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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Isolation and identification of avian leukosis virus in Shouguang chickens

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Abstract

Serum and cloacal samples from suspected Shouguang chickens were collected for detection of avian leukosis. The percentage of anti-ALV-A/B was 6.73, 2.27 and 1.28% in parental lines, hybrid lines and one day old birds of Shouguang chicken respectively. Moreover, high percentages of p27 antigen-positive chickens were found in all 3 lines. Proviral DNA samples of the ALV-B extracted from the chickens and the virus-infected DF-1 cells were used as templates for PCR. Through the sequenced of ALV-B-SG12-gp85 and compared the results with 12 strains of ALV-A and 6 strains of ALV-B. It was confirmed that the ALV isolated from Shouguang chickens was ALV-B and might be a common origin to JS-B1203 (GenBank: KC282891.1) in China. One or more small tumour nodules were observed on the surface of livers in the early stage in Shouguang chickens while the scattered tumour nodules were found on enlarged livers, spleens and kidneys in the late stage. Besides, tumour nodules were also detected in other viscera. Histologically, visceral tissues mainly contained myeloid cells with acidophilic granules with densely-arranged lymphoblast while normal cells were squeezed and even destroyed by these tumour cells. The increased number of tumour cells and intra and extra-cellular hydropic degeneration might be responsible for the enlargement of viscera organs. We concluded that the ALV isolated from Shouguang chickens was ALV-B and have a high common origin to JS-B1203 (GenBank: KC282891.1) in China.

Keywords: Avian leucosis; serology; isolation; identification; pathology

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Introduction

Avian leukosis virus (ALV) is the most common naturally occurring avian retrovirus infection, causing neoplastic diseases and other production problems in chickens. There are ten well characterized chicken subgroups of ALV (A to J), which are defined on the basis of host range, antibody neutralization, and receptor interference studies (Pandiri et al., 2009; Payne et al., 2012).

Lymphoid leukemia is the most common under natural conditions. ALV (subgroup A to J) is founded in almost every breed of chickens in the world (Malkinson, et al., 2004; Pandiri et al., 2009), including China (Cui et al., 2003; Chen et al., 2005; Chai et al., 2010). ALV causes heavy losses and spreads primarily through vertical transmission. Because there is no commercial vaccine for the virus, some farms have to take different measures to eliminate it. The harm of avian leukosis covers three aspects: tumour-associated deaths, reduced productivity and immunosuppression. The epidemic of the disease has become increasingly serious in recent years, especially since 2007 outbreak when suspected cases in laying hens have been very frequent in Shandong district.

Limited studies are available regarding the investigation of ALV in Shandong breed in China. Therefore a study was designed to find the prevalence of avian leukosis in Shandong breed in China.

Materials and Methods

Investigation of the epidemic strains of ALV

A total 341 vaginal and serum samples were taken from Shouguang chickens. The vaginal samples were stored at -20^{o}C and thawed before using while the

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blood samples were placed at 4°C to clot and the serum was obtained. Both vaginal and the serum samples were tested for the P27 antigen of ALV (ALV-P27-Ag), anti-ALV-A/B antibody (ALV-A/B-Ab) and anti-ALV-J antibody (ALV-J-Ab) via ELISA kits (IDEXX Company) according to the manufacturer's instructions.

Separation of the pathogens

Spleen samples from the suspected chickens were collected, added four fold sterile PBS, then the supernatant containing ALV was purified by low speed centrifugation and filtration through cellulose acetate (0.22 μm pore-size). Penicillin-streptomycin mixture was added at the rate of 100U/ml each. DF-1 cells were cultivated in the cell culture flask and when the coverage of cells rose to 70~80%, added the filtered solution with the virus. The inoculated DF-1 cultures were kept in a 5% CO_2 incubator at 37°C for 7 days. The supernatant of each well was detected for ALV-P27-Ag via ELISA.

