

## Serological Survey of the Avian Leukosis Virus Infection in China Native Chickens

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**Abstract:** To investigate the Avian leukosis virus infection status in China native chicken flocks, 2530 serum samples from 26 kinds of China native chickens were collected and detected using the Avian Leukosis Virus Antibody Test kit (ALV-A/B) and Avian Leukosis Virus Antibody Test kit-Subgroup J (ALV-J). The results showed that among 2530 sera samples 118 samples were positive for ALV-A/B, 332 samples were positive for ALV-J and 35 samples were positive for both ALV-A/B and ALV-J. The positive rate for ALV-A/B, ALV-J and their co-infection was 0-16.67, 0-59.60 and 0-5.05%, respectively. This investigation suggests that the infection of ALV-A/B, ALV-J and their co-infection do exist or even very popular in some native chickens flocks in China and the prevention of ALV should be given more attention.

**Key words:** China native chickens, Avian Leukosis Virus (ALV), ALV-J, ALV-A/B, serological survey

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### INTRODUCTION

Avian Leukosis Virus (ALV) is a common avian retrovirus associated with neoplastic diseases (Payne, 1998). Exogenous Avian Leukosis Viruses (ALV) are classified into A, B, C, D and J subgroups based on their host range, cross-neutralization and viral interference, they can induce different pathotypes of neoplastic diseases in chickens (Coffin, 1992). Among these subgroups, subgroup J, A and B are more common than other subgroups in chickens and usually isolated and reported in China (Cui *et al.*, 2002, 2003; Du *et al.*, 2000; Xu *et al.*, 2004).

Avian Leukosis Virus subgroup J (ALV-J) was first isolated in 1988 from meat-type chickens in Great Britain (Payne *et al.*, 1991), it mainly induced myelocytomatosis and nephromas in meat-type chickens. During the last 10 years, ALV-J has been reported in many areas of the world (Cui *et al.*, 2003; Du *et al.*, 2000; Fadly and Smith, 1999; Malkinson *et al.*, 2004; Payne *et al.*, 1993; Thapa *et al.*, 2004; Thu and Wang, 2003).

Avian leukosis virus subgroup A and B infect mainly egg-type chickens usually leading to typical symptoms of lymphoid leukosis and the most common B-cell lymphoma of chickens (Payne, 1992; Gavora, 1987). Although, low mortality occurs in chickens with lymphoid leukosis, severe economic losses are caused mainly by decreased productivity which includes reduction of weight gain, egg production and so on (Gavora, 1987).

In this short communication, antibodies to ALV-A/B and ALV-J of 2530 serum samples from 26 kinds of China native chickens were detected and analyzed to investigate the ALV-A/B and ALV-J infection status.

### MATERIALS AND METHODS

**China native chickens investigated in this study:** In this study, 26 kinds of China native chickens was chosen as the investigation object such as Taihe chicken, Xianju chicken, Gushi chicken, Xiaoshan chicken, Beijing chicken, Langshan chicken, White ear chicken, Luyuan chicken, Big bone chicken, Chahua chicken, New Langshan chicken, Shiqi chicken, Youxi chicken, Chongren chicken, Tibetan chicken, Green-shell layer chicken and so on. All these kinds of chickens were protected as National Gene Pool for Native Poultry Breeds in China, according to the requirements of manufacturers it was marked with No. 01-26 instead of the chicken's farms as shown in Table 1. All flocks investigated in this study are >150 days old and began laying. A 1 mL blood was drawn from the vein for each chicken and the sera was installed in -40°C.

**Detection of antibodies to ALV-A/B and ALV-J:** The sera were assayed for antibodies to ALV-A/B by enzyme-linked immunosorbent assay tests using the Avian Leukosis Virus Antibody Test kit (IDEXX Laboratory) according to the manufacturer's instructions, serum

samples with S/P ratios >0.40 should be considered positive. The sera were also assayed for antibodies to ALV-J by enzymes-linked immunosorbent assay tests using the Avian Leukosis Virus Antibody Test kit-subgroup J (ALV-J) (IDEXX Laboratory) according to the manufacturer's instructions, S/P ratios >0.60 indicate the presence of antibody to ALV-J. In order to ensure the accuracy of results, each sample was repeated twice.

