Control of Avian Reticuloendotheliosis

Ching-Ho Wang[1]*, Wan-Hsing Cheng[1] and Henry D. Hunt[2]

[1]Department of Veterinary Medicine, National Taiwan University
No 1 Sec 4, Roosevelt Rd, Taipei 10617, Taiwan ROC
[2] Avian Disease and Oncology Laboratory, ARS-USDA, East-Lansing, MI, U.S.A.
*Corresponding author (E-mail: chingho@ntu.edu.tw)

ABSTRACT

Reticuloendotheliosis (REV) infection is common, but not ubiqutous. In order to understand the REV infection status, antibody determination and virus isolation were performed for chickens in Taiwan. The results showed that REV infection is quite common in chickens. Two REV viruses were isolated from infected chickens and one from a fowl poxvirus vaccine. The phylogenetic analysis of those REV isolates will be presented.

Key words: gp85 protein, Monoclonal antibody, Subgroup J avian leukosis virus, Surface plasmon resonance.

INTRODUCTION

Reticuloendotheliosis viruses (REVs) belong to the Gammaretrovirus genus and have a genomic structure consisting of the group-specific antigen (gag), protease (pro), polymerase (pol), and envelope (env) regions flanked by long terminal repeats (LTRs). Birds infected with REV after hatching, a transient viremia followed by antibody formation. Infection of older birds rarely results in clinical diseases (Witter, 1985).The prevalence of seropositive flocks and the proportion of seropositive birds in a flocks increase with birds' ages. In certain instances, some infected chickens show chronic lymphomas and an immunosuppressive runting disease. Two types of tumors are found in infected chickens, bursal and non-bursal. The bursal type tumor originates in the cloacal bursa, and later spreads to liver and other organs in 17-43 weeks after inoculation with nondefective chick syncytial or T strains of REV (Witter, 1981). A second type of chronic tumor, nonbursal lymphoma, in chickens occurs by 6 weeks after inoculation with spleen necrosis or chick syncytial strains of nondefective REV (Witter, 1986). Some birds show an immunosuppressive runting disease, this developed by 3-5 weeks after infection (Mussman, 1971).

Although REV infection is common, natural outbreaks of this disease in commercial poultry are rare. One possibility is from contaminated vaccines, especial fowl poxvirus or Marek's disease virus vaccines (Fadly, 1996; Koyama, 1976). Those vaccines have been used extensive in chickens. The impact of vaccination and the relationship between infection and vaccination are not clear for the present. This paper describes the REV infection status in chickens and compares the REVs from infected chickens and vaccines.

MATERIALS AND METHODS

Case history

Case 3122/03 was from a breeder farm in Yilan with two kinds of breed, breed A and breed B, 3 flocks each. The ages and the breed of those chickens in this farm were 10, 38, 66 weeks old of breed A and 13, 39, 63 weeks old of breed B in December 2003. Besides ordinary vaccination for commercial broiler breeder chickens, those chickens were vaccinated with live herpes virus of turkey and live SB1 strain of Marek's disease virus vaccines at one day of age and vaccinated two times with live fowl poxvirus vaccines at the age of 1 and 13 weeks of age, respectively. Those chickens were considered to be normal because their weekly mortality ranged from 0 to 0.15%. Blood samples were taken from those chickens for REV antibody determination and virus isolation.

Case 3295/04 was from a color breeder farm with 15,000 chickens. The weekly mortality of this farm was less than 0.2%, considered to be normal. The blood samples were taken for REV antibody determination and virus isolation.

REV antibody determination

Plasma samples from different flocks were tested for the presence of anti-REV antibody by using a commercial ELISA kit (Idexx, Westbrook, ME) according to the manufacturer's instructions.

REV virus isolation from chickens

Blood samples were taken from Whole blood was collected with heparin (Becton Dickinson, Franklin Lakes, NJ) and put on ice immediately. Buffy coat from whole blood was co-cultured with DF1 cells (ATCC CRL-12203, American Type Culture Collection, Manassas, VA) and incubated at 39 C for 7 days each passage. The presence of virus was examined by RT-PCR in supernatant with LTR primers for long terminal repeat (Aly, 1993). Virus isolation was attempted three passages in cell culture before considering negative.

REV gene detection in vaccines by PCR

Eighteen commercial fowl poxvirus vaccines and 18 Marek's disease vaccines sent for inspection at Animal Health Research Institute, Taiwan were collected for REV gene detection in 2003 and 2004, respectively. The REV LTR gene in fowl poxvirus vaccines was with primer, TR-1/TR-2 (Singh, 2003) and that in Marek's disease vaccines was detected by polymerase chain reaction by using LTR primer (Aly, 1993).

REV virus isolation from vaccines

All the collected vaccines were re-suspended with PBS and co-cultrued with DF1 cells. After changing medium at the second day, those cells were culture for 1 week in one passage. The presence of virus was examined by RT-PCR in supernatant with LTR primers for long terminal repeat (Aly, 1993). Virus isolation was attempted three passages in cell culture before considering negative.

REV env gene sequencing

The env genes of those REV isolates were amplified by RT-PCR with env5/env6 primer (new designed), amplifying the whole env gene and cloned into yT&A vector (Yeastern Biotech, Taipei, Taiwan) and sequenced.