Extraction of proviral DNA and PCR

DNA was extracted using the DNAiso Reagent (Takara, Dalian, China) from spleen samples and DF-1 cells that were ALV-P27-Ag positive. According to the sequence of ALV-B on the genebank, a pair of primers was designed (Qiu yuyu et al., 2011). Genomic DNA PCR amplification was performed according to the ExTaq kit (Biomiga, Shanghai, China). The PCR conditions involved an initial denaturation for 5 min at 95°C, followed by 95°C for 30s, 55°C for 30s, 72°C for 1 min for 35 cycles, and a final extension at 72°C for 10 min.

Sequence analysis

The PCR products were examined on 1% agarose-TBE gels and purified by DNA Gel Extraction Kit. The gel-purified PCR products were ligated into the pMD18-T vector, and transformed into DH5α *E. coli* competent cells. DNA sequences from the positive clones were determined by a kit (Sangon Biotech Company). DNAMAN and Mega5.0 software were used to carry out the sequences analysis. The related sequences were gathered from the Gene bank. It contained 12 strains of ALV-A and 6 strains of ALV-B which have been reported in China and abroad. The trees were constructed using the software MEGA5.0 with neighbouring-joining method, with 500 replication bootstrap analysis.

Pathological observation

The chickens infected with ALV were selected and the gross lesions were recorded. The heart, liver, spleen, kidney, lung and jejunal tissues were collected and fixed in 10% neutral formalin solution to make the HE staining slice for the optical microscope observation.

Results

Positive rate of ALV in Shouguang chickens

The prevalence of ALV infection in Shouguang strain is shown in Table 1. The positive rate of ALV-P27-Ag in parental lines was the highest (75%) while in hybrid chickens ALV-A/B-Ab was the highest (6.73%). The result of the random-sampled flocks revealed that the epidemic strain of ALV in this study was subgroup A/B.

Isolation and identification of ALV

Extracted proviral DNA from cells, through specific primers amplified target fragment by PCR, were 1038bp (Fig. 1). We sequenced the ALV-B-SG12-gp85 and the percentage of the homology was above 90% when compared with the 6 strains ALV-B gene sequences. However, the percentage was 85% for the homology between ALV-B-SG12-gp85 and the listed ALV-A strains. Amino acid analysis showed that the sequences were extremely similar with the RAV-2, WB11080, RSV-SCHMIDT-RUPPIN-B and JS-B1203. The ALV-B-SG12 was subject to the subgroup B of ALV which was demonstrated in phylogenetic trees (Fig. 2).

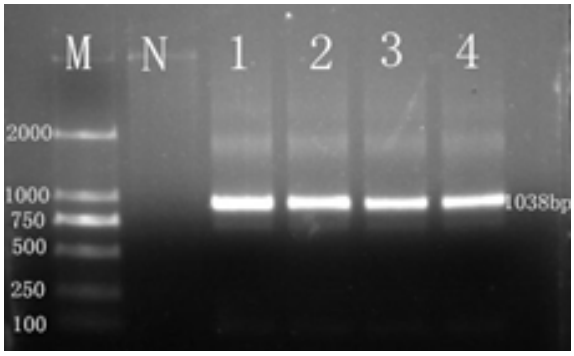

Fig. 1: Detection of ALV-B-SG12-gp85 by PCR; M: Marker; N: Negative control; No.1-4: PCR products of ALV-B-SG12-gp85

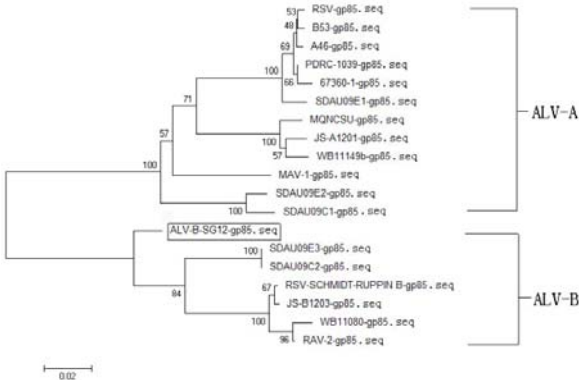

Fig. 2: Phylogenetic trees for ALV-B-SG12-gp85 and the amino acid sequences of ALV-A/B strains

