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Original Research Article

Porcine circovirus type 2 (PCV2) and *Campylobacter* infection induce diarrhea in piglets: Microbial dysbiosis and intestinal disorder

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ABSTRACT

Diarrhea is considered to be associated with microbial dysbiosis caused by infection of pathogens but poorly understood. We herein characterized the colonic microbiota of diarrheal early-weaning piglets infected with porcine circovirus type 2 (PCV2) and *Campylobacter*. *Campylobacter* infection significantly decreased species richness and Shannon diversity index of colonic microbiota together with a significant increase in the proportion of *Campylobacter* and Enterobacteriaceae, whereas no significant difference on the above indexes was observed in piglets infected with PCV2 compared with healthy piglets. PCV2 and *Campylobacter* infection could disturb the homeostasis of colonic microbiota through deterioration of ecological network within microbial community, and specially *Campylobacter* performed as a module hub in ecological networks. The microbial dysbiosis caused metabolic dysfunction and led to a remarkable reduction in production of short chain fatty acids, following by a higher pH level in colon cavity. *Campylobacter* infection disturbed the function of colonic tract barrier observed in terms of significant lower relative expression of claudin-1, occluding, and zonula occludens protein-1 genes, and PCV2 infection induced intestinal inflammation together with a higher permeability of colon. Generally, these results suggested that PCV2 and *Campylobacter* infection could induce microbial dysbiosis and metabolic dysfunction, and cause intestinal disorder, all of which finally were associated to contribute to the diarrhea of early-weaning piglets.

1. Introduction

Diarrhea is the leading cause of child death in many parts of the world and increases mortality in neonatal and early-weaning piglets (Bik and Relman, 2014; Yang et al., 2017b). The invasion of pathogens has the greatest potential to cause microbial dysbiosis as seen in many animal models (Bik and Relman, 2014; Giallourou et al., 2018; Kamada et al., 2013). Diarrhea is considered to be associated with intestinal disorders in terms of microbial dysbiosis and an unbalanced interaction between intestinal microbiota and mucosal immune system (Gresse et al., 2017), whereas the underlying mechanisms involved in these processes are poorly understood.

It is well known that intestinal commensal microbiota plays a crucial role in the nutrition metabolism and immune organ maturation of host and can prevent the invasion of pathogens by competing for receptors and enteric nutrients, stimulating the innate immune system, and producing antimicrobial compounds (Sommer et al., 2017; Valdes et al., 2018). Accordingly, the homeostasis of intestinal microbiota is critical to host health. Abnormal shift of intestinal microbiota is related to many intestinal diseases and numerous studies have reported the disturbance in intestinal microbiota of humans and piglets suffering from

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diarrhea, whereas most of these studies focused on species richness and abundance (Gresse et al., 2017; Maharshak et al., 2018; Pop et al., 2014). However, the stability of microbial ecology system depends on the number of species and their abundance, as well as the complex interactions within the community (Coyte et al., 2015; Yang et al., 2017a). A complicated ecological network is obtained through interspecific interactions, by which microbiota maintains a dynamic balance and accomplishes systemic function which contributes to host health (Montoya et al., 2006). Additionally, the intestinal microbiome serves as an auxiliary metabolic organ and can express more than 100-fold genes of the host, and these genes are involved in carbohydrate, energy, lipid, and amino acid metabolisms (Clarke et al., 2014). During these metabolic processes, microbial fermentation of complex plant-derived polysaccharides can acquire short chain fatty acids (SCFA) which contribute to the health of host (Morrison and Tom, 2016). Thus, disturbance of intestinal microbial homeostasis can cause microbial metabolic dysfunction, and previous studies have revealed that bacterial and viral infections could cause the disruption of beneficial function of microbial community (Munyaka et al., 2016; Ward et al., 2016).

As we known, the infection of pathogenic bacteria *Campylobacter* species and virus porcine circovirus type 2 (PCV2) can cause gastroenteritis and then further trigger the diarrhea (Kim et al., 2004; Kirk et al., 2016). *Campylobacter* infection is one of the main bacterial causes of diarrhea and of 'environmental enteropathy' and body growth failure. *Campylobacter* species like *Campylobacter jejuni* and *Campylobacter concisus* could cause human diarrhea diseases which originate from piglets via consumption of contaminated meat products (de Vries et al., 2017; Rosner et al., 2017). Also, PCV2 infection is widespread in swine industry (Baró et al., 2015). Though numerous studies have reported the intestinal microbiota of piglets infected with *Campylobacter* and PCV2, these studies mainly focused on its composition (Burrough et al., 2013; van Sambeek et al., 2016; Yang et al., 2017b). This study systemically reports the colonic microbiota through the assessment of microbial composition, interspecific interactions, functional gene composition, and metabolite of microbes, and also further explores the relevance with colonic inflammation of early-weaning piglets infected with *Campylobacter* and PCV2.

2. Materials and methods

All the experimental procedures used in the study were agreed by the Committee on Research Ethics of the Department of Laboratory Animal Science, Nanchang University.

2.1. Animals and sample collection

The colonic content samples were collected from healthy ($n = 10$, control group) and diarrheal ($n = 17$) piglets, within 1 week after weaning, in a piglets farm (Qiyi, Jiangxi province, China) which reared 30,000 piglets. The selection of diarrheal piglets was based on a variety of clinical signs including inappetence, vomiting, loss of weight, and severe watery diarrhea. Among these diarrheal piglets, 8 and 9 individuals respectively came from 2 different rooms with obvious difference in fecal moisture. The pH in colon cavity was detected by a pH meter (Mettler toledo, FE28). All the piglets were sacrificed by euthanasia. The content in the middle of colon from each piglet was collected as one sample and frozen at −80 °C.

The presence of pseudorabies virus (PRV), PCV2, classical swine fever virus (CSFV), porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), porcine group A rotavirus (GAR), and porcine deltacoronavirus (PDCoV) in colon content samples was detected based on the gene copies of virus using multiplex and real-time PCR. According to the determining results, PRV, PCV2, and PDCoV were detected, and the copy number of PRV and PDCoV was very low in the piglets (Appendix Table 1). Whereas the copy number of PCV2 from 9 piglets in the same room was remarkably higher than that in other piglets ($P < 0.05$), and the value for copy number from 9 piglets was 26- and 17-fold in healthy piglets and another batch of diarrheal piglets, respectively. Accordingly, these 9 piglets were confirmed to be infected with PCV2, thus this group is named VI group. Another 8 diarrheal individuals were classified as unknown pathogen group (PB group). The healthy piglets were classified as control group.

2.2. 16S rRNA sequencing and colonic microbiota analysis

Qiagen PowerFecal Kit was used to extract DNA from the collected digesta. The 16S rRNA V3 to V4 region was amplified and barcoded with fusion primers of 341F and 805R followed by High-throughput sequencing using the Illumina MiSeq platform. The raw sequences were sorted according to the barcodes, then chimera sequences were filtered with UCHIME. Classification of sequences into operational taxonomic units (OTU) was done at 97% similarity level using QIIME version 1.9.1 after the removal of low-quality scores (Q score, 20). The UCLUST method was then used to assign taxonomies to each OTU. Alpha diversity (number of OTU; Chao1 estimator of richness; abundance-based coverage estimator [ACE]; Shannon diversity indices) and beta diversity (Weight UniFrac principal coordinates analysis [PCoA]) analyses were also performed using QIIME version 1.9.1. Statistical Analysis of Metagenomics Profiles (STAMP) and Analysis of Similarity (ANOSIM) were respectively employed to analyze statistical difference in the relative abundance of microbiota and the whole microbial community by Welch's *t*-test.