**RESULTS AND DISCUSSION**

**Detection of antibodies to ALV-A/B:** Among 2530 sera samples 118 samples were positive for ALV-A/B and the positive rate was 4.66%. Among 26 kinds of strains only 3 strains show quite negative to ALV-A/B. The positive rate for different flocks ranging from 0-16.67% (Table 1). The antibody titre for different positive samples is quite different while most of them is in a comparative low level and their S/P value is in the range of 0.4-1.0 (Table 2).

Table 1: Detection of ALV antibody in serum from 26 native chicken flocks (random collection)

Farms No.	No. of chicks for ALV-AB antibody positive/Total (%)	No. of chicks for ALV-J antibody positive/Total (%)	No. of chicks positive both for ALV-J and ALV-AB/Total (%)
01	9/100 (9.00)	32/100 (32.00)	3/100 (3.00)
02	9/99 (9.09)	59/99 (59.60)	4/99 (4.04)
03	10/99 (10.10)	6/99 (8.70)	0/99 (0.00)
04	2/99 (2.02)	10/99 (10.10)	1/99 (1.01)
05	2/101 (1.98)	13/101 (12.87)	0/101 (0.00)
06	8/100 (8.00)	17/100 (17.00)	5/100 (5.00)
07	9/99 (9.09)	19/99 (19.20)	5/99 (5.05)
08	0/100 (0.00)	20/100 (20.00)	0/100 (0.00)
09	1/101 (0.99)	9/101 (8.91)	0/101 (0.00)
10	16/98 (16.33)	10/98 (10.20)	3/98 (3.06)
11	8/100 (8.00)	20/100 (20.00)	5/100 (5.00)
12	2/99 (2.02)	2/99 (2.02)	0/99 (0.00)
13	1/98 (1.02)	3/98 (3.06)	0/98 (0.00)
14	8/100 (8.00)	13/100 (13.00)	2/100 (2.00)
15	7/42 (16.67)	2/42 (4.76)	1/42 (2.38)
16	3/98 (3.06)	14/98 (14.29)	2/98 (2.04)
17	1/100 (1.00)	24/100 (24.00)	0/100 (0.00)
18	0/97 (0.00)	0/97 (0.00)	0/97 (0.00)
19	3/99 (3.03)	8/99 (8.08)	2/99 (2.17)
20	0/100 (0.00)	0/100 (0.00)	0/100 (0.00)
21	4/100 (4.00)	4/100 (4.00)	0/100 (0.00)
22	5/101 (4.95)	8/101 (7.92)	0/101 (0.00)
23	5/101 (4.95)	16/101 (15.84)	0/101 (0.00)
24	2/96 (2.08)	7/96 (7.29)	0/96 (0.00)
25	1/100 (1.00)	6/100 (6.00)	0/100 (0.00)
26	2/103 (1.94)	10/103 (9.71)	2/103 (1.94)
Total	118/2530 (4.66)	332/2530 (13.12)	35/2530 (1.38)

All flocks investigated in this table are >150 days old

Table 2: Analysis of different S/P value for ALV-AB positive serum

S/P values	Number	Percentage
0.400-1.000 (1.000 included)	89	75.42
1.000-2.000 (2.000 included)	20	16.95
2.000-3.000 (3.000 included)	5	4.24
3.000-4.000 (4.000 included)	1	0.85
4.000-5.000 (5.000 included)	2	1.69
5.000-6.000 (6.000 included)	1	0.85
Total	118	100.00

**Detection of antibodies to ALV-J:** Among 2530 sera samples 332 samples were positive for ALV-J, the positive rate was 13.12%. Only 2 flocks show negative to ALV-J. The positive rate for different flocks ranging from 0-59.60% (Table 1). The S/P value of about 48% positive samples is in the range of 0.6-1.0 and 42% positive samples is in the range of 1.0-2.0 (Table 3). It indicates the ALV-J infection is more common than ALV-A/B in these native flocks.

**Co-infection of ALV-A/B and ALV-J:** Antibodies positive both for ALV-A/B and ALV-J in the same sera indicate the chicken was co-infected by ALV-A/B and ALV-J, among 2530 sera samples 35 samples were positive both for ALV-A/B and ALV-J. Although, the total positive rate for co-infection of ALV-A/B and ALV-J was only 1.38%, the co-infection exist in 12 flocks (Table 1).

