Avian Leukosis Virus Subgroup J Infection Alters Viral Composition in the Chicken Gut

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Abstract

Background Chicken is one of the economically important poultry species. ALV-J has emerged as a serious cause of mortality and suboptimal performance of domestic chickens. The changes in the virome may contribute to pathogenesis. Thus, it is important to investigate the effects of ALV-J infection on the composition of virome in chicken.

Results Our results indicated that the chicken gut virome contained a diverse range of viruses, and at the order, family, genus, and species levels, there was a significant difference in virome between the ALV-J-infected chickens and controls. The predominant order was *Herpesvirales*, accounting for more than 96% of the chicken gut virome. Furthermore, the relative abundance of *Caudovirales* in the controls was higher than that in the AVL-J-infected chickens. At the family level, the relative abundance of *Herpesviridae*, *Myoviridae*, *Alloherpesviridae*, and *Genomoviridae* was significantly altered in the AVL-J-infected chickens compared with the controls. Additionally, the relative abundance of 15 genera showed a significant difference between the AVL-J-infected chickens and controls. Interestingly, the relative abundance of 366 species showed significant differences between the AVL-J-infected chickens and controls.

Conclusions Our results indicated that the chicken gut virome contained a diverse range of viruses from invertebrates, vertebrates, plants, and phages. Furthermore, at the order, family, and genus levels, AVL-J infection significantly altered the chicken gut virome composition. The results will increase our understanding of the viral diversity and the changes in chicken virome, with implications in chicken health.

Background

Chicken is one of the economically important poultry species. Avian leukosis virus (ALV) is one of the major causes of disease in chickens. Avian leucosis virus subgroup J (ALV-J) has a higher pathogenicity and transmission ability than the other subgroups. ALV-J was found to cause, in addition to myelocytomatosis, a wide variety of other tumors [1]. During the past decade, the host range of ALV-J has gradually expanded to commercial layers and Chinese domestic breeds [2–4]. More seriously, ALV-J infection results in pathogenic effects, such as immunosuppression, secondary infection [5], and increased mortality, in chickens.

The gastrointestinal tract of chicken harbors various bacteria [6, 7] and viruses. A previous study reported that the microbial communities inhabiting the gastrointestinal tract of chickens significantly affected their health [8]. Therefore, host health is strongly correlated with the maintenance of stasis of the gut microflora [9–11]. An increasing number of studies have indicated that viral infections disrupts the normal microbiota in the chicken gut [12–14]. However, little is known about the effects of viral infections on the virome within the chicken gut. Recent studies have highlighted the role of the virome in maintaining either health or disease [15–17]. These studies raised the possibility that changes in the virome may contribute to pathogenesis. As the largest habitat of microbiota in the body, the intestines are

the most affected by the virome [18]. Thus, it is important to investigate the effects of ALV-J infection on the composition of virome in chicken. Currently, viral metagenomic sequencing has been used to investigate the virome associated with complex disease syndromes in farm animals [19, 20]. In the present study, we utilized metagenomic sequencing to characterize the virome in fecal samples collected from AVL-J-infected chickens and healthy controls, and then explored the effects of ALV-J infection in the chicken gut virome.

