632/zwr6x3f4vy.2)). For example, the abundance of an OTU (GreenGeneID #332919) assigned to *Lactobacillus agilis* was significantly increased only in the WBH group. Of note, an OTU (GreenGene_ID #86812) assigned to the genus *Campylobacter* was significantly increased by infection in both the proximal colon and fecal microbial communities, especially in the WBH group (Fig. 3).

Table 1
Gut microbial diversity indices affected by *Ascaris suum* infection in pigs.

Index	Control	WBL	WBH
PD whole tree	40.54 ± 1.92	34.03 ± 1.87[a]	34.08 ± 4.48
Chao1	1158.00 ± 88.13	802.0 ± 69.77[a]	780.80 ± 121.20[a]
Shannon	5.96 ± 0.28	5.06 ± 0.19[a]	5.06 ± 0.33
Simpson	0.94 ± 0.01	0.91 ± 0.01[a]	0.91 ± 0.01
Pielou's evenness	0.55 ± 0.02	0.47 ± 0.01[a]	0.51 ± 0.02

WBL, worm burden low; WBH, worm burden high; PD, phylogenetic diversity.
[a] $P < 0.05$ based on an unadjusted t test.

3.3. Ascaris suum affected the metabolic potential of the porcine colon microbial community

The PICRUSt algorithm was used to assess the metabolite potential of gut microbial communities. The mean number of KEGG gene families predicted was 4423.36 ± 370.96 in the colon and 4823.79 ± 161.05 in feces. The five most abundant KEGGs in the proximal colon luminal contents of control pigs were RNA

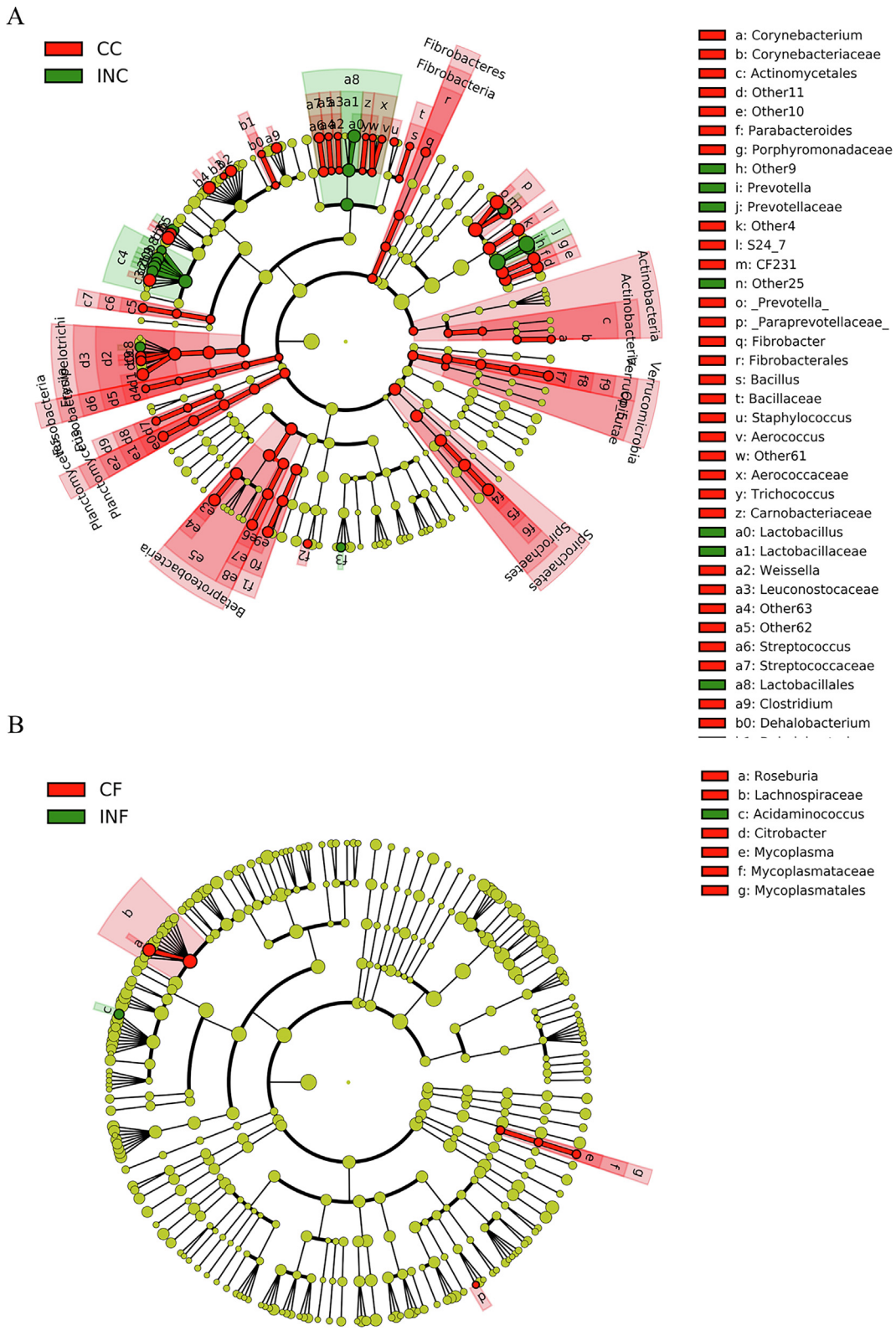

Fig. 1. Graphical representation of the taxa significantly impacted by infection of pigs with *Ascaris suum*. (A) The proximal colon microbiome. (B) The fecal microbial community.

polymerase sigma-70 factor, the extracytoplasmic function (ECF) subfamily (K03088, abundance = 0.71%), iron complex outer membrane receptor protein (K02014, 0.52%), phosphoribosyl aminoimidazole carboxamide formyl transferase/inosine monophosphate (IMP) cyclohydrolase (K00602, 0.38%), penicillin-binding protein 1A (K05366, 0.38%), and outer membrane protein (K06142, 0.37%). The relative abundances of approximately 118 KEGGs identified were significantly altered as a result of infection, either in the WBL or the WBH group (LDA > 2.0). For example, the hits assigned to RNA polymerase sigma-70 factor, ECF subfamily (K03088)