RESULTS

The seropositive prevalence and virus isolation in the broiler breeder farm

The seropositive prevalence and the ages of case 3122/03 farm in breed A were 0% (0/16) at the age of 10 weeks, 24% (4/17) at the age of 38 weeks, and 93% (15/16) at the age of 66 weeks and those in breed B were 0% (0/13) at the age of 13 weeks, 92% (12/13) at the age of 39 and 100% at the age of 63 weeks. The seropositive prevalence increased with ages but not immediately after Marek's disease or fowl pox vaccinations. A REV, 3122/03, was isolated from a 31-week-old female broiler breeder chicken of breed A from that farm. The total virus isolation rate from those chickens was 1% (1/88).

The seropositive prevalence and virus isolation in the color breeder farm

The seropositive prevalence and the ages of case 3259/04 farm were 0% (0/15) at the age of 12 weeks, 93% (13/14) at the age of 25 weeks, 67% (10/15) at the age of 26 weeks, 86% (12/14) at the age of 28 and 100% at the age of 50 weeks. One REV virus, 3295/04, was isolated from a 12-week-old chicken.

Detection and virus isolation of REV in vaccines

Seven of the tested 18 fowl poxvirus vaccines had approximate 290 bp PCR product. However, only one REV was isolated from those vaccines. The vaccine with positive virus isolation had not this PCR band. No PCR product was found in Marek's disease vaccines with the primer used. After two passages of virus isolation from fowl poxvirus vaccines in DF1 cells, a faint band was found. After 13 times of passage, the PCR became more clear and finally a REV, 3134/03 was isolated from that vaccine.

REV env gene analysis

The identity of the env genes from the two chicken REV isolates, 3122/03 and 3259/04, was 99.7%. The REV isolate, 3134/03, from fowl poxvirus vaccine showed 461 n.t. deletion in its env gene when compared with the chicken isolates. Thus, the identities of this REV and those two chicken isolates were 67% and 67.1% respectively. Another clones from the same virus will be performed for this deletion. The identities of a reference strain, FPV-REV (AF246698) and these chicken isolates were 99.7% and 99.8%, respectively. The identity of strain A REV with the present two chicken isolates (X01455) was 95.5%.

DISCUSSION

Although REV infection is common but not ubiquitous (Witter and Fadly, 2003). Data presented in this paper document the presence of REV in the field is quite common. Almost hundred percent of chickens get infection at old ages. However, those chickens showed neither clinical signs nor REV-induced tumors. Like avian leucosis virus infection, chicken showed resistant to REV infection after 1 week of age (Witter and Fadly, 2003). From the late seroconversion of those flocks (after 16 weeks old), those chickens were infected at the later ages. This means those chickens established their resistance but not immune tolerance getting infection before hatching.

Infection of those chickens with REV was not caused by contaminated vaccines since the santibody didn't appear immediately after Marek's disease and fowl pox vaccinations. In addition, the low prevalence at the beginning of sero-conversion (at the age 20 weeks, 7 weeks after the second fowl pox vaccination) revealed some chickens got infection at the beginning because vaccination likely caused almost the whole seroconversion but not only some.

More than 1/3 of fowl poxvirus vaccines contained small part of REV LTR gene and failed to give virus in culture. This means a small LTR fragment remains in the vaccine genes but fail to produce intact virion. REV LTR incorporated in the genomes of fowl poxvirus vaccines without indication of the presence of infectious REV (Moore, 2000). Large part of the REV provirus may be lost from host DNA over time, whereas LTRs remaining (Robinson, 1986).

ACKNOWLEDGMENTS

We gratefully express our appreciation to Dr. Aly Fadly at the ADOL, ARS-USDA for his help and advice. This study was supported by the research grant [93AS-1.4-CI-01] from Council of Agriculture, Executive Yuan, Taiwan, ROC.

REFERENCES

Aly, M. M., E. J. Smith and A. M. Fadly. 1993. Detection of reticuloendotheliosis virus infection using the polymerase chain reaction. Avian Pathol. 22:543-554.

Fadly, A. M., R. L. Witter, E. J. Smith, R. F. Silva, W. M. Reed, F. J. Hoerr and M. R. Putnam. 1996. An outbreak of lymphomas in commercial boriler breeder chickens vaccinated with a fowlpox vaccine contaminated with reticuloendotheliosis virus. Avian Dis. 25:35-47.

Koyama, H., Y. Suzuki, Y. Ohwada and Y. Saito. 1976. Reticuloendotheliosis group virus pathgenic to chicken isolated from material infected with turkey herpesvirus (HVT). Avian Dis. 20:429-434.

Moore, K. M., J. R. Davis, T. Sato and A. Yasuda. 2000. Reticuloendotheliosis virus (REV) long terminal repeats incorporated in the genomes of commercial fowl poxvirus vaccines and pigeon poxviruses without indication of the presence of infectious REV. Avian Dis. 44:827-841.

Mussman, H. C. and M. J. Twiehaus. 1971. Pathogenesis of reticuloendothelial virus disease in chicks-an acute runting syndrome. Avian Dis. 15:483-502.

Robinson, H. L. and G. C. Gagnon. 1986. Ptterns of proviral insertion and deletion in avian leukosis virus-induced lymphomas. J. Virol. 57:28-36.

Singh, P., W. M. Schnitzlein and D. N. Tripathy. 2003. Reticuloendotheliosis virus sequences within the genomes of field strains of fowlpox virus display variability. J. Virol. 77:5855-5862.

Witter, R. L. and A. M. Fadly. 2003. Reticuloendotheliosis. In: Diseases of poultry, 11th ed. Saif, Y. M. *et. al.* eds. Amer. Assoc. Avian Pathol., Iowa State Press, a Blackwell Publishing Co., Ames, IA. p. 517-536.

Witter, R. L. and D. C. Johnson. 1985. Epidemiology of reticuloendotheliosis virus in broiler breeder flocