Avian leukosis virus subgroup J infection influences the gut microbiota composition in chicken

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Research article

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Abstract

Background: Avian leukosis virus (ALV) is a major cause of disease in poultry. Probiotics play a critical role in maintaining animal health. Studies have indicated that viral infection can alter the composition of the chicken gut microbiota. We hypothesized that ALV-J infection alters the probiotics composition in the chicken fecal bacterial microbiome. We performed high-throughput 16S rRNA gene sequencing and evaluated the gut microbiota profiles using feces from ALV-J-infected and healthy chickens.

Results: The relative abundance at the phylum and species levels was calculated. The phylum *Proteobacteria* was more abundant in ALV-J-infected chickens than in healthy chickens. Additionally, the abundance of the opportunistic pathogen *Propionibacterium acnes* was significantly increased in ALV-J-infected chickens. Interestingly, ALV-J infection tended to be significantly decreased by the probiotics *Lactobacillus helveticus* and *Lactobacillus reuteri*

Conclusions: The study indicates that ALV-J infection significantly altered the gut microbiota distribution in chickens. Additionally, ALV-J infection significantly influenced the abundance of *L. helveticus* and *L. reuteri* in the chicken gut.

Background

Avian leukosis virus (ALV) is a major cause of disease in poultry and commonly produces tumors and immunosuppression in infected chickens. Subgroup J ALV (ALV-J) shows greater pathogenicity and transmission ability than the other subgroups **[1]**. ALV-J is primarily associated with myeloid leukosis in broiler breeders. ALV-J infection of broilers was first detected in China in 1999. In the past decade, the host range of ALV-J has gradually expanded to the Chinese local breed of commercial layers **[2-4]**, suggesting that ALV-J infection is a major problem in China.

The gastrointestinal tract of chicken harbors various bacteria **[5,6]**, which affect the animals' health **[7]**. However, the delicate balance of the gut microbiome can be disrupted by many factors, including chronic viral infections **[8,9]**. An increasing number of studies has indicated that viral infection can alter the composition of the chicken gut microbiota. For example, Marek's disease virus changes the core gut microbiome of chicken during different phases of viral replication **[10]**. Infection with bursal disease virus significantly influences microbiota composition **[11]**. Influenza A virus subtype H9N2 infection disrupts the composition of the intestinal microbiota of chickens **[12]**.

ALV-J-infected chickens at 21 days of age have been shown to have a large number of notable pathogens from *Proteobacteria* and other phyla of conditional pathogens **[13]**. However, little is known about the effect of ALV-J infection on probiotics within the chicken microbiota. Probiotics have been defined by the United Nations and World Health Organization Expert Panel as "live microorganisms which when administered in adequate amounts confer a health benefit on the host." Probiotics ferment undigested carbohydrate residues to produce high levels of short-chain fatty acids, which create an acidic environment that is not conducive for the growth of pH-sensitive pathogenic bacteria. *In vivo* administration of lactobacilli can improve antibody-mediated immune responses in chicken **[14]**. Strains of *Lactobacillus acidophilus* interfere with a wide range of pathogens **[15-17]**. Thus, probiotics play a role in maintaining animal health. We hypothesized that ALV-J infection alters the probiotics composition in the chicken fecal bacterial microbiome. We conducted an extensive microbial diversity survey to evaluate the differences in the gut microbiota between chickens infected with ALV-J and healthy chickens by high-throughput 16S rRNA gene sequencing.

Results

Phyla composition exhibited significant microbial differences between ALV-J-infected chickens and controls

Each sample was rarefied to 17,595 sequences; using a threshold of 97% sequence identity, 16,740 unique operational taxonomic units (OTUs) were identified in the samples. Total sequences were assigned to 38 phyla (3 archaeal phyla and 35 bacterial phyla). Bacterial phyla isolated from the samples included *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria.* The distribution of the four phyla in group A (viral control) showed that the gut microbiota was dominated by *Firmicutes* (average relative abundance: 98.03%), whereas other phyla were present in smaller quantities including *Proteobacteria* (average relative abundance: 1.03%), *Bacteroidetes* (average relative abundance: 0.27%), and *Actinobacteria* (average relative abundance: 0.16%). In group B (ALV-J-infected chickens), the gut microbiota was dominated by both *Firmicutes* (average relative abundance: 52.51%) and *Proteobacteria* (average relative abundance: 38.69%), with other phyla found in smaller quantities including *Bacteroidetes* (average relative abundance: 1.46%), *Actinobacteria* (average relative abundance: 0.68%) and a few other unknown phyla (**Fig 1, S1**).