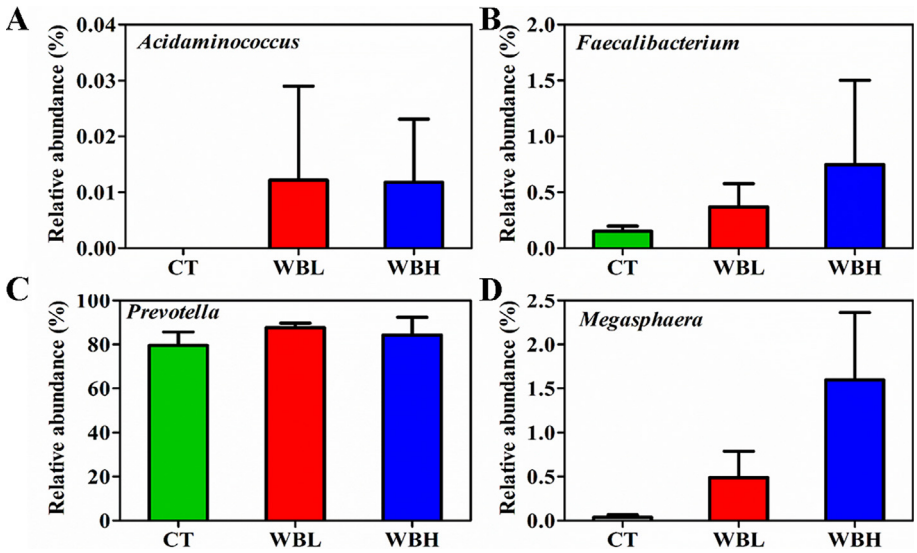

Fig. 2. Select genera significantly impacted by *Ascaris suum* in the porcine proximal colon microbial community. (A) *Acidaminococcus*; (B) *Faecalibacterium*; (C) *Megasphaera*; (D) *Prevotella*. CT, control uninfected pigs; WBL, the infected pigs with low worm burdens; WBH, the infected pigs with high worm burdens. The error bars represent S.D.

Table 2
Select operational taxonomic units in the porcine colon microbiome significantly impacted by infection regardless of worm burden. The number denotes relative abundance (mean ± S.D.).

OTU ID	Control	WBL	WBH	LDA score	Annotation (phylum/taxon)
547854	4.73 ± 1.11[b]	1.59 ± 0.81	1.13 ± 0.96	4.22	Bacteroidetes/*Prevotella*
353459	2.47 ± 1.25[b]	0.64 ± 0.51	0.54 ± 0.67	3.96	Bacteroidetes/*Prevotella*
856235	2.23 ± 0.50[b]	0.51 ± 0.53	0.26 ± 0.28	3.95	Bacteroidetes/*Prevotella*
300853	1.63 ± 0.61[b]	0.32 ± 0.25	0.30 ± 0.33	3.81	Bacteroidetes/CF231
343443	1.51 ± 0.99[a]	0.25 ± 0.41	0.16 ± 0.33	3.81	Bacteroidetes/*Prevotella*
1033345	1.09 ± 1.10[b]	0.01 ± 0.01	0.01 ± 0.02	3.72	Bacteroidetes/ *[Prevotella]*
28970	0.83 ± 0.91[b]	0.05 ± 0.05	0.04 ± 0.04	3.63	Bacteroidetes/*Prevotella*
509621	0.49 ± 0.15[b]	0.26 ± 0.04	0.20 ± 0.10	3.06	Bacteroidetes/*Prevotella copri*
605577	0.45 ± 0.05[b]	0.09 ± 0.07	0.09 ± 0.11	3.24	Firmicutes/Erysipelotrichaceae/p-75-a5
527874	0.43 ± 0.15[b]	0.97 ± 0.27	1.17 ± 0.50	3.53	Bacteroidetes/*Prevotella copri*
987581	0.40 ± 0.28[a]	0.06 ± 0.05	0.02 ± 0.04	3.25	Bacteroidetes/*Prevotella*
28974	0.40 ± 0.35[b]	0.01 ± 0.01	0.02 ± 0.02	3.30	Bacteroidetes/*Parabacteroides*
198502	0.31 ± 0.04[b]	0.53 ± 0.09	1.23 ± 1.18	3.42	Bacteroidetes/*Prevotella copri*
302909	0.24 ± 0.17[b]	0.01 ± 0.01	0.01 ± 0.01	3.05	Firmicutes/Lachnospiraceae
812596	0.21 ± 0.10[b]	0.04 ± 0.07	0.03 ± 0.04	2.93	Firmicutes/*Anaerovibrio*
272587	0.17 ± 0.23[b]	0.93 ± 0.20	1.18 ± 0.16	3.63	Firmicutes/*Dialister*
918187	0.16 ± 0.10[b]	0.01 ± 0.00	0.01 ± 0.01	2.87	Bacteroidetes/*Parabacteroides*
332831	0.15 ± 0.19[b]	0.00 ± 0.00	0.00 ± 0.00	2.85	Firmicutes/Ruminococcaceae
655793	0.13 ± 0.10[b]	0.02 ± 0.01	0.01 ± 0.01	2.81	Firmicutes/Ruminococcaceae/*Oscillospira*
196800	0.13 ± 0.18[b]	0.00 ± 0.00	0.01 ± 0.02	2.80	Bacteroidetes/*Prevotella*

[a] $P < 0.05$
[b] $P < 0.01$ (uninfected control versus infected animals). All OTUs had a significantly different abundance when comparing uninfected control to either worm burden low (WBL) or worm burden high (WBH) groups (absolute $\log_{10}$ Linear Discriminant Analysis (LDA) score >2.0 and $P < 0.05$ based on the Kruskal–Wallis test).

decreased from 0.71% in the control uninfected pigs to 0.64% in the infected WBH pigs. The abundance of 14 KEGGs was significantly changed by infection independent of worm burden. For example, compared with uninfected controls, the abundances of β-galactosidase (K01190), lactoylglutathione lyase (K01759), and putative family 31 glucosidase (K01811) were significantly decreased in both WBL and WBH groups, while the abundances of aspartate ammonia-lyase (K01744), sucrose-6-phosphatase (K07024) and serine/threonine protein kinase, bacterial (K08884) were significantly increased regardless of worm burden. On the other hand, phosphomethylpyrimidine kinase (K00941) and anaerobic C4-dicarboxylate transporter DcuA (K07791) were among the KEGGs with a significantly increased abundance only in WBH and WBL, respectively.

Approximately 58 KO pathways identified in the proximal colon microbial community were associated with infection. As shown in Table 3, infection may have a significant impact on a broad range of biological functions such as amino acid metabolism and biosynthesis, carbohydrate metabolism, DNA repair and recombination, and fatty acid biosynthesis. Of the 58 pathways involved, eight were significantly impacted by infection independent of worm burden (Table 3). For example, compared with uninfected controls, the number of hits assigned to the pathways named "Other ion-coupled transporters" and "Thiamine metabolism" was significantly increased, while the hits assigned to Energy metabolism and Fructose and mannose metabolism were significantly reduced in both WBL and WBH groups. However, the reduced number of the hits assigned to Carbohydrate metabolism by infection was

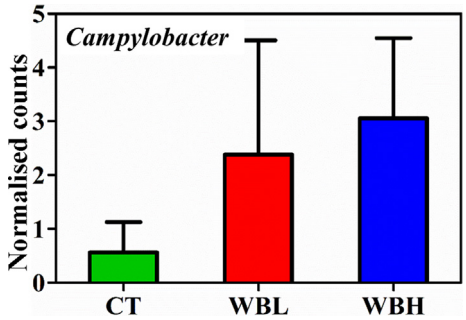

Fig. 3. Normalized hit counts of an operational taxonomic unit assigned to the genus *Campylobacter*. Y-axis values represent the normalized counts of sequence hits positively assigned to the operational taxonomic unit (GreenGene ID #86812) that belongs to *Campylobacter* (per 10,000 total assigned hits). CT, control uninfected pigs; WBL, the infected pigs with low worm burdens; WBH, the infected pigs with high worm burdens. The error bars represent S.D.

observed only in the WBH group. Moreover, the number of hits assigned to two pathways related to Carbohydrate metabolism, Fructose and mannose metabolism and Starch and sucrose metabolism, were significantly reduced in microbial communities from both the proximal colon and feces.

3.4. Microbial co-occurrence network and keystone species identified in the microbial community in the proximal colon of infected pigs

The global microbial co-occurrence network obtained the RMT-based molecular ecological network analysis (MENA) pipeline from the infected samples included 535 nodes and 910 links (Fig. 4). The network consisted of 53 subnetworks or modules with a high modularity value = 0.735, significantly higher than those of 100 random networks. The mean number of nodes per module was 10.1 with the largest modules containing 79 members. Individual modules differed in the number of nodes (size) and shape (connections). Greater than 76.6% of all nodes (OTUs) were included in the top 12 modules. However, more than half of all modules, 33 in total, contained only two to four members which were generally isolated with no links to the remaining network.

The majority of the nodes (>95%) in the network were peripherals according to the Olesen classification, with low Z and low P values (Fig. 5). Among those, approximately 80% of all nodes had no links outside of their own modules ($P = 0$). A total of 15 nodes may act as module hubs in the network and were highly connected and tended to link to other nodes within their own module (a high $Z > 2.5$ and a low $P \leq 0.62$). These nodes were likely important to the stable co-occurrence of other microbial taxa within their own modules and may support the establishment and growth of other microbes within the module. For example, two OTUs (GreenGene ID #43305 and #520643) from the family Lachnospiraceae were sole module hubs for Modules# 6 and 7, respectively. One OTU (GreenGene ID #347875), assigned to *Prevotella copri*, was one of the four module hubs in Module #1. Module #2, the largest module with a total of 79 members, also had three module hubs, all from the phylum Bacteroidetes. Nine OTUs had low Z ($\leq$2.5) and high P (>0.62) values from the scatter plot (Fig. 5). These OTUs linked several modules together and acted as connector species. For example, an OTU that belongs to Lachnospiraceae (GreenGene ID #205148) and a novel OTU assigned to Ruminococcaceae, both from the class Clostridia, may act as connectors. The two OTUs (GreenGene ID #370183 and #578240) from the genera *Blautia* and *Treponema*, respectively, also acted as connectors. Both module hubs and connectors likely functioned as habitat generalists, microorganisms with broad environmental tolerances or those able to metabolize a large diversity of substrates, in the microbial community, unlike peripherals which were generally specialists (i.e., those with restricted habitat ranges). No super generalists or network hubs, i.e., the nodes with high values for both Z (>2.5) and P (>0.62), were identified in the infection network in this study.

3.5. The correlations between modules and traits

The correlation analyses between module-based eigengenes and several biochemical measurements were conducted to understand the response of individual modules to infection and subsequent physiological changes. In the proximal colon microbial community of the infected pigs, at least six modules were significantly correlated with SCFA or one of their three major components, acetate, propionate, and butyrate. Module #5 with 45 members negatively correlated with fecal acetate as well as SCFA levels, while Module #15 was negatively correlated with fecal propionate and SCFA levels ($P < 0.05$). Module #11 had a strong correlation with both acetate and propionate as well as SCFA levels ($r = -0.75$; $P = 0.01$). On the other hand, both Modules #7 and #10 were strongly correlated with fecal butyrate concentrations ($P < 0.05$). The phylum Firmicutes was a predominant feature in Module #10. Among 20 members in this module, 18 were assigned to the class Clostridia in the phylum Firmicutes, while nine of the 18 members belong to the family Ruminococcaceae. In another module that was strongly correlated with butyrate levels (Module #7), Firmicutes, especially the class Clostridia, also played an important role. Among the OTUs acting as generalists, two of them were assigned to the family Lachnospiraceae in the class Clostridia. However, no modules displayed a significant correlation with the parasitological parameters of EPG and worm burden.

The relationships between network topology and physiological and parasitological traits were also assessed by calculating the correlation between the OTU significance (GS), r^2 (the square of Pearson correlation coefficient) of OTU abundance profiles with traits, and node connectivity. In infected pigs, the node connectivity of the OTUs assigned to the phylum Tenericutes and the class Mollicutes was significantly ($P < 0.05$) correlated with GS of the EPG

Table 3
Altered abundance of eight Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with *Ascaris suum* infection in the porcine proximal colon microbiome regardless of the worm status. The P value was calculated based on the Kruskal–Wallis test. The $\log_{10}$ Linear discriminant analysis score was derived from the Linear discriminant analysis effect size algorithm. The number denotes the normalized counts of hits positively assigned to a given pathway (mean ± S.D.).

Pathway	Control	Worm burden low	Worm burden high	LDA score	P value
Energy metabolism	1.15 ± 0.02	1.11 ± 0.02	1.08 ± 0.01	2.4700	0.0072
Fructose and mannose metabolism	0.92 ± 0.01	0.89 ± 0.01	0.88 ± 0.01	2.1346	0.0047
Histidine metabolism	0.53 ± 0.06	0.44 ± 0.04	0.42 ± 0.05	2.7229	0.0109
Phenylalanine metabolism	0.19 ± 0.02	0.16 ± 0.01	0.15 ± 0.01	2.2202	0.0072
Aminoacyl-tRNA biosynthesis	1.38 ± 0.03	1.42 ± 0.01	1.43 ± 0.02	2.4591	0.0109
Other ion-coupled transporters	1.32 ± 0.01	1.36 ± 0.01	1.38 ± 0.01	2.2515	0.0047
Restriction enzyme	0.22 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	2.1278	0.0072
Thiamine metabolism	0.42 ± 0.01	0.45 ± 0.01	0.46 ± 0.00	2.0924	0.0072

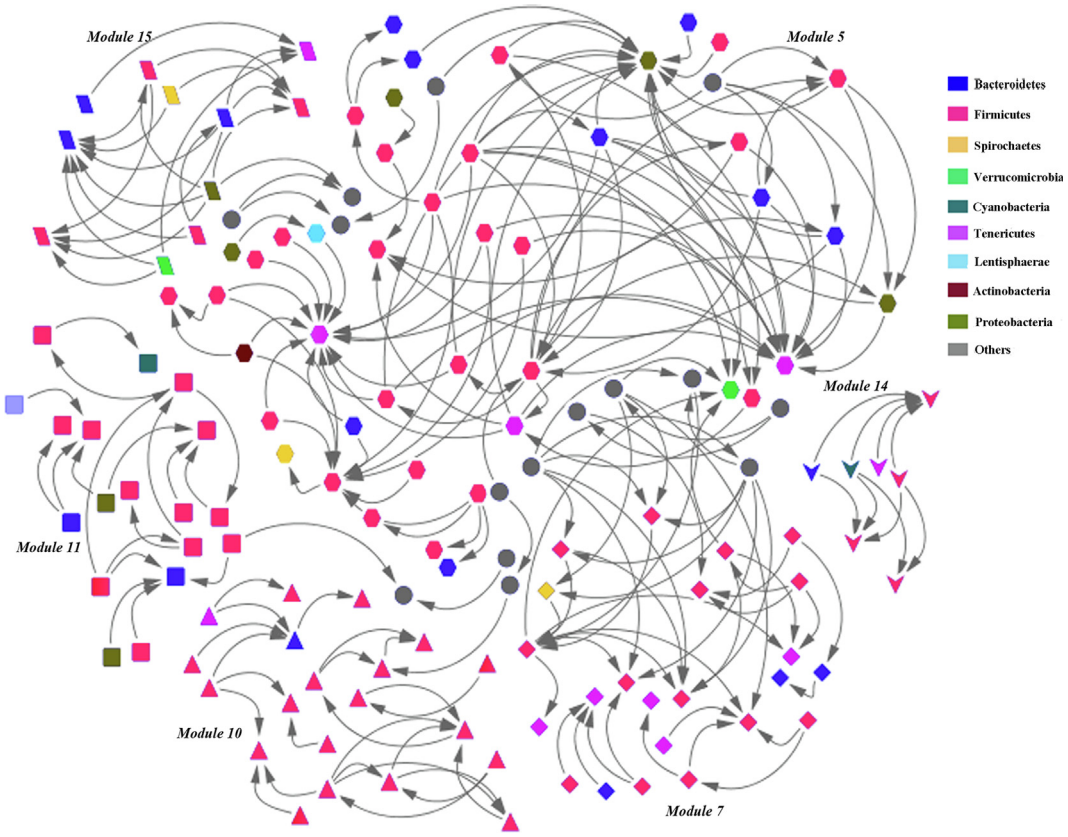

Fig. 4. Visualization of a partial microbial co-occurrence network identified using the fast greedy modularity optimization method in the pigs with *Ascaris suum*. Select modules with correlative traits are shown. Nodes represent an operational taxonomic unit Edge (links) with solid lines represent positive connections. Dashed lines represent negative connections. The colors of nodes indicate the phylum to which the operational taxonomic units belong.

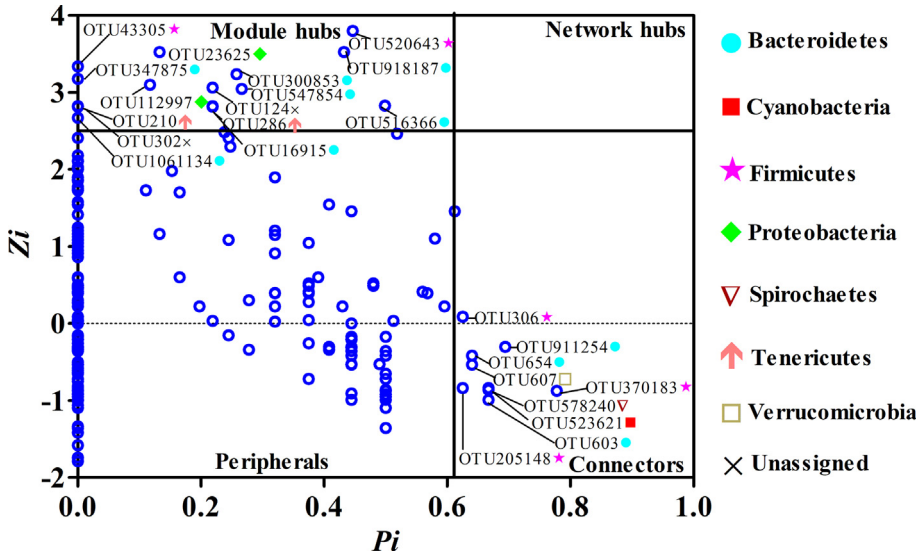

Fig. 5. The scatter plot showing the distribution of operational taxonomic units based on their topological roles in the network in pigs infected with *Ascaris suum*. Each dot represents an operational taxonomic unit. *Z*, within-module connectivity; *P*, among-module connectivity. The symbols of selected OTUs after the OTU _ID# denote the phylum to which the OTU was assigned.

value ($r = 0.31$ and 0.29, respectively) (Table 4). At the phylum levels, the node connectivity of the OTUs assigned to Bacteroidetes and Verrucomicrobia had a significant but marginal correlation with fecal propionate concentrations ($r = 0.16$ and 0.50, respectively; $P < 0.05$). Moreover, the node connectivity of the OTUs in the class β-proteobacteria (especially the order Burkholderiales and the family Alcaligenaceae) was strongly correlated with fecal acetate, propionate, and total SCFA levels ($r > 0.75$; $P < 0.05$). No correlation was detected for worm burden and butyrate in the study.

Table 4
A partial Mantel test identified strong correlations of select trait operational taxonomic units (OTU) significance (GS) values and node connectivity of the OTUs assigned to certain taxa in the proximal colon microbial community of *Ascaris suum*-infected pigs. GS is defined as the square of Pearson correlation coefficient (r^2) of OTU abundance profile with traits.

Trait	Taxon	*r*	*P*
Acetate	Betaproteobacteria (Class)	0.8159	0.0167
	Burkholderiales (Order)	0.8159	0.0167
	S24-7 (Family)	0.5555	0.0120
	Alcaligenaceae (Family)	0.8159	0.0167
Propionate	Bacteroidetes (Phylum)	0.1638	0.0100
	Verrucomicrobia (Phylum)	0.4962	0.0333
	Bacteroidia (Class)	0.1638	0.0090
	Betaproteobacteria (Class)	0.7420	0.0333
	Bacteroidales (Order)	0.1638	0.0110
	Burkholderiales (Order)	0.7420	0.0333
	Lachnospiraceae (Family)	0.2070	0.0060
	S24-7 (Family)	0.4665	0.0390
	Alcaligenaceae (Family)	0.7420	0.0333
Total SCFA	Betaproteobacteria (Class)	0.8497	0.0167
	Burkholderiales (Order)	0.8497	0.0167
	Lachnospiraceae (Family)	0.1019	0.0470
	S24-7 (Family)	0.5798	0.0190
	Alcaligenaceae (Family)	0.8497	0.0167
Fecal egg count (EPG)	Tenericutes (Phylum)	0.3124	0.0230
	Mollicutes (Class)	0.2924	0.0280
	RF39 (Order)	0.2924	0.0280
	Porphyromonadaceae (Family)	0.5550	0.0417

EPG, eggs per gram of feces.

4. Discussion

Helminth parasites have evolved specific mechanisms that affect host immunity to ensure their survival (Maizels et al., 2004). The interaction between gastrointestinal helminth infection and the intestinal microbiome further complicates the host-parasite relationship. Recent studies show that *Ascaris suum* excretory-secretory products and body fluid possess a broad spectrum of antimicrobial properties and play a direct role in shaping the host gut microbiome (Midha et al., 2018). Furthermore, while successful parasite infection requires a host microbiota, infection-induced alterations in the host microbiota in turn control parasite numbers and may promote the long-term survival of parasites, as documented in the mouse – *Trichuris muris* system (White et al., 2018). To better understand host-parasite interactions and the pathophysiological relevance of the infection-induced changes in the pig gut microbiome, we characterized the structure and function of the proximal colon and fecal microbial communities in response to infection with *A. suum* to identify microbial co-occurrence patterns and keystone species.

Previous studies demonstrated that parasitic helminths significantly alter the structure and function of the host gut microbiome (Li et al., 2012, 2016; Wu et al., 2012; Williams et al., 2017). Infection of pigs with the whipworm *Trichuris suis* lead to changes in the relative abundance of 13% of all genera detected in the proximal colon microbiome, approximately 26% of related metabolic pathways (Li et al., 2012), and 29.8% of gut luminal metabolites. Moreover, the parasite-induced changes in the gut microbiome appear to be independent of worm burden; changes persist weeks after worms are spontaneously cleared from the host (Wu et al., 2012). In this study, a 54 day infection by *A. suum* was shown to be associated with a profound change in the microbial composition of the proximal colon microbiome, altering abundance of three phyla, Spirochaetes, Planctomycetes and Verrucomicrobia, and approximately 29% of all genera identified in this study. At the species level, the abundances of 105 OTUs were significantly impacted by infection with a low worm burden while 125 OTUs were changed by infection in the group with a high worm burden. Among 106 OTUs consisting of the core colon microbiome, 25 OTUs, or ~24%, were significantly altered by infection. Notably, fewer changes in the abundance of various taxa in the microbial community were observed in the feces compared with the proximal colon contents, probably due to disruption by parasitic larvae and proximity to the site of adult worms in the small intestine.

The pathophysiological relevance of *A. suum* infection-associated changes in the microbial composition remains unknown. It is likely that the changes result in a shift in the metabolism of the gut microbiome, which could affect the metabolic needs of the host during infection. The abundance of two phyla, *Spirochaetes* and *Verrucomicrobia,* was significantly reduced in WBL and WBH groups, respectively (LDA > 2.0). These two phyla, while not among the most predominant in the gut microbial community, play an important role in the gut microbial ecosystem. The *Spirochaetes* spp. are capable of degrading polymers (such as xylan, pectin and arabinogalactan), and are found to be positively correlated with the apparent hemicellulose digestibility of pigs (Niu et al., 2015). Species from this phylum invade the colonic epithelium of pigs with swine dysentery (Harris and Glock, 1971), and pigs experimentally infected with *T. suis* (Rutter and Beer, 1975). On the other hand, while not particularly abundant, the species from Verrucomicrobia persistently reside in the gut and in environmental samples. Some members from the phylum Verrucomicrobia express polysaccharide hydrolase activities and are considered potential glycoside hydrolysis generalists in the environment (Cardman et al., 2014).

The significant alterations in the proximal colon microbial community by *A. suum* infection was also evident at the genus level. The genus *Prevotella* was the most dominant genus in the proximal colon (Fig. 2), contributing to 79.58% of total sequences in this study, in agreement with published reports (Lamendella et al., 2011; Poroyko et al., 2010; Looft et al., 2014; Ramayo-Caldas et al., 2016; Yang et al., 2016). *Ascaris* infection tended to increase *Prevotella* abundance, but only in the WBL group. This may help explain a published observation in which *A. suum* infection induced an increase in the relative abundance of *Prevotella* but failed to reach a statistically significant level when the worm burden was very high (Williams et al., 2017). *Prevotella* spp. are generally considered beneficial bacteria and play a key role in carbohydrate metabolism with a unique mucin glycoprotein degradation capability. For example, *Prevotella* is positively correlated with luminal secretory IgA concentrations (Mach et al., 2015) and body weight (Mach et al., 2015; Ramayo-Caldas et al., 2016). Some species utilize xylan, xylose and carboxy-methylcellulose to produce high levels of SCFAs (Flint et al., 2008; Wu et al., 2011). Previous studies demonstrated that the relative abundance of *Prevotella* is associated with increased fiber intake (De Filippo et al., 2010; Wu et al., 2011; Koeth et al., 2013). Of the OTUs significantly impacted by infection, 43 belong to *Prevotella* while 19 of those can be assigned to *Prevotella copri*, which is associated with insulin resistance and improved glucose tolerance (Kovatcheva-Datchary et al., 2015; Pedersen et al., 2016). A diet rich in non-starch polysaccharides tends to increase the abundance of *P. copri* in healthy individuals, resulting in an increase in polysaccharide fermenting capacities (Nilsson et al., 2006, 2008; Johansson et al., 2013; Kovatcheva-Datchary et al., 2015). Together, our findings support a hypothesis that *A. suum* infection may have a broad impact on gut microbial metabolism, and especially carbohydrate metabolism.

Data on pathway analysis provided another piece of evidence that carbohydrate metabolism in the proximal colon microbial community may be significantly impacted by *A. suum* infection. As many as nine repressed KEGG pathways related to carbohydrate

metabolism were associated with infection (Table 4), including starch and sucrose metabolism, and fructose and mannose metabolism. Together, these results suggested that *A. suum* infection was associated with impaired abilities of the proximal colon microbiome to utilize carbohydrates. While subsequent experimental validation using global metabolomics is still needed, we hypothesize that dietary supplements with readily fermentable carbohydrates may help restore or even enhance the function of the microbiota in parasite-infected pigs. Indeed, feeding pigs with probiotic bacteria during *A. suum* infection can prevent the reduced glucose absorption in the small intestine that normally facilitates worm expulsion (Solano-Aguilar et al., 2008). Furthermore, the means to restore microbiome function may contribute to the repair process of the colonic epithelium damaged by infection. This in turn could enhance protective immunity and reduce inflammation through a restored capacity for mucin biosynthesis (Van der Sluis et al., 2006) and subsequent worm expulsion through epithelial cell turnover (Zaiss et al., 2006). These findings further support the use of diets rich in highly fermentable carbohydrates as a nematode control strategy in pigs (Petkevicius et al., 2007).