Results

Viral metagenomic overview between the AVL-J-infected chickens and controls

The gut virome composition was investigated at the order, family, genus, and species levels in the AVL-J-infected chickens and controls. At the order level in the two groups, the top six orders included *Herpesvirales*, *Caudovirales*, *Picornavirales*, *Bunyavirales*, *Tymovirales*, and *Ortervirales* (Fig. 1A). The predominant order was *Herpesvirales*, accounting for more than 96% in the two groups. The relative abundance of *Caudovirales* in the controls was higher than that in the AVL-J-infected chickens ($P < 0.01$). Viral families represented by only one or two individuals of each group were excluded from the analysis to avoid spurious associations. In both the chicken groups, the four dominant phyla were *Herpesviridae*, *Alloherpesviridae*, *Genomoviridae*, and *Partitiviridae* (Fig. 1B). The relative abundance of *Herpesviridae* and *Myoviridae* showed an obvious decrease in the AVL-J-infected chickens compared with the controls ($P < 0.05$). Conversely, the relative abundance of *Alloherpesviridae* and *Genomoviridae* significantly increased in the AVL-J-infected chickens compared with the controls ($P < 0.01$). At the genus level, there was a significant difference in the relative abundance of the top four genera between the two groups, namely, *Varicellovirus*, *Cyprinivirus*, *Roseolovirus*, and *Simplexvirus (*$P < 0.05$). Additionally, the relative abundance of *Cyprinivirus*, *Roseolovirus*, *Mardivirus*, *Gemykibivirus*, *Percavirus*, and *Pahexavirus* was significantly increased, but there was a decrease in the relative abundance of *Varicellovirus*, *Simplexvirus*, *Limestonevirus*, *Rhadinovirus*, *Bixzunavirus*, *Prasinovirus*, *Polerovirus*, *Cheoctovirus*, and *Cyclovirus* in the AVL-J-infected chickens compared with the controls (Fig. 1C). Overall, the chicken gut virome contained a diverse range of viruses. At the order, family, and genus levels, the results indicated that AVL-J infection significantly altered the chicken gut virome composition.

Host sources of these viruses included bacteria, archaea, fungi, protozoa, algae, invertebrates, vertebrates, and plants. In the AVL-J-infected chickens, the most abundant species found were *Bubaline alphaherpesvirus 1*, *Cyprinid herpesvirus 1*, *Human betaherpesvirus 7*, and *Suid alphaherpesvirus 1.* Whereas, *Bubaline alphaherpesvirus 1*, *Cyprinid herpesvirus 1*, *Suid alphaherpesvirus 1*, and *Macacine alphaherpesvirus 1* were the most abundant species in the controls (Fig. 1D). The relative abundance in 366 species showed significant differences between the two groups ($P < 0.05$) (S1). Interestingly, among the 366 species, 44 species were detected only in the controls, whereas 33 species were found only in the AVL-J-infected chickens. Among the 77 species, *Caudovirales* species showed the most significant

differences between the two groups. Figure 2 shows the phylogenetic analysis of the 77 species based on the taxonomy data from the NCBI (Fig. 2). Moreover, the predominant species detected only in the AVL-J-infected chickens or controls did not show a close phylogenetic relationship. In summary, the AVL-J-infected chickens showed a significant alteration in the diversity, evenness, and richness of species compared with the healthy control gut, suggesting that AVL-J infection significantly altered the composition of the gut virome in chickens.

Dominant taxa between the ALV-J-infected chickens and controls

Figure 3A depicts the PLS-DA scatter plots for the ALV-J-infected chickens and controls. The first PLS component is used to discriminate between the ALV-J-infected chickens and controls, whereas the second PLS component separates each sample within the two groups. These taxa showed a large variation within the groups, and they were different between the two groups. Furthermore, we used the LEfSe for the quantitative analysis of biomarkers within the two groups (Fig. 3B). The LEfSe provided two main outputs, describing the effect sizes of differences observed between the ALV-J-infected chickens and controls. The LEfSe revealed 19 viral clades showing statistically significant and biologically consistent differences in the ALV-J-infected chickens, whereas, 16 viral clades were detected in the controls. The four most abundant taxa in the ALV-J-infected chickens were *Alloherpesviridae*, *Cyprinivirus*, *Roseolovirus*, and *Cyprinid herpesvirus 1*. They were used as biomarkers at the family, genus, and species levels in the ALV-J-infected chickens. In contrast to this, in the controls, the predominant taxa were *Caudovirales*, *Myoviridae*, *Eptesicus fuscus gammaherpesvirus*, and *Suid alphaherpesvirus 1*. They were used as biomarkers at the order, family, genus, and species levels in the controls.

