

Re-emergence of Avian leukosis virus subgroup J in the rooster of Hy-line brown layers and its transmission pattern in flocks

Fanfeng Meng (✉ fanfengmeng@126.com)

Shandong Agricultural University <https://orcid.org/0000-0002-3773-840X>

Qiuchen Li

China Agricultural University

Xintao Gao

Biotechnology research Institute,caas

Fubing Luo

beijing center for animal disease control and prevention

Guangnian Shen

beijing center for animal disease control and prevention

Xiaodong Liu

Centers for Disease Control and Prevention

Zhifang Zhang

Biotechnology Research Center

Peng Zhao

Shandong Agricultural University

Guozhong Zhang

China Agricultural University

Research Article

Keywords: ALV-J, Rooster, Semen, transmission pattern

Posted Date: July 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-644696/v1>

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

[Read Full License](#)

Abstract

Avian leukosis virus subgroup J (ALV-J) is the most prevalent subgroup in chickens and exhibits increasing pathogenicity and stronger horizontal and vertical transmission ability among different kinds of chickens. Although vertical transmission of ALV-J from hens infected through artificial insemination was reported before by the detection of swabs and serum, but there was no further research on the transmission pattern of ALVs in the roosters. In the present study, the introduction of Hy-line brown roosters significantly increased the p27 positive rate of ALV in an indigenous flock detected by ELISA and virus isolation. Sequence analysis and IFA showed that it is classified into ALV-J subgroup, locating in a new branch compared with the domestic and foreign referential sequences. Meanwhile, the *gp85* gene of the ALV-J isolated in the hens and its albumens had a homology of 94.1–99.7% with that in the roosters, which means that the strain is quite likely transmitted to the hens and their offspring through insemination of the roosters. In addition, there are four ALV-J infection status in plasma and semen of rooster (V + S+, V-S+, V + S-, V-S-), so the eradication of ALV in rooster requires simultaneous virus isolation of semen and plasma. Additionally, compared with ALV detection in samples by DF-1 cells, directly detecting ALV in semen by ELISA exists some false positives. Collectively, our results suggested that the incomplete eradication process of roosters leads to the sporadic findings of ALV-J in laying hens

1 Introduction

Avian leukosis (AL), inducing malignant or benign tumorigenic diseases by avian leukosis virus (ALV), has caused immense economic losses all over the world in the poultry industry since its emergence [1, 2]. ALVs were divided into 11 different subgroups (designated A to K) based on cross neutralization and *gp85* sequences [3]. Among those, avian leukosis virus subgroup J (ALV-J) is the most prevalent subgroup in chickens and exhibits increasing pathogenicity and stronger vertical transmission ability in recent years [4–6]. ALV-J infection used to be a common problem in poultry industry during 2003–2010, causing myelocytoma of broilers and haemangioma of layers when its widespread [7–11]. Because of the widespread distribution of the ALV-J strains, together with the less organized character of the poultry industry especially among the local breeds of chickens, control and eradication of ALV in China remain a major challenge. Since 2018, a more tumorigenic ALV-J strain has appeared in the imported broiler breeders in China, inducing severe osteomas in keel and ribs, causing a massive pandemic, and hence becoming a major concern of avian health [3, 11]. Therefore, many domestic farms have started self-checking for ALV infection [3].

Rubin and his colleagues defined four serological classes of susceptible birds: viraemia, no antibody (V + A-); no viraemia, with antibody (V - A+); viraemia, with antibody (V + A+); and no viraemia, no antibody (V - A-)[2, 12]. Another study showed that roosters could spread ALV-J to hens by insemination and cause vertical transmission [13]. Yet the researchers did not elaborate on the ALV infection status of semen from male or the role of males in the transmission of ALV. In the present, roosters may be selected for freedom from ALV by ELISA tests of cloacal swabs or semen sampling, as well as virus isolation. However, the accuracy of these detection methods remains to be defined.

In this study, it was discovered that ALV-J re-emerged in Hy-line brown roosters in recent years. Moreover, the hens were infected by insemination, and virus can be transmitted vertically to the breeding eggs. Several infection states of ALV-J in roosters were clarified and the reliability of different methods for detecting samples of males were compared. All those data may provide more detailed basis for the eradication of ALV.

2 Materials And Methods

2.1 Sample origin

The positive rates of virus isolation in flocks and p27 antigen in breeding eggs of an indigenous chicken were both within 1% for two consecutive generations. In order to introduce new genes, the introduction of 46 Hy-line brown fast feathering roosters and artificial insemination were performed. Ever since, about 7.61% (7 from 92 samples) were found to be p27 positive in the albumen samples in this generation. Therefore, semen and plasma samples from the 46 roosters, plasmas from 92 hens and 10 albumen samples of breeding eggs from the five ALV positive hens (2 samples/hens) were collected for virus isolation and identification.

2.2 virus isolation and identification

Blood and semen were collected aseptically from the Hy-Line brown roosters and indigenous hens. The semen samples were diluted 5 times with PBS containing 2% penicillin and streptomycin. After mixing upside down gently, the supernatant was centrifuged to precipitate cells at 3000×g for 2 min. Albumens of 10 breeding eggs were collected aseptically from 5 ALV positive chickens, and diluted 4 times with DMEM in 5ml syringes. Virus isolation from plasma, semen and albumens were performed by inoculating DF1 cells as previously described [9]. The ALV group-specific antigen p27 in the culture supernatant was detected by enzyme-linked immunosorbent assay (ELISA, IDEXX, USA(#99-0925)).

2.3 Indirect immunofluorescence assay (IFA)

The cell were washed with PBS and fixed with cold acetone–alcohol mixture (3:2) for 5 min. Then, the cells were incubated with mouse anti-ALV-J monoclonal antibody JE9 [24] at 37°C for 60 min, following by incubation with goat anti-mouse IgG antibody conjugated with fluorescein isothiocyanate (Sigma, California, USA) at 37°C for another 60 min. Finally, the cells were observed under fluorescence microscope.

2.4 Primers and PCR Amplification

A pair of universal primers (The length of the amplification is about 2200bp), targeting env and LTR, was adopted to detect exogenous ALVs as previously reported (ALV-F:GATGAGGCGAGCCCTCTCTTTG/ALV-R: TGTGGTGGGAGGTAAATGGCGT) [11]. DNA was extracted from the DF-1 cells. The conditions for PCR with primers ALV-F/R were as follows: 95°C for 5 min; followed by 31 cycles of 95°C for 50 s, 55°C for 40 s, and 72°C for 140 s; with a final elongation step of 10 min at 72°C. The PCR product was analyzed by

electrophoresis in 0.8% agarose in Tris-acetate-EDTA buffer. Molecular cloning of positive amplicons was performed and positive clones were confirmed and subjected to Sanger sequencing.

2.5 Sequence Analysis

Sequence alignments were assembled with other ALV-J referential sequences retrieved from the National Center for Biotechnology Information database (Table 1). Phylogenetic analysis was based on the neighbor-joining method with 1000 bootstrap replicates by MEGA ver. 6.0. The GenBank accession numbers of the strains used in this study are listed in Table 1.

Table 1
The referential strains for the comparison of *gp85* gene.

Strain	Year	Origin	Source	Genbank	Subgroup
RAV-1	1980	USA	/	MF926337	A
MAV-2	1993	CAN	/	L10922	B
RSR B	1998	USA	/	AF052428	B
Prague C	1977	USA	/	J02342	C
RSR D	1992	JPN	/	D10652	D
EV-1	2000	USA	/	AY013303	E
SD0501	2007	CHN	CEF	EF467236	E
JS11C1	2014	CHN	Gallus	KF746200	K
JS14CZ01	2017	CHN	Gallus	KY490695	K
QL1	2020	EGY	Layer	MN496121	J
HPRS-103	1989	UK	Brioler	Z46390	J
NX0101	2001	CHN	Brioler	DQ115805	J
ADOL-7501	1997	UK	CEF	AY027920	J
ADOL-HC1	1993	UK	Brioler	AF097731	J
JS09GY3	2009	CHN	Layer	GU982308	J
HuB09WH02	2009	CHN	Layer	HQ634804	J
HN1001-1	2010	CHN	Layer	HQ260974	J
GD1109	2011	CHN	Layer	JX254901	J
NG_VX29	2017	CHN	Vaccine	MH669345	J
EO59	2017	NGA	Layer	MF926336	J
SVR807	2008	RUS	Gallus	HM776937	J
10022-2	2006	USA	Gallus	GU222396	J
SDGM1801	2018	CHN	Broiler	MN413674	J
GD14J2	2014	CHN	Yellow	KU500032	J
YZ9902	2010	CHN	Broiler	HM235670	J
CLB908U	2009	RUS	Gallus	JQ935966	J
HB201101	2020	CHN	Layer-P	MW476816	J

Strain	Year	Origin	Source	Genbank	Subgroup
HB201101-1	2020	CHN	Layer-E	MW476817	J
HB201102	2020	CHN	Layer-P	MW476818	J
HB201102-1	2020	CHN	Layer-S	MW476819	J
HB201103	2020	CHN	Layer-P	MW476820	J
HB201103-1	2020	CHN	Layer-S	MW476821	J
HB201104	2020	CHN	Layer-P	MW476822	J
HB201104-1	2020	CHN	Layer-S	MW476823	J
HB201105	2020	CHN	Layer-P	MW476824	J
HB201105-1	2020	CHN	Layer-S	MW476825	J
HB201106	2020	CHN	Layer-P	MW476826	J
HB201106-1	2020	CHN	Layer-S	MW476827	J
HB201107	2020	CHN	Layer-P	MW476828	J
HB201107-1	2020	CHN	Layer-S	MW476829	J

3 Results

3.1 The introduction of Hy-line brown roosters with ALV-J increased the positive rate of ALV in the flock

Before the introduction of Hy-line brown roosters, the positive rate of virus isolation and p27 in the albumens in the flock was within 1% for two consecutive generations, and the peak egg production was about 70%. However, after the introduction of the roosters, the positive rate of virus isolation and p27 in the albumens increased to 5.4% (5/92) and 7.6% (7/92), respectively. The peak egg production dropped to about 65% from 70%. The albumen of two breeding eggs from each positive hen were collected for virus isolation, and only one albumen showed positive result. Collectively, our results indicated that the introduction of Hy-line brown roosters with ALV-J increased the positive rate of ALV in the flock.