Functional prediction of microbial communities was performed by PICRUSt (Langille et al., 2013). And then, the non-metric multidimensional scaling (NMDS) and ANOSIM were employed to evaluate the overall differences in predicted microbial functional composition. Statistical analysis of microbial function was performed using STAMP. All of the sequencing data are available in the Sequence Read Archive (SRA) database at NCBI under BioProject ID PRJNA549283 and accession number SRR9312921, SRR9312920, and SRR9312919.

2.3. Molecular ecological network construction and visualization

Random Matrix Theory (RMT)-based approach was used to construct molecular ecological network through an open-accessible pipeline (Deng et al., 2012). The steps involved in construction of 3 networks are as follows. First, the absolute values of the correlation matrix are taken to obtain a similarity matrix, and this allows measurement of the degree of consistency between the abundance distributions of the individual OTU in different samples. Second, in order to obtain an adjacency matrix, an appropriate threshold is used for defining network structure, s_t, which encodes the strength of the connection between each pair of nodes. Third, the sub-modules within a large module are detected by fast-greedy modularity optimization. The visualization of network was performed by Circos and Cytoscape 3.2.0, respectively.

2.4. SCFA analysis and relative expression of inflammation and tight junction protein genes

Concentrations of acetate, propionate, butyric, and valeric acids were analyzed in colonic content by gas chromatography as previously described (Zhou et al., 2013). Total RNA was extracted from

Table 1
Diversity indices used in this study.

Groups[1]	Diversity index				ECS, %
	OTU	Chao1	ACE	Shannon	
Control	6,168 ± 655[b]	14,392 ± 1,523[b]	15,488 ± 1,657[b]	8.02 ± 0.31[b]	95.0 ± 0.52
PB	3,908 ± 418[a]	9,690 ± 1,031[a]	10,306 ± 1,091[a]	6.05 ± 0.39[a]	96.9 ± 0.36
VI	4,540 ± 375 [ab]	10,738 ± 823 [ab]	11,519 ± 909 [ab]	7.14 ± 0.34 [ab]	96.3 ± 0.28

OTU = operational taxonomic unit; ACE = abundance-based coverage estimator; ECS = Good's estimated sample coverage.
[a, b] Values with different superscripts, within the same column, are significantly different ($P < 0.05$).
[1] Control group, healthy piglets; PB group, piglets infected with *Campylobacter*; VI group, piglets infected with porcine circovirus type 2.

colonic tissue and then reverse-transcribed to cDNA by PrimeScript RT reagent Kit (Takara, Japan). Gene expression of interferon-γ (*INF-γ*), tumor necrosis factor-α (*TNF-α*), interleukin-6 (*IL-6*), Claudin-1, Occludin, Zonula occludens protein-1 (*ZO-1*), and junctional adhesion molecule-1 (*JAM1*) genes was measured by real-time PCR (Mastercycler ep realplex; Eppendorf, Germany). The quantitative primer of reference genes is listed in Appendix Table 2. Based on the cycle threshold (CT) values, $2^{-\Delta\Delta CT}$ method was used to calculate the genes expression, and the value stood for n-fold difference relative to the calibrator.

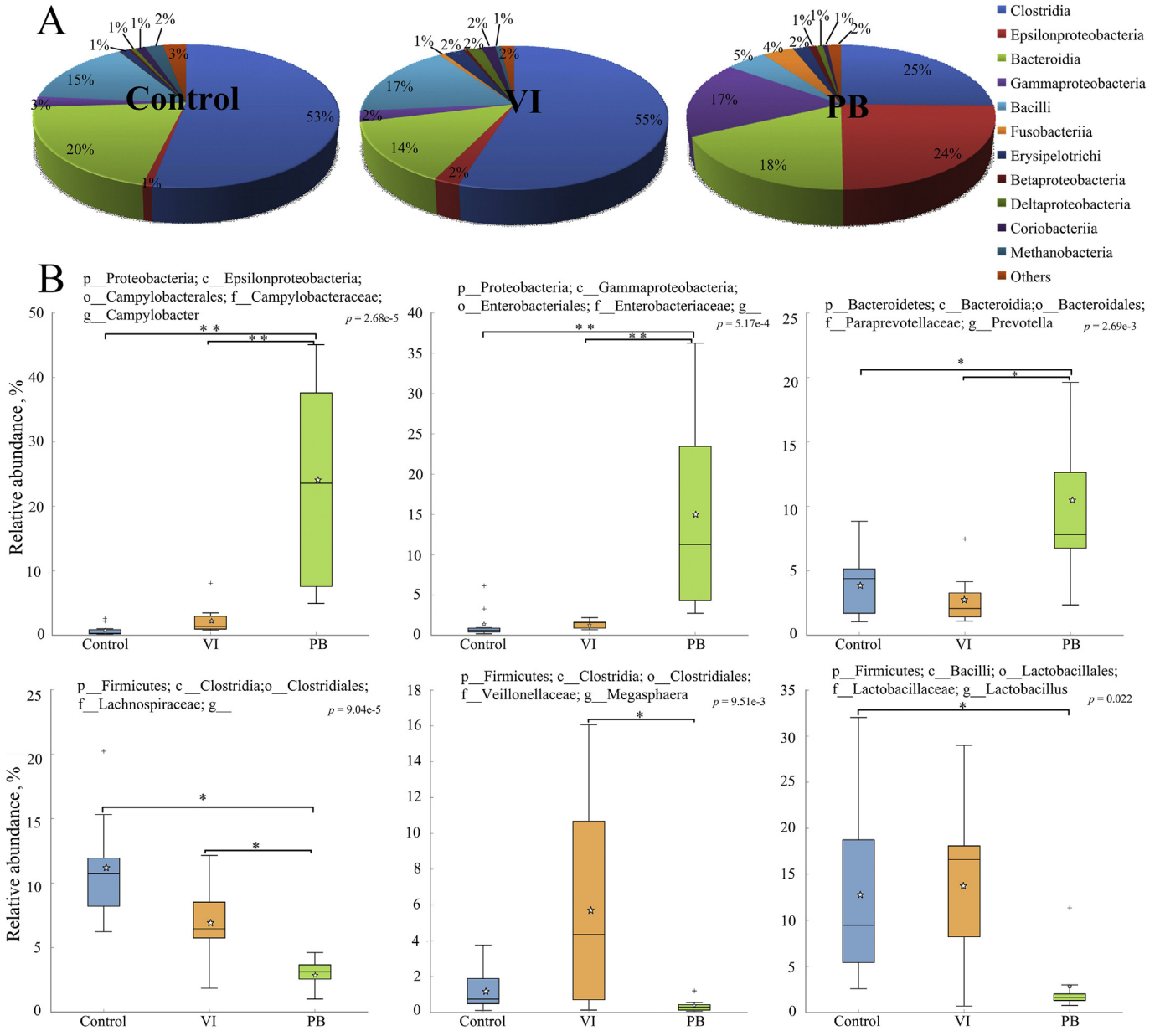

Fig. 1. Relative abundance (A) of different microbial classes (≥a cutoff value of 0.6%) and box plots (B) showing significant variations of relative abundances of microbiota in the colon of piglets. p = phylum; c = class; o = order; f = family; g = genus; s = species. Control group, healthy piglets; VI group, piglets infected with porcine circovirus type 2; PB group, piglets infected with *Campylobacter*. The median value is shown as a line within the box; +, outlier; *, $P < 0.05$; **, $P < 0.01$; ☆, mean value.

