Investigations of Avian Leukosis Virus Subgroup J and Reticuloendotheliosis Virus Infections in Broiler Breeders in China

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ABSTRACT

Avian leukosis virus subgroup J (ALV-J) and reticuloendotheliosis virus (REV) were detected in Arbor Acres (AA) parent broiler breeders between September 2008 and December 2009 in China. Samples from blood (n=1331) and sick chickens (n=152) were obtained from 19 flocks in China for serology, histopathology and PCR analysis. Serologic analysis revealed that the anti-ALV-J, -REV or -ALV-J and REV antibody responses were 10.2%, 42.6% and 6%, respectively. Fifty-seven chickens showed signs of neoplasia based on gross and microscopic examination. The type of tumors included myelocytomas, hemangiomas, lymphosarcomas and fibrosarcoma. Seropositivity of tumor bearing chickens for ALV-J, REV or ALV-J and REV was 19.3%, 42.1% and 3.5%, respectively. PCR results showed that the positive rate was 43.9%, 14% and 5.3% for ALV-J, REV or ALV-J and REV respectively, in 57 tumor bearing chickens. The data presented in this report demonstrate that ALV-J or REV infections (or co-infections) rates in AA flocks are relative high in China.

Key words: Avian leukosis virus subgroup J, reticuloendotheliosis virus, serology, pathology, PCR.

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INTRODUCTION

Avian leukosis virus subgroup J (ALV-J) and avian reticuloendotheliosis virus (REV) are classified as retroviruses that are the most common causes of avian retroviral infections, causing neoplastic diseases and reproductive complications in poultry (1,2). Furthermore, the transmissibility of the ALV-J subgroup is much higher than other ALV subgroups (3), making control and eradication of this subgroup more difficult.

ALV-J was first reported in the United Kingdom (4) and was found to be associated with myeloid leukosis in chickens (5) and infections have had a severe economic impact on the poultry industry. REV is an avian retrovirus that is structurally and antigenically unrelated to the leukosis-sarcoma group of viruses, discovered in 1958 from a turkey with gross leukemic lesions (6). REV infections have been shown to transform pre-B and pre-T lymphocytes, causing bursal and T-cell lymphomas in chickens and turkeys and infections can result in a variety of non-neoplastic lesions collectively referred to as runting disease syndrome (7,8).

Although avian mortality due to ALV-J or REV infections as a result of tumor formation and immunosuppression can result in significant economic losses, an additional economic concern is losses related to REV infection acquired from the use of avian vaccines contaminated with REV since administration of the vaccine can result in an infection. There have also been reports of live poultry vaccines of Marek's disease virus (MDV) and fowlpox virus (FPV) contaminated with REV (8,9) and ALV-A (10). Davidson (1) reported that co-infection with REV and ALV-J was most common between 1994 and 1997 although no cases were reported

between 1998 and 2006 in Israel where the study was carried out.

In an attempt to eradicate retroviruses in broiler breeders we investigated the status of single and concurrent avian leukosis virus subgroup J infections with REV in broiler breeder flocks in China. Sera and sick chickens from nineteen flocks in five regions of China where screened for ALV-J or REV infections or co-infections.

MATERIALS AND METHODS

Sample collection

Between September 2008 and December 2009, nineteen (designated 1-19) AA parent broiler breeder flocks from five different regions in China were tested for infections with either (or both) ALV-J or REV. Flocks 1-3 were located in northern China, flocks 4-6 in southern China, flocks 7-12 in southwestern China, flocks 13-16 in central China and flocks 17-19 were located in eastern China. The age of flocks ranged from 28-450 days. One thousand three hundred and thirty one blood samples from chickens in the 19 flocks were examined by ELISA for the presence of anti-ALV-J or -REV antibodies (IDEXX, Westbrook, ME). One hundred fifty-two clinically sick chickens from 19 flocks (mean 8/flock) were examined. The chickens that were diagnosed as tumor bearing, based on gross and microscopic examination were used for serology and PCR examination. Liver samples (containing tumor tissues) were tested for ALV-J and REV in cultured chicken embryo fibroblasts (CEF) by PCR. Tissues were fixed in 10% buffered formalin, stained with hematoxylin and eosin (HE) and examined for microscopic lesions.