3.2 The infection status of ALV in semen and plasma of a rooster

To determine whether the rooster infected the ALV, 46 semen and plasma samples from the 46 roosters were collected and detected by different methods. In 46 roosters, 14 roosters were tested to be positive for ALV in plasma or semen (30.43%). Among those, 12 roosters were positive in plasma (26.09%) and 8 roosters were positive in semen (17.39%). According to the results of virus isolation from semen and plasma of the 46 roosters, there are 4 infection status of Avian leukosis virus (Table 2). 6 roosters

showed viraemia, with semen positive (V + S+, 13.04%); 6 roosters showed viraemia, with semen negative (V + S-, 13.04%); 2 roosters showed no viraemia, with semen positive (V-S+, 4.35%), while the remaining 32 roosters were double-negative (V-S-, 69.56%).

Table 2
The results of virus isolation and ELISA with different samples.

Number	Genbank No.	Status	Plasma VI	Semen VI	Semen ELISA
Roosters					
1-8,10,12-16,18-19,22-23,32-33,37-38,40-43,82	/	P(-)S(-) (27/46) 58.69%	Neg	Neg	Neg
9	/	P(-)S(-) (5/46) 10.87%	Neg	Neg	Pos
11	/		Neg	Neg	Pos
20	/		Neg	Neg	Pos
21	/		Neg	Neg	Pos
39	/		Neg	Neg	Pos
24	/	P(+)S(-) (6/46) 13.04%	Pos	Neg	Pos
27	/		Pos	Neg	Pos
28	/		Pos	Neg	Neg
35	/		Pos	Neg	Neg
51	/		Pos	Neg	Neg
55	/		Pos	Neg	Neg
17	HB201102/-1	P(+)S(+) (6/46) 13.04%	Pos	Pos	Pos
25	HB201103/-1		Pos	Pos	Pos
26	HB201104/-1		Pos	Pos	Pos
30	HB201105/-1		Pos	Pos	Pos
31	HB201106/-1		Pos	Pos	Neg
57	HB201107/-1		Pos	Pos	Neg
29	/	P(-)S(+) (2/46) 4.35%	Neg	Pos	Neg
34	/		Neg	Pos	Neg
Hens					
E-4	/	Plasma	Pos	/	/
E-7	/	Plasma	Pos	/	/

Note: The numbers are the original number in the flock. P = Plasma, S = Semen; VI = Virus isolation; Pos = Positive, Neg = Negative, "/" = no result.

Number	Genbank No.	Status	Plasma VI	Semen VI	Semen ELISA
E-9	/	Plasma	Pos	/	/
E-20	/	Plasma	Pos	/	/
E-63	HB201101	Plasma	Pos	/	/
E-63	HB201101-1	Albumen	Pos	/	/

Note: The numbers are the original number in the flock. P = Plasma, S = Semen; VI = Virus isolation; Pos = Positive, Neg = Negative, "/" = no result.

3.3 High proportion of false positives in semen directly detected by ELISA

In addition, the semen were detected directly by ELISA, and the results showed that 11 semen samples were positive, but only 6 corresponding semen samples showed positive from virus isolation by DF1 cells in plasmas or semen. while 5 semen samples showed positive in the semen by ELISA, which were negative, due to no virus isolated by DF-1 cells. Therefore, Our data suggested that the ALV was directly detected in semen by ELISA is not inaccurate, which due to unknown material in the semen binding the p27 antigen.

3.4 Identification of ALV-J isolated from roosters, hens and albumens

14 positive samples from roosters, hens, and albumens were went through virus isolation and sequence analysis. Phylogenetic analysis of the *gp85* gene, suggested that all of the isolated strains belonged to ALV-J. However, these strains are located in a new branch of the subgroup J, and do not show obvious homology through the comparison with domestic and foreign referential sequences (Fig. 1). In addition, we performed the IFA experiments, and consistent with phylogenetic analysis, the obvious green fluorescence could be observed using the monoclonal antibody JE9 (Fig. 2).

3.5 The homology analysis of ALV-J strains in roosters, hens and albumens

The viruses isolated from roosters, hens, as well as albumens, show a homology between 94.1–99.7%, and are located in the same branch of the phylogenetic tree (Fig. 1), which demonstrate that they may be derived from the same source. The comparison of the *gp85* sequence between semen and plasma sample of the same rooster shows that the nucleotide homology in most roosters (5/6) is more than 98.6%, but the other one (1/6) only has a homology of 96%, and its homology with that of the other 5 chicken is as low as 94.1%.

