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Investigation of Co-Infection of REV with CAV in Chicken Flocks of Live Poultry Market in Shan Dong Province of China

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Abstract: To investigate the co-infection status of Reticuloendotheliosis Virus (REV) with Chicken Anemia Virus (CAV) in chicken flocks of live poultry market, 370 spleen samples from six 817 broiler chicken flocks were collected and it's DNA was detected by nucleic acid hybridization with the REV-specific and CAV-specific probes. Out of 370, 20 samples could be detected by REV probe and 87 samples detected by CAV probe. The positive rate of co-infection of REV with CAV was 3.2%. These results indicated that co-infection of REV and CAV also existed in healthy broiler chicken flocks in the live poultry market. A subclinical infection of REV and CAV might be an important channel for cross-infection in different chicken flocks.

Key words: Reticuloendotheliosis (REV), Chicken Anemia Virus (CAV), ucleic acid hybridization, co-infection, flocks, hybridization

INTRODUCTION

Immunosuppression in chickens can be induced by many viruses including Marek's Disease Virus (MDV), Avian Leukosis Viruses (ALV), Chicken Anemia Virus (CAV) and Reticuloendotheliosis Viruses (REV). All of them could induce different symptoms and lesions from subclinical infection to growth retardation, immunosuppression and tumors and always they are indistigwishable from each other and made diagnosis more difficult in the field.

CAV was first isolated in Japanese (Yuasa *et al*., 1979) it is a member of the newly categorized family Circoviridae, a family of circular negative single-stranded DNA viruses that also includes the porcine circovirus and the beak and feather disease virus of parrots (Todd *et al*., 1991). The disease may pose a significant economic threat to the broiler industry (Witter and Fadly, 2003).

REV is a group of avian retroviruses belonging to the genus mammalian C-type tumor retrovirus. The REV virus strain (REV-T) was isolated from tumors in turkeys in 1958 then it continued to be isolated from chickens, ducks, goose, quails and other wild birds (Witter and Fadly, 2003). By sero-epidemiological surveys and laboratory studies on field samples in recent years it was recognized that REV infection has become very common in China (Cui *et al*., 2000; Jin *et al*., 2001; Zhang *et al*., 2003; Jiang *et al*., 2005).

In recent years, more and more studies have found that REV genome components could be integrated into other viral genome. As earlier reported *REV* gene fragment could be integrated into the genome of MDV (Davidson and Borenshtain, 2001). The same situation was also found in the Fowl Pox Virus (FPV) with REV partial or whole genome (Wang *et al*., 2010). The earlier study have proved the co-infection of MDV and REV in Marek's tumor samples and some recombinant field MDV strains with partial REV genome were identified (Zhang *et al*., 2004; Zhang and Cui, 2005). The phenomena of natural genetic recombinations between REV and MDV warned that the co-infection and recombination of REV with other viruses would speed up evolution of some viruses. So, REV infection not only causes tumors and immunosuppression in chickens but also has other negative potentials by accelerating other viral mutations. At the same time, the recombinant phenomenon for REV with MDV or FPV can increase the transmission probability and make the detection using the molecular biological methods more difficult.

In China, the co-infection of REV and CAV has been reported in some samples with obvious symptoms and lesions (Jin *et al*., 2001; Jiang *et al*., 2005) however the co-infection status of REV with CAV in healthy chicken flocks in live poultry market is not clear so far. In this study, 370 spleen samples from six 817 broiler flocks were

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collected and their DNA was detected by nucleic acid hybridization with the REV-specific probe and CAV-specific probe.

MATERIALS AND METHODS

Samples: A total of 370 spleen samples of 817 broiler chickens were collected from six live poultry market in suburb of Taian in Shandong province of China. All samples were preserved at -80°C. All these chickens were apparently normal and have no obvious tumor or clinical symptoms.

Specific nucleic acid probes for REV and CAV detection: The detection kit included specific nucleic acid probes for REV (775 bp DNA of *pol* gene) and CAV (842 bp DNA of part *VP1* gene and part *VP3* gene). These were produced using the PCR DIG Probe Synthesis kit (Roche, Germany) and preserved in -20°C. At least 1 pg positive DNA could be detected by this probe. It has been applied for a patent (Patent No. 2010101422731) in State Intellectual Rroperty Office of the People's Republic of China.

Genomic DNA extraction and preparation of membranes: DNA of spleen samples was extracted by DNA Extraction kit (BIO-OMEGA, China) according to the manufacturer's instructions. Each sample DNA was finally dissolved into 20 μL elution buffer and stored at -20°C. Four nylon membranes (Roche, Germany) of 8×8 cm were cut and each sample DNA (2 μL) was drawn to the membrane. The membrane was incubated in degeneration buffer (0.5 mol L^{-1} NaOH, 0.5 mol L^{-1} NaCl) for 10 min and then transferred into neutralizing liquid buffer (0.5 mol L^{-1} Tris-HCl, 1.5 mol L^{-1} NaCl, pH 7.4) for 5 min. Finally the nylon membrane was dried at room temperature for 30 min and then kept at 80°C for 2 h to fix the sample DNA. Four samilar nylon membranes were prepared for REV and CAV detection and detection was repeated twice.

Detection of DNA samples by dot blot hybridization: The membrane prepared above was subjected to dot-blot hybridization with the DIG Nucleic Acid Detection kit (Roche, Germany) following the report (Jiang *et al*., 2005). The membrane was incubated in 10 mL freshly prepared color substrate solution (with NBT and BCIP mixture) in a appropriate container in the dark for 8 h. Upon obtaining of desiresd spot or bands the reaction was stopped with 30 mL of sterile double distilled water or TE buffer. The results were documented by photography.

RESULTS AND DISCUSSION

Detection results for REV or CAV infection alone: Among 370 spleen samples, 20 samples showed positive to REV and the positive rate was 5.4%, the positive control show typical purple color after hybridization with REV specific probe while the negative control was quite clean as white blank (Fig. 1). Another 87 samples showed positive to CAV and the positive rate was 23.5% for CAV infection alone (Fig. 2).

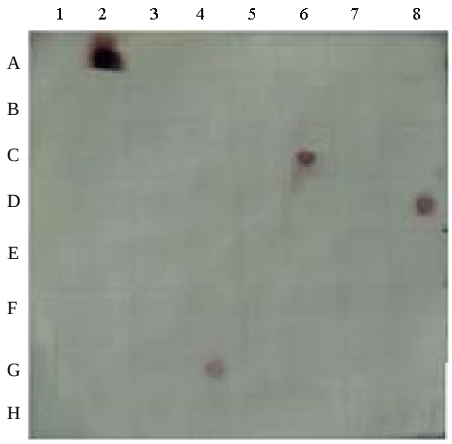

Fig. 1: Results of chicken spleen samples by dot hybridization with REV-specific probeA1:Elution Buffer in the DNA Extraction kit as a blank control; A2: DNA of CEF infected by REV as positive control; A3-H6: sample DNA; H7, H8: no any substances as blank film control

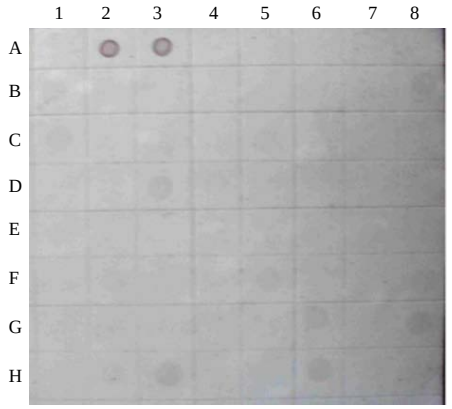

Fig. 2: Results of chicken spleen samples by dot hybridization with CAV-specific probeA1: Elution Buffer in the DNA Extraction kit as a blank control; A2: CAV genome DNA (1 pg); A3: CAV genome DNA (0.1 pg); A4-H6: sample DNA; H7, H8: no any substances as blank film control

Table 1: The detection result of 370 spleen samples by Dot-blot hybridization

Virus	Sample number	Number (positive)	Positive rate (%)
REV	370	20	5.40
CAV	370	87	23.50
REV+CAV	370	12	3.20

Co-infection of REV and CAV: Out of 370 samples, the number of the samples positive for both REV and CAV as identified by REV probe and CAV probe was 12, co-infection rate of REV and CAV was 3.2% (Table 1).

Earlier serology surveys and pathogen epidemiology investigations showed that the phenomenon of multiple immunosuppressive virus infection in chicken flocks of China has been becoming more and more serious (Jin *et al*., 2001; Jiang *et al*., 2005). Both CAV and REV can be transmitted vertically as no commercial vaccine was available in china up to now, the control of RVE and CAV mainly depend on detection and elimination of positive flocks for blocking vertical transmission of these two viruses.

In recent years, many molecular biology methods have been applied widely in animal infectious disease detection. Compared to conventional technology such as virus isolation, PCR and dot hybridization detection can not only shorten the detection time but also make the detection more sensitive. Relatively, the dot hybridization with the ability to perform on many samples simultaneously and high sensitivity is particularly suitable for large-scale epidemiological surveys.

In this study, 370 spleen samples from six 817 broiler chicken flocks were collected and it's DNA was detected by nucleic acid hybridization with the REV-specific and CAV-specific probe. The results showed that positive rate for REV, CAV and their co-infection was 5.4, 23.5 and 3.2%, respectively. The results indicated that co-infection of REV with CAV also exist in healthy broiler chicken flocks in the live poultry market and the subclinical infection of REV with CAV might be an important channel for cross-infection in different chicken flocks.

Although, the chicks investigated have REV and CAV infections, no significant tumor or death was found in these flocks. According to the report, early REV infection in chickens could not only cause growth retardation but also severely suppressed immune responses to vaccinations against new castale disease and Avian influenza virus (Sun *et al*., 2006). When chicken was infected by REV combined with other immunosuppressive virus this effect could be more obvious. Just as repoted that CAV or REV alone could significantly inhibit the antibody response to H5 and H9 subtype avian influenza viruses inactivated vaccines and that this effect was more apparent if the chicken were co-infected by CAV and REV (Li *et al*., 2008).

CONCLUSION

The result of this study showed that REV contamination in some live poultry vaccines such as FPV and MDV (Davidson and Borenshtain, 2001; Wang *et al*., 2010) however, it is very difficult to identify these infection is induced by field strain or contamination of live vaccines in this study. So, it is very important and urgent for the breeding corporations to do well in choosing good and safety live vaccines in China.

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