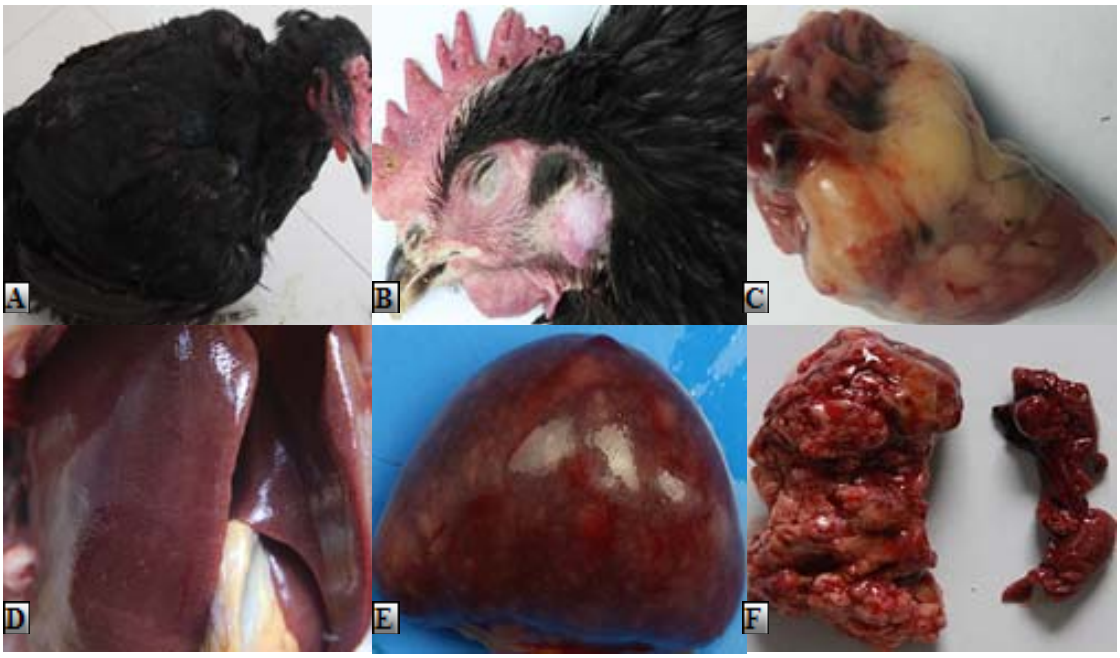

Fig. 3: A: The sick chicken with messy matt feather. B: The comb with granulation-like protrusions. C: The heart with fat accumulation D: The enlarged liver with nodules on the surface and ventral surface. E: The spleen with tumour nodules on the surface. F: The kidney enlargement with tumour nodules