ALV is a retrovirus that induces malignant neoplasms in poultry. Lymphoid leukosis induced by subgroups ALV-A and ALV-B is one of the most common tumour diseases in chickens (Fadly *et al.*, 1989). Although, some effective eradication measures have been taken to eliminate ALV-A/B, lymphoid leukosis has still spread widely in egg-producing chickens (Gavora, 1987; Payne and Fadly, 1997). A few reports have been confirmed that meat-producing chickens can also be infected by the A subgroup of ALV (Li *et al.*, 2009a, b). In the last 10 years, more and more attentions were given to contaminations with ALV in some poultry live vaccines, just as the ALV-A/B contamination in some Marek's disease live vaccines has been confirmed by many report (Barbosa *et al.*, 2008; Hussain *et al.*, 2003; Zavala and Cheng, 2006). Some breeder farms always faced an overall reduction on growth of chicken after using the contaminated vaccines.

For another important subgroup, ALV-J has been identified and reported from meat-type breeders and commercial broilers with myeloid tumors from different provinces of China since, 1999 (Cui *et al.*, 2002, 2003; Du *et al.*, 2000). In the field, it causes both late-onset myeloid tumors and acute tumors even in 4-5 weeks old birds (Fadly and Smith, 1999; Payne, 1992). It suggested that the existence of ALV-J infection in the field might be a long-term problem for the poultry industry in China.

Table 3: Analysis of different S/P value for ALV-J positive serum

S/P values	Number	Percentage
0.600-1.000 (1.000 included)	161	48.49
1.000-2.000 (2.000 included)	138	41.57
2.000-3.000 (3.000 included)	21	6.33
3.000-4.000 (4.000 included)	11	3.31
4.000-5.000 (5.000 included)	1	0.30
Total	332	100.00

China has very rich resources of native chickens, there are 48 kinds of native chickens in China as a Chinese report (without English abstract). Although, they are the important protection subject of the government, these native chickens also face a lot of viral infectious disease threats, especially for immunosuppressive viruses such as ALV, CAV, REV and so on. While for a long time, the infection status for immunosuppressive viruses especially for ALV in these native strains is not clear and have not enough data.

To detect the ALV infection, different methods have been developed and promoted such as virus isolation, indirect immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA) (Payne *et al.*, 1993; Spencer *et al.*, 1984). Commercial ELISA kit aimed to antigen is usually the first choice for the identification of ALV in the poultry industry but it is dependent on the group specific antigen p27 which is shared by both exogenous and endogenous ALV (Chesters *et al.*, 2002). For there is no commercial vaccine to control ALV up to now, it is reasonable to reflect the ALV infection status by serological method. So, in this study, >2500 serum samples from 26 kinds of China native chickens were detected their antibody to ALV-A/B and ALV-J to investigate the ALV infection status in these native chicken flocks. The results showed that the infection of ALV-A/B, ALV-J and their co-infection do exist or even very popular in some native chickens flocks in China.

In recent years, avian leukemia in breeder flocks in China has been more and more popular and some breeder farms have taken different eradication measures to eliminate ALV and the ALV infection is on the decline in some commercial breeders. This investigation reminds us the prevention of ALV to China native strains should also be given more attention and some effective measures should be conducted to eradicate the ALV urgently.

### CONCLUSION

The infection of ALV-A/B, ALV-J and their co-infection do exist or even very popular in some native chickens flocks in China, the native chickens may be a very important natural host for ALV and this makes the eradication of ALV in both local breeders and commercial chickens becoming more difficult. The prevention of ALV for native chickens in China should be given more attention too.