4 Discussion

Since the first strain of ALV-J, HPRS-103, was isolated from meat-type breeder chickens in the UK in 1988, it has rapidly spread around the world, causing severe economic losses to the poultry industry[4, 14]. Being the most pathogenic subgroup of ALV, ALV-J could induce malignant or benign tumorigenic diseases and immunosuppression in chicken, including hemangiomas, myelomas and fibrosarcomas [2, 15–17]. The outbreak of hemangioma associated with ALV-J was reported between 2006 and 2010 in China in commercial layer chickens[7, 18]. However, with the implementation of the ALV purification project by a number of major breeder companies, successful eradication has been achieved at pedigree and multiplier levels. Therefore, there is few reports of ALV-J infection in layers in recent years. Zhao discovered that co-infection with avian hepatitis E virus and avian leukosis virus subgroup J is the cause of an outbreak of hepatitis and liver hemorrhagic syndromes in a Hy-line brown layer chicken flock in China recently[19]. This study also found subclinical infection of ALV-J in the roosters of Hy-line layers, which reminded us that the purification of ALV can not be overlooked, and needs to be continued through monitoring by proportional sampling.

Different methods were used to detect various samples of roosters and hens in the same flock. The ELISA method is used to detect semen directly, which showed that only 50% samples are consistent with the results of virus isolation. Virus isolation is used as the gold standard for detecting ALV, so the results achieved by ELISA has false positives and false negatives, resulting in missed and false mistaken of chickens. At the same time, different infection status in semen and plasma of the same rooster were clarified, namely, viraemia, with semen positive (V + S+, 13.04%), no viraemia, but semen positive (V-S+, 4.35%), viraemia, but semen negative (V + S-, 13.04%), double-negative (V-S-, 69.56%). The positive rate of virus isolation from plasma is higher than that of semen, but the two are not one-to-one. Therefore, this suggests that we need to choose both semen and plasma for virus isolation when performing ALV purification of roosters in order to obtain a better purification effect.

Li previously found that ALV-J in semen could be transmitted to hens by insemination through animal experiments in SPF chicken[13], but they only discovered the antibody of ALV-J, without viraemia in the hens and their offspring. This study is based on the infected clinical flocks. The ALV-J was isolated not only in the hens, but also in its breeding eggs. Although the positive rate of virus isolation in albumens was low, one strain of ALV-J was found and sequenced. The homology analysis showed that the strain had a homology of 94.1–99.7% with that in the roosters. Up to 100% similarity means that the strain is quite likely transmitted to the hens and their offspring through insemination of the roosters. This study comprehensively described that ALV-J from roosters can be transmitted to hens and their offspring through insemination.

Previous studies have demonstrated that ALV-J displays a high level of genetic variation and recombination [20, 21], which allows the development of new variants with changes in antigenicity, tissue tropism, host range and pathopoiesis [22]. Gao found that ALV-J is subject to greater selective pressure in the hen's follicles, which can promote the evolution of the virus[23]. In this study, we found that the ALV-J in the semen of the same rooster had only 96% homology with that in the plasma, which indicated that semen of roosters may suffer heavy selective pressure that promoted the evolution of ALV-J;and that is

why the isolated strains located in a new branch in the phylogenetic tree compared with the referential stains.

In summary, we isolated ALV-J from Hy-line brown layers, and discovered the complete chain of the transmission of ALV-J from roosters to hens and then to the offspring through insemination and vertical transmission. Semen are detected by ELISA method is not completely accurate. There are four ALV-J infection status in plasma and semen of rooster, so the purification of ALV in rooster requires simultaneous virus isolation of semen and plasma. Therefore, we speculate that the reason why there are still some sporadic findings of ALV-J in laying hens is probably due to the incomplete purification process of roosters.

Declarations

Date Availability

The data used to support the findings of this study are included within the article.

Funding

This work was supported by the National Key and Development Plan of China (Grant NO. 2017YFD0500706).

Compliance with ethical standards

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval

This article does not include experiments with human participants or animals performed by any of the authors.

References

1. Weiss RA, Vogt PK (2011) 100 years of Rous sarcoma virus. *J Exp Med* 208(12):2351–2355
2. Payne LN, Nair V (2012) The long view: 40 years of avian leukosis research. *Avian Pathol* 41(1):11–19
3. Dong X, Zhao P, Xu B, Fan J, Meng F, Sun P, Ju SD, Li Y, Chang S, Shi WF, Cui Z (2015) Avian leukosis virus in indigenous chicken breeds. *China Emerg Microbes Infect* 4(12):e76
4. Payne LN, Howes K, Gillespie AM, Smith LM (1992) Host range of Rous sarcoma virus pseudotype RSV(HPRS-103) in 12 avian species: support for a new avian retrovirus envelope subgroup.