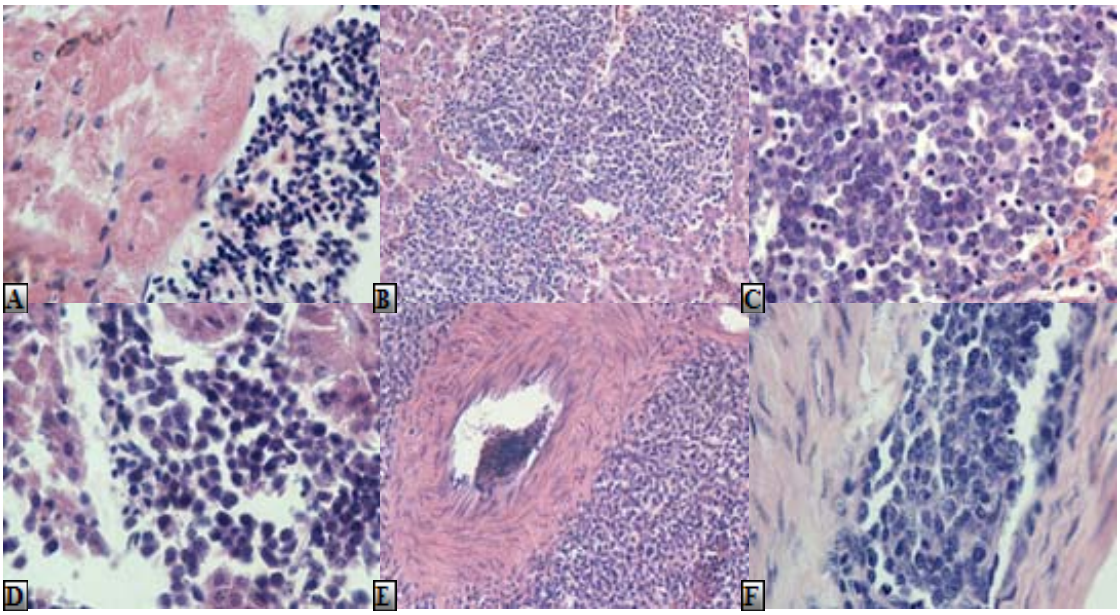

Fig. 4: Histological lesions of the infected chickens (HE staining). A: The hyperplasia of tumour cells in heart (1000×). B: The typical lymphoid tumour cells in liver (400×). C: The tumour cells in the spleen (1000×). D: The tumour cells in the kidney (1000×). E: The tumour cells in the lungs (400×). F: The tumour cells in the jejunal tissue (1000×)

Gross lesions of the infected chickens

The suspected samples showed dysplasia, the laying rate was low and the death rate was high. The clinical symptoms include diahorrhea and loss of appetite (Fig. 3A). The sick chicken showed emaciation and dull feathers. The granulation-like protrusions and hemorrhagic spots were seen on the surface of some atrophied pale combs (Fig. 3B).

Small tumours nodules were observed on the surface of the hearts (Fig. 3C). The grey tumours nodules were observed on the ventral surface (Fig. 3D) of the enlarged livers. Similar lesions were also seen on

Table 1: Prevalance of avian leukosis virus in Shouguang chickens

	Parental lines		Hybrid lines		Commercial chickens
	145 d	340 d	145 d	340 d	1 d
ALV-J-Ab	0	0	0	0	0
ALV-A/B-Ab	0	6.73%	0	2.27%	1.28%
ALV -p27-Ag	75%	25%	45.16%	15.91%	62.82%

the spleen (Fig. 3E). No significant lesions were found in the kidneys (Fig. 3F).

Histological lesions of the infected chickens

The hyperplasia of tumour cells was observed in some myocardial tissue (Fig. 4A). The tumour cells in liver had similar morphology with a round large cell body, slightly basophilic cytoplasm and pathological mitotic such asymmetry, irregular and cyclic nucleus and the normal liver lines were squeezed to destruction (Fig. 4B). Similar lesions were also observed in the spleen section (Fig. 4C). Some tubular epithelial cells were swollen and degenerated. Lymphocyte-like tumour cells between the renal tubules and corpuscles were observed (Fig. 4D). The proliferation of tumour cells in the lungs also could be observed easily (Fig. 4E). The tumour cells could be observed in the jejunal tissue (Fig. 4F).

Discussion

In the present epidemiological investigation, avian leukosis occurred not only in parent breeds but also in hybrid and commercial chickens of Shandong. It has been previously reported that Shandong local chicken is susceptible breeds to AV infection (Wang et al., 2011) and it has a variety of endogenous and exogenous subtypes. The endogenous is less pathogenic or non-pathogenic. The ALV p27 antigen was high in the present study; however, the P27 ELISA can not distinguish ALV subgroup, causing some difficulty in the survey. Thus an investigation of the gp85 genes in ALV-B cloned was to make a final decision on the type of ALV.

After infection of ALV, the chickens do not necessarily express clinical symptoms. Generally, incubation period is very long (Lei et al., 2004; Akihiro et al., 2012). Due to the presence of multiple serotypes and intermittent detoxification phenomenon, the chickens may not be in the period of positive antibody or antigen when detected via ELISA (Li et al., 2013). The susceptibility to ALV declined significantly with increasing age of birds and ALV-A/B-Ab gradually increased. ALV-B mainly infects the bone marrow and its precursor cells, so the typical tumour nodules were found in lots of the infected chickens and the proliferation of tumour cell also can be observed. These are the typical signs of avian leukosis disease. These cells grow faster than the normal bone marrow cell, and they could also enter into the blood stream and then to every organ, to grow to tumour metastases (Pandiri et al., 2009; Cheng et al., 2010). As spleen, liver and glandular stomach had affluent blood, when infected, tumour metastases appeared at the 1st time, and most was located surround the vessel. The increasing number of tumour cells and intra-and extra-cellular hydropic degeneration might be responsible for the enlargement of viscera organs.