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### REFERENCES

- Barbosa, T., G. Zavala and S. Cheng, 2008. Molecular characterization of three recombinant isolates of avian leukosis virus obtained from contaminated Marek's disease vaccines. *Avian Dis.*, 52: 245-252.
- Chesters, P.M., K. Howes, L. Petherbridge, S. Evans, L.N. Payne and K. Venugopal, 2002. The viral envelope is a major determinant for the induction of lymphoid and myeloid tumours by avian leukosis virus subgroups A and J, respectively. *J. Gen. Virol.*, 83: 2553-2561.
- Coffin, J.M., 1992. Structure and Classification of Retroviruses. In: *Retroviridae*, Levy, J.A. (Ed.), Plenum Press, New York, pp: 19-49.
- Cui, Z., Y. Du, Z. Zhang and R.F. Silva, 2003. Comparison of Chinese field strains of avian leukosis subgroup J viruses with prototype strain HPRS103 and United States strains. *Avian Dis.*, 47: 1321-1330.
- Cui, Z., Z. Zhang and Y. Du, 2002. Epidemiological studies of subgroup J avian leukosis in China. *Chin. J. Prev. Vet. Med.*, 24: 292-295.
- Du, Y., Z.Z. Cui, A. Qin, R. Silva and L. Lee, 2000. Isolation of subgroup J avian leukosis viruses and their partial sequence comparison. *Chin. J. Virol.*, 16: 341-346.
- Fadly, A.M. and E.J. Smith, 1999. Isolation and some characteristics of a subgroup J-like avian leukosis virus associated with myeloid leukosis ALV-J strain NX0101 decreases HI antibody titers to NDV in meat-type chickens in the United States. *Avian Dis.*, 43: 391-400.
- Fadly, A.M., T.F. Davison, L.N. Payne and K. Howes, 1989. Avian leukosis virus infection and shedding in brown leghorn chickens treated with corticosterone or exposed to various stressors. *Avian Pathol.*, 18: 283-298.
- Gavora, J.S., 1987. Influence of avian leukosis virus infection on production and mortality and the role of genetic selection in the control of lymphoid leukosis. *Dev. Vet. Virol.*, Vol., 241/260.
- Hussain, A.I., J.A. Johnson, M. da Silva Freire and W. Heneine, 2003. Identification and characterization of avian retroviruses in chicken embryo-derived yellow fever vaccines: investigation of transmission to vaccine recipients. *J. Virol.*, 77: 1105-1111.
- Li, X., K. Shi and M. Luo, 2009a. Diagnosis of avian leukosis virus subgroup A and avian leukosis virus subgroup J infection in breeding chicken. *Avian Dis.*, 21: 13-17.

- Li, Y., X. Huang, B. Xia and C. Zheng, 2009b. Development and validation of a duplex quantitative real-time RT-PCR assay for simultaneous detection and quantitation of foot-and-mouth disease viral positive-stranded RNAs and negative-stranded RNAs. *J. Virol. Methods*, 161: 164-167.
- Malkinson, M., C. Banet-Noach, I. Davidson, A.M. Fadly and R.L. Witter, 2004. Comparison of serological and virological findings from subgroup J avian leukosis virus-infected neoplastic and non-neoplastic flocks in Israel. *Avian Pathol.*, 33: 281-287.
- Payne, L.N. and A.M. Fadly, 1997. Leukosis/Sarcoma Group. In: *Diseases of Poultry*, Calnek, B.W., H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif (Eds.). Iowa State University, Ames, Iowa, pp: 414-464.
- Payne, L.N., 1992. Developments in avian leukosis research. *Leukemia*, 3: 150-152.
- Payne, L.N., 1998. Retrovirus-induced disease in poultry. *Poult. Sci.*, 77: 1204-1212.
- Payne, L.N., A.M. Gillespie and K. Howes, 1993. Recovery of acutely transforming viruses from myeloid leukosis induced by the HPRS-103 strain of avian leucosis virus. *Avian. Dis.*, 37: 438-450.
- Payne, L.N., S.R. Brown, N. Bumstead, K. Howes, J.A. Frazier and M.E. Thouless, 1991. A novel subgroup of exogenous avian leukosis virus in chickens. *J. Gen. Virol.*, 72: 801-807.
- Spencer, J.L., F. Gilka, J.S. Gavor and P.F. Wright, 1984. Distribution of lymphoid leukosis virus and p27 group-specific antigen in tissues from laying hens. *Avian Dis.*, 28: 358-373.
- Thapa, B.R., A.R. Omar, S.S. Arshad and M. Hair-Bejo, 2004. Detection of avian leukosis virus subgroup J in chicken flocks from Malaysia and their molecular characterization. *Avian Pathol.*, 33: 359-363.
- Thu, W.L. and C.H. Wang, 2003. Phylogenetic analysis of subgroup J avian leucosis virus from broiler and native chickens in Taiwan during 2000-2002. *J. Vet. Med. Sci.*, 65: 325-328.
- Xu, B., W. Dong, C. Yu, Z. He and Y. Lv *et al.*, 2004. Occurrence of avian leukosis virus subgroup J in commercial layer flocks in China. *Avian Pathol.*, 33: 13-17.
- Zavala, G. and S. Cheng, 2006. Detection and characterization of avian leukosis virus in Marek's disease vaccines. *Avian Dis.*, 50: 209-215.