designated J J Gen Virol 73(Pt 11):2995–2997

5. Venugopal K, Howes K, Flannery DM, Payne LN (2000) Isolation of acutely transforming subgroup J avian leukosis viruses that induce erythroblastosis and myelocytomatosis. *Avian Pathol* 29(4):327–332
6. Li XJ, Lin WC, Chang S, Zhao P, Zhang XH, Liu Y, Chen WG, Li BH, Shu DM, Zhang HM, Chen F, Xie Q (2016) Isolation, identification and evolution analysis of a novel subgroup of avian leukosis virus isolated from a local Chinese yellow broiler in South. *China Arch Virol* 161(10):2717–2725
7. Lai HZ, Zhang HN, Ning ZY, Chen RL, Zhang WY, Qing AJ, Xin CA, Yu KZ, Cao WS, Liao M (2011) Isolation and characterization of emerging subgroup J avian leukosis virus associated with hemangioma in egg-type chickens. *Vet Microbiol* 151(3–4):275–283
8. Pan W, Gao YL, Qin LT, Ni W, Liu ZS, Yun BL, Wang YQ, Qi XL, Gao HL, Wang X (2012) Genetic diversity and phylogenetic analysis of glycoprotein GP85 of ALV-J isolates from Mainland China between 1999 and 2010: coexistence of two extremely different subgroups in layers. *Vet Microbiol* 156(1–2):205–212
9. Li Y, Liu XM, Liu HX, Xu CG, Liao YL, Wu XC, Cao WS, Liao M (2013) Isolation, identification, and phylogenetic analysis of two avian leukosis virus subgroup J strains associated with hemangioma and myeloid leukosis. *Vet Microbiol* 166(3–4):356–364
10. Cui Z, Du Y, Zhang Z, Silva RF (2003) Comparison of Chinese field strains of avian leukosis subgroup J viruses with prototype strain HPRS-103 and United States strains. *Avian Dis* 47(4):1321–1330
11. Li J, Meng F, Li W, Wang Y, Chang S, Zhao P, Cui Z (2018) Characterization of avian leukosis virus subgroup J isolated between 1999 and 2013 in China. *Poult Sci* 97(10):3532–3539
12. Rubin H, Fanshier L, Cornelius A, Hughes WF (1962) Tolerance and immunity in chickens after congenital and contact infection with an avian leukosis virus. *Virology* 17:143–156
13. Li Y, Cui S, Li W, Wang Y, Cui Z, Zhao P, Chang S (2017) Vertical transmission of avian leukosis virus subgroup J (ALV-J) from hens infected through artificial insemination with ALV-J infected semen. *BMC Vet Res* 13(1):204
14. Zhang YW, Su Q, Zhang ZH, Cui ZZ, Chang S, Zhao P (2020) Molecular characteristics of the re-emerged avian leukosis virus in China, 2018–2019. *Transbound Emerg Dis* 67(3):1141–1151
15. Sun Y, Du T, Liu B, Syed SF, Chen Y, Li H, . . Zhao Q (2016) Seroprevalence of avian hepatitis E virus and avian leucosis virus subgroup J in chicken flocks with hepatitis syndrome. *China BMC Vet Res* 12(1):261
16. Wang YX, Li J, Li Y, Fang LC, Sun XL, Chang S, Zhao P, Cui ZZ (2016b) Identification of avian leukosis virus subgroup J-associated acutely transforming viruses carrying the v-src oncogene in layer chickens. *J Gen Virol* 97(5):1240–1248
17. Wang YX, Li J, Li Y, Fang LC, Sun XL, Chang S, Zhao P, Cui ZZ (2016a) Identification of ALV-J associated acutely transforming virus Fu-J carrying complete v-fps oncogene. *Virus Genes* 52(3):365–371

18. Cheng Z, Liu J, Cui Z, Zhang L (2010) Tumors associated with avian leukosis virus subgroup J in layer hens during 2007 to 2009 in China. *J Vet Med Sci* 72(8):1027–1033
19. Sun Y, Lu QZ, Zhang JF, Li XX, Zhao JK, Fan WQ, Ji PP, Wang K, Zhou EM, Zhao Q (2020) Co-infection with avian hepatitis E virus and avian leukosis virus subgroup J as the cause of an outbreak of hepatitis and liver hemorrhagic syndromes in a brown layer chicken flock in. *China Poult Sci* 99(3):1287–1296
20. Meng F, Dong X, Hu T, Chang S, Fan J, Zhao P, Cui Z (2016) A deep sequencing reveals significant diversity among dominant variants and evolutionary dynamics of avian leukosis viruses in two infectious ecosystems. *BMC Vet Res* 12(1):287
21. Dong X, Meng F, Hu T, Ju SD, Li Y, Sun P, Wang YX, Chen WQ, Zhang FS, Su HQ, Li SF, Cui H, Chen JX, Xu SZ, Fang LC, Luan HB, Zhang ZJ, Chang S, Li JL, Wang L, Zhao P, Shi WF, Cui Z (2017) Dynamic Co-evolution and Interaction of Avian Leukosis Virus Genetic Variants and Host Immune. Responses *Front Microbiol* 8:1168
22. Lupiani B, Hunt H, Silva R, Fadly A (2000) Identification and characterization of recombinant subgroup J avian leukosis viruses (ALV) expressing subgroup A ALV envelope *Virology* 276(1): 37–43
23. [1] RA, Weiss PK, Vogt (2011) 100 years of Rous sarcoma virus. *The Journal of experimental medicine* 208:2351–2355. 10.1084/jem.20112160
24. Payne LN, Nair V, The long view: 40 years of avian leukosis research, *Avian pathology: journal of the W.V.P.A* 41 (2012) 11–19. 10.1080/03079457.2011.646237
25. Dong X, Zhao P, Xu B, Fan J, Meng F, Sun P, Ju S, Li Y, Chang S, Shi W, Cui Z, Avian leukosis virus in indigenous chicken breeds, China, *Emerging microbes & infections* 4 (2015) e76. 10.1038/emi.2015.76
26. Payne LN, Howes K, Gillespie AM, Smith LM, Host range of Rous sarcoma virus pseudotype RSV(HPRS-103) in 12 avian species: support for a new avian retrovirus envelope subgroup, designated J, *The Journal of general virology* 73 (Pt 11) (1992) 2995–2997. 10.1099/0022-1317-73-11-2995
27. Venugopal K, Howes K, Flannery DM, Payne LN, Isolation of acutely transforming subgroup J avian leukosis viruses that induce erythroblastosis and myelocytomatosis, *Avian pathology: journal of the W.V.P.A* 29 (2000) 327–332. 10.1080/03079450050118458
28. Li X, Lin W, Chang S, Zhao P, Zhang X, Liu Y, Chen W, Li B, Shu D, Zhang H, Chen F, Xie Q (2016) Isolation, identification and evolution analysis of a novel subgroup of avian leukosis virus isolated from a local Chinese yellow broiler in South China. *Archives of virology* 161:2717–2725. 10.1007/s00705-016-2965-x
29. Lai H, Zhang H, Ning Z, Chen R, Zhang W, Qing A, Xin C, Yu K, Cao W, Liao M (2011) Isolation and characterization of emerging subgroup J avian leukosis virus associated with hemangioma in egg-type chickens. *Veterinary microbiology* 151:275–283. 10.1016/j.vetmic.2011.03.037

