

# Avian leukosis virus subgroup J infection influencing composition of gut microbiota within chicken

Yuan Chen

Huizhou University

Jiajia Ni

Southern Medical University

Hongwei Li (✉ [lhwcau@163.com](mailto:lhwcau@163.com))

school of life science, Huizhou University, China <https://orcid.org/0000-0001-9993-6607>

---

## Research article

**Keywords:** Avian leukosis virus subgroup J , gut microbiota , 16S rRNA gene sequencing , chickens , Infection , probiotics

**Posted Date:** May 23rd, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-16010/v2>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

**Background:** Avian leukosis virus (ALV) is a major cause of disease in poultry. Probiotics play a critical role in animal health maintenance. 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 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 commercial layers, the Chinese local breeds [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 have indicated that viral infection can alter the composition of chicken gut microbiota. For example, Marek's disease virus changes the core gut microbiome of chicken during different phases of viral replication [10]. Infectious bursal disease virus infection significantly influences microbiota composition [11]. Influenza A virus subtype H9N2 infection disrupts the composition of the intestinal microbiota of chickens [12].

A previous study showed that ALV-J-infected chickens at 21 days old were characterized by a larger number of notable pathogens from *Proteobacteria* and other 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]. So, probiotics play a role in animal health maintenance. We hypothesized that the ALV-J infection alters the probiotics composition in the chicken fecal bacterial microbiome. To test this, an extensive microbial diversity survey was conducted to evaluate the differences in the gut microbiota of chickens infected with ALV-J compared to in 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*, whereas other phyla appeared in smaller quantities including *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria*. In group B (ALV-J-infected chickens), the gut microbiota was dominated by both *Firmicutes* and *Proteobacteria*, with other phyla found in smaller quantities including *Bacteroidetes*, *Actinobacteria*, and a few other unknown phyla (Fig 1).

Interestingly, two phyla, *Firmicutes* and *Proteobacteria*, were found to be 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 impacted 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 B2 clustered together. This indicates that ALV-J infection significantly altered the gut microbiota distribution in chickens.

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 detected 15 bacterial taxonomic clades showing significant differences among the two groups ( $P < 0.05$ ). In group B, the key phylotypes were *Proteobacteria*, *Helicobacter*, *Helicobacteraceae*, *Comamonas*, *Betaproteobacteria*, *Burkholderiales*, *Gammaproteobacteria*, *Comamonadaceae*, *Actinomycetales*, *Stenotrophomonas*, *Methylobacterium*,

*Xanthomonadaceae*, *Rickettsiales*, *Corynebacteriaceae*, and *Propionibacterium*, whereas *Lactobacillales* 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 with 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 in chicken gut microbes.

## **Discussion**

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

An increasing number of studies have 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 related to 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, and class levels, whereas a few taxa were markers for healthy chickens, most prominently members of the family *Lactobacillaceae*.

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

The abundance of *P. acnes* significantly increased in the chicken gut microbiota after ALV-J infection. *P. acnes* is an opportunistic pathogen and 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 in 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 production of interleukin-10 [24]. Further, *L. paracasei* can degrade highly inflammatory interferon  $\gamma$ -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*, were significantly decreased in the chicken gut microbiota following ALV-J infection.

## Methods

### Animal and fecal samples collection

Female Huiyang bearded chickens approximately 25- weeks old were used in the 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

chickens were not artificially infected by ALV-J. To investigate the rate of natural ALV-J infection as described previously [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 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 using liquid nitrogen, and transported to the laboratory, where they were stored at  $-80^{\circ}\text{C}$  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 4 pools to produce 3 DNA samples per pool. DNA concentration and purity were determined using the Nanodrop 2000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Extracted DNA was diluted to  $10\text{ ng}/\mu\text{L}$  for PCR amplification. The universal primers 515F and 909R 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 using 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 through the QIIME pipeline. Taxonomy was assigned by the Ribosomal Database Project classifier using Greengenes 13\_8 [36]; ([http://qiime.org/home\\_static/dataFiles.html](http://qiime.org/home_static/dataFiles.html)) as a reference database. Statistical comparisons of microbial communities between treatments were determined using the linear discriminant analysis effect size (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 test used to test the different significance between groups using R 3.5.1 software.  $P < 0.05$  was considered statistically significant.

