Case Report

Infection of subgroup J avian leukosis induces erythroblasts and lymphoblasts in Chinese local breed chickens

Jie Yang[1] Ziqiang Cheng[1] Chengui Li[1] Guihua Wang[1*]

Abstract

Anaemia, emaciation, increased mortality and decreased egg production were observed in 21 birds in a Chinese local breed flock infected naturally at 240 days old. Macroscopically, the spleen and kidneys were enlarged. There were diffuse, multifocal white foci on the surface of the liver, lungs and ovaries. Histopathological examination demonstrated typical lymphosarcoma cells and erythroblasts in the lungs, spleen, liver, kidneys and ovaries. The results of polymerase chain reaction (PCR) showed that avian leukosis virus subgroup J (ALV-J) was positive in the liver, spleen and ovaries. Immunofluorescence assay results further confirmed the presence of ALV-J antigen in the liver, kidneys and ovaries. Sequence analysis of the specific nucleotides fragments demonstrated that the viruses were closest to the United Kingdom (UK) prototype strain virus HPRS-103 (97.8% to 98.2%). This is the first report of erythroblasts and lymphoblasts caused by ALV-J in Chinese local breed chickens.

Keywords: avian leukosis virus subgroup J, erythroblasts, lymphoblasts, Chinese local breed chickens

[1]*Department of Fundamental Veterinary, College of Veterinary Medicine, Shandong Agricultural University, Tai'an, China*
***Correspondence**: wguihua1126@163.com* (G. Wang)

Introduction

The avian leukosis virus subgroup J (ALV-J) is a retrovirus that induces immunosuppression and tumor formation. The virus was first reported in commercial meat-type chickens in the UK and mainly caused myelocytoma (Payne *et al*., 1991). Subsequently, ALV-J spread to other chicken types and induced hemangioma (Meng *et al*., 2018a), myeloma and fibrosarcoma (Dong *et al*., 2015). ALV-J shows a more extensive host range and tumorigenicity because of its high pathogenicity and mutability, which causes significant economic loss for the poultry industry. Although an eradication program of ALV-J has been successfully applied in China (Zhou *et al*., 2019), ALV-J infection in laying hens and broiler chickens were rarely but still frequently detected in local chickens (Dong *et al*., 2015; Meng *et al*., 2018b). This report describes a case of ALV-J infection in Chinese local breed chickens causing erythroblastoma and lymphoma simultaneously.

Case description

In May 2018, 20 000 chickens of a 240 day old Chinese local breed flock in Heze in Shandong Province infected naturally presented with anaemia, emaciation and depression. The mortality was severe and increased over 10%. The average egg production decreased by up to 70%. A total of 21 birds were necropsied and tissue including heart, liver, spleen, lung, kidney, ovary and proventriculus were collected and rapidly fixed in 10% formalin for histopathological examination.

There were diffuse, multifocal white foci on the surface of (Figure 1A) the lungs (Figure 1B) and liver (Figure 1C). Proventriculus nipples were flattened (Figure 1D), kidneys were swollen (Figure 1E) and spleens (Figure 1F) were severely enlarged. No gross lesions were observed in other organs. Microscopically, lymphoblasts and erythroblast were observed in different tissue sections simultaneously. There were numerous erythroblasts at different growth stages in the blood vessels of the ovaries (Figure 2A), lungs (Figure 2B), liver (Figure 2C) and kidneys (Figure 2E). The erythroblasts replaced the normal erythrocyte in the splenic pulp of the spleen (Figure 2F). The erythroblasts had polymorphism (spherical, ellipse and irregular shape), greater cellularity, loosened chromatin and a bigger volume than normal erythrocytes. About 2-4 cells with mitotic figures were seen in one high-powered field. Otherwise, a large number of lymphoblasts replaced the normal architecture of the ovaries (Figure 2A), lungs (Figure 2B), livers (Figure 2D) and spleen (Figure 2F). The normal organizational structures of these tissues were destroyed by tumor cells. The lymphoblasts had uniform morphous, large vesicular nuclei and faint basophilic cytoplasm. The large nuclei contained one or two nucleoli.