30. Pan W, Gao Y, Qin L, Ni W, Liu Z, Yun B, Wang Y, Qi X, Gao H, Wang X (2012) Genetic diversity and phylogenetic analysis of glycoprotein GP85 of ALV-J isolates from Mainland China between 1999 and 2010: coexistence of two extremely different subgroups in layers. *Veterinary microbiology* 156:205–212. 10.1016/j.vetmic.2011.10.019
31. Li Y, Liu X, Liu H, Xu C, Liao Y, Wu X, Cao W, Liao M (2013) Isolation, identification, and phylogenetic analysis of two avian leukosis virus subgroup J strains associated with hemangioma and myeloid leukosis. *Veterinary microbiology* 166:356–364. 10.1016/j.vetmic.2013.06.007
32. Cui Z, Du Y, Zhang Z, Silva RF (2003) Comparison of Chinese field strains of avian leukosis subgroup J viruses with prototype strain HPRS-103 and United States strains. *Avian Dis* 47:1321–1330. 10.1637/6085
33. Li J, Meng F, Li W, Wang Y, Chang S, Zhao P, Cui Z, Characterization of avian leukosis virus subgroup J isolated between 1999 and 2013 in China, *Poultry science* 97 (2018) 3532–3539. 10.3382/ps/pey241
34. Rubin H, Fanshier L, Cornelius A, Hughes WF (1962) Tolerance and immunity in chickens after congenital and contact infection with an avian leukosis virus. *Virology* 17:143–156. 10.1016/0042-6822(62)90091-0
35. Li Y, Cui S, Li W, Wang Y, Cui Z, Zhao P, Chang S (2017) Vertical transmission of avian leukosis virus subgroup J (ALV-J) from hens infected through artificial insemination with ALV-J infected semen. *BMC Vet Res* 13:204. 10.1186/s12917-017-1122-4
36. Zhang Y, Su Q, Zhang Z, Cui Z, Chang S, Zhao P, Molecular characteristics of the re-emerged avian leukosis virus in China, 2018–2019, *Transboundary and emerging diseases* 67 (2020) 1141–1151. 10.1111/tbed.13440
37. Sun Y, Du T, Liu B, Syed SF, Chen Y, Li H, Wang X, Zhang G, Zhou EM, Zhao Q, Seroprevalence of avian hepatitis E virus and avian leucosis virus subgroup J in chicken flocks with hepatitis syndrome, China, *BMC veterinary research* 12 (2016) 261. 10.1186/s12917-016-0892-4
38. Wang Y, Li J, Li Y, Fang L, Sun X, Chang S, Zhao P, Cui Z (2016) Identification of avian leukosis virus subgroup J-associated acutely transforming viruses carrying the v-src oncogene in layer chickens. *J Gen Virol* 97:1240–1248. 10.1099/jgv.0.000420
39. Wang Y, Li J, Li Y, Fang L, Sun X, Chang S, Zhao P, Cui Z (2016) Identification of ALV-J associated acutely transforming virus Fu-J carrying complete v-fps oncogene. *Virus Genes* 52:365–371. 10.1007/s11262-016-1301-6
40. Cheng Z, Liu J, Cui Z, Zhang L (2010) Tumors associated with avian leukosis virus subgroup J in layer hens during 2007 to 2009 in China. *The Journal of veterinary medical science* 72:1027–1033. 10.1292/jvms.09-0564
41. Sun Y, Lu Q, Zhang J, Li X, Zhao J, Fan W, Ji P, Wang K, Zhou EM, Zhao Q (2020) Co-infection with avian hepatitis E virus and avian leukosis virus subgroup J as the cause of an outbreak of hepatitis and liver hemorrhagic syndromes in a brown layer chicken flock in China. *Poultry science* 99:1287–1296. 10.1016/j.psj.2019.10.067

42. Meng F, Dong X, Hu T, Chang S, Fan J, Zhao P, Cui Z (2016) A deep sequencing reveals significant diversity among dominant variants and evolutionary dynamics of avian leukosis viruses in two infectious ecosystems. *BMC Vet Res* 12:287. 10.1186/s12917-016-0902-6
43. Dong X, Meng F, Hu T, Ju S, Li Y, Sun P, Wang Y, Chen W, Zhang F, Su H, Li S, Cui H, Chen J, Xu S, Fang L, Luan H, Zhang Z, Chang S, Li J, Wang L, Zhao P, Shi W, Cui Z (2017) Dynamic Co-evolution and Interaction of Avian Leukosis Virus Genetic Variants and Host Immune Responses. *Frontiers in microbiology* 8:1168. 10.3389/fmicb.2017.01168
44. Lupiani B, Hunt H, Silva R, Fadly A (2000) Identification and characterization of recombinant subgroup J avian leukosis viruses (ALV) expressing subgroup A ALV envelope. *Virology* 276:37–43. 10.1006/viro.2000.0539
45. Gao X, Li Q, Fang L, Song H, Cui Z, Meng F, Zhang Z, The follicle promotes the evolution of variants of avian leukosis virus subgroup J in vertical transmission, *Biochemical and biophysical research communications* 521 (2020) 1089–1094. 10.1016/j.bbrc.2019.11.042