Interestingly, two phyla, *Firmicutes* and *Proteobacteria*, were proportionally significantly different between groups A and B ($P < 0.05$). Among the two phyla, the *Proteobacteria* concentration was much higher in group B than in group A (**Fig 1**). These results indicate that ALV-J infection significantly affected the proportion of *Firmicutes* and *Proteobacteria* at the phylum level.

Bacterial taxonomic clades showed significant differences between ALV-J-infected chickens and controls

Principal coordinate analysis was conducted based on weighted UniFrac distances to assess the microbial distribution between the two groups. The results of weighted UniFrac analysis revealed a notable distribution difference for PC2; However, no difference in the distribution of PC1 was observed. The gut microbial community of group A was substantially separated from that of group B (**Fig. 2A**). In group A (control), all samples clustered together, whereas in group B (ALV-J-infected chickens), all samples except for B2 clustered together. This indicates that ALV-J infection significantly altered the gut microbiota distribution in chickens. Although the microbiome of B2 was rather different from that of the other samples in group B, a higher abundance of *Proteobacteria* was observed in all samples in group B than in group A. Notably, B2 was characterized by a higher abundance of the phylum *Proteobacteria* (60.89% of relative abundance) and lower abundance of the phylum *Firmicutes* (13.48% of relative abundance) than those in the other chickens in group B, resulting in an obvious separation of B2 from the other chickens in group B (Fig. 2A).

To investigate which OTUs can serve as biomarkers in an unbiased manner, we used the linear discriminant analysis effect size (LEfSe) classification tool. The analysis revealed that 15 bacterial taxonomic clades significantly differed among the two groups ($P < 0.05$). In group B, the key phylotypes were *Proteobacteria*, *Helicobacter*, *Helicobacteraceae*, *Comamonas*, *Betaproteobacteria*, *Burkholderiales*, *Gammaproteobacteria*, *Comamonadaceae*, *Actinomyc* and *Lactobacillaceae* were present in group A (**Fig. 2B**). The heat map displayed a similar pattern, as shown in **Fig. 2C**. These results suggest that the composition of the chicken gut microbiota was significantly altered by ALV-J infection.

Difference in composition of probiotics in chicken gut microbiota in ALV-J-infected chickens and controls

We further identified the dominant species in the gut microbiota between the two groups. The results revealed significant differences ($P < 0.05$) between eight species including *Propionibacterium acnes*, *Lactobacillus coleohominis*, *Lactobacillus helveticus*, *Lactobacillus reuteri*, and rarely identified species such as *Mycoplana* spp., *Comamonas* spp., *Delftia* spp., and *Helicobacter* spp. (**Figure 3**). In group B (ALV-J-infected chickens), three species exhibited a significant reduction in abundance including *L. coleohominis*, *L. helveticus*, and *L. reuteri* compared to in group A (control). Two of these species, *L. helveticus* and *L. reuteri*, are probiotics. These results suggest that at the species level, ALV-J infection significantly altered the composition of probiotics among the chicken gut microbes.

Discussion

Effect of ALV-J infection on composition of chicken gut microbiota

An increasing number of studies has indicated that viral infection can alter the composition of the chicken gut microbiota **[10-13]**; these results are consistent with our research. Our results also agreed with a recent study of microbial diversity in chickens showing that *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes* were the top four phyla in the intestinal tract of chickens **[18]**.

The defined taxa are potential biomarkers for ALV-J-infected and healthy chickens. For example, *Proteobacteria* can serve as biomarkers for ALV-J infection in chickens at the phylum, order, class, family and genus levels (S1,S2, S3, S4 and S5), whereas a few taxa were markers for healthy chickens, most prominently members of the family *Lactobacillaceae*.

Further, at the species level, 8 species exhibited significant differences between ALV-J-infected and healthy control chickens. The abundance of *L. coleohominis*, *L. helveticus*, and *L. reuteri* was significantly decreased in ALV-J-infected chickens, whereas significant increases were observed in the abundance of *P. acnes* and four unidentified species, *Mycoplana* spp., *Comamonas* spp., *Delftia* spp., and *Helicobacter* spp. Our results clearly illustrate that this viral infection can significantly alter the composition of the host gut microbiota, which is consistent with previous findings **[19-21]**. However, further studies are required to evaluate whether changes in the microbiome play a role in disease complications.