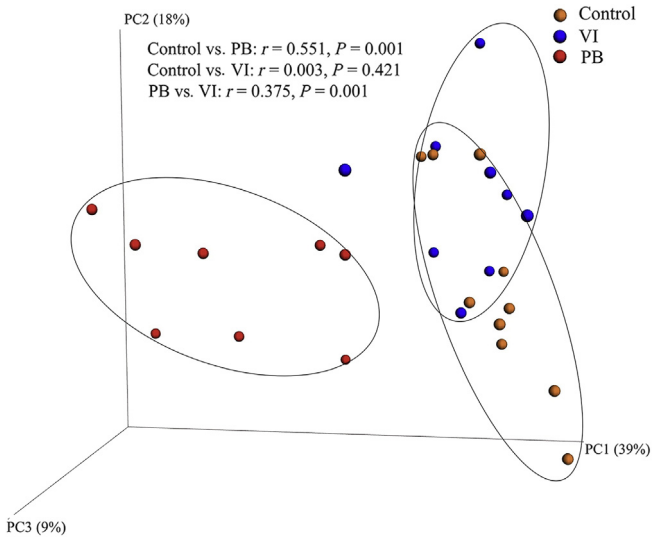

Fig. 2. Weight UniFrac principal coordinates (PC) analysis of the microbial communities. Control group, healthy piglets; VI group, piglets infected with porcine circovirus type 2; PB group, piglets infected with *Campylobacter*.

2.5. Statistical analysis

Data from alpha diversity indices of microbes, pH, SCFA, and gene expression were subjected to a one-way ANOVA and the differences among the means were tested by Duncan's multiple range test (SPSS 16.0). The level of significance was set at $P < 0.05$.

3. Results

3.1. Colonic microbial change in diarrheal piglets

A total of 2,068,064 high-quality sequences were obtained from the colonic content collected from healthy piglets (control group) and piglets infected with PCV2 (VI group) and *Campylobacter* (PB group), respectively. As shown in Table 1, control group presented the greatest number of OTU and the highest Chao1, ACE and Shannon diversity indexes, all of which remarkably differed from those in PB group ($P < 0.05$), whereas no significant difference was observed between VI and control groups.

Nearly all reads were from 5 phyla (relative abundance $\geq$ 1%), among which Firmicutes and Bacteroidetes were 2 predominant phyla in control (69.2% and 20.2%, respectively), VI (74.2% and 13.9%, respectively), and PB (32.3% and 18.1%, respectively) groups (Appendix Fig. 1). Additionally, Proteobacteria predominated in PB group (increased from 4.5% to 43.6% compared with control group). Specifically, control and VI groups were enriched with classes of Clostridia (53.0% and 54.9%, respectively), Bacteroidia (20.2% and 13.9%, respectively), and Bacilli (14.9% and 16.8%, respectively), and the predominant classes in PB group were Clostridia (25.3%), Epsilonproteobacteria (24.5%), Bacteroidia (18.2%), and Gammaproteobacteria (17.1%) (Fig. 1A).

The proportions of *Campylobacter* (increased from 2.3% to 24.1%), Enterobacteriaceae (increased from 1.4% to 14.8%) and *Prevotella* (increased from 3.9% to 10.6%) in PB group were remarkably higher than those in the other 2 groups (Fig. 1B, $P < 0.05$). There was also a significant reduction in the proportion of Lachnospiraceae (decreased from 6.6% to 3.0%), and *Lactobacillus* (decreased from 12.6% to 2.3%) in PB group compared with control group ($P < 0.05$), and a significant increase in *Megasphaera* (increased from 0.4% to 5.7%) presented in VI group compared to PB group ($P < 0.05$). As shown in Fig. 2, PCoA analysis suggested that the microbial community in PB and control groups clustered separately into 2 groups, whereas the microbial community in VI group was similar with that in control group. ANOSIM analysis further confirmed the significant difference in whole microbial community between PB and control ($r = 0.340$, $P = 0.003$), or VI ($r = 0.352$, $P = 0.003$) groups.

3.2. Ecological network of colonic microbes in diarrheal piglets

The circos plot and ecology network exhibited taxonomic composition and interspecific interactions within the microbial community in the colon of piglets (Fig. 3A). The control, VI and PB networks comprised OTU from 17 classes, among which the dominant classes were Clostridia, Bacteroidia, and Bacilli (Appendix Table 3). Numerous edges observed within the circos and networks presented the interspecies interactions between different OTU, and gray and red edges respectively indicated cooperative and competitive microbial interactions. As shown in Fig. 3B and Appendix Tables 3 and 4, 22 sub-modules ($\geq$5 nodes) were observed in control network, which consisted of 697 nodes (OTU) and 3,037 edges (including 2,930 Gy and 107 red edges, respectively), and C1 (131 OTU), C2 (98 OTU), C7 (64 OTU), and C4 (53 OTU) were 4 largest sub-modules ($\geq$50 OTU). VI network contained 740 nodes and 3,160 edges (including 3,140 gray and 20 red edges, respectively) and had 23 sub-modules ($\geq$5 nodes), among which V1(143 OTU), V2 (122 OTU), and V6 (55 OTU) were 3 largest sub-modules. In PB network, 13 sub-modules ($\geq$5 nodes) were observed in the network, which consisted of 694 nodes and 2,765 edges (including 2,166 gray and 599 red edges, respectively) had 3 largest sub-modules, P1 (140 OTU), P2 (120 OTU), and P3 (118 OTU). In these 3 networks, 81.3% OTU belonged to Clostridia, Bacteroidia, and Bacilli, and many OTU from the same class were found to be clustered within one module. The positive interaction predominated in these 3 networks, whereas the proportion of negative interaction was remarkably increased in PB network compared to control and VI networks, especially in the sub-module P1 and P4.

In the network, the majority of OTU were peripherals, and some of them served as connectors or module hubs (Fig. 4). As shown in Table 2, the total number of connectors and module hubs in the VI and PB networks was much fewer than that in the control network, and the fewest number was observed in the VI network. There were 31 OTU from Bacteroidia, Bacilli, and Clostridia serving as module hubs, and 3 OTU from Clostridia and Bacteroidia serving as connectors in the control network. In the VI network, the module hubs and connectors were performed by 14 OTU from Bacteroidia and Clostridia serving as module hubs. In the PB network, the module hubs and connectors were performed by 24 OTU from Clostridia, Bacteroidia, Epsilonproteobacteria, Actinobacteria, Coriobacteriia, Bacilli, Betaproteobacteria, Spirochaetes and unclassified; notably, 2 OTU (OTU6849 and 14,570) from *Campylobacter* served as module hubs, and lots of negative interaction were found between OTU14570 and another 102 OTU.

3.3. Functional predictions of colonic microbiota

NMDS ordination and ANOSIM analysis suggested that microbial functional compositions in the colon of piglets from PB group significantly differed from those from control ($r = 0.551$, $P = 0.001$) and VI ($r = 0.375$, $P = 0.001$) groups (Appendix Fig. 2). Control and VI groups had similar microbial function, and only several microbial functional genes proportion related to amino acid metabolism (taurine and hypotaurine metabolism and tryptophan metabolism), and carbohydrate metabolism (fructose and mannose metabolism) were remarkably increased in VI group ($P < 0.05$), which had less