In this study, our data demonstrated that *A. suum* infection was associated with a significant change in the relative abundance of dozens of taxa in the proximal colon microbial community. Moreover, the infection also had a significant impact on gut microbial diversity. Infection was associated with a significant reduction in species richness such as Chao1, which was in a good agreement with previous observations in other host-parasite systems (Walk et al., 2010; Lee et al., 2014; Giacomin et al., 2015). The decline in microbial diversity was worm burden-independent and became evident even in the worm-free pigs, which result from immunity-mediated strong worm expulsion (Roepstorff et al., 2011). The contemporary concept of biodiversity encompasses not just traditional species-centric indices such as richness and evenness as well as phylogenetic diversity (PD), but also species interactions (Olesen et al., 2007). This paradigm shift has led to the use of network analysis to identify microbial co-occurrence patterns and species interactions.

The myriad microbial species in a complex microbial community are linked by phylogenetically or functionally related species or modules. Regrettably, the OTUs detected in <50% of samples were excluded from this study due to a drastic effect of OTU sparsity on the precision and sensitivity of network inference (Weiss et al., 2016). Among the 778 input OTUs, the majority (523) had one or more interactions with other OTUs, forming a global network with 910 links. This network was highly modular, with a total of 53 modules, displaying typical small-world behaviorial characteristics. These distinct modules, likely resulting from close interactions of phylogenetically or functionally related OTUs, may have important implications in understanding functional diversity in the gut microbial community in helminth-infected pigs. Indeed, at least six modules that displayed strong correlations with SCFA concentrations were identified. One of the notable features of these modules was the concentration of OTUs belonging to the class Clostridia, which are known to produce SCFA. For example, Module #10 was strongly correlated with butyrate. Seventeen of the 20 OTU nodes from this module were from Clostridia, including one of the most important butyrate-producing bacteria, *Faecalibacterium prausnitzii*.

The network analysis also identified OTUs with different topological roles in the network (Fig. 5). The classification based on two of the topological properties, within-module degree (Z) and among-module connectivity (*P*), may facilitate an understanding of the ecological function of certain keystone species in the gut microbial community. A targeted manipulation of generalists, such as those module hubs or connectors, using pre- or probiotics or antibiotics, may have a higher chance of success as one of the microbial community engineering strategies. For example, a partial Mantel test identified a strong correlation between node connectivity of the OTUs assigned to the family Porphyromonadaceae (in the class Mollicutes) with EPG (Table 4). It is conceivable that the EPG trait can be modulated by targeted network elimination or disruption of OTUs assigned to Porphyromonadaceae.

Accumulated evidence has established a solid link between parasitic infection and subsequent bacterial or viral infections, in addition to direct pathophysiological damage to the host by the former (Mansfield and Urban, 1996; Li et al., 2012; Wu et al., 2012). For example, T. suis initiates an interaction with gut resident bacteria to induce muco-hemorrhagic enteritis (Mansfield and Urban, 1996). Moreover, worm-induced modulation of mucosal immunity may directly contribute to the accumulation or infectivity of bacteria at the site of worm attachment. In humans, Campylobacter *jejuni* colitis is associated with concomitant whipworm infection (Shin et al., 2004). Furthermore, the T. suis-induced increase in Campylobacter in the proximal colon of pigs is worm count-dependent (Wu et al., 2012). In this study, we provided further evidence that *A. suum* infection may be associated with an increased risk of secondary bacterial infection. The relative abundance of an OTU assigned to the genus *Campylobacter* was significantly increased in pigs with high worm burdens, suggesting that worms are able to modulate host immunity to increase the local colonization of pathogenic bacteria. Furthermore, understanding the mechanisms of interactions between parasitic infection and subsequent bacterial and viral infections has important practical implications as previous reports indicate that *A. suum* infection compromises the efficacy of a bacterial vaccine (Steenhard et al., 2009). Together, these findings enhance our understanding of the pathophysiological consequences of helminth infection in humans and animals.

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References

Broadhurst, M.J., Ardeshir, A., Kanwar, B., Mirpuri, J., Gundra, U.M., Leung, J.M., Wiens, K.E., Vujkovic-Cvijin, I., Kim, C.C., Yarovinsky, F., Lerche, N.W., McCune, J. M., Loke, P., 2012. Therapeutic helminth infection of macaques with idiopathic chronic diarrhea alters the inflammatory signature and mucosal microbiota of the colon. PLoS Pathog. 8, e1003000.

Cardman, Z., Arnosti, C., Durbin, A., Ziervogel, K., Cox, C., Steen, A.D., Teske, A., 2014. Verrucomicrobia are candidates for polysaccharide-degrading bacterioplankton in an arctic fjord of svalbard. Appl. Environ. Microb. 80, 3749–3756.

Dawson, H.D., Beshah, E., Nishi, S., Solano-Aguilar, G., Morimoto, M., Zhao, A., Madden, K.B., Ledbetter, T.K., Dubey, J.P., Shea-Donohue, T., Lunney, J.K., Urban Jr., J.F., 2005. Localized multigene expression patterns support an evolving Th1/Th2-like paradigm in response to infections with *Toxoplasma gondii* and *Ascaris suum*. Infect. Immun. 73, 1116–1128.

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J.B., Massart, S., Collini, S., Pieraccini, G., Lionetti, P., 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc. Natl. Acad. Sci. U S A 107, 14691–14696.

Deng, Y., Jiang, Y.H., Yang, Y.F., He, Z.L., Luo, F., Zhou, J.Z., 2012. Molecular ecological network analyses. BMC Bioinform. 13.

Flint, H.J., Bayer, E.A., Rincon, M.T., Lamed, R., White, B.A., 2008. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. Nat. Rev. Microbiol. 6, 121–131.

Giacomin, P., Zakrzewski, M., Croese, J., Su, X., Sotillo, J., McCann, L., Navarro, S., Mitreva, M., Krause, L., Loukas, A., Cantacessi, C., 2015. Experimental hookworm infection and escalating gluten challenges are associated with increased microbial richness in celiac subjects. Sci. Rep. 5, 13797.

Harris, D.L., Glock, R.D., 1971. Swine dysentery: a review. Iowa State Univ. Vet. 33, 4–11.

Hooper, L.V., Littman, D.R., Macpherson, A.J., 2012. Interactions between the microbiota and the immune system. Science 336, 1268–1273.