DNA was extracted from the livers, kidneys, spleen, lungs and ovaries using DNA extraction kit (TaKaRa, Bio, Inc., China). The polymerase chain reaction (PCR) amplifications were performed using provirus DNA as templates with specific primers for ALV-J (Smith *et al*., 1998), avian leukosis virus subgroup A/B (ALV-A/B) (Silva et al., 2007), Marek's disease virus (MDV) (Silva and Smith, 1997), reticuloendotheliosis virus (REV) (Silva *et al*., 2007) and avian erythroblastosis virus (AEV) (Wang *et al*., 2013) respectively (Table 1). Three of the nine samples were positive for ALV-J with a PCR product of 924 bp (Figure 3). The three DNA samples were extracted from the ovaries, liver and kidneys, respectively. All samples were negative for other viruses (data not shown).

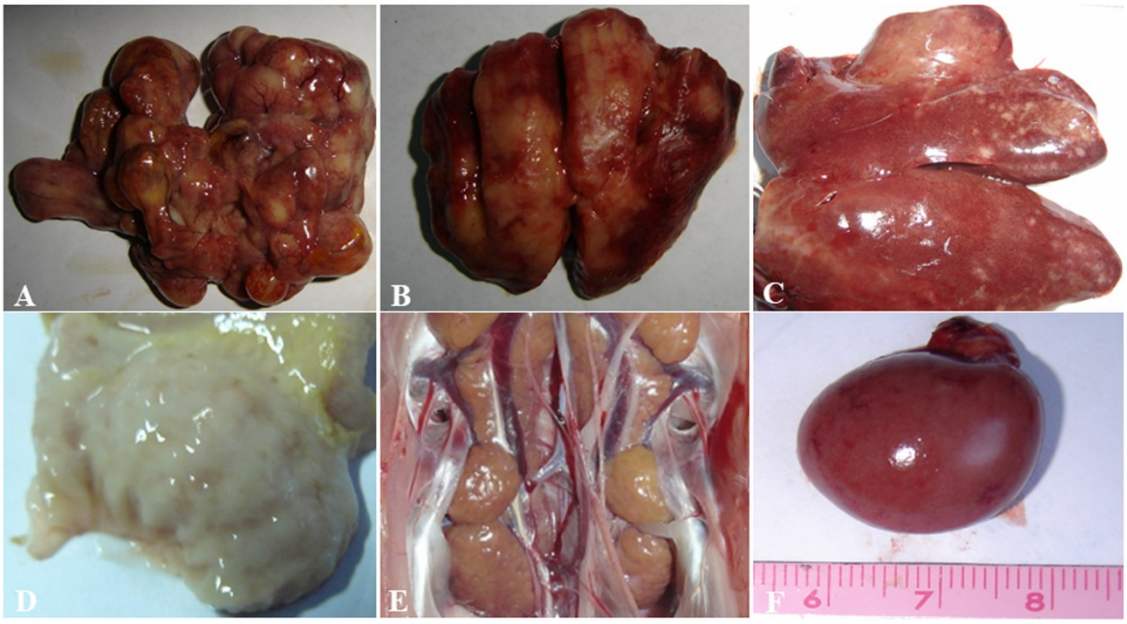

Figure 1 Gross lesion features. Diffuse, multifocal white foci on the surface of ovaries (A), lungs (B) and liver (C), proventriculus nipples flattened (D), kidney speckled (E) and spleen enlarged (F).