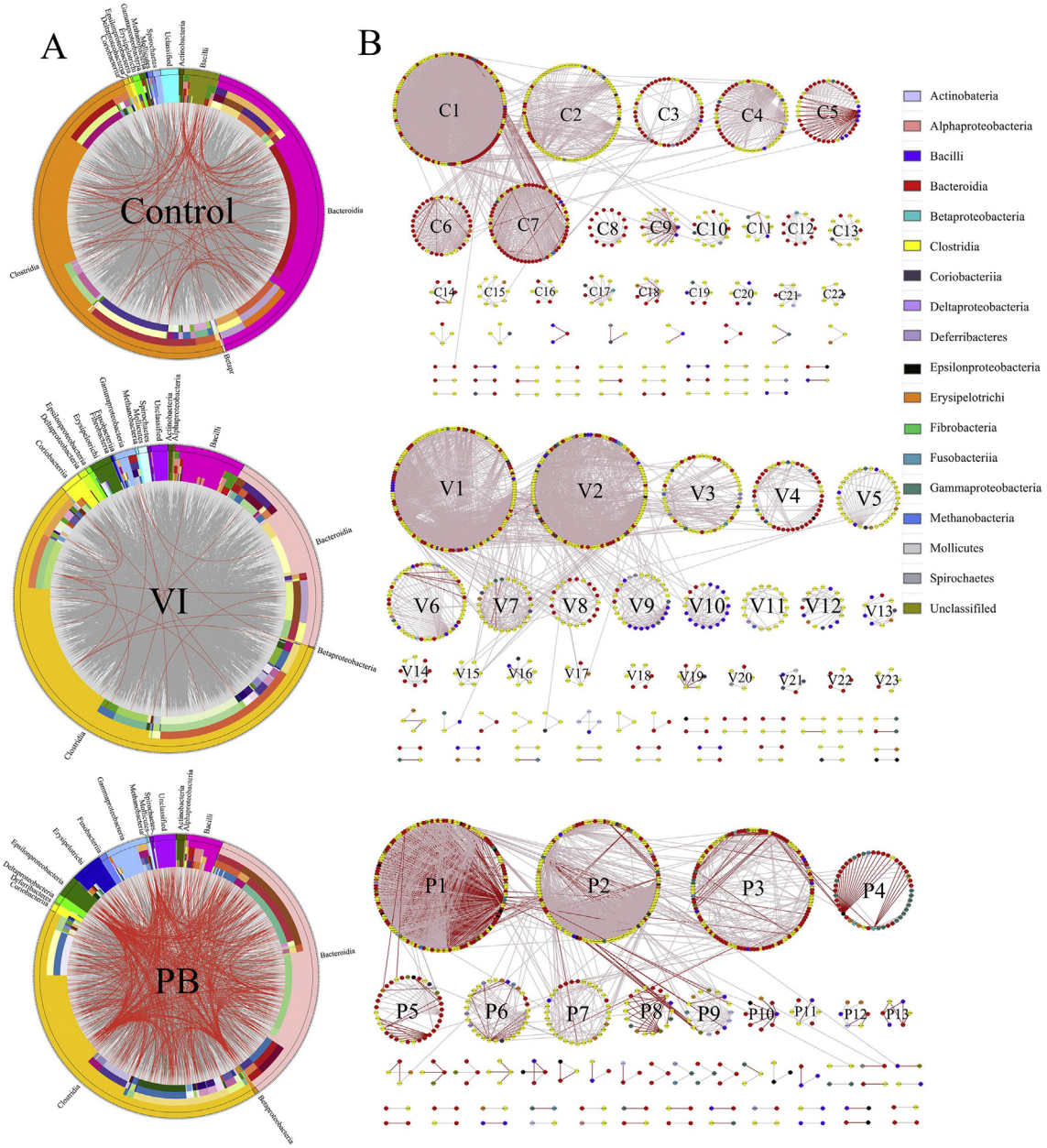

Fig. 3. Circular plot (A) shows the distribution of OTU at different classification levels. Ecological network (B) describes the sub-modules and the interspecific interaction within the colonic microbial community of piglets. Control group, healthy piglets; VI group, piglets infected with porcine circovirus type 2; PB group, piglets infected with *Campylobacter*. The taxonomic levels were class, order, family, genera, and species from the outside to the inside of the circle, respectively.

proportion of genes involved in cyanoamino acid metabolism, phenylpropanoid biosynthesis, and starch and sucrose metabolism (Fig. 5A). Compared to control and VI groups, the colonic microbiota in PB group had more genes involved in glycan biosynthesis and metabolism (lipopolysaccharide biosynthesis and lipopolysaccharide biosynthesis proteins), energy metabolism (carbon fixation pathways in prokaryotes, nitrogen metabolism, and oxidative phosphorylation), carbohydrate metabolism (citrate cycle [TCA], glyoxylate and dicarboxylate metabolism, and propanoate metabolism), and amino acid metabolism (glutathione metabolism, lysine degradation, and tryptophan metabolism) ($P < 0.05$); a significant decrease was observed in genes related to carbohydrate metabolism (amino sugar and nucleotide sugar metabolism, galactose metabolism, glycolysis/gluconeogenesis, pentose and glucuronate interconversions, pentose phosphate pathway), amino acid metabolism (cyanoamino acid metabolism, arginine and proline metabolism, and cysteine and methionine metabolism), methane metabolism, peptidases, and pyrimidine metabolism ($P < 0.05$, Fig. 5B and C). Additionally, fructose and mannose metabolism in PB group was remarkably suppressed than that in VI group ($P < 0.05$).

3.4. SCFA level and inflammation-related and tight junction protein gene expression

As shown in Table 3, PB group had the lowest concentration of acetic acid, propionic acid, and butyric acid, all of which remarkably differed from that in the other 2 groups ($P < 0.05$). Although control group had the highest concentration of acetic acid and butyric acid, it presented no significant difference from VI group ($P > 0.05$). A

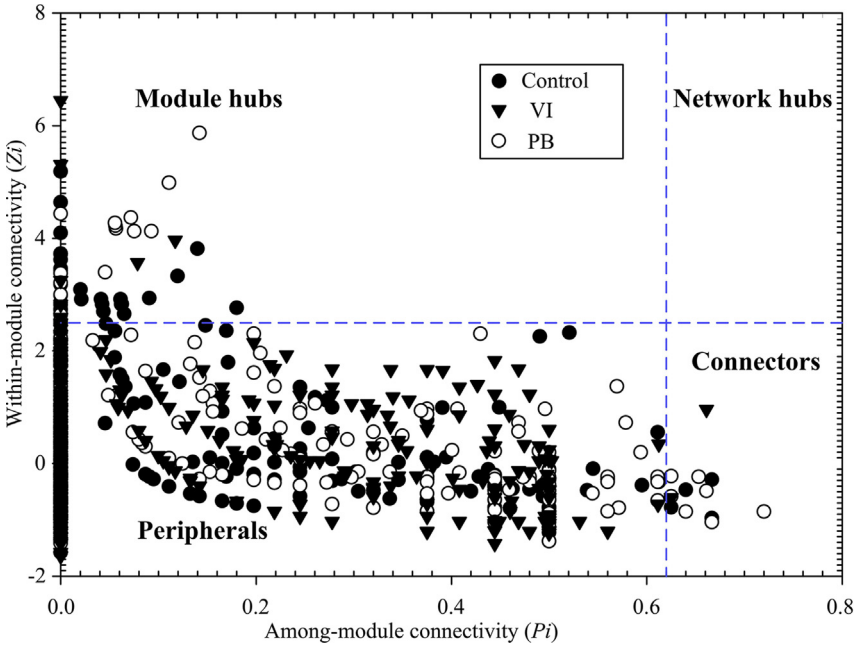

Fig. 4. *Zi-Pi* plot showing the distribution of operational taxonomic units (OTU) based on their topological roles. Control group, healthy piglets; VI group, piglets infected with porcine circovirus type 2; PB group, piglets infected with *Campylobacter*.

significantly higher content of valeric acid was detected in VI group compared to control and PB groups ($P < 0.05$). The highest and lowest pH in colonic cavity were respectively observed in PB and control groups, and pH significantly differed from each other among all groups ($P < 0.05$). As shown in Fig. 6A, significantly higher relative expression level of *IFN-γ* and *IL-6* was recorded in VI group compared to control group ($P < 0.05$). The relative expression level of the claudin-1, occluding, and *ZO-1* genes in VI and PB groups was detected remarkably lower than that in control group ($P < 0.05$), and PB group had a higher relative expression level of *ZO-1* than VI group ($P > 0.05$, Fig. 6B).