## **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 has given its consent to use the 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.

## **Funding**

This study was supported by the Educational Commission of Guangdong Province, China (grant 2018KTSCX217), and the Project of Huizhou University, China (2019X0701012), and International training project for outstanding young scientific research talents in Guangdong Universities. All funders supported the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

## **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

## **Acknowledgements**

We would like to thank Danlong Zhong at Guangdong Jinzhong Agriculture and Animal Husbandry Technology Co., Ltd, for help with collection of chicken gut content.

## **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

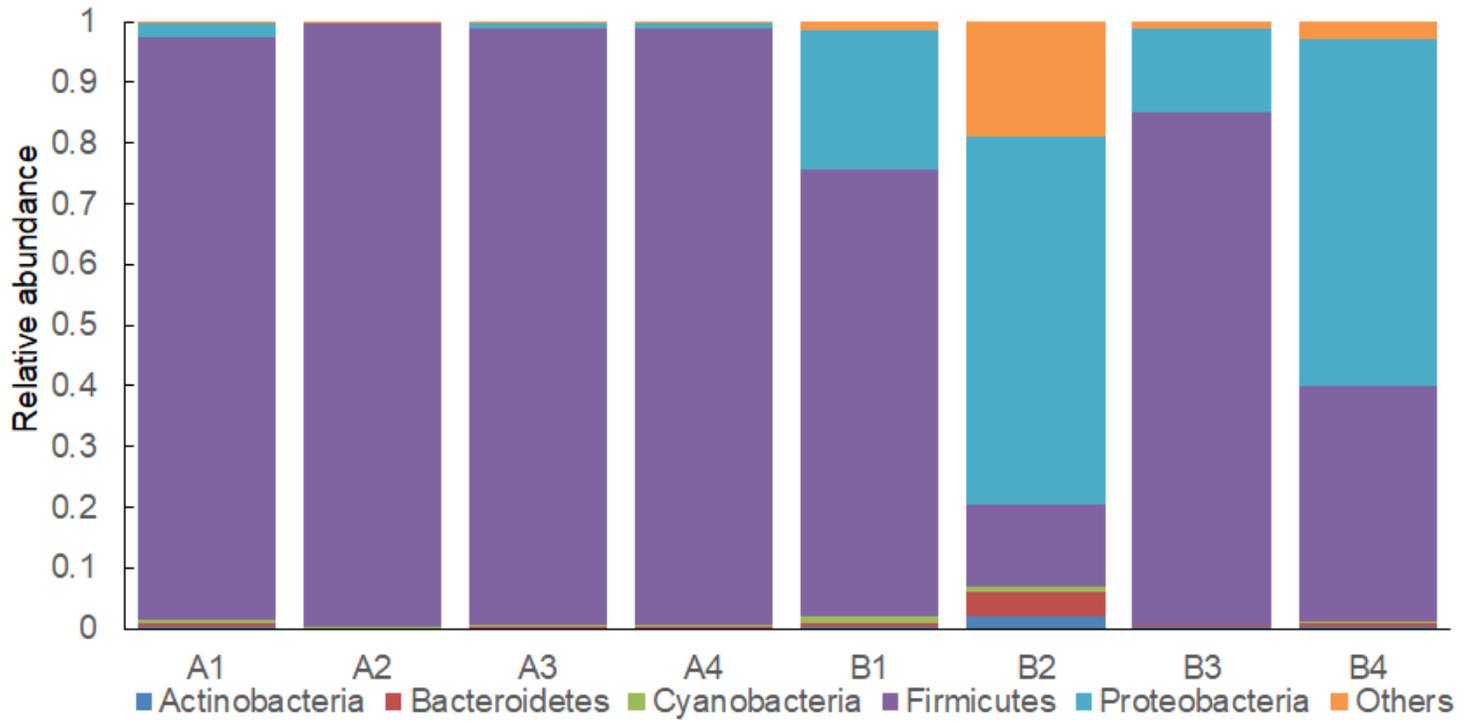
## References

1. Payne LN, Nair V. The long view: 40 years of avian leukosis research. *Avian Pathol.* 2012;41(1):11-9. DOI: 10.1080/03079457.2011.646237
2. Meng F, Li Q, Zhang Y, Zhang Z, Tian S, Cui Z, Chang S, Zhao P. Characterization of subgroup J avian Leukosis virus isolated from Chinese indigenous chickens. *Virol J.* 2018;15(1):33. DOI: 10.1186/s12985-018-0947-1
3. Lin L, Wang P, Yang Y, Li H, Huang T, Wei P. Full-length genome sequence analysis of four subgroup J avian leukosis virus strains isolated from chickens with clinical hemangioma. *Virus Genes* 2017;53(6):868-75. DOI: 10.1007/s11262-017-1490-7
4. Lin W, Li X, Dai Z, Zhang X, Chang S, Zhao P, Zhang H, Chen F, Xie Q. Molecular epidemiology of J-subgroup avian leukosis virus isolated from meat-type chickens in southern China between 2013 and 2014. *Arch Virol.* 2016;161(11):3039-46. DOI: 10.1007/s00705-016-3003-8
5. Yeoman CJ, Chia N, Jeraldo P, Sipos M, Goldenfeld ND, White BA. The microbiome of the chicken gastrointestinal tract. *Anim Health Res Rev.* 2012;13(1):89–99. DOI: 10.1017/S1466252312000138
6. Mohd Shaufi MA, Sieo CC, Chong CW, Gan HM, Ho YW. Deciphering chicken gut microbial dynamics based on high-throughput 16S rRNA metagenomics analyses. *Gut Pathog.* 2015;7:4. DOI: 10.1186/s13099-015-0051-7
7. Borda-Molina D, Seifert J, Camarinha-Silva A. Current Perspectives of the Chicken Gastrointestinal Tract and Its Microbiome. *Comput Struct Biotechnol J.* 2018;16:131-9. DOI: 10.1016/j.csbj.2018.03.002
8. Maccaferri S, Biagi E, Brigidi P. Metagenomics: Key to human gut microbiota. *Dig Dis.* 2011;29:525–30. DOI: 10.1159/000332966
9. Vyboh K, Jenabian MA, Mehraj V, Routy JP. HIV and the gut microbiota, partners in crime: breaking the vicious cycle to unearth new therapeutic targets. *J Immunol Res.* 2015:614127. DOI: 10.1155/2015/614127
10. Perumbakkam S, Hunt HD, Cheng HH. Marek's disease virus influences the core gut microbiome of the chicken during the early and late phases of viral replication. *FEMS Microbiol Ecol.* 2014;90(1):300-12. DOI: 10.1111/1574-6941.12392
11. Li L, Pielsticker C, Han Z, Kubasová T, Rychlik I, Kaspers B, Rautenschlein S. Infectious bursal disease virus inoculation infection modifies *Campylobacter jejuni*–host interaction in broilers. *Gut Pathog.* 2018;10:13. DOI: 10.1186/s13099-018-0241-1
12. Yitbarek A, Weese JS, Alkie TN, Parkinson J, Sharif S. Influenza A virus subtype H9N2 infection disrupts the composition of intestinal microbiota of chickens. *FEMS Microbiol Ecol.* 2018;94(1):1-10. DOI: 10.1093/femsec/fix165
13. Ma XX, Wang Q, Li HM, Xu CT. 16S rRNA genes Illumina sequencing revealed differential cecal microbiome in specific pathogen free chickens infected with different subgroup of avian leukosis viruses. *Vet Microbiol.* 2017;207:195–204. DOI: 10.1016/j.vetmic.2017.05.016

