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Avian leukosis virus subgroup A and B infection in wild birds of Northeast China

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ABSTRACT

To analyze the status of wild birds infected with avian leukosis virus (ALV) in China, we collected 300 wild birds from various areas. Virus isolation and PCR showed that wild birds were infected by ALV-A and ALV-B. Two ALV-A and 4 ALV-B env sequences were obtained by PCR using primers designed to detect ALV-A and -B respectively. Our results showed that the gp85 genes of the 2 ALV-A strains have the highest homology with RAV-1, 99.8%, and more than 92% homology with other American strains. However, the gp85 genes of the two ALV-A strains showed slightly lower homology with Chinese strains (87.2–92.6%). Additionally, the 4 ALV-B strains have high homology with the prototype strain (RAV-2), from 99.1 to 99.4%, but they have slightly lower identity with Schmidt-Ruppin B and Prague subgroup B, from 93.3 to 98.4%. The 4 ALV-B strains showed the lowest identity with SDAU09C2 and SDAU09E3 (90%). In total, these results suggested that avian leukosis virus has infected wild birds in China.

1. Introduction

Avian leukosis viruses (ALVs), which belong to the genus alpharetrovirus of the Retroviridae family, cause neoplastic diseases and other reproduction problems in the poultry industry worldwide (Gao and Wang, 2012). Avian leukosis viruses are divided into 10 well-characterized subgroups, A to J, based on receptor usage group and host range in chickens. The subgroup specificity of these viruses has been mapped to the viral surface (SU) domain of the envelope (Env) glycoprotein, which is responsible for receptor binding (Siliciano, 1996). ALV-A and ALV-B strains were widely prevalent in the 1970s–1980s. Large poultry breeding companies took several years to eradicate ALV in an attempt to reduce the economic loss it caused. To date, several studies have examined the prevalence of ALV strains and its association with diseases in poultry. Burstein et al. (1984) reported that ALV-A could induce the hemangioma in layer chickens. Fenton et al. (2005) found that ALV-A was highly prevalent in meat-type chickens in Australia. Qiao and Pang (2008) isolated one ALV-A strain in a poultry breeding farm in Beijing, indicating that ALV-A had infected the layer chickens in China. ALV-B was reported by both Graf (1972) and Kawai and Hanafusa (1972) in the 1970s and successively reported around the world. In 2002, Gingerich et al. (2002) reported a recombined virus of ALV-A and ALV-B in commercial white leghorns. Spencer et al. (2003) detected

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both ALV-A and ALV-B in the commercial layer chicken in 2003. In 2006, Lupiani et al. (2006) studied the molecular biological characteristics of a recombined virus of ALV-B and ALV-J. The past molecular epidemiology studies were mainly focused on poultry and waterfowl, and reports of infection in wild birds were rare. Our study represents the first report of the ALV-A and ALV-B strains in wild birds and, furthermore, we sequenced and analyzed the env genes to elucidate the epidemiology of avian leukosis of wild birds in China.

2. Materials and methods

2.1. Clinical samples

Clinical samples were collected from several provinces in China (Heilongjiang, Jilin and Liaoning). The species collected ranged from Northern pintail, Green-winged Teal to Baikal Teal, including approximately 10 species in total. Most of the birds collected were dead, having been trapped in the nets from the bird banding stations. Livers and spleens were collected from 300 wild birds autopsied in the biological safety lab.