According to the result of sequence analysis, we concluded that the ALV isolated from Shouguang chickens was ALV-B and have a high common origin to JS-B1203 (GenBank: KC282891.1) in China.

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References

Akihiro, O., OKenji, Sayuri, N., Akiko, K., Yuji, S., and Takashi, U., 2012. Molecular characteristics and pathogenicity of an avian leukosis virus isolated from avian neurofibrosarcoma. *Avian Diseases*, 56: 35-43.

Chai, J.Q., Wu, Z.N. and Yin, Q., 2010. Studies on histopathology and ultrastructure of subgroup J avian leukosis of egg-type chicken breeder. *China Journal of Animal and Veterinary Science*, 41: 735-740.

Chen, Z., Zhang, L., Liu, S., Zhang, L. and Cui, Z. (2005). Emerging of Avian leukosis virus subgroup J in a flock of Chinese local breed [in Chinese with English abstract]. *Acta Microbiologica Sinica*, 4: 99-102.

Cheng, Z., Liu, J., Cui, Z. and Zhang, L., 2010. Tumors associated with avian leukosis virus subgroup J in layer hens during 2007 to 2009 in China. *Journal of Veterinary and Medical Science*, 72: 1027-1033.

Cui, Z., Zhang, Z. and Du, Y., 2002. Epidemiological studies of subgroup J avian leukosis in Chinese meat type chickens [J]. *Chinese Journal of Preventive Veterinary Medicine*, 24: 293-294.

Cui, Z., Du, Y., Zhang, Z. and Silva, R.F. 2003. Comparison of Chinese field strains of avian leukosis subgroup J viruses with prototype strain HPRS-103 and United States strains. *Avian Diseases*, 47: 1321-1330.

Lai, H., Zhang, H., Ning, Z., Chen, R., Zhang, W., Qing, A., Xin, C., Yu, K., Cao, W. and Liao, M. 2011. Isolation and characterization of emerging subgroup J avian leukosis virus associated with hemangiorna in egg-type chickens. *Veterinary Microbiology*, 151: 275-283.

Li, Y.H., Liu, X.M., Liu, H.X., Xu, C.G., Liao, Y.L., Wu, X.C., Cao, W.S. and 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.

Malkinson, M., Banet-Noach, C., Davidson, I., Fadly, A.M. & Witter, R.L. 2004. Comparison of serological and virological findings from subgroup J avian leukosis virus-infected neoplastic and non-neoplastic flocks in Israel. *Avian Pathology*, 33: 281-287.

Pandiri, A.R., Gimeno, I.M., Reed, W.M., Fitzgerald, S.D. and Fadly, A.M., 2009. Subgroup J avian leukosis virus-induced histiocytic sarcomatosis occurs only in persistently viremic but not immunotolerized meat-type chickens. *Veterinary Pathology*, 46: 282-287.

Pandiri, A.R., 2010. Subgroup J avian leukosis virus neutralizing antibody escape variants contribute to viral persistence in Meat-Type chicken. *Avian Diseases*, 54: 848-856

Payne, L.N. and Nair, V., 2012. The long view: 40 years of avian leukosis research. *Avian Pathology*, 41: 11-19.

Yuyu, Q., Xiaoxia, L., Zhuanchang, W., Zhizhong, C., Shuhong, S. and Xianzhong, Z. 2011. Preparation and characterization of a subgroup A and B avian leukosis virus-specific monoclonal antibodies. *Chinese Journal of Immunology*, 7: 639-647.

Thu, W.L. and Wang, C.H. 2003. Phylogenetic analysis of subgroup J avian leucosis virus from broiler and native chickens in Taiwan during 2000-2002. *Journal of Veterinary Medical Science*, 65: 325-328.

Wang, J., Yang, F. and Sun, H.L. 2011. Investigation of contagious tumor disease in Luhua chicken. *China Journal of Veterinary Science*, 31: 1573-1577.