Figures

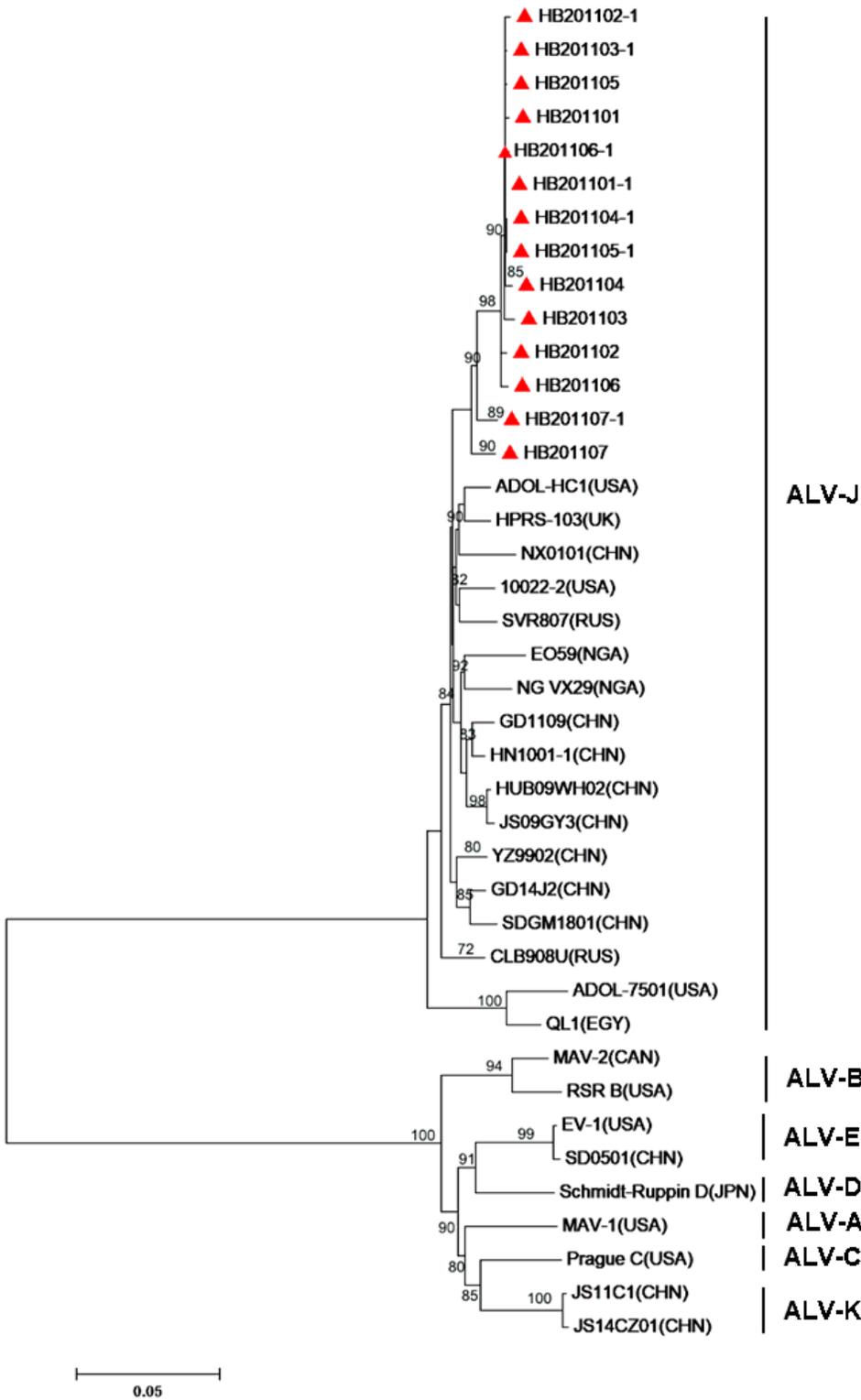


Figure 1

The phylogenetic tree of gp85 gene in the isolates and the referential strains. The phylogenetic tree is generated using the neighbor-joining method using MEGA5.1 software (bootstrap method with 1000 replicates). ALV-A to E, K and J were divided and marked on the right. The source of ALV-J reference strains is in parentheses. Purple rhombus represent the strains were isolated in the plasmas of the rooster. Green triangles represent the strains were isolated in the semens of the rooster. The other strains

in this study are also highlighted by the red figure. The sources of reference and isolated strains are marked after each strain. The following indicates the abbreviations used for country names: USA=United States, UK=United Kingdom, CHN=China, RUS=Russia, EGY=Egypt, NGA=Nigeria.

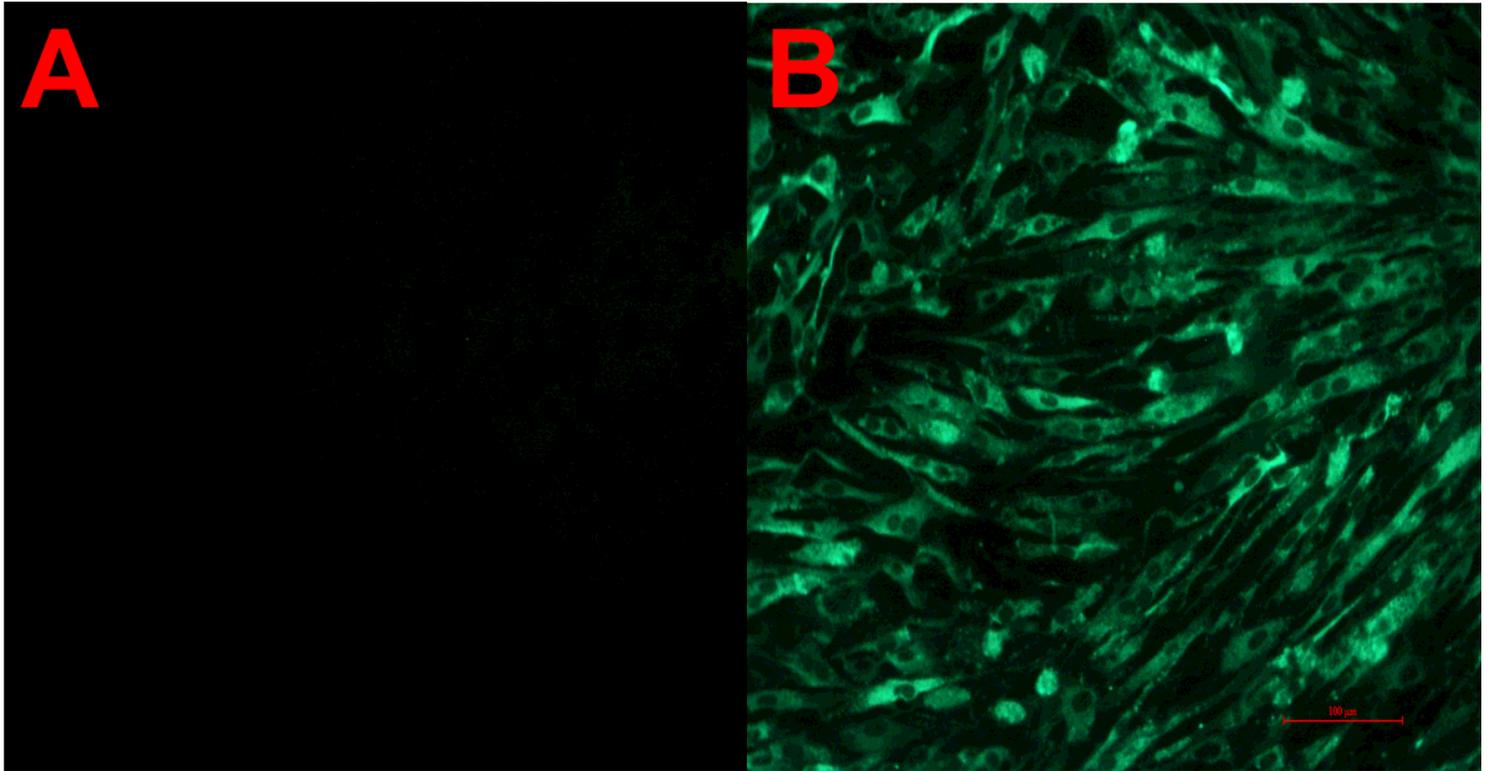


Figure 2

The results of immunofluorescence assay on the DF1 cells with JE9 monoclonal antibody. (A) Negative control, 100×; (B) DF-1 cells infected with semens, 100×.