14. Alizadeh M, Shojadoost B, Astill J, Taha-Abdelaziz K, Karimi SH, Bavananthasivam J, Kulkarni RR, Sharif S. Effects of in ovo inoculation of multi-Strain Lactobacilli on cytokine gene expression and antibody-mediated Immune responses in chickens. *Front Vet Sci.* 2020;7:105. DOI: 10.3389/fvets.2020.00105
15. Coconnier MH, Liévin V, Lorrot M, Servin AL. Antagonistic activity of *Lactobacillus acidophilus* LB against intracellular *Salmonella enterica* serovar typhimurium infecting human enterocyte-like Caco-2/TC-7 cells. *Appl Environ Microbiol.* 2000;66:1152-7. DOI: 10.1128/aem.66.3.1152-1157.2000
16. Coconnier MH, Bernet MF, Kernéis S, Chauvière G, Fourniat J, Servin AL. Inhibition of Adhesion of Enteroinvasive Pathogens to Human Intestinal Caco-2 Cells by *Lactobacillus acidophilus* Strain LB Decreases Bacterial Invasion. *FEMS Microbiol Lett.* 1993;110:299-305. DOI: 10.1111/j.1574-6968.1993.tb06339.x
17. Fayol-Messaoudi D, Berger CN, Coconnier-Polter MH, Liévin-Le Moal V, Servin AL. pH-, lactic acid and non-lactic acid-dependent activities of probiotic Lactobacilli against *Salmonella enterica* serovar typhimurium. *Appl Environ Microbiol.* 2005;71:6008-13. DOI: 10.1128/AEM.71.10.6008-6013.2005
18. Huang P, Zhang Y, Xiao KP, et al. The chicken gut metagenome and the modulatory effects of plant-derived benzylisoquinoline alkaloids. *Microbiome* 2018;6:211. DOI: 10.1186/s40168-018-0590-5
19. Stanley D, Keyburn AL, Denman SE, Moore RJ. Changes in the caecal microbiota of chickens following *Clostridium perfringens* challenge to induce necrotic enteritis. *Vet Microbiol.* 2012;159:155–162. DOI: 10.1016/j.vetmic.2012.03.032
20. Matsumoto H, Nomura S, Hayakawa Y. Changes of RNA virus infection rates and gut microbiota in young worker *Apis mellifera* (Hymenoptera: Apidae) of a chalkbrood-infected colony after a pollination task in a greenhouse. *Appl Entomol Zool.* 2014;49:395–402. DOI: 10.1007/s13355-014-0261-3
21. Liu S, Zhao L, Zhai Z, Zhao W, Ding J, Dai R, Sun T, Meng H. Porcine epidemic diarrhea virus infection induced the unbalance of gut microbiota in piglets. *Curr Microbiol.* 2015;71:643–9. DOI: 10.1007/s00284-015-0895-6
22. Alexeyev OA, Marklund I, Shannon B, Golovleva I, Olsson J, Andersson C, Eriksson I, Cohen R, Elgh F. Direct visualization of *Propionibacterium acnes* in prostate tissue by multicolor fluorescent in situ hybridization assay. *J Clin Microbiol.* 2007;45(11):3721–8. DOI: 10.1128/JCM.01543-07
23. Fassi Fehri L, Mak TN, Laube B, Brinkmann V, Ogilvie LA, Mollenkopf H, Lein M, Schmidt T, Meyer TF, Brüggemann H. Prevalence of *Propionibacterium acnes* in diseased prostates and its inflammatory and transforming activity on prostate epithelial cells. *Int J Med Microbiol.* 2011;301(1):69–78. DOI: 10.1016/j.ijmm.2010.08.014
24. Konstantinov SR, Smidt H, de Vos WM, Bruijns SC, Singh SK, Valence F, Molle D, Lortal S, Altermann E, Klaenhammer TR, van Kooyk Y. S layer protein A of *Lactobacillus acidophilus* NCFM regulates immature dendritic cell and T cell functions. *Proc Natl Acad Sci USA.* 2008;105(49):19474–9. DOI: 10.1073/pnas.0810305105