The abundance of *P. acnes* was significantly increased in the chicken gut microbiota after ALV-J infection. *Propionibacterium acnes* is an opportunistic pathogen that may play a role in other conditions, including inflammation of the prostate leading to cancer **[22,23]**. These results suggest that ALV-J infection can result in increased expression of opportunistic pathogenic bacteria, which is consistent with a previous study **[13]**.

Effect of ALV-J infection on composition of probiotics in the chicken gut microbiota

Our results indicate that ALV-J infection significantly influenced the composition of probiotics in the chicken gut microbiota. This also is the first study to investigate the potential effects of ALV-J infection on the composition of probiotics in the fecal bacterial microbiome of chickens. The mechanisms by which probiotics affect infection, disease, and immunity are an active area of study. Different strains of lactobacilli can decrease inflammation in the gastrointestinal tract. For example, *L. acidophilus* interacts with dendritic cells to induce the production of interleukin-10 **[24]**. Further, *L. paracasei* can degrade highly inflammatory interferon γ-induced protein 10 **[25]**. Probiotics are being developed as a nonpharmacological means for preventing or ameliorating gastroenteritis caused by enteropathogens. *Lactobacillus rhamnosus* GG-supplemented pigs showed a significant reduction in diarrhea following rotavirus challenge **[26,27]**. Moreover, *L. reuteri* and *L. acidophilus* with human rotavirus infection produced an additive effect on TLR2- and TLR9-expressing antigens in Gn pigs **[28]**. *Lactobacillus reuteri* strains produced an array of antimicrobial compounds that inhibited pathogens *in vitro* **[29]**. Increasing evidence has shown that strains belonging to *L. helveticus* species have health-promoting properties **[30]**. Interestingly, our study indicated that ALV-J infection inhibits the growth of beneficial bacteria such as *Lactobacillus*.

The abundance of the probiotics *L. helveticus* and *L. reuteri* was significantly decreased in the chicken gut microbiota following ALV-J infection; this may be useful for assisting with the diagnosis of this illness post-mortem. Moreover, further studies are required to understand the mechanism by which ALV-J infection significantly decreased the abundance of these two probiotics. Our study indicates that to relieve avian leucosis, ALV-J multiplication must be prevented and microbiota-targeted therapies such as probiotic supplements are required.

Conclusion

ALV-J infection significantly altered the gut microbiota distribution in chickens. The abundance of two probiotics, *L. helveticus* and *L. reuteri*, was significantly decreased in the chicken gut microbiota following ALV-J infection.

Methods

Animal and fecal sample collection

Female Huiyang bearded chickens at approximately 25 weeks of age were used in this study. The chickens were obtained from a local broiler in Huizhou City, China. They were randomly collected from the national Huiyang bearded chicken breeding ground at Guangdong Jinzhong Agriculture and Animal Husbandry Technology Co., Ltd. (Huizhou, China). The birds were housed in a commercial caging system (each cage was 40 × 40 × 30 cm in height, width, and depth, respectively). Chickens were randomly assigned to the cages, with three chickens in each unit. Water was supplied via two “on-demand” nipples per cage. The company used eradication programs to minimize the transmission of ALV-J from hens to their progenies. To evaluate the eradication programs, based on a previously described method [31], a Taq Man-based real-time PCR method was performed to detect and quantify ALV-J with proviral DNA from 400 swab samples. Using a real-time PCR method to detect ALV-J, only 12 chickens were found to be ALV-J-infected (B group), and 12 uninfected chickens were randomly selected as controls (A group). All 24 experimental chickens were sacrificed by cervical dislocation. Their gut contents were instantly collected from the ceca within 5 min of sacrifice, immediately placed in cryogenic vials, frozen in liquid nitrogen, and transported to the laboratory, where they were stored at −80°C until DNA extraction.

DNA extraction, PCR, and 16S rRNA gene sequencing

A genomic DNA extraction kit, the TIANamp Stool DNA Kit, was utilized to extract DNA from the gut contents (TIANGEN, Beijing, China). Twelve DNA samples from each group were randomly divided into four pools to produce three DNA samples per pool. The DNA concentration and purity were determined using a Nanodrop 2000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Extracted DNA was diluted to 10 ng/μL for PCR amplification. The universal primers 515F and 909R, which have been described previously, were used to amplify the V4–V5 hypervariable region of the microbial 16S rRNA gene **[32]**. The PCR amplifications and purification procedures were performed as described in our previous study **[33]**. All amplicons were sequenced on an Illumina MiSeq system (San Diego, CA, USA) at Guangdong Meilikang Bio-Science, Ltd. (Shaoqing, China).