Johansson, E.V., Nilsson, A.C., Ostman, E.M., Bjorck, I.M., 2013. Effects of indigestible carbohydrates in barley on glucose metabolism, appetite and voluntary food intake over 16 h in healthy adults. Nutr. J. 12, 46.
Koeth, R.A., Wang, Z., Levison, B.S., Buffa, J.A., Org, E., Sheehy, B.T., Britt, E.B., Fu, X., Wu, Y., Li, L., Smith, J.D., DiDonato, J.A., Chen, J., Li, H., Wu, G.D., Lewis, J.D., Warrier, M., Brown, J.M., Krauss, R.M., Tang, W.H., Bushman, F.D., Lusis, A.J., Hazen, S.L., 2013. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat. Med. 19, 576–585.
Kovatcheva-Datchary, P., Nilsson, A., Akrami, R., Lee, Y.S., De Vadder, F., Arora, T., Hallen, A., Martens, E., Bjorck, I., Backhed, F., 2015. Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of *Prevotella*. Cell Metab. 22, 971–982.
Lamendella, R., Domingo, J.W., Ghosh, S., Martinson, J., Oerther, D.B., 2011. Comparative fecal metagenomics unveils unique functional capacity of the swine gut. BMC Microbiol. 11, 103.
Lee, S.C., Tang, M.S., Lim, Y.A.L., Choy, S.H., Kurtz, Z.D., Cox, L.M., Gundra, U.M., Cho, I., Bonneau, R., Blaser, M.J., Chua, K.H., Loke, P., 2014. Helminth colonization is associated with increased diversity of the gut microbiota. PLoS Negl. Trop. Dis. 8.
Li, R.W., Wu, S., Li, W., Huang, Y., Gasbarre, L.C., 2011. Metagenome plasticity of the bovine abomasal microbiota in immune animals in response to *Ostertagia ostertagi* infection. PLoS ONE 6, e24417.
Li, R.W., Wu, S., Li, W., Navarro, K., Couch, R.D., Hill, D., Urban Jr., J.F., 2012. Alterations in the porcine colon microbiota induced by the gastrointestinal nematode *Trichuris suis*. Infect. Immun. 80, 2150–2157.
Li, R.W., Li, W., Sun, J., Yu, P., Baldwin, R.L., Urban, J.F., 2016. The effect of helminth infection on the microbial composition and structure of the caprine abomasal microbiome. Sci. Rep. 6, 20606.
Liu, F., Zhang, N., Li, Z., Wang, X., Shi, H., Xue, C., Li, R.W., Tang, Q., 2017. Chondroitin sulfate disaccharides modified the structure and function of the murine gut microbiome under healthy and stressed conditions. Sci. Rep. 7, 6783.
Looft, T., Allen, H.K., Cantarel, B.L., Levine, U.Y., Bayles, D.O., Alt, D.P., Henrissat, B., Stanton, T.B., 2014. Bacteria, phages and pigs: the effects of in-feed antibiotics on the microbiome at different gut locations. ISME J. 8, 1566–1576.
Mach, N., Berri, M., Estelle, J., Levenez, F., Lemonnier, G., Denis, C., Leplat, J.J., Chevaleyre, C., Billon, Y., Dore, J., Rogel-Gaillard, C., Lepage, P., 2015. Early-life establishment of the swine gut microbiome and impact on host phenotypes. Environ. Microbiol. Rep. 7, 554–569.
Maizels, R.M., Balic, A., Gomez-Escobar, N., Nair, M., Taylor, M.D., Allen, J.E., 2004. Helminth parasites–masters of regulation. Immunol. Rev. 201, 89–116.
Mansfield, L.S., Urban, J.F., 1996. The pathogenesis of necrotic proliferative colitis in swine is linked to whipworm induced suppression of mucosal immunity to resident bacteria. Vet. Immunol. Immunopathol. 50, 1.
Masure, D., Vlaminck, J., Wang, T., Chiers, K., Van den Broeck, W., Vercruysse, J., Geldhof, P., 2013a. A role for eosinophils in the intestinal immunity against infective *Ascaris suum* larvae. PLoS Negl. Trop. Dis. 7, e2138.
Masure, D., Wang, T., Vlaminck, J., Claerhoudt, S., Chiers, K., Van den Broeck, W., Saunders, J., Vercruysse, J., Geldhof, P., 2013b. the intestinal expulsion of the roundworm *Ascaris suum* is associated with eosinophils, intra-epithelial T cells and decreased intestinal transit time. PLoS Negl. Trop. Dis. 7, e2588.
Midha, A., Janek, K., Niewienda, A., Henklein, P., Guenther, S., Serra, D.O., Schlosser, J., Hengge, R., Hartmann, S., 2018. The intestinal roundworm *Ascaris suum* releases antimicrobial factors which interfere with bacterial growth and biofilm formation. Front. Cell. Infect. Microbiol. 8, 271.
Miller, L.A., Colby, K., Manning, S.E., Hoenig, D., McEvoy, E., Montgomery, S., Mathison, B., de Almeida, M., Bishop, H., Dasilva, A., Sears, S., 2015. Ascariasis in humans and pigs on small-scale farms, Maine, USA, 2010–2013. Emerg. Infect. Dis. 21, 332–334.
Nilsson, A., Granfeldt, Y., Ostman, E., Preston, T., Bjorck, I., 2006. Effects of GI and content of indigestible carbohydrates of cereal-based evening meals on glucose tolerance at a subsequent standardised breakfast. Eur. J. Clin. Nutr. 60, 1092–1099.
Nilsson, A., Ostman, E., Preston, T., Bjorck, I., 2008. Effects of GI vs content of cereal fibre of the evening meal on glucose tolerance at a subsequent standardized breakfast. Eur. J. Clin. Nutr. 62, 712–720.
Niu, Q., Li, P., Hao, S., Zhang, Y., Kim, S.W., Li, H., Ma, X., Gao, S., He, L., Wu, W., Huang, X., Hua, J., Zhou, B., Huang, R., 2015. Dynamic distribution of the gut microbiota and the relationship with apparent crude fiber digestibility and growth stages in pigs. Sci. Rep. 5, 9938.
Olesen, C., Picard, M., Winther, A.M.L., Gyrup, C., Morth, J.P., Oxvig, C., Moller, J.V., Nissen, P., 2007. The structural basis of calcium transport by the calcium pump. Nature 450, 1036–U1035.
Olesen, J.M., Stefanescu, C., Traveset, A., 2011. Strong, long-term temporal dynamics of an ecological network. PLoS One 6.
Osborne, L.C., Monticelli, L.A., Nice, T.J., Sutherland, T.E., Siracusa, M.C., Hepworth, M.R., Tomov, V.T., Kobuley, D., Tran, S.V., Bittinger, K., Bailey, A.G., Laughlin, A.L., Boucher, J.L., Wherry, E.J., Bushman, F.D., Allen, J.E., Virgin, H.W., Artis, D., 2014. Coinfection. Virus-helminth coinfection reveals a microbiota-independent mechanism of immunomodulation. Science 345, 578–582.