Virus isolation

Liver (containing tumor tissue) samples were inoculated onto CEF cell cultures prepared from specific pathogen free embryos and samples were then incubated for two serial passages of 5-7 days each at 37°C. After incubation, genomic DNA was extracted and analyzed by PCR for ALV-J and REV.

Polymerase chain reaction (PCR)

PCR was used to identify the proviral gp85 and (long terminal repeat) LTR sequences specific to ALV-J and the REV, respectively, from cultured CEF inoculated with liver samples. The primers used for PCR are described in Table 1. The PCRs were performed as described previously (11,12).

RESULTS

Serologic profiles

The humoral anti-ALV-J and -REV profiles in AA broiler breeders from the nineteen flocks examined are shown in Table 2. 89.5% (17/19), 100% (19/19) and 81.3% (16/19) of the chicken flocks examined were positive for ALV-J, REV or ALV-J and REV antibodies, respectively, using an indirect ELISA assay. The average seropositivity for ALV-J, REV or ALV-J and REV was 10.2%, 42.6% and 6%, respectively. For each site examined, the percentage of seropositive animals ranged from 2-30% for ALV-J, 7-80% for REV, and 1-19% positive for both ALV-J and REV, indicating a significantly high incidence of singularly or co-infected broiler breeder flocks. Anti-ALV-J antibody responses correlated with chicken age i.e., antibody positive rates increased significantly after 154 days (Table 2). In contrast, anti-REV antibody responses were independent of age. Seropositivity of tumor bearing chickens for ALV-J, REV or ALV-J and REV was 19.3%, 42.1% and 3.5%, respectively (Table 3).

Clinical examination and gross pathology

One hundred fifty-two sick chickens from 19 flocks (mean 8 per flock) were examined. Upon arrival to the laboratory

Table 1: PCR primer sequences.

Target	Primer	Sequence	Product size (bp)
ALV-J gp85	Forward P1	5'-CTGGATCCATGGGAGTTCATCTATTGCAACAACCAG-3'	924
	Reverse P2	5'-TACTGCAGTTAGCGCCTGCTACGGTGGTGACC-3'	
REV-LTR	Forward P3	5'-CATACTGGAGCCAATGGTT-3'	293
	Reverse P4	5'-AATGTTGTAGCAAGTACT-3'	
	Forward P5-nest	5'-GTAAAGGGAGATGCTA-3'	260
	Reverse P6-nest	5'-TACTACGGATTCAGTCC-3'	

Table 2: Detection of anti-ALV-J and -REV antibodies in broiler breeders of various ages and locations in China.

Location	Flock	Age (d)	No. of samples	Anti-ALV-J (+)	Anti-REV (+)	Co-infected
North	Flock 1	220	59	7(11.9%)	24(40.7%)	4(6.8%)
	Flock 2	400	63	3(4.8%)	20(31.8%)	3(4.8%)
	Flock 3	400	64	5(7.8%)	15(23.4%)	5(7.8%)
South	Flock 4	56	57	0(0%)	4(7.0%)	0(0%)
	Flock 5	70	53	3(5.7%)	8(15.1%)	1(1.9%)
	Flock 6	420	67	20(29.9%)	14(20.9%)	11(16.4%)
South- west	Flock 7	50	53	1(1.9%)	10(18.9%)	0(0%)
	Flock 8	90	74	2(2.7%)	28(37.8%)	2(2.7%)
	Flock 9	154	90	2(2.2%)	15(16.7%)	2(2.2%)
	Flock 10	245	91	6(6.6%)	73(80.2%)	5(5.5%)
	Flock 11	320	62	16(25.8%)	12(19.4%)	12(19.4%)
	Flock 12	450	64	11(17.2%)	48(75.0%)	7(10.9%)
Central	Flock 13	242	61	13(21.3%)	32(52.5%)	7(11.5%)
	Flock 14	260	90	14(15.6%)	61(67.8%)	8(8.9%)
	Flock 15	170	68	8(11.8%)	24(35.3%)	5(7.4%)
	Flock 16	300	120	15(12.5%)	48(40.0%)	7(5.8%)
East	Flock 17	28	49	0(0%)	33(67.4%)	0(0%)
	Flock 18	56	75	5(6.7%)	46(61.3%)	1(1.3%)
	Flock 19	98	71	5(7.0%)	52(73.2%)	2(2.8%)
Total	19		1331	136(10.2%)	567(42.6%)	80(6.0%)

some chickens were inactive, reluctant to stand and had ruffled feathers. All birds were bled, serum collected and then euthanized for gross pathology observation. All birds showed no or minimal ovarian development, indicating that they were not reproductively viable. Twenty-three birds showed signs of gross neoplasia at necropsy. The most common lesion sites were the liver (n=23) and spleen (n = 23). Tumors were also found in the heart (n = 2), kidneys (n = 4), ovaries (n = 4), the proventriculus (n = 1) and intestine (n = 1). No gross lesions were seen in nerve tissues, brains, eyes, or bursas of Fabricius.

Histopathology and PCR

Histological examination confirmed that thirty four additional chickens had neoplasia and/or hyperplasia in various visceral organs, resulting in a total of fifty seven chickens with neoplasia and/or hyperplasia. Different types of tumors or hyperplasia were found in the 57 chickens examined (Table

Table 3: Comparison between the antibody response and PCR analysis in tumor bearing chickens.

Samples/ flock	Tumor Type and [f]N	ELISA (%)			PCR		
		ALV-J	REV	ALV-J+REV	ALV-J	REV	ALV-J+REV
19/flock1	[a]ML:14; [b]HG:6	5	9	0	11	3	1
14/flock6	ML:6; [c]ECH:10	3	7	1	4	1	1
13/flock11	ML:8;HG:1; [d]FS:5	2	5	0	4	3	1
11/flock19	ML:1;[e]LL:10	1	3	1	3	1	0
57		11(19.3%)	24(42.1%)	2(3.5%)	25(43.9%)	8(14%)	3(5.3%)

[a]ML, Myelocytomatosis; [b]HG, Heamangioma; [c]ECH: endothelial cell hyperplasia; [d]FS, Fibrosacoma; [e]LL, Lymphosarcoma; [f]N, Number.