Bioinformatics analysis

The raw reads were merged using FLASH-software **[34]**. QIIME Pipeline-Version 1.9.0 was used to process the merged sequence data. The UCHIME algorithm was used to filter the clean data **[35]**. Effective sequences were grouped into OTUs at a user-defined level of sequence similarity (such as e.g., 97% to approximate species-level phylotypes). The alpha diversity indices and weighted UniFrac distance metrics were determined using the QIIME pipeline. Taxonomy was assigned by the Ribosomal Database Project classifier using Greengenes 13_8 **[36]** (http://qiime.org/home_static/dataFiles.html) as a reference database. Statistical comparisons of microbial communities between treatments were determined using the LEfSe.

Data analysis

Principal coordinate analysis was conducted based on weighted UniFrac distances using QIIME Pipeline-Version 1.9.0. LEfSe analysis was conducted using the Galaxy platform **[37]**. Wilcoxon rank-sum test was used to test the significance of differences between groups using R 3.5.1 software. $P < 0.05$ was considered as statistically significant.

Abbreviations

ALV: Avian leukosis virus; ALV-J: subgroup J ALV; LEfSe: linear discriminant analysis effect size; OTUs: operational taxonomic units; QIIME: quantitative insights into microbial ecology

Declarations

Ethics approval and consent to participate

This study was performed by strictly following Animal management regulations of the People’s Republic of China. The study was approved by Huizhou University. The protocol was approved by the Committee of the Experimental Animal Management of Huizhou University. The animals were owned by Guangdong Jinzhong Agriculture and Animal Husbandry Technology Co., Ltd. The owner gave its consent to the use of animals in the study.

Consent for publication

Not applicable.

Availability of data and materials

The 16S rRNA gene sequencing raw data of the dataset were deposited at the BIG Sequence Read Archive with BioSample accessions CRA002114.

Competing interest

The authors declare that they have no competing interests.

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Authors’ contributions

HL contributed to the study design and prepared the manuscript. HL and YC participated in all experiments and contributed to data interpretation. HL and JN contributed to the bioinformatics analysis. All authors have read and approved the manuscript

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Figures

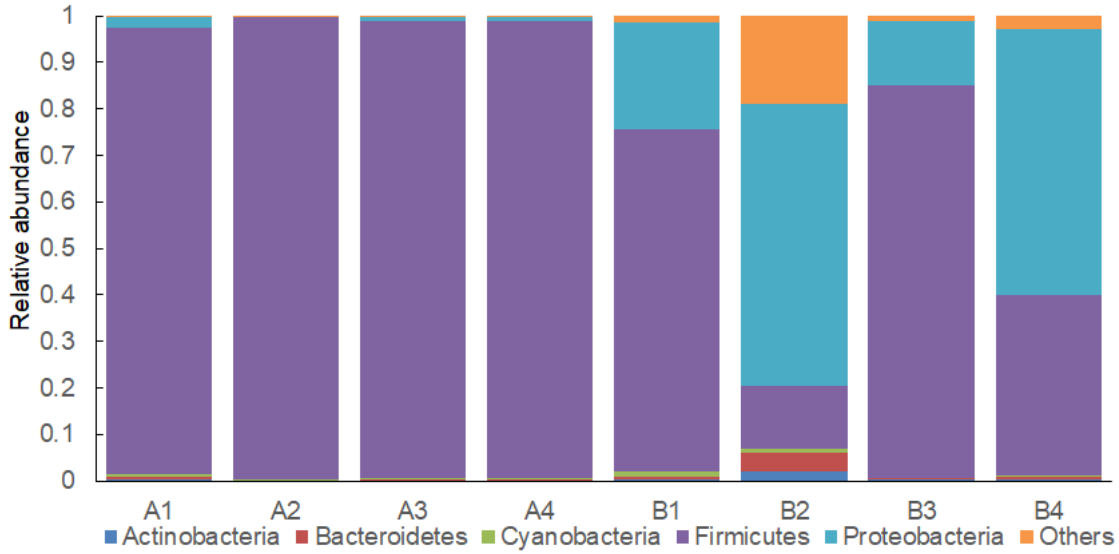
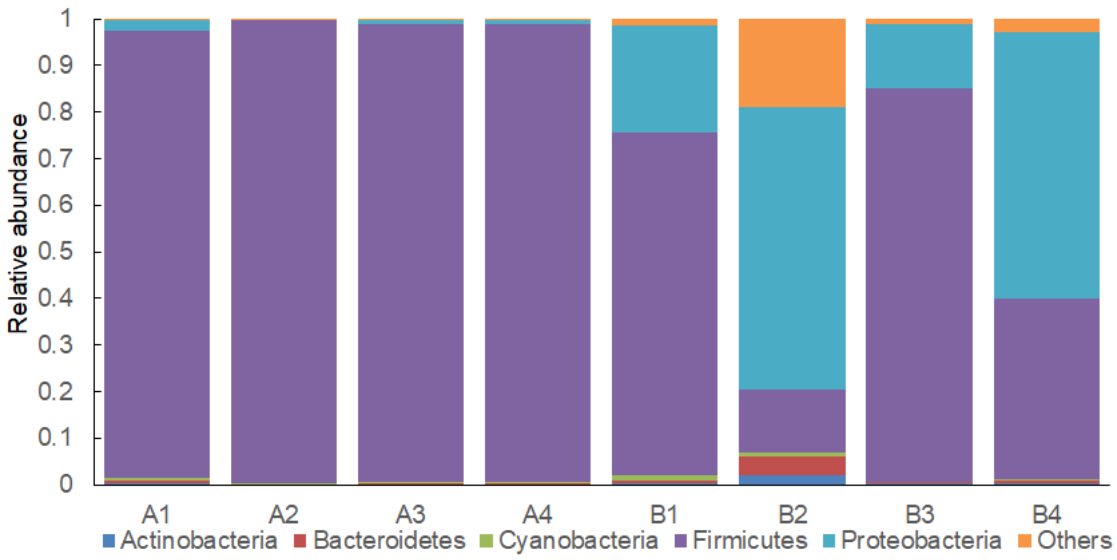


Figure 1

Dominant phyla in gut microbiota of chicken. Across all samples, total sequences were assigned to 38 phyla. The percentage bar diagram shows the composition of the dominant phyla in the chicken gut microbiota in different groups. A and B groups represent healthy control and avian leukosis virus subgroup J infection chickens, respectively.



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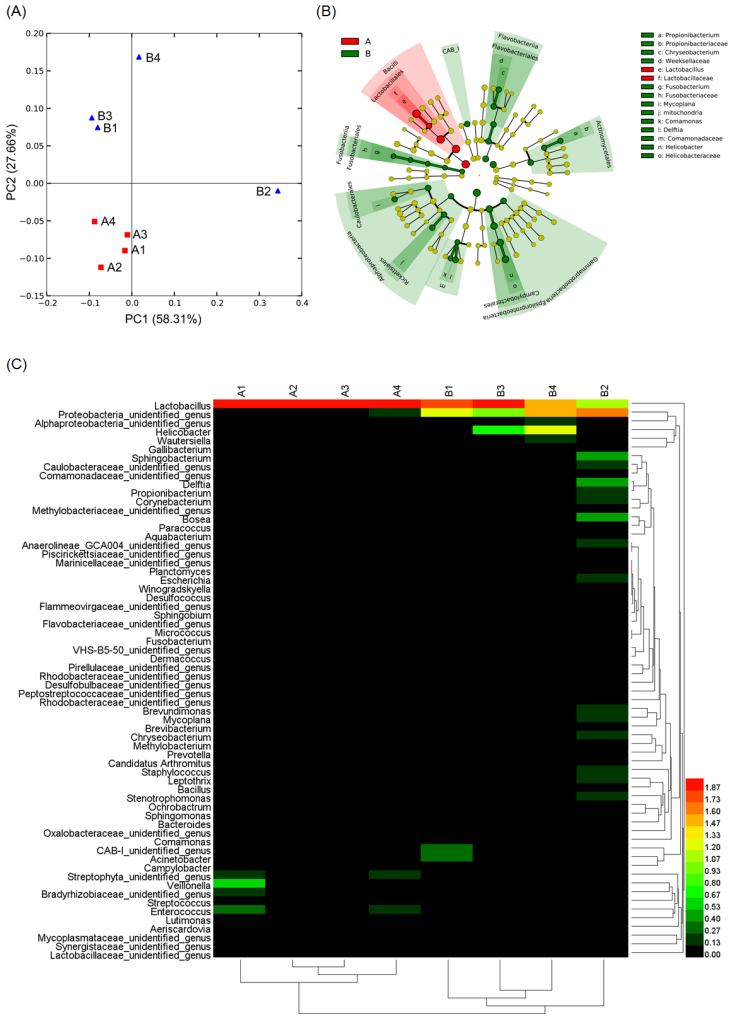


Figure 2

Gut microbiota differentiation of chickens infected with ALV-J (B group) and healthy chickens (A group) A and B groups represent healthy control and ALV-J-infected chickens, respectively. (A) Principal component analysis plot based on weighted UniFrac distance, showing the distance between bacterial communities. (B) Phylogenetic profiles of specific bacterial taxa and predominant bacteria among the two different groups, as determined by linear discriminant analysis effect size analysis. Biomarker taxa are shown as colored circles and shaded areas. Each circle's diameter is relative to the abundance of the taxa in the community (C) heat map profile of dominant OTUs in the gut microbiota of chicken. Heat map of hierarchical clustering results for the abundance of genera in feces. Colors reflect relative abundance from low (green) to high (red) (color figure online).

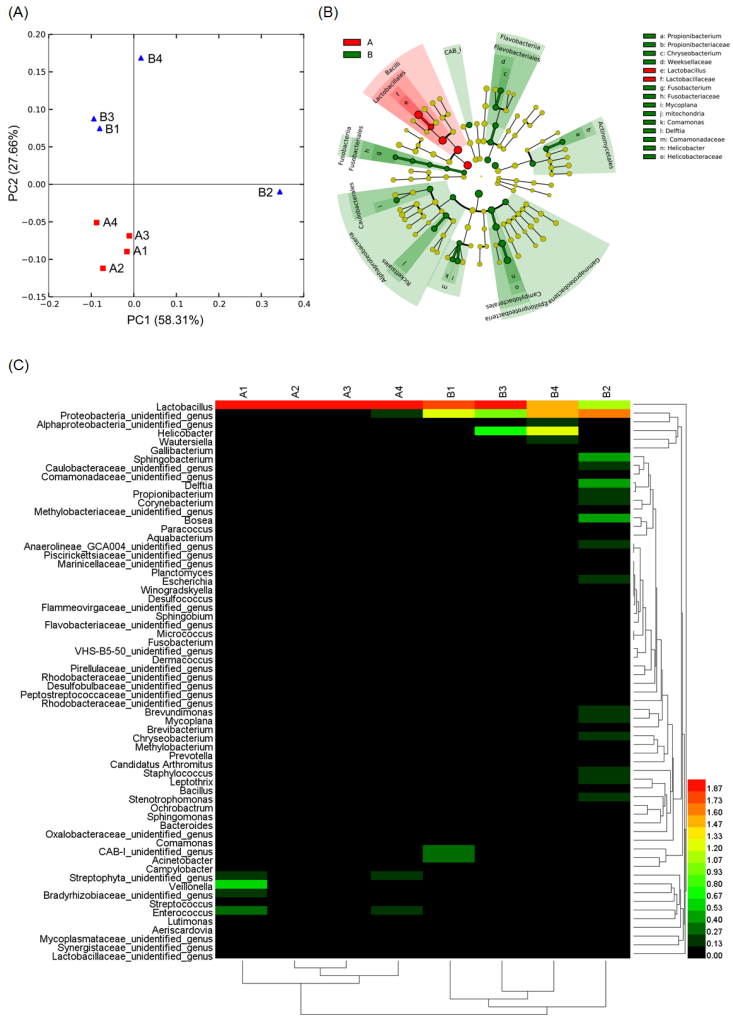


Figure 2

Gut microbiota differentiation of chickens infected with ALV-J (B group) and healthy chickens (A group) A and B groups represent healthy control and ALV-J-infected chickens, respectively. (A) Principal component analysis plot based on weighted UniFrac distance, showing the distance between bacterial communities. (B) Phylogenetic profiles of specific bacterial taxa and predominant bacteria among the two different groups, as determined by linear discriminant analysis effect size analysis. Biomarker taxa are shown as colored circles and shaded areas. Each circle's diameter is relative to the abundance of the taxa in the community (C) heat map profile of dominant OTUs in the gut microbiota of chicken. Heat map of hierarchical clustering results for the abundance of genera in feces. Colors reflect relative abundance from low (green) to high (red) (color figure online).