4. Discussion

Early-weaning piglets have an immature digestive system, and thus are susceptible to disease, especially diarrhea, when food converts from digestible watery milk to a less digestible solid (Adams et al., 2019; Kiers et al., 2003). Growing evidences from animal and humans supported that the complex and dynamic microbial community played a key role in host health (Marchesi et al., 2016; Sommer et al., 2017; Valdes et al., 2018). Previous studies have demonstrated that pathogens infection induced host acute intestinal diseases associated with microbial dysbiosis (Giallourou et al., 2018; Minamoto et al., 2014). *Campylobacter* species like *C. jejuni* and *C. concisus,* Gram-negative bacteria, are the most frequently isolated bacteria in piglets and children under 5 years with diarrhea (de Vries et al., 2017; Rosner et al., 2017), and PCV2-induced diarrhea is popular in piglet industry worldwide (Baró et al., 2015). In this study, *Campylobacter* (increased from 2.3% to 24.1%) was observed to predominate in the colon of piglets in the PB group, suggesting that *Campylobacter* infection was responsible for piglet diarrhea. Besides, the proportion of Enterobacteriaceae was also observed to increase in the colon together with a significant reduction in *Lactobacillus*, consistent with those finding in the previous studies (Hermann-Bank et al., 2015; Yang et al., 2017b). However, in the present study, our results suggested that PCV2 infection did not cause a remarkably change in the composition of microbial community compared with that in heathy piglets, consistent with the study in piglets inoculated with PCV2 (van Sambeek et al., 2016). Fecal-oral and oral-nasal were confirmed to be the most efficient infectious routes of pathogens, including *Campylobacter* and PCV2, due to the simultaneous exposure of susceptible early-weaning piglets to contaminated respiratory, digestive, and urinary secretions (Nathues et al., 2013; Patterson et al., 2011; Sinha et al., 2011). Accordingly, fecal-oral route may be one way of the transmission of *Campylobacter* and PCV2 infection in early-weaning piglets farming in this study.

Microbial community, a complicated ecology system, can from a complex ecological network through interspecies interactions and maintains its dynamic balance by this network in host gut (Coyte et al., 2015). Within an ecological network, microbial interactions may occur because they perform similar or complementary functions or share environmental conditions (Zhou et al., 2011). In the present study, microbial cooperation dominated in the colon, which is an important place for microbial fermentation, and cooperative interaction of microbes could promote the fermentation metabolism of plant-derived polysaccharides, consistent with the study on grass carp (Yang et al., 2019). However, mass proliferation of *Campylobacter* inhibited many other bacterial species, and this is why the percentage of other taxa decreased in the colon of piglets infected with *Campylobacter*. Consistent with our results, *Campylobacter* species was observed to be the predominant bacterial agent in diarrheal patients infected with *Campylobacter* (Chi et al., 2018; de Wit et al., 2001).

In network, peripherals may represent specialists whereas connectors and module hubs may be related to generalists and network hubs as super-generalists based on ecological viewpoint (Olesen et al., 2007). Various species performed their characteristic roles in the micro-ecology system, in which the keystone strains served as connectors or module hubs and contributed to maintaining the stability of network (Kaiser-Bunbury et al., 2010; Ramos-Jiliberto et al., 2012). Thus, the entire network would be fragmented if the key species like generalists disappear. In the present study, a remarkable reduction in the total number of generalists was observed in ecological network of diarrheic piglets. Accordingly, our results suggested that the infection of PCV2

Table 2
Topological roles of colonic microbiota.

Groups[1]	Topological roles	OTU	Module number	SpeciesPhylogenetic associations
Control	Module hub	OTU32050	C6	c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__
	Module hub	OTU24026	C7	c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__
	Module hub	OTU23910	C1	c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__
	Module hub	OTU26246	C6	c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__
	Module hub	OTU4283	C3	c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__copri
	Module hub	OTU24053	C7	c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__
	Module hub	OTU4421	C1	c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__
	Module hub	OTU26054	C1	c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__
	Module hub	OTU23764	C7	c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__
	Module hub	OTU30540	C7	c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__
	Module hub	OTU13831	C5	c__Bacilli; o__Bacillales; f__Bacillaceae; g__Bacillus; s__cereus
	Module hub	OTU39348	C5	c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Lactococcus; s__
	Module hub	OTU795	C1	c__Clostridia; o__Clostridiales; f__; g__; s__
	Module hub	OTU12118	C4	c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__; s__
	Module hub	OTU24509	C4	c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Clostridium; s__
	Module hub	OTU27959	C8	c__Clostridia; o__Clostridiales; f__Lachnospiraceae
	Module hub	OTU26235	C4	c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
	Module hub	OTU9606	C2	c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
	Module hub	OTU2044	C3	c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
	Module hub	OTU32308	C1	c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__
	Module hub	OTU6466	C2	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
	Module hub	OTU15446	C2	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
	Module hub	OTU34926	C1	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
	Module hub	OTU33464	C13	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
	Module hub	OTU27901	C1	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
	Module hub	OTU23766	C9	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__
	Module hub	OTU25632	C1	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__
	Module hub	OTU24620	C1	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__
	Module hub	OTU6745	C2	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__
	Connector	OTU23082	C9	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__
	Connector	OTU4140	C6	c__Clostridia; o__Clostridiales; f__; g__; s__
	Connector	OTU18686	C2	c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__
	Connector	OTU6252	C7	c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__
VI	Module hub	OTU899	V14	c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__
	Module hub	OTU15904	V1	c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__
	Module hub	OTU34296	V4	c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__copri
	Module hub	OTU6252	V1	c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__
	Module hub	OTU11210	V4	c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__
	Module hub	OTU38933	V2	c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
	Module hub	OTU20799	V2	c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
	Module hub	OTU25353	V1	c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospira; s__
	Module hub	OTU30446	V4	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
	Module hub	OTU31253	V12	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Faecalibacterium; s__prausnitzii
	Module hub	OTU4632	V5	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__
	Module hub	OTU28229	V6	c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Mitsuokella
	Connector	OTU26279	V8	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__
	Connector	OTU1722	V3	c__Clostridia; o__Clostridiales; f__; g__; s__
PB	Module hub	OTU28900	P9	c__Actinobacteria; o__Bifidobacteriales; f__Bifidobacteriaceae; g__Bifidobacterium; s__
	Module hub	OTU7457	P5	c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__
	Module hub	OTU20810	P5	c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__fragilis
	Module hub	OTU30683	P3	c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__
	Module hub	OTU17670	P3	c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__
	Module hub	OTU26626	P3	c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__copri
	Module hub	OTU6252	P1	c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__
	Module hub	OTU34520	P10	c__Bacilli; o__Lactobacillales; f__Enterococcaceae; g__Enterococcus; s__cecorum
	Module hub	OTU28884	P2	c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__
	Module hub	OTU15258	P1	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
	Module hub	OTU14922	P1	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
	Module hub	OTU38258	P1	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
	Module hub	OTU30678	P1	c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Phascolarctobacterium; s__
	Module hub	OTU5386	P1	c__Betaproteobacteria; o__Burkholderiales; f__Comamonadaceae; g__Comamonas; s__
	Module hub	OTU6849	P4	c__Epsilonproteobacteria; o__Campylobacterales; f__Campylobacteraceae; g__Campylobacter; s__
	Module hub	OTU14570	P1	c__Epsilonproteobacteria; o__Campylobacterales; f__Campylobacteraceae; g__Campylobacter; s__
	Module hub	OTU10968	P8	Unclassified
	Connector	OTU31745	P5	c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__; s__
	Connector	OTU14218	P5	c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__
	Connector	OTU8947	P7	c__Clostridia; o__Clostridiales; f__; g__; s__
	Connector	OTU5328	P3	c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__; s__
	Connector	OTU21769	P4	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
	Connector	OTU39111	P7	c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__
	Connector	OTU11716	P3	c__Spirochaetes; o__Sphaerochaetales; f__Sphaerochaetaceae; g__Sphaerochaeta; s__

OTU = operational taxonomic unit; c = class; o = order; f = family; g = genus; s = species.
[1] Control group, healthy piglets; VI group, piglets infected with porcine circovirus type 2; PB group, piglets infected with *Campylobacter*.

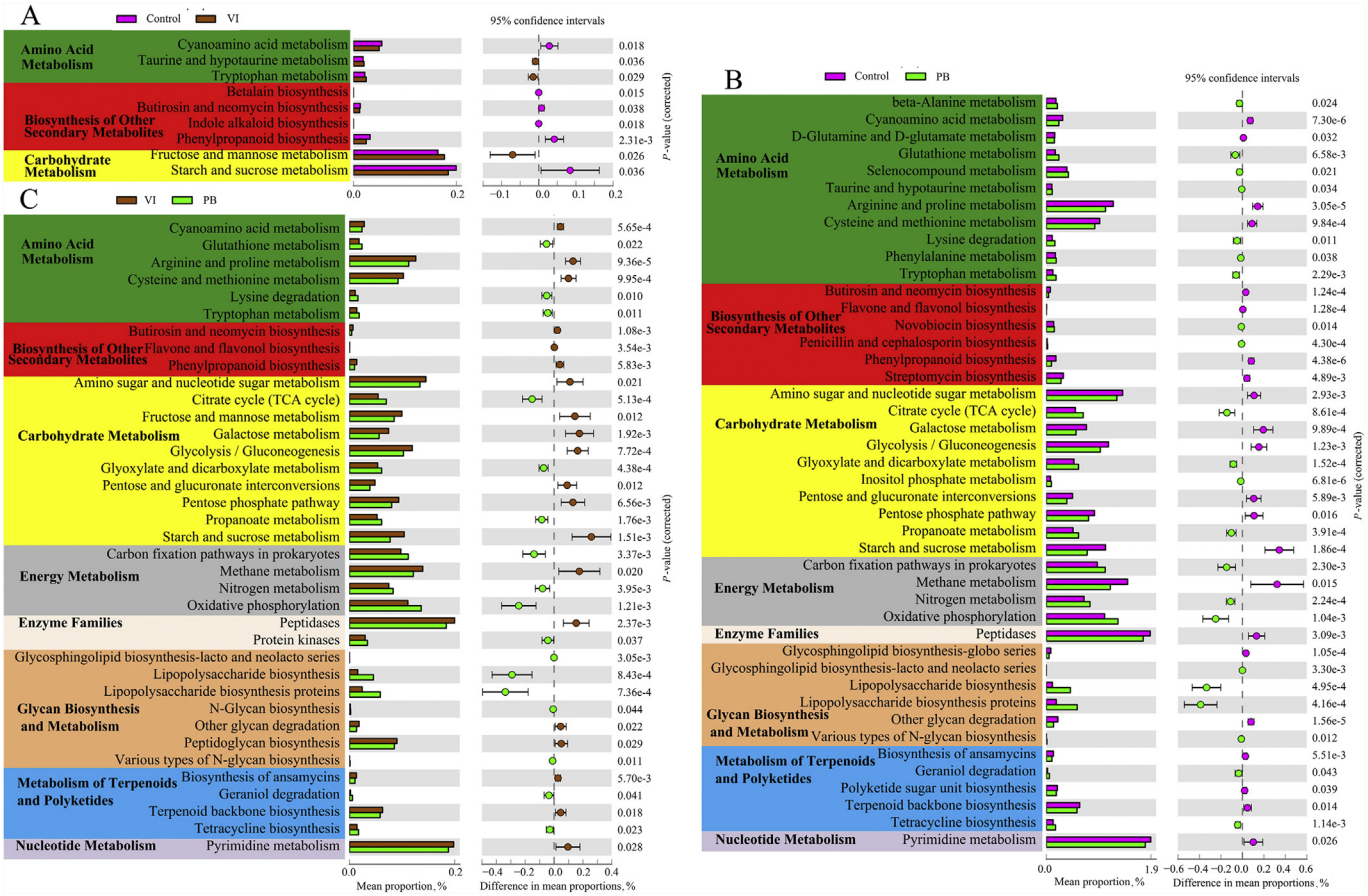

Fig. 5. Significant changes in bacterial Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways using the response ratio method at a 95% confidence interval (CI). (A) Control vs. VI; (B) control vs. PB; (C) VI vs. PB. Control group, healthy piglets; VI group, piglets infected with porcine circovirus type 2; PB group, piglets infected with *Campylobacter*.

and *Campylobacter* notably reduced the number of generalists, caused the deterioration of the entire network, and finally led to the dysbiosis of colonic microbiota. Evidence from a recent study has suggested that ulcerative colitis in human exhibited a biologically relevant dysbiosis of microbial community (Chen et al., 2017). Furthermore, 2 OTU from *Campylobacter* performed as module hubs in the network of piglets infected with *Campylobacter*, and this again confirmed the key role of *Campylobacter* in inducing diarrhea.

The colon is one important place that microbes ferment the non-digestible carbohydrate (Gratz et al., 2019; Flint et al., 2012). It is well known that microbial fermentation could increase the efficiency of nutrient nutrition for host, nevertheless microbial dysbiosis could cause its metabolic dysfunction (Bouter et al., 2017; Haro et al., 2017). As shown here, the carbohydrate metabolism of colonic microbiome significantly decreased in piglets infected with *Campylobacter* due to a mass reduction in relative abundance of bacteria species from Bacteroidia and Clostridia, both of which were the main bacteria species fertilizing the non-digestible carbohydrate. This result was confirmed by the reduction of SCFA, the main metabolites of intestinal microbiome, which could promote barrier function and maintain a healthy and slightly acidic environment in the colon (Akira et al., 2003). The reduction of SCFA led to a significantly higher pH in the colon cavity of piglets infected with *Campylobacter* in this study. Furthermore, the highest pH was found in the colon cavity of piglet infected with PCV2, indicating that its microbial metabolism function also significantly differed from that in the healthy individuals. Nevertheless, compared to the healthy piglets, no significant difference in predicted function of colonic microbiota was observed in piglets infected with PCV2 because the microbial function prediction based on its microbial compositions, which was similar with that in healthy piglets. However, the fermentation process depends on a consortium of the microbiota, which is interdependent with respect to the efficiency of fermentation (Dearing and Kohl, 2017). Therefore, in the case of the microbial dysbiosis, the disturbances in species–species interactions resulted in metabolic dysfunction of colonic microbiome in diarrheic piglets in this study.

Table 3
Short chain fatty acid metabolites and pH in colon of piglets ($n = 5$).

Groups[1]	Acetic acid, mmol/L	Propionic acid, mmol/L	Butyric acid, mmol/L	Valeric acid, mmol/L	pH
Control	4.58 ± 0.29^{b}	1.46 ± 0.12^{b}	0.48 ± 0.04^{b}	0.09 ± 0.01^{a}	6.11 ± 0.12^{a}
VI	3.81 ± 0.50^{b}	1.47 ± 0.22^{b}	0.37 ± 0.10^{b}	0.21 ± 0.05^{b}	6.60 ± 0.14^{b}
PB	1.07 ± 0.17^{a}	0.32 ± 0.10^{a}	0.14 ± 0.03^{a}	0.05 ± 0.01^{a}	7.01 ± 0.10^{c}

a, b, c Values with different superscripts, within the same column, are significantly different at $P < 0.05$.
[1] Control group, healthy piglets; VI group, piglets infected with porcine circovirus type 2; PB group, piglets infected with *Campylobacter*.

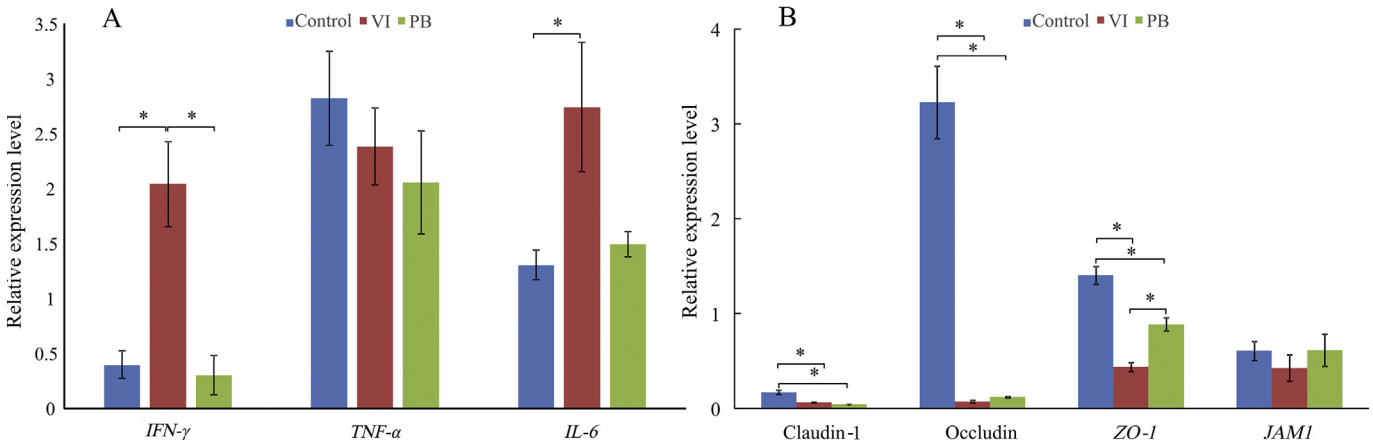

Fig. 6. Relative expression of inflammation-related (A) and tight junction protein genes (B) in colon tissue. *IFN-γ* = interferon-γ; *TNF-α* = tumor necrosis factor-α; *IL-6* = interleukin-6; *ZO-1* = Zonula occludens protein-1; *JAM1* = junctional adhesion molecule-1. Control group, healthy piglets; VI group, piglets infected with porcine circovirus type 2; PB group, piglets infected with *Campylobacter*. *, $P < 0.05$; **, $P < 0.01$.

Except for nutrient absorption, intestinal epithelial cells also form an effective barrier against pathogen invasion. However, microbial dysbiosis could disturb the protective capacity of the intestinal barrier, concurrent with an increase in risk of pathogen invasion (Leclercq et al., 2014; Spiljar et al., 2017). Here, PCV2 infection did induce the inflammation of colon accompanied by a significant increase in colonic permeability in terms of reduction of Claudin-1, Occludin, and ZO-1, consistent with the previous studies on piglets (Kim et al., 2004; Meng, 2013). Similarly, *Campylobacter* infection significantly increased intestinal permeability by affecting the tight junctions, in accordance with previous findings in mouse and humans (Liu et al., 2018; Mortensen et al., 2016). And thus, the increase in intestinal permeability in turn would further aggravate the invasion of PCV2 and *Campylobacter*, and then led to the diarrhea in piglets.

5. Conclusion

Our results suggested that the infection of PCV2 and *Campylobacter* would induce the microbial dysbiosis in the colon of piglets by disturbing the interspecific interaction and reducing the number of generalists within the ecological network, rather than fundamentally changing its composition. In particular, *Campylobacter* predominated in the colon and served as module hubs in the network. The microbial dysbiosis caused the metabolic dysfunction, together with dysfunction of colonic barrier. Therefore, these findings indicated that the infection of PCV2 and *Campylobacter* disturbed colonic microbiota homeostasis and function of colonic barrier, both of which contributed to intestinal disorders and led to the diarrhea of piglets.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix

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References

Adams S, Che D, Hailong J, Zhao B, Rui H, Danquah K, Qin G. Effects of pulverized oyster mushroom (*PleurOTU ostreatus*) on diarrhea incidence, growth performance, immunity, and microbial composition in piglets. J Sci Food Agric 2019;99:3616–27.
Akira A, Tomoyuki T, Yoshihide F. Role of dietary fiber and short-chain fatty acids in the colon. Curr Pharmaceut Des 2003;9:347–58.
Baró J, Segalés J, Martínez J. Porcine circovirus type 2 (PCV2) enteric disease: an independent condition or part of the systemic disease? Vet Microbiol 2015;176:83–7.
Bik EM, Relman DA. Unrest at home: diarrheal disease and microbiota disturbance. Genome Biol 2014;15:120.
Bouter KE, Raalte DHV, Groen AK, Nieuwdorp M. Role of the gut microbiome in the pathogenesis of obesity and obesity-related metabolic dysfunction. Gastroenterology 2017;152:1671.
Burrough E, Terhorst S, Sahin O, Zhang Q. Prevalence of *Campylobacter* spp. relative to other enteric pathogens in grow-finish pigs with diarrhea. Anaerobe 2013;22:111–4.
Chen G, Zhang Y, Wang W, Ji X, Meng F, Xu P, Yang N, Ye F, Bo X. Partners of patients with ulcerative colitis exhibit a biologically relevant dysbiosis in fecal microbial metacommunities. World J Gastroenterol 2017;23:4624–31.
Chi CY, Liao LN, Ho CM, Chou CH, Ho MW, Wang JH. Epidemiology, clinical features, and microbiology of patients with diarrhea in community clinics in Taiwan. J Microbiol Immunol 2018;51:527–34.
Clarke G, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, Dinan TG. Minireview: gut microbiota: the neglected endocrine organ. Mol Endocrinol 2014;28:1221–38.
Coyte KZ, Schluter J, Foster KR. The ecology of the microbiome: networks, competition, and stability. Science 2015;350:663–6.
de Vries SP, Linn A, Macleod K, Maccallum A, Hardy SP, Douce G, Watson E, Dagleish MP, Thompson H, Stevenson A. Analysis of *Campylobacter jejuni* infection in the gnotobiotic piglet and genome-wide identification of bacterial factors required for infection. Sci Rep 2017;7:44283.
de Wit dA, Koopmans MP, Kortbeek LM, van Leeuwen NJ, Vinjé J, van Duynhoven YT. Etiology of gastroenteritis in sentinel general practices in The Netherlands. Clin Infect Dis 2001;33:280–8.
Dearing MD, Kohl KD. Beyond fermentation: other important services provided to endothermic herbivores by their gut microbiota. Integr Comp Biol 2017;54:723–31.
Deng Y, Jiang Y, Yang Y, He Z, Luo F, Zhou J. Molecular ecological network analyses. BMC Bioinf 2012;13:113.
Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. Gut Microb 2012;3:289–306.
Giallourou N, Medlock GL, Bolick DT, Medeiros PH, Ledwaba SE, Kolling GL, Tung K, Guerry P, Swann JR, Guerrant RL. A novel mouse model of *Campylobacter jejuni* enteropathy and diarrhea. PLoS Pathog 2018;14:e1007083.
Gratz SW, Hazim S, Richardson AJ, Scobbie L, Johnstone AM, Fyfe C, Holtrop G, Lobley GE, Russell WR. Dietary carbohydrate rather than protein intake drives colonic microbial fermentation during weight loss. Eur J Nutr 2019;58:1147–58.