Discussion

Currently, viral metagenomics is used to obtain information on the composition of animal viromes, thus providing candidates for the identification of the etiology of infectious diseases in animals as well as identification of zoonotic and emerging viruses [21]. To the best of our knowledge, this study is the first to describe viral communities in the feces of chickens. Our results indicated that viral infection had a significant impact on host gut virome, which is consistent with a previous study [22].

Our study shows a preliminary view of viral diversity present in the virome of chickens. In both groups of chicken, the most abundant order was *Herpesvirales*, accounting for 96.34–96.75% of the gut virome in the ALV-J-infected chickens and controls, respectively. This is not consistent with a previous study in Duck [23]. Furthermore, in the chicken gut virome, *Caudovirales* contained a diverse range of phage sequences, including members from *Siphoviridae*, *Myoviridae*, and *Podoviridae*. Overall, the chicken gut virome contained a diverse range of viruses from invertebrates, vertebrates, plants, and phages, which likely reflected the diet and social habits of the chickens. Moreover, the PLS-DA analysis indicated diversity in the individual gut virome. Studies of twins have also revealed that the virome was diverse among individuals [24, 25].

At the order, family, genus, and species levels, there were significant differences between the ALV-J-infected chickens and controls, suggesting that ALV-J infection had a significant effect on the gut virome. We observed a significant alteration in the abundance of *Herpesvirales* families, *Alloherpesviridae* and *Herpesviridae* in the ALV-J-infected chickens compared with the controls. Members of the family *Herpesviridae* comprise the mammal, bird, and reptile viruses, and those of the new family *Alloherpesviridae* comprise the fish and frog viruses [26]. Herpesviruses, identified in all vertebrates [27], is one of the largest and most complex viruses. At least one major disease in each domestic animal species, including infectious Marek's Disease, except in sheep, is caused by a herpesvirus. However, a significant effort is required to elucidate the effect of the alteration in diversity of the herpesviruses on chicken health.

Interestingly, in the ALV-J-infected chickens, the relative abundance of *Caudovirales* significantly decreased compared with that in the controls in the present. At the species level, our study indicated that the AVL-J-infected chickens and controls showed significant differences in diversity, suggesting that AVL-J-infection significantly changed the diversity and richness of the *Caudovirales* phage in the chicken gut virome. This was consistent with the findings of a study, which reported that there was a decrease in the abundance of *Caudovirales* phage in the simian immunodeficiency viruses from the gut of infected gorillas compared with uninfected individuals [22]. This implies that viral infections might negatively affect the richness of *Caudovirales* phage in the gut virome.

Caudovirales phage comprises a large proportion of bacteriophages in the gut virome [18]. A previous study indicated that bacteriophages that infect commensal bacteria are diverse and likely to have a substantial effect on the host [28]. A significant increase in the richness of *Caudovirales* bacteriophages was observed in the intestinal diseases, including inflammatory bowel disease, Crohn's disease, and ulcerative colitis [29]. Contrary to the findings of these studies, our results showed a significant decrease in the relative abundance of *Caudovirales* in the AVL-J-infected chickens compared with the healthy controls, and this could a reason that ALV-J infection results in secondary infection in chickens. However, further research is required to determine the direct interactions that occur between the changes in the abundance of bacteriophages and their implications in the pathogenesis of AVL-J infection in chickens.

Conclusions

To the best of our knowledge, this study is the first to describe viral communities in the feces of ALV-J-infected chickens in comparison with the healthy controls. Our results indicated that the chicken gut virome contained a diverse range of viruses from invertebrates, vertebrates, plants, and phages. Furthermore, at the order, family, and genus levels, AVL-J infection significantly altered the chicken gut virome composition. Thus, this study increases our understanding of viral diversity in the enteric tract of chickens and of the changes in chicken virome, with implications in chicken health.

Methods

Animal and fecal sample collection

Based on a previously described method [30], female Huiyang bearded chickens aged around 25 week were divided into the following two groups: viral control (DB group) and naturally ALV-J-infected groups (JB group), with five individuals per group. The chickens were a local broiler in Huizhou City, China. They were collected from the National Huiyang Bearded Chicken Breeding Ground, Guangdong Jinzhong Agriculture and Animal Husbandry Technology Co., Ltd., Huizhou, China. The chickens were housed in a modern, nationally certified animal facility under the supervision of board-certified veterinarians. The chickens were euthanized by cervical dislocation, and then their gut contents were instantly collected from the cecum within 5 min of euthanasia and immediately stored at -80 °C.

Virus enrichment, purification, and nucleic acid extraction

The gut samples were thawed, and then approximately 0.2 g of feces from each sample was transferred into a 1.5-mL Eppendorf tube containing 1 mL of SM Buffer. The contents were vortexed, and then centrifuged (10 000 × g, 10 min, 4 °C) to precipitate the particulate material. The supernatant was filtered through a 0.45-μm filter (Millipore) to remove eukaryotic and bacterial cell-sized particles. The filtrate enriched with viral particles were treated with DNase and RNase to digest the unprotected nucleic acids at 37 °C for 60 min. The total nucleic acid (TNA) was extracted from 250 μL of the filtered supernatant using the TGuide S32 Automatic Nucleic Acid Extractor (Tiangen, China).

Viral metagenomic sequencing

The TNA extracts (12 μL) were reverse transcribed using SuperScript III (Thermo, Fisher, Waltham, MA, USA) with random hexamers. The cDNA was RNase H treated before the second strand synthesis using a Klenow fragment (New England Biolabs, Ipswich, MA, USA). The treated DNA samples were quantified (Nanodrop), and 1 μg of DNA was randomly fragmented by ultrasonication (Covaris) followed by library construction. The qualified libraries were amplified on cBot to generate a cluster on the flow cell. The amplified flow cell was pair-end sequenced on the Illumina NovaSeq 6000 System (Novogene, Beijing, China).

Virome data analysis

The paired-end reads of 150 bp were generated using the Illumina NovaSeq and debarcoded using software from Illumina during bioinformatic analysis. An in-house analysis pipeline running on a 32-node Linux cluster was used to process the data. Clonal reads were removed, and low sequencing quality tails were trimmed using a Phred quality score of 10 as the threshold. Adaptors were trimmed using the default parameters of VecScreen. The cleaned reads were de novo assembled using Megahit, which is an NGS de novo assembler for assembling large and complex metagenomic data in a time- and cost-efficient manner. The assembled contigs, along with singlets, were aligned to an in-house viral proteome database using BLASTx with an E-value cutoff of $< 10^{-5}$. R package mixOmics was employed to perform Partial least squares-discriminant analysis (PLS-DA) [31]. Statistical comparisons of viral communities between treatments were determined using the linear discriminant analysis (LDA) effect size (LEfSe). The

LEfSe analysis was performed using Galaxy website [32]. The phylogenetic analyses (the analysis was performed using https://www.ncbi.nlm.nih.gov/Taxonomy/CommonTree/wwwcmt.cgi and https://itol.embl.de)were performed using the predicted amino acid or nucleotide sequences.

Abbreviations

ALV
Avian leukosis virus ; ALV-J:subgroup J ALV ; LEfSe:linear discriminant analysis (LDA) effect size; PLS-DA:Partial least squares-discriminant analysis

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 protocol was approved by the Committee of the Experimental Animal Management of Huizhou University. The owner has given its consent to use the animals in the study

Consent for publication

Not applicable.

Availability of data and materials

The original sequence data are available at the SRA by accession number PRJNA613285 (https://www.ncbi.nlm.nih.gov/sra/PRJNA613285**).**

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. YC participated in all experiments and contributed to data interpretation. FC and YL participated in some experiments. All authors have read and approved the manuscript

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Figures

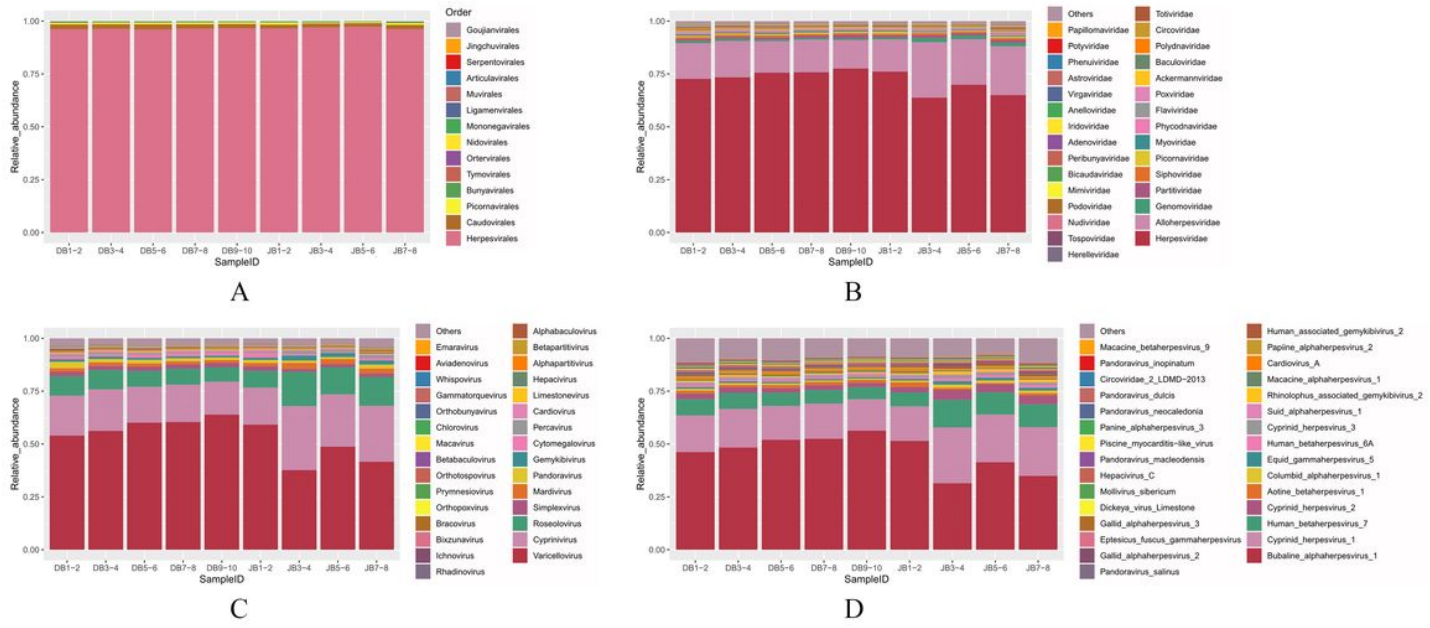

Figure 1

Predominant taxa in the gut virome between the AVL-J-infected chickens and controls. DB and JB representing the controls and AVL-J-infected chickens, respectively. The following letters represent an overview of the gut virome at the order level (A), family level (B), genus level (C), and species level (D).

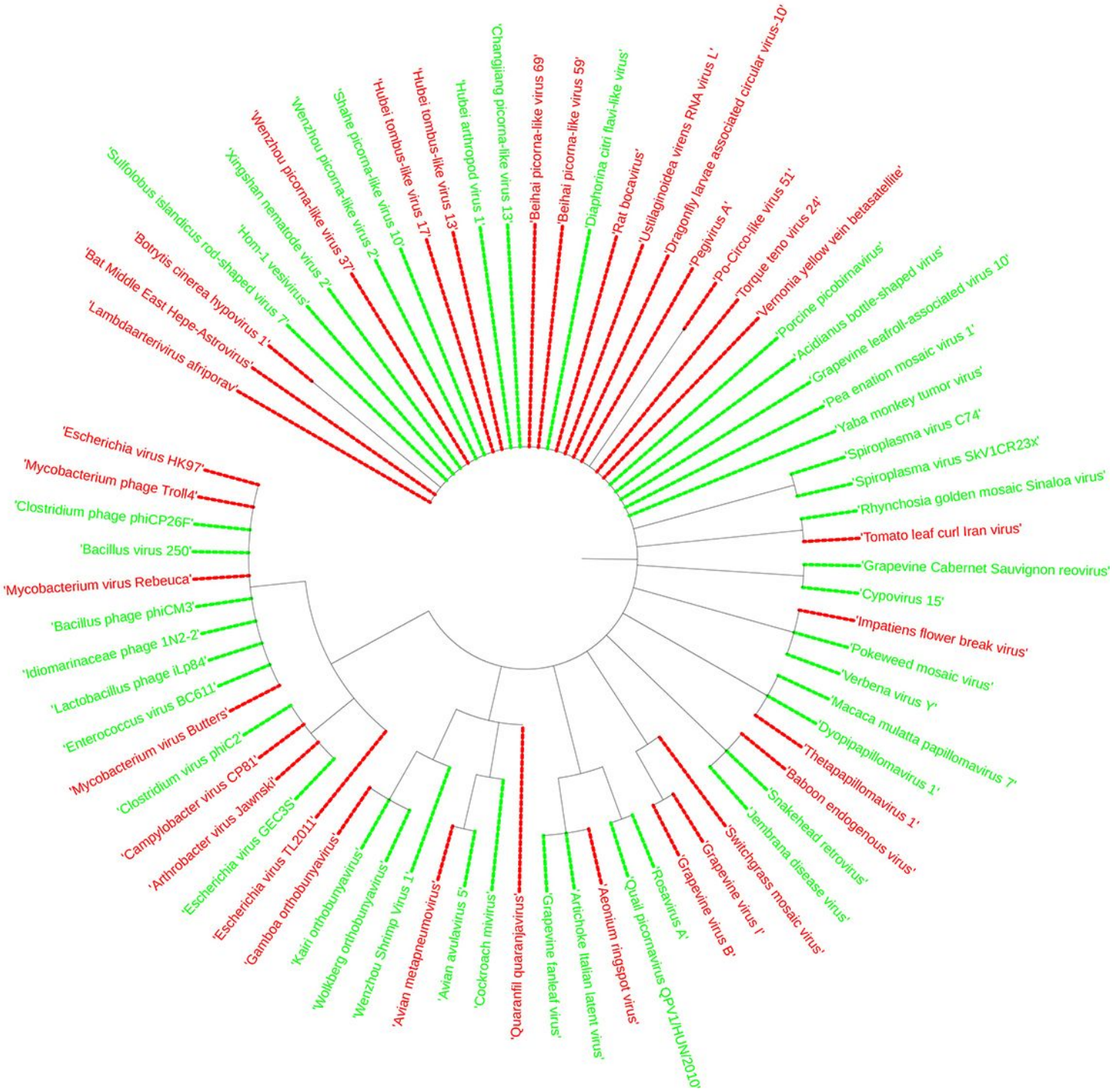

Figure 2

Phylogenetic analysis of the species detected only in the AVL-J-infected chickens or controls using the NCBI. DB and JB representing the controls and AVL-J-infected chickens, respectively. Species detected only in the AVL-J-infected chickens are indicated in green and the species detected only in the controls are indicated in red.

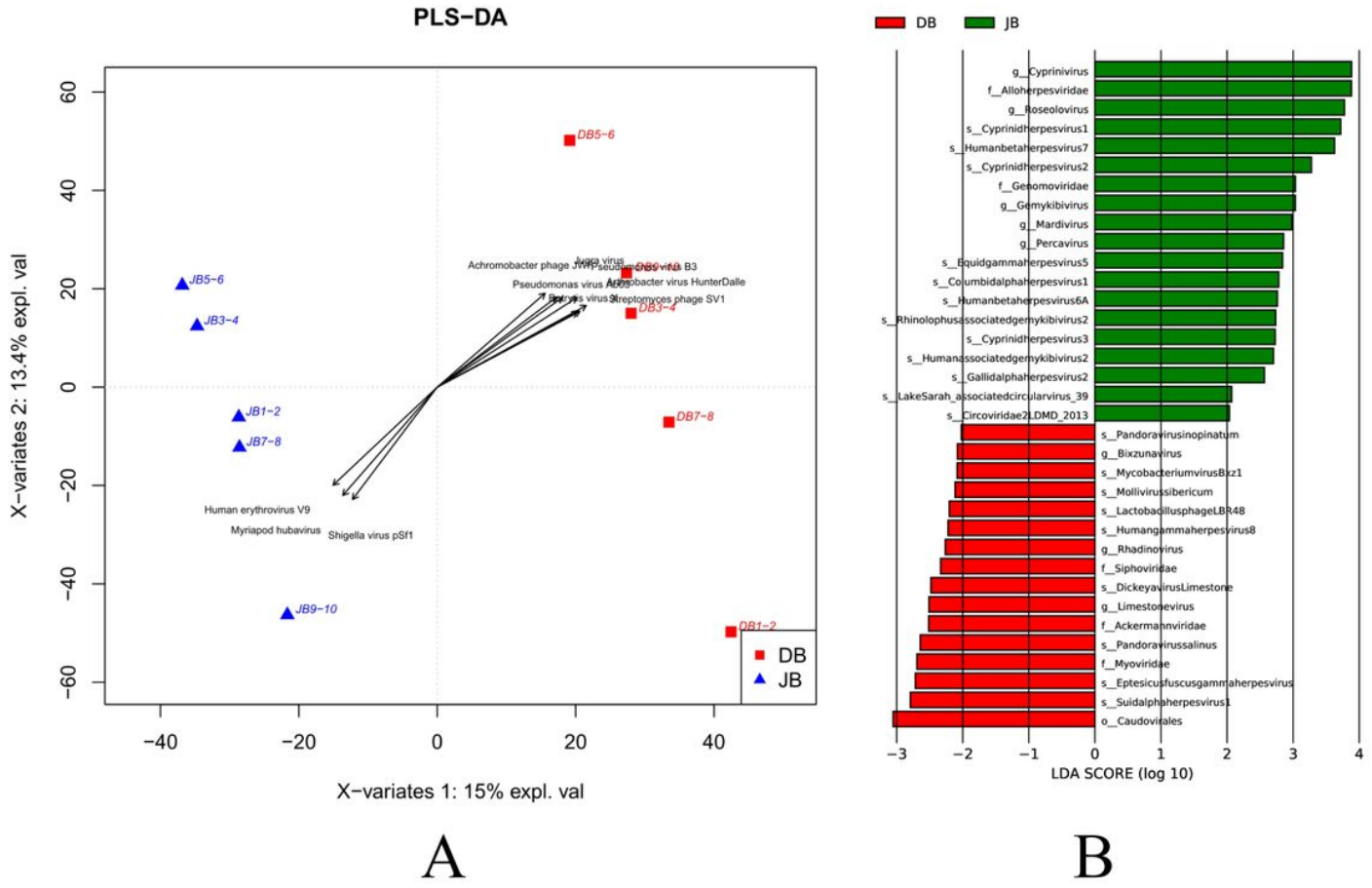

Figure 3

Biomarkers analysis for the ALV-J infected chickens and controls. DB and JB representing the controls and AVL-J-infected chickens, respectively. (A) The PLS-DA scatter plots for the ALV-J-infected chickens and controls. (B) LEfSe were selected for biomarkers analysis on viral profile for the ALV-J-infected chickens and controls. The most abundant classes in the two groups are represented in different colors (red indicates the controls, blue indicates the controls). 35viruses were identified as biomarkers.

Supplementary Files

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