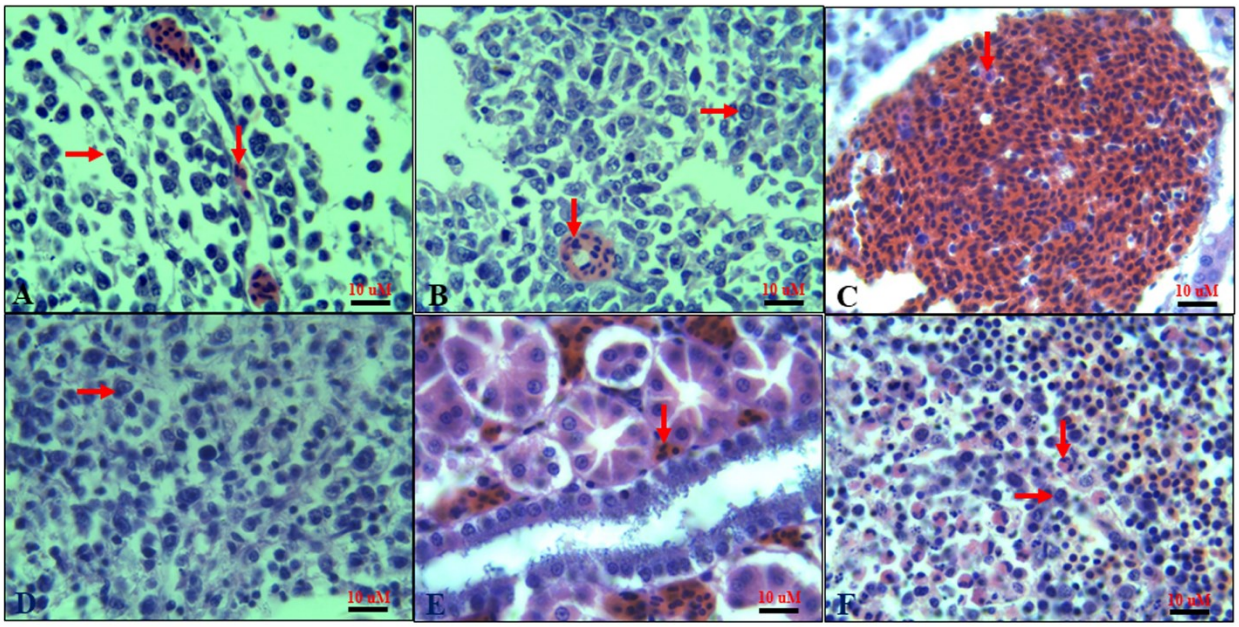

Figure 2 Histopathological features. Erythroblasts were in ovaries (A), lungs (B), liver (C), kidneys (E) and spleen (F) (arrow). Lymphoblasts were in ovaries (A), lungs (B), liver (D) kidneys and spleen (F) (arrow). Bar= 10 uM

Table 1 Primers used for PCR diagnosis

Primers	sequences	Fragment sizes
ALV-A(env)	F:5′-CGAGAGTGGCTCGCGAGATGG-3′ R:5′-CCCATTTGCCTCCTCTCCTTGTA-3′	1300bp
ALV-B(env)	F:5′-CGAGAGTGGCTCGCGAGGATGG-3′ R:5′-AGCCGGACTATCGTATGGGGTAA-3′	1100bp
ALV-J(env)	F:5′-ATGGGAGTTCATCTATTGCAACAACCAG-3′ R:5′-TTAGCGCCTGCTACGGTGGTGACC-3′	924bp
MDV	F:5′-TACTTCCTATATAGATTGAGACGT-3′ R:5′-GAGATCCTCGTAAGGTGTAATATA-3′	132bp
REV(env)	F:5′-AGCTAGGCTCGTATGAA-3′ R:5′-TATTGACCAGGTGGGTTG-3′	138bp
AEV(env)	F:5′-AGAAGAACCTGCACCCCACCTAC-3′ R:5′-AAAGACCGATGCCTAGACCAACC-3′	1981bp