Gresse R, Chaucheyrasdurand F, Fleury MA, Van dWT, Forano E, Blanquetdiot S. Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. Trends Microbiol 2017;25:851–73.
Haro C, García-Carpintero S, Rangel-Zúñiga OA, Alcalá-Díaz JF, Landa BB, Clemente JC, Pérez-Martínez P, López-Miranda J, Pérez-Jiménez F, Camargo A. Consumption of two healthy dietary patterns restored microbiota dysbiosis in obese patients with metabolic dysfunction. Mol Nutr Food Res 2017;61:1700300.
Hermann-Bank ML, Skovgaard K, Stockmarr A, Strube ML, Larsen N, Kongsted H, Ingerslev H-C, Mølbak L, Boye M. Characterization of the bacterial gut microbiota of piglets suffering from new neonatal porcine diarrhoea. BMC Vet Res 2015;11:139.
Kaiser-Bunbury CN, Muff S, Memmott J, Müller CB, Caflisch A. The robustness of pollination networks to the loss of species and interactions: a quantitative approach incorporating pollinator behaviour. Ecol Lett 2010;13:442–52.
Kamada N, Seo SU, Chen GY, Núñez G. Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol 2013;13:321–35.
Kiers J, Meijer J, Nout M, Rombouts F, Nabuurs M, Van der Meulen J. Effect of fermented soya beans on diarrhoea and feed efficiency in weaned piglets. J Appl Microbiol 2003;95:545–52.
Kim J, Ha Y, Jung K, Choi C, Chae C. Enteritis associated with porcine circovirus 2 in pigs. Can J Vet Res 2004;68:218–21.
Kirk KF, Nielsen HL, Thorlaciussussing O, Nielsen H. Optimized cultivation of *Campylobacter concisus* from gut mucosal biopsies in inflammatory bowel disease. Gut Pathog 2016;8:27.
Langille MGI, Zaneveld J, Caporaso JG, Mcdonald D, Dan K, Reyes JA, Clemente JC, Burkepile DE, Thurber RLV, Knight R. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 2013;31:814–21.
Leclercq S, Matamoros S, Cani PD, Neyrinck AM, Jamar F, Stärkel P, Windey K, Tremaroli V, Bäckhed F, Verbeke K. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. Proc Natl Acad Sci USA 2014;111:E4485–93.
Liu F, Ma R, Wang Y, Zhang L. The clinical importance of *Campylobacter concisus* and other human hosted *Campylobacter* species. Front Cell Infect Microbiol 2018;8:234.
Maharshak N, Ringel Y, Katibian D, Lundqvist A, Sartor RB, Carroll IM, Ringel-Kulka T. Fecal and mucosa-associated intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. Dig Dis Sci 2018;63:1–10.
Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G, Quraishi MN, Kinross J, Smidt H, Tuohy KM. The gut microbiota and host health: a new clinical frontier. Gut 2016;65:330–9.
Meng X. Porcine circovirus type 2 (PCV2): pathogenesis and interaction with the immune system. Annu Rev Anim Biosci 2013;1:43–64.
Minamoto Y, Dhanani N, Markel ME, Steiner JM, Suchodolski JS. Prevalence of *Clostridium perfringens, Clostridium perfringens* enterotoxin and dysbiosis in fecal samples of dogs with diarrhea. Vet Microbiol 2014;174:463–73.
Montoya JM, Pimm SL, Solé RV. Ecological networks and their fragility. Nature 2006;442:259.
Morrison DJ, Tom P. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microb 2016;7:189–200.
Mortensen NP, Mercier K, Mcritchie S, Cavallo TB, Pathmasiri W, Stewart D, Sumner S. Microfluidics meets metabolomics to reveal the impact of *Campylobacter jejuni* infection on biochemical pathways. Biomed Microdevices 2016;18:51.
Munyaka PM, Sepehri S, Ghia JE, Khafipour E. Carrageenan gum and adherent InvasiveEscherichia coliin a piglet model of inflammatory bowel disease: impact on intestinal mucosa-associated microbiota. Front Microbiol 2016;7:462.
Nathues C, Grüning P, Fruth A, Verspohl J, Blaha T, Kreienbrock L, Merle R. *Campylobacter* spp., *Yersinia enterocolitica*, and *Salmonella enterica* and their simultaneous occurrence in German fattening pig herds and their environment. J Food Protect 2013;76:1704–11.
Olesen JM, Bascompte J, Dupont YL, Jordano P. The modularity of pollination networks. Proc Natl Acad Sci USA 2007;104:19891–6.
Patterson A, Madson D, Halbur P, Opriessnig T. Shedding and infection dynamics of porcine circovirus type 2 (PCV2) after natural exposure. Vet Microbiol 2011;149:225–9.
Pop M, Walker AW, Paulson J, Lindsay B, Antonio M, Hossain MA, Oundo J, Tamboura B, Mai V, Astrovskaya I. Diarrhea in young children from low-income countries leads to large-scale alterations in intestinal microbiota composition. Genome Biol 2014;15:R76.
Ramos-Jiliberto R, Valdovinos FS, Moisset de Espanés P, Flores JD. Topological plasticity increases robustness of mutualistic networks. J Anim Ecol 2012;81:896–904.
Rosner BM, Schielke A, Didelot X, Kops F, Breidenbach J, Willrich N, Gölz G, Alter T, Stingl K, Josenhans C. A combined case-control and molecular source attribution study of human *Campylobacter* infections in Germany, 2011-2014. Sci Rep 2017;7:5139.
Sinha A, Shen H, Schalk S, Beach N, Huang Y, Meng X, Halbur P, Opriessnig T. Porcine reproductive and respiratory syndrome virus (PRRSV) influences infection dynamics of porcine circovirus type 2 (PCV2) subtypes PCV2a and PCV2b by prolonging PCV2 viremia and shedding. Vet Microbiol 2011;152:235–46.
Sommer F, Anderson JM, Bharti R, Raes J, Rosenstiel P. The resilience of the intestinal microbiota influences health and disease. Nat Rev Microbiol 2017;15:630.
Spiljar M, Merkler D, Trajkovski M. The immune system bridges the gut microbiota with systemic energy homeostasis: focus on TLRs, mucosal barrier, and SCFAs. Front Immunol 2017;8:1353.
Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. BMJ 2018;361:k2179.
van Sambeek D, Tran H, Fernando S, Ciobanu D, Miller P, Burkey T. Alteration of the pig intestinal microbiome when vaccinated against or inoculated with porcine circovirus 2 using a multivariate analysis model. J Anim Sci 2016;94:387–90.
Ward DV, Scholz M, Zolfo M, Taft DH, Schibler KR, Tett A, Segata N, Morrow AL. Metagenomic sequencing with strain-level resolution implicates uropathogenic *E. coli* in necrotizing enterocolitis and mortality in preterm infants. Cell Rep 2016;14:2912–24.
Yang G, Jian SQ, Cao H, Wen C, Hu B, Peng M, Peng L, Yuan J, Liang L. Changes in microbiota along the intestine of grass carp (*Ctenopharyngodon idella*): community, interspecific interactions, and functions. Aquaculture 2019;498:151–61.
Yang G, Peng M, Tian X, Dong S. Molecular ecological network analysis reveals the effects of probiotics and florfenicol on intestinal microbiota homeostasis: an example of sea cucumber. Sci Rep 2017a;7:4778.
Yang Q, Huang X, Zhao S, Sun W, Yan Z, Wang P, Li S, Huang W, Zhang S, Liu L. Structure and function of the fecal microbiota in diarrheic neonatal piglets. Front Microbiol 2017b;8:502.
Zhou J, Deng Y, Luo F, He Z, Yang Y. Phylogenetic molecular ecological network of soil microbial communities in response to elevated CO2. mBio 2011;2.e00122–11.
Zhou X, Ruan Z, Huang X, Zhou Y, Liu S, Yin Y. The prebiotic lactosucrose modulates gut metabolites and microbiota in intestinal inflammatory rats. Food Sci Biotechnol 2013;23:157–63.