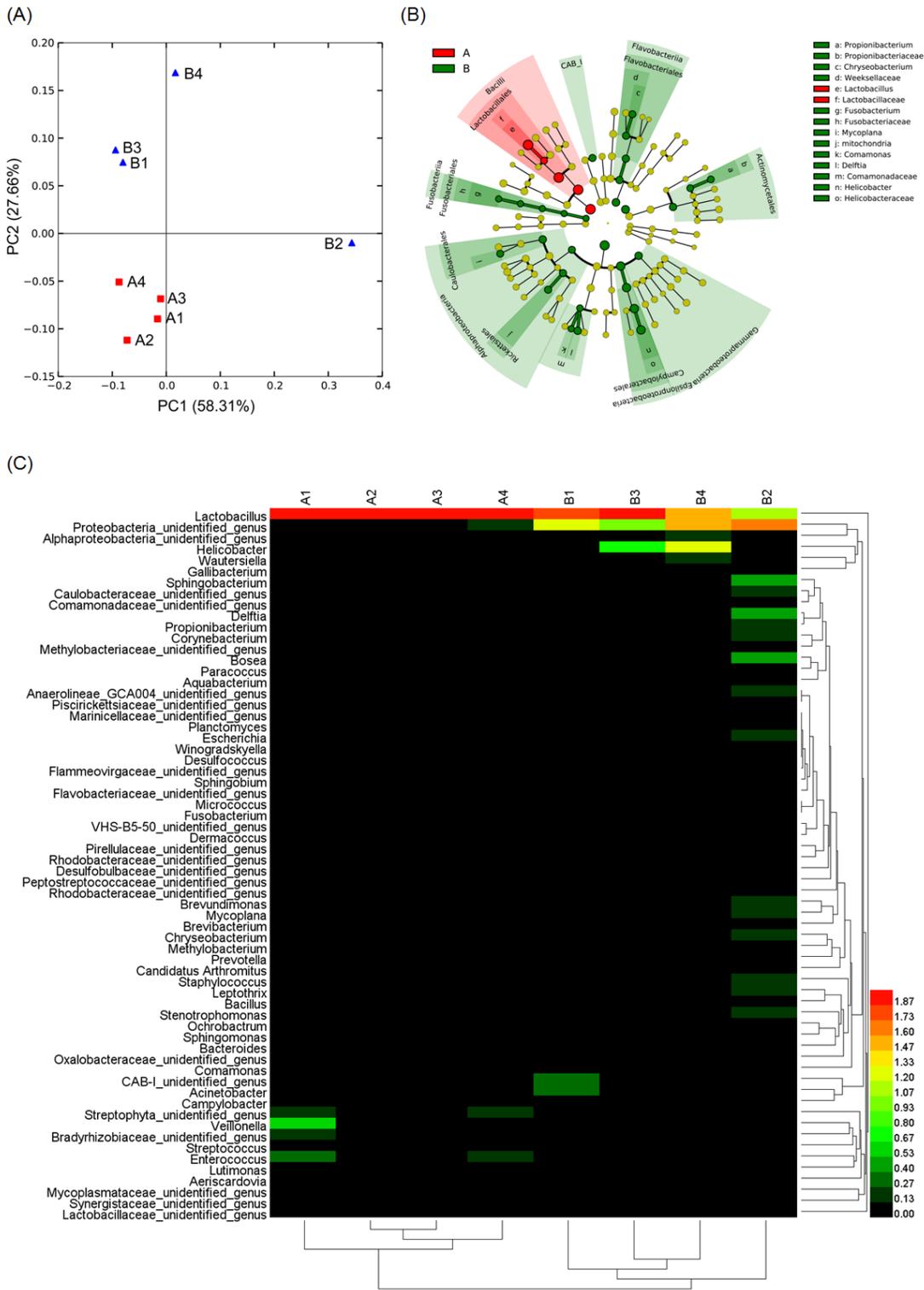
25. von Schillde MA, Hörmannspurger G, Weiher M, Alpert CA, Hahne H, Bäuerl C, van Huynegem K, Steidler L, Hrnir T, Pérez-Martínez G, Kuster B, Haller D . Lactocepin secreted by *Lactobacillus* exerts anti-inflammatory effects by selectively degrading proinflammatory chemokines. *Cell Host Microbe*. 2012;11(4):387–96. DOI: 10.1016/j.chom.2012.02.006
26. Mao X, Gu C, Hu H, Tang J, Chen D, Yu B, He J, Yu J, Luo J, Tian G. Dietary *Lactobacillus rhamnosus* GG supplementation improves the mucosal barrier function in the intestine of weaned piglets challenged by porcine rotavirus. *PLoS One*. 2016;11(1):e0146312. DOI: 10.1371/journal.pone.0146312
27. Liu F, Li G, Wen K, Wu S, Zhang Y, Bui T, Yang X, Kocher J, Sun J, Jortner B, Yuan L . *Lactobacillus rhamnosus* GG on rotavirus-induced injury of ileal epithelium in gnotobiotic pigs. *J Pediatr Gastroenterol Nutr*. 2013;57(6):750–8. DOI: 10.1097/MPG.0b013e3182a356e1.
28. Wen K, Azevedo MS, Gonzalez A, Zhang W, Saif LJ, Li G, Yousef A, Yuan L. Toll-like receptor and innate cytokine responses induced by lactobacilli colonization and human rotavirus infection in gnotobiotic pigs. *Vet Immunol Immunopathol*. 2009;27(3–4):304–15. DOI: 10.1016/j.vetimm.2008.10.322
29. Spinler JK, Taweechotipatr M, Rognerud CL, Ou CN, Tumwasorn S, Versalovic J. Human-derived probiotic *Lactobacillus reuteri* demonstrate antimicrobial activities targeting diverse enteric bacterial pathogens. 2008;14:166–71. DOI: 10.1016/j.anaerobe.2008.02.001