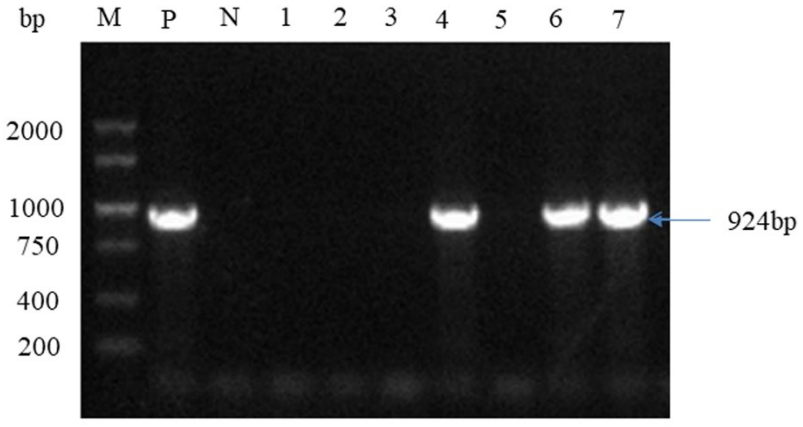

Figure 3 The result of PCR for ALV-J detection. M: marker DL2000. P: Positive control. N: Negative control. Lane 1-7: DNA templates from spleen, lungs, brain, ovaries, proventriculus, liver and kidneys.

To further confirm the presence of virus, tissues extracted from the liver, lungs, spleen and ovaries of the ill chickens were inoculated onto DF-1 cells. Then indirect immunofluorescence assay (IFA) was performed using specific primary antibodies against ALV-A/B (1:200, Santa Cruz, USA), ALV-J (1:500, prepared by our lab), REV (1:200, Santa Cruz, USA), MDV (1:200, Santa Cruz, USA) and AEV (1:200, prepared by our lab). The cells were incubated with FITC secondary antibody (1:1000, Santa Cruz, USA). Green fluorescence was observed in the cytoplasm of DF-1 cells incubated with antibody against ALV-J

(Figure 4B-F). There was no staining in the negative control cells (Figure 4A). These results indicated the ALV-J infection on cells. No fluorescent signals for other virus were observed (data not shown).

Lastly, the sequence of PCR product was sent to BGI for analysis. The sequence alignment performed with DNAStar software was compared with other strains. From phylogenetic analysis of the whole *gp85* gene, homology comparing among ALV-J family further indicated that the three strains sequences were closest to the United Kingdom (UK) prototype virus, HPRS-103 (≥98%) (Figure 5).

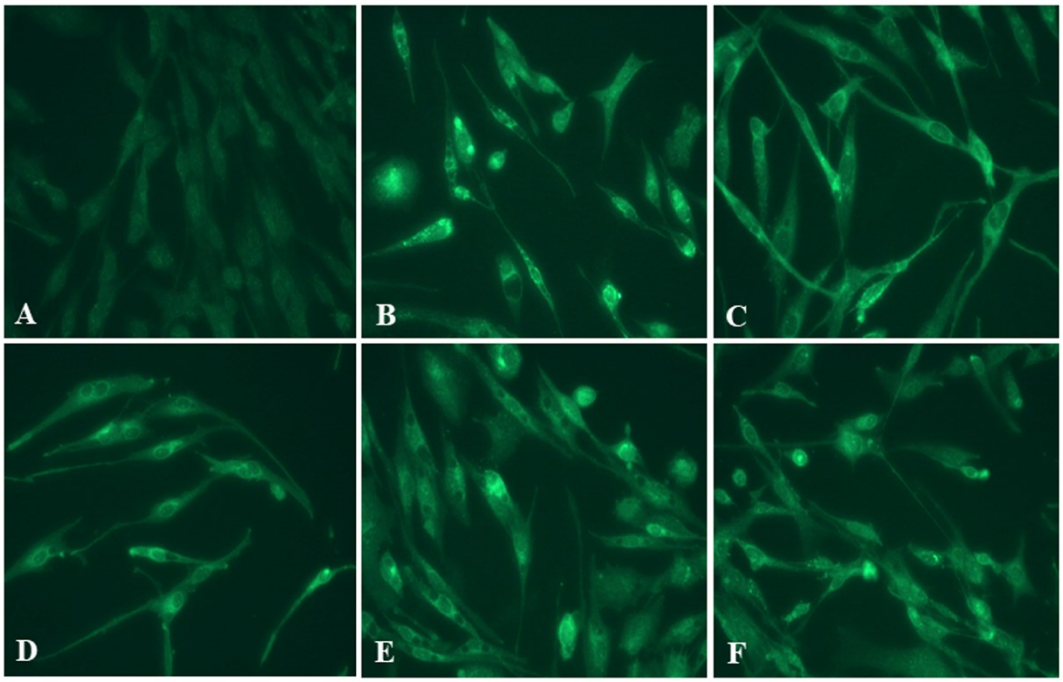

Figure 4 Detection of ALV-J in DF-1 cells by IFA incubated with antibody against ALV-J. A: Negative control. B: Positive control, DF-1 cell infected with ALV-J. DF-1 cell incubated with the inoculum of liver (C), lungs (D), ovaries (E) and spleen (F).

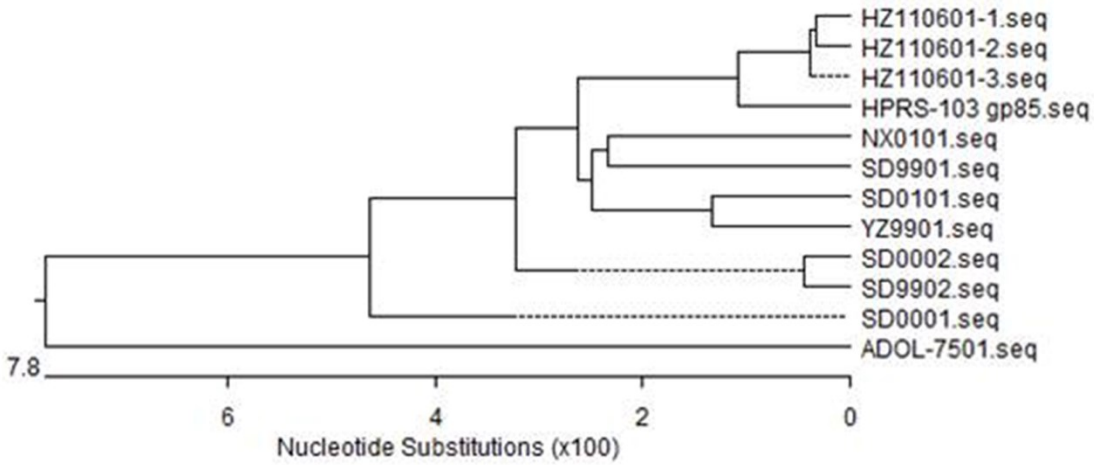

Figure 5 Phylogenetic relationships among the gp85 sequences of isolated ALV-J strains and other 9 known ALV-J strains. The sequences of HZ110601-1, HZ110601-2, and HZ110601-3 were obtained in this study. HZ110601-1 was from ovaries. HZ110601-2 was from liver. HZ110601-3 was from kidneys.

Discussion

In this study, the infected chickens were diagnosed as erythroblasts and lymphoblasts with a possible pathogen of ALV-J based on the epidemiology, clinical signs, histopathology and the shape and structural character of tumor cells. This diagnosis result was confirmed by PCR for ALV-J and sequences analysis of the PCR product.

Disease associated with ALV-J has been described for more than 20 years(Payne *et al*., 1991). The first reported in China was in 1999 and became a major disease that led to enormous economic losses. According to the Chinese government's 11th Five-Year plan (2011-2015), ALV-J would be eradicated from commercial chicken farms (Meng *et al*., 2018b). However, ALV-J is a recombinant exogenous ALV, which shows a more extensive host range in comparison with other subgroups, especially in Chinese local chickens (Qiu *et al*., 2018).