Pedersen, H.K., Gudmundsdottir, V., Nielsen, H.B., Hyotylainen, T., Nielsen, T., Jensen, B.A., Forslund, K., Hildebrand, F., Prifti, E., Falony, G., Le Chatelier, E., Levenez, F., Dore, J., Mattila, I., Plichta, D.R., Poho, P., Hellgren, L.I., Arumugam, M., Sunagawa, S., Vieira-Silva, S., Jorgensen, T., Holm, J.B., Trost, K., Meta, H.I.T.C., Kristiansen, K., Brix, S., Raes, J., Wang, J., Hansen, T., Bork, P., Brunak, S., Oresic, M., Ehrlich, S.D., Pedersen, O., 2016. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature 535, 376–381.
Peng, W., Criscione, C.D., 2012. Ascariasis in people and pigs: new inferences from DNA analysis of worm populations. Infect. Genet. Evol. 12, 227–235.
Petkevicius, S., Thomsen, L.E., Bach Knudsen, K.E., Murrell, K.D., Roepstorff, A., Boes, J., 2007. The effect of inulin on new and on patent infections of *Trichuris suis* in growing pigs. Parasitology 134, 121–127.
Poroyko, V., White, J.R., Wang, M., Donovan, S., Alverdy, J., Liu, D.C., Morowitz, M.J., 2010. Gut microbial gene expression in mother-fed and formula-fed piglets. PLoS ONE 5, e12459.
Pullan, R.L., Smith, J.L., Jasrasaria, R., Brooker, S.J., 2014. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasite Vector 7.
Ramayo-Caldas, Y., Mach, N., Lepage, P., Levenez, F., Denis, C., Lemonnier, G., Leplat, J.J., Billon, Y., Berri, M., Dore, J., Rogel-Gaillard, C., Estelle, J., 2016. Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits. ISME J. 10, 2973–2977.
Rausch, S., Held, J., Fischer, A., Heimesaat, M.M., Kuhl, A.A., Bereswill, S., Hartmann, S., 2013. Small intestinal nematode infection of mice is associated with increased enterobacterial loads alongside the intestinal tract. PLoS One 8.
Roepstorff, A., Mejer, H., Nejsum, P., Thamsborg, S.M., 2011. Helminth parasites in pigs: new challenges in pig production and current research highlights. Vet. Parasitol. 180, 72–81.
Rutter, J.M., Beer, R.J., 1975. Synergism between *Trichuris suis* and the microbial flora of the large intestine causing dysentery in pigs. Infect. Immun. 11, 395–404.
Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12, R60.
Shin, N.Y., Dise, R.S., Schneider-Mergener, J., Ritchie, M.D., Kilkenny, D.M., Hanks, S. K., 2004. Subsets of the major tyrosine phosphorylation sites in Crk-associated substrate (CAS) are sufficient to promote cell migration. J. Biol. Chem. 279, 38331–38337.
Solano-Aguilar, G., Dawson, H., Restrepo, M., Andrews, K., Vinyard, B., Urban, J.F., 2008. Detection of Bifidobacterium animalis subsp lactis (Bb12) in the intestine after feeding of sows and their piglets. Appl. Environ. Microb. 74, 6338–6347.
Steenhard, N.R., Jungersen, G., Kokotovic, B., Beshah, E., Dawson, H.D., Urban Jr., J.F., Roepstorff, A., Thamsborg, S.M., 2009. *Ascaris suum* infection negatively affects the response to a Mycoplasma hyopneumoniae vaccination and subsequent challenge infection in pigs. Vaccine 27, 5161–5169.
Taylor, H.L., Spagnoli, S.T., Calcutt, M.J., Kim, D.Y., 2016. Aberrant *Ascaris suum* nematode infection in cattle, Missouri, USA. Emerg. Infect. Dis. 22, 339–340.
Urban Jr., J.F., Alizadeh, H., Romanowski, R.D., 1988. *Ascaris suum*: development of intestinal immunity to infective second-stage larvae in swine. Exp. Parasitol. 66, 66–77.
Van der Sluis, M., De Koning, B.A.E., De Bruijn, A.C.J.M., Velcich, A., Meijerink, J.P.P., Van Goudoever, J.B., Buller, H.A., Dekker, J., Van Seuningen, I., Renes, I.B., Einerhand, A.W.C., 2006. Muc2-deficient mice spontaneously develop colitis, indicating that Muc2 is critical for colonic protection. Gastroenterology 131, 117–129.
Walk, S.T., Blum, A.M., Ewing, S.A., Weinstock, J.V., Young, V.B., 2010. Alteration of the murine gut microbiota during infection with the parasitic helminth *Heligmosomoides polygyrus*. Inflamm. Bowel Dis. 16, 1841–1849.
Weiss, Y., Class, C., Goldstein, S.L., Hanyu, T., 2016. Key new pieces of the HIMU puzzle from olivines and diamond inclusions. Nature 537, 666.
White, E.C., Houlden, A., Bancroft, A.J., Hayes, K.S., Goldrick, M., Grencis, R.K., Roberts, I.S., 2018. Manipulation of host and parasite microbiotas: survival strategies during chronic nematode infection. Sci. Adv. 4, eaap7399.
Williams, A.R., Krych, L., Fauzan Ahmad, H., Nejsum, P., Skovgaard, K., Nielsen, D.S., Thamsborg, S.M., 2017. A polyphenol-enriched diet and *Ascaris suum* infection modulate mucosal immune responses and gut microbiota composition in pigs. PLoS ONE 12, e0186546.
Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.Y., Keilbaugh, S.A., Bewtra, M., Knights, D., Walters, W.A., Knight, R., Sinha, R., Gilroy, E., Gupta, K., Baldassano, R., Nessel, L., Li, H., Bushman, F.D., Lewis, J.D., 2011. Linking long-term dietary patterns with gut microbial enterotypes. Science 334, 105–108.
Wu, S., Li, R.W., Li, W., Beshah, E., Dawson, H.D., Urban Jr., J.F., 2012. Worm burden-dependent disruption of the porcine colon microbiota by *Trichuris suis* infection. PLoS ONE 7, e35470.
Yang, H., Huang, X.C., Fang, S.M., Xin, W.S., Huang, L.S., Chen, C.Y., 2016. Uncovering the composition of microbial community structure and metagenomics among three gut locations in pigs with distinct fatness. Sci Rep-UK, 6.
Zaiss, M.M., Rapin, A., Lebon, L., Dubey, L.K., Mosconi, I., Sarter, K., Piersigilli, A., Menin, L., Walker, A.W., Rougemont, J., Paerewijck, O., Geldhof, P., McCoy, K.D., Macpherson, A.J., Croese, J., Giacomin, P.R., Loukas, A., Junt, T., Marsland, B.J., Harris, N.L., 2015. The intestinal microbiota contributes to the ability of helminths to modulate allergic inflammation. Immunity 43, 998–1010.
Zaiss, D.M., Yang, L., Shah, P.R., Kobie, J.J., Urban, J.F., Mosmann, T.R., 2006. Amphiregulin, a TH2 cytokine enhancing resistance to nematodes. Science 314, 1746.
Zhou, J.Z., Deng, Y., Luo, F., He, Z.L., Yang, Y.F., 2011. Phylogenetic molecular ecological network of soil microbial communities in response to elevated CO_2. Mbio 2.