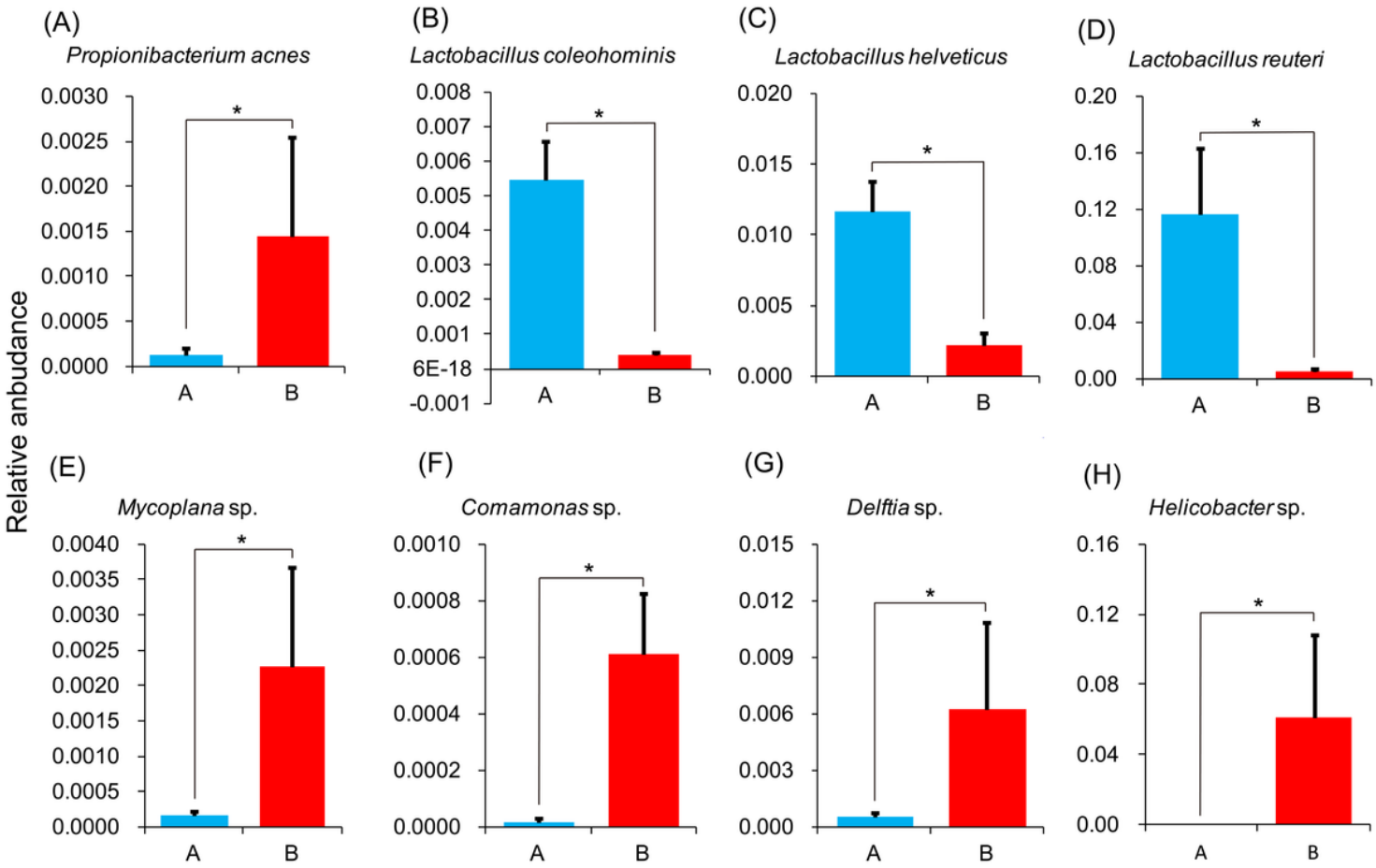
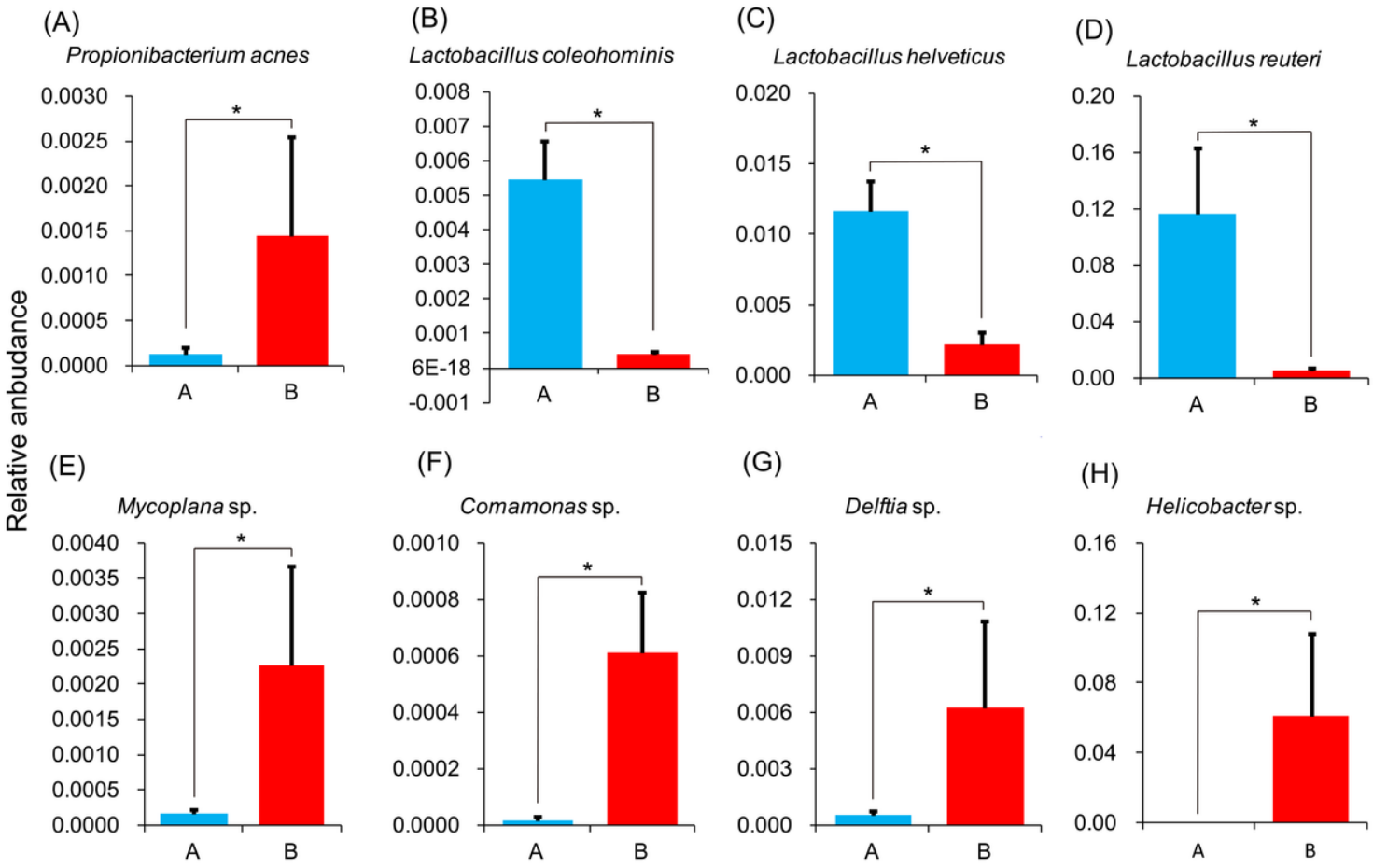


Figure 3

Dominant species of gut microbial microbiota found in groups A and B Dominant species found in the gut microbiota between the two groups. Eight species showed significant differences ($P < 0.05$) between the two groups. Colors represent different groups; group A is blue and group B is red. *indicates significant differences between the two indicated groups ($P < 0.05$).



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Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- NC3RsARRIVEGuidelinesChecklist2014.docx
- NC3RsARRIVEGuidelinesChecklist2014.docx
- renamed4e11e.xlsx
- renamed4e11e.xlsx
- renamed95e99.xlsx
- renamed95e99.xlsx
- S3Therelativeabundanceoforderinallsamples.xlsx
- S3Therelativeabundanceoforderinallsamples.xlsx
- S4Therelativeabundanceoffamilyinallsamples.xlsx
- S4Therelativeabundanceoffamilyinallsamples.xlsx
- S5Therelativeabundanceofgenusinallsamples.xlsx
- S5Therelativeabundanceofgenusinallsamples.xlsx