30. Taverniti V and Guglielmetti S. Health-promoting properties of *Lactobacillus helveticus*. *Front Microbiol*. 2012;3:392. DOI: 10.3389/fmicb.2012.00392
31. Qin L, Gao Y, Ni W, Sun M, Wang Y, Yin C, Qi X, Gao H, Wang X. Development and application of real-time PCR for detection of subgroup J avian leukosis virus. *J Clin Microbiol*. 2015;51(1):149-54. DOI: 10.1128/JCM.02030-12
32. Tamaki H, Wright CL, Li X, Lin Q, Hwang C, Wang S, Thimmapuram J, Kamagata Y, Liu WT. Analysis of 16S rRNA amplicon sequencing options on the Roche/454 next-generation titanium sequencing platform. *PLoS One* 2011;6:e25263. DOI: 10.1371/journal.pone.0025263
33. Chen Y, Ni JJ, Li HW. Effect of green tea and mulberry leaf powders on the gut microbiota of chicken. *BMC Vet Res*. 2019;15:77. DOI: 10.1186/s12917-019-1822-z
34. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*. 2011;27:2957–63. DOI: 10.1093/bioinformatics/btr507
35. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*. 2011;27:2194–200. DOI: 10.1093/bioinformatics/btr381
36. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol*. 2007;73:5261–7. DOI: 10.1128/AEM.00062-07
37. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. Metagenomic biomarker discovery and explanation. *Genome Biol*. 2011;12:R60. DOI: 10.1186/gb-2011-12-6-r60