Sequence analysis showed the pathogen was a variant strain of HPRS-103. It has been reported that ALV-J infection induced erythroblasts in Chinese local breed chickens (Wang *et al*., 2013). This is the first report of naturally simultaneously occurring lymphoblasts and erythroblasts caused by ALV-J in Chinese local breed stock. It has been reported that the HPRS-103 strain does not induce lymphoid leukosis associated with low bursal tropism (Arshad *et al*., 1997). The virus titers, expression levels of tumor-related genes and host responses influence host susceptibility and tumor classification in ALV-J infection (Qu *et al*., 2016). Our results demonstrated that the isolated strain was a multipotential oncogenic virus. Multipotential oncogenicty maybe associated with the mutation of virus and host.

References

Arshad SS, Howes K, Barron GS, Smith LM, Russell PH and Payne LN 1997. Tissue Tropism of the HPRS-103 Strain of J Subgroup Avian Leukosis Virus and of a Derivative Acutely Transforming Virus. Vet Pathol. 34(2):127-137.

Dong X, Zhao P, Li W, Chang S, Li J, Li Y, Ju SD, Sun P, Meng FF, Liu J and Cui ZZ 2015. Diagnosis and sequence analysis of avian leukosis virus subgroup J isolated from Chinese Partridge Shank chickens. Poul Sci. 94(4):668-672.

Meng FF, Li QC, Zhang YB, Cui ZZ, Chang S and Zhao P 2018a. Isolation and characterization of subgroup J Avian Leukosis virus associated with hemangioma in commercial Hy-Line chickens. Poul Sci. 97(8):2667-2674.

Meng FF, Li QC, Zhang YW, Zhang ZH, Tian SB, Cui ZZ, Chang S and Zhao P 2018b. Characterization of subgroup J avian Leukosis virus isolated from Chinese indigenous chickens. Virol J. 15(1):33-40.

Payne L, Brown S, Bumstead N, Howes K, Frazier JA and Thouless ME. 1991. A novel subgroup of exogenous avian leukosis virus in chickens. J Gen Virol. 72(Pt4):801-807.

Qiu LL, Chang GB, Bi YL, Liu XP and Chen GH 2018. Circular RNA and mRNA profiling reveal competing endogenous RNA networks during avian leukosis virus, subgroup J-induced tumorigenesis in chickens. PloS One. 213(10): e0204931.

Qu YJ, Liu LT, Niu YJ, Qu Y, Li N, Sun W, Lv CW, Wang PF, Zhang GH and Liu SD. 2016. Viral proliferation and expression of tumor-related gene in different chicken embryo fibroblasts infected with different tumorigenic phenotypes of avian leukosis virus subgroup J. Poul Sci. 95(10):2383-2390.

Silva RF and Smith EJ, 1997. PCR as a tool for the diagnosis of avian tumor viruses and tumors. Avian Tumor Viruses symposium, 40th Ammual Meeting Reno. Nevada: AAAP Inc, 19-22.

Silva RF, Fadly AM and Taylor SP. 2007. Development of a polymerase chain reaction to differentiate avian leukosis virus (ALV) subgroups: detection of an ALV contaminant in commercial Marek's disease vaccines. Avian Dis. 51(3):663-667.

Smith EJ, Williams SM, Fadly AM. 1998. Detection of avian leukosis virus subgroup J using the polymerase chain reaction. Avian Dis. 42(2):375-380.

Wang GH, Jiang YP, Yu LL, Wang Y, Zhao XM and Cheng ZQ 2013. Avian leukosis virus subgroup J associated with the outbreak of erythroblastosis in chickens in China. Virol J. 10(1):1-6.

Zhou DF, Xue JW, Zhang Y, Wang GH, Feng YS, Hu LP, Shang YL and Cheng ZQ 2019. Outbreak of myelocytomatosis caused by mutational avian leukosis virus subgroup J in China, 2018. Transbound Emerg Dis. 66(2):622-626.