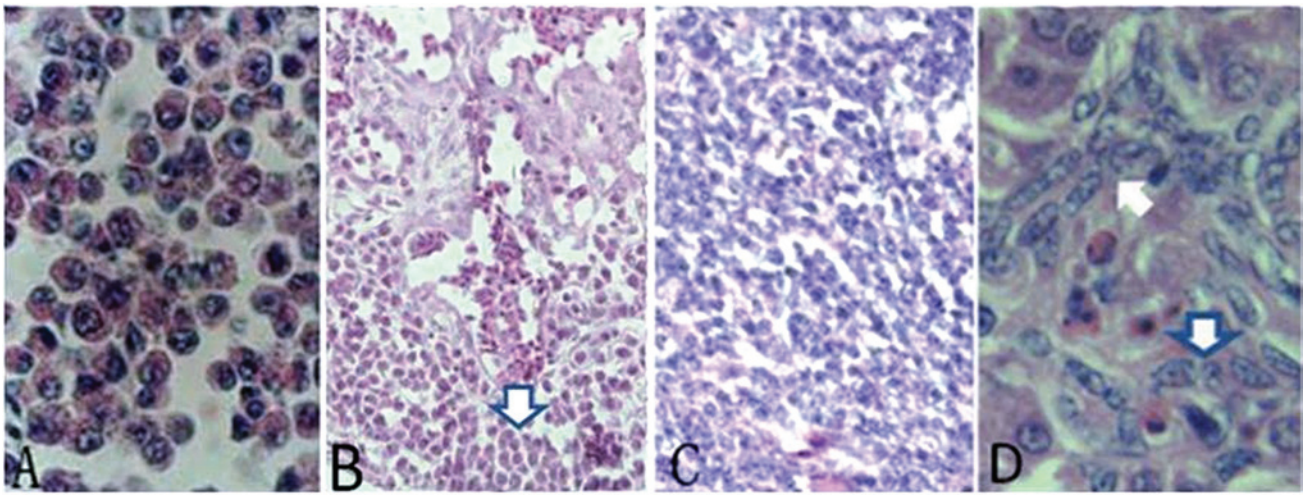

Figure 1. Histopathology. Myelocytoma cells in liver (1000X) (A), hemangioma with infiltration of myeloid cells (100X) (B); lymphosarcoma (400X) (C); Liver sinusoid endothelial cells hyperplasia (1000X) (D).

3): myelocytomas (29/57) (Fig. 1A), hemangiomas (7/57) (Figure 1B), lymphosarcomas (10/57) (Fig. 1C), sinusoids endothelial cell hyperplasia (10/57) (Fig. 1D) and fibrosarcomas (5/57).

Thus, myelocytomas were common tumor in AA parent Broiler Breeders. Two kinds of tumors or hyperplasia were found in same chicken, including myelocytomas and heamangiomas, myelocytomas and sinusoids endothelial cell hyperplasia, myelocytomas and fibrosarcomas. PCR results showed that the positive rate was 43.9%, 14% and 5.3% for ALV-J, REV or ALV-J and REV, respectively (Table 3).

DISCUSSION

Avian retroviral infections produce symptoms that set them apart from other avian viral diseases, such as immunosuppression and growth retardation. These viruses can be transmitted vertically, thereby facilitating cross-generational contamination (13). To assess the ALV-J and REV infection rates and tumor type in Broiler Breeders, we investigated blood samples and clinically sick chickens. The data presented in this report demonstrate that ALV-J and REV infections in AA parent broiler breeder flocks in China were quite prevalent.

Seropositivity of anti-ALV-J antibody in broiler breeders was 10.2% and the anti-REV antibody response was 42.6% which were similar to those reported by Cheng (12%) (14) and Qin (39.6%) (15). The dual infection rates in our study were 6%. In contrast to our study, Davidson's results showed that there were no singularly or co-infected chickens in a study carried out in Israel (1). A high rate of ALV-J and REV co-infection was found in normal flocks (6%) and tumor bearing chickens populations (5.3%). The co-infection rates in chickens were higher than reported previously (1).

Comparing the results of ELISA and PCR, we found that PCR positivity is much higher compared with ALV-J seropositivity (19.3%), and is much lower compared with REV seropositivity (42.1%), but is similar with dual infection rate in tumor bearing chickens (5.3% and 3.5%). The data suggests that there are birds infected with ALV-J who are immunologically tolerant and probably were infected in ovo or at a very young age but with REV the opposite is true, that is, most birds were likely infected when immunocompetent, resulting in greater seropositivity.

This report demonstrated that ALV-J or REV infections (or co-infections) in AA flocks are quite common in China. Due to the severe economic impact that these infections can have on the poultry industry, defined methods for screening needs to be put into practice.

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Abbreviations: ALV-J = subgroup J avian leukosis virus; ALV-A= subgroup A avian leukosis virus; REV = reticuloendotheliosis virus; AA = Arbor Acres; HE = hematoxylin and eosin; PCR = polymerase chain reaction; CEF =chicken embryo fibroblasts; MDV =Marek's disease virus ; FPV= fowlpox virus; ML= myelocytomatosis; HG=heamangioma; ECH=endothelial cell hyperplasia; FS=fibrosacoma; LL=lymphosarcoma; LTR=long terminal repeat.