# Figures



**Figure 1**

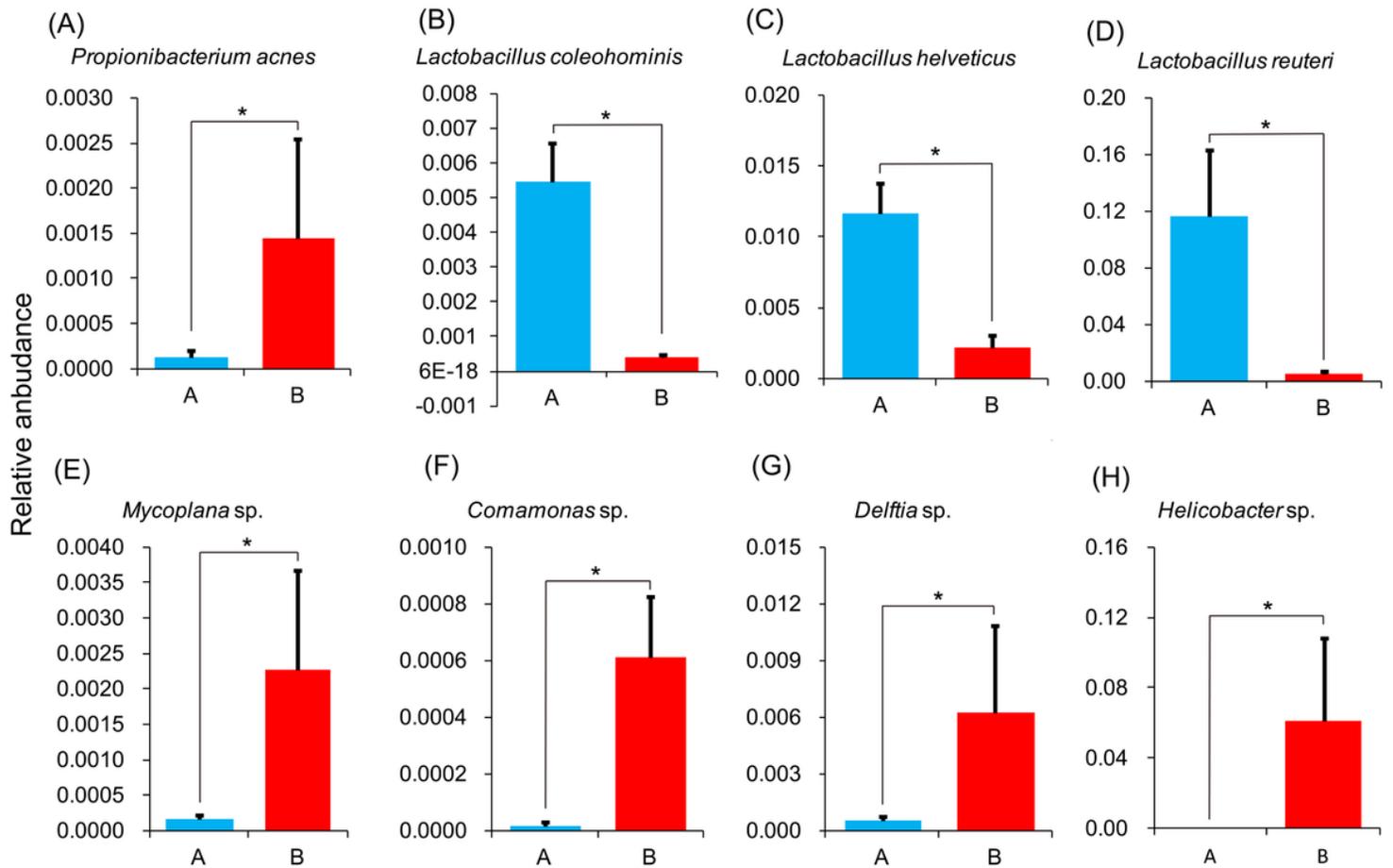
Dominant phyla in gut microbiota of chicken. Across all samples, total sequences were assigned to 38 phyla. The percentage bar diagram shows the composition of the dominant phyla in the chicken gut microbiota in different groups. A and B groups represent healthy control and avian leukosis virus subgroup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



**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 represented different group, red for group B, blue for group A. \*indicates significant differences between the two indicated groups ( $P < 0.05$ ).

## Supplementary Files

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

- [NC3RsARRIVEGuidelinesChecklist2014.docx](#)