2.2. Virus isolation and proviral DNA extraction

All virus isolations were performed in DF-1 cells, which are known to be susceptible only to exogenous ALVs (Maas et al., 2006). The procedures for the isolation and identification of ALV in cell culture were performed according to previously described studies (Bagust et al., 2004). Briefly, filtered liver and spleen tissues were inoculated into DF-1 cells, which were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, CA) supplemented with 10% fetal bovine serum (FBS) for two serial passages (5 days for each passage) at 37 °C in a 5% CO_2 incubator with daily monitoring. After the incubation, the infected DF-1 cells were tested for the ALV group-specific antigen (p27) by an antigen-capture enzyme-linked immunosorbent assay (AC-ELISA) with anti-p27 antibody-coated plates (IDEXX Inc., MA). The positive samples detected by ELISA were harvested for DNA extraction and PCR amplification. The DNA was directly extracted from the ELISA-positive cultured DF-1 cells or tumors using an established method. Briefly, the cultured cells were lysed in tissue lysis buffer (4 M guanidine hydrochloride, 25 mM sodium citrate, and 1% Triton X-100) and extracted twice with phenol-chloroform-isoamyl alcohol (25:24:1). The DNA was precipitated with absolute isopropanol, washed with 70% isopropanol and dried at room temperature. Subsequently, the DNA was resuspended in nuclease-free water and stored at −80 °C.

2.3. PCR cloning and sequencing

PCR was used to test genomic DNA from the cultured DF-1 cells or tumors for the presence of envelope sequences specific for ALV-A and ALV-B, as previously described (Smith et al., 1998). The primer set ALVAF (5′-GGTTGGTCTAGACAGGAAGC-3′) and ALVAR (5′-CATTGCCACAGCGGTAC-3′) was used for the specific detection of ALV-A proviral DNA, which generates a 180-bp PCR product. According to the sequence of the ALV-A strain, RSA (GenBank accession number M37980), the primer pair ALVAENV F (5′-CGAGAGTGGCTCGCGAGATGG-3′) and ALVAENV R (5′-TATACTATTCTGCTTTCAGGCTG-3′) was designed for the amplification of the ALV-A env gene. The primer set ALVBF (5′-CATACGATAGTCCGGCTG-3′) and ALVBR (5′-CCCCACACATCCTGACA-3′) was used for the specific detection of ALV-B proviral DNA, which generates a 260-bp PCR product (Lupiani et al., 2006). According to the sequence of the ALV-B strain, Schmidt-Ruppin B (GenBank accession number AF052428), the primer pair ALVBENV F (5′-CGAGAGTGGCTCGCGAGATGG-3′) and ALVBENV R (5′-AGCGCTATACCACCACCCATGTA-3′) was designed for the amplification of the ALV-B env gene. The PCR conditions included an initial denaturation cycle of 4 min at 94 °C, followed by 30 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 54 °C, and an extension for 2 min at 72 °C, with a final extension of 7 min at 72 °C. All PCRs were carried out with PrimerSTAR HS DNA high-fidelity polymerase (TaKaRa, Dalian, China). The PCR products were excised from a 1.0% agarose gel, purified using an AxyPrep DNA gel extraction kit (Axygen Scientific, Inc., CA), and cloned into the TA vector pMD18-T (TaKaRa). Three independent clones of each ALV-J isolate were sequenced by the Beijing Genomics Institute (Beijing, China).

2.4. DNA alignments and phylogenetic analysis

The nucleotide sequences were aligned using the ClustalX program, version 1.83 (Kumar et al., 2004). A neighbor-joining tree was drawn using the MEGA program, version 4.0 (Thompson et al., 1997), with confidence levels assessed using 1,000 bootstrap replications. The GenBank sequences of the ALV-A and ALV-B strains that were isolated from meat-type and layer chickens were included in the multiple-sequence alignment and are summarized in Tables 1 and 2.

2.5. Nucleotide sequence accession numbers

The sequences obtained in this study have been submitted to GenBank, and the accession numbers are provided in Tables 1 and 2.

3. Results

3.1. Virus isolation and identification of ALV-A and ALV-B

A total of 6 samples yielded positive results on virus isolation, and they were all tested using the primers designed to detect ALV-A and ALV-B. Finally, 2 ALV-A and 4 ALV-B strains (detailed information is summarized in Tables 1 and 2) were identified as positive samples. The PCR of DNA extracted from DF-1 cells infected with the 2 ALV-A and 4 ALV-B isolates produced an ALV-A specific 180-bp fragment with primers ALVAF and ALVAR and an ALV-B specific 260-bp fragment with primers ALVBF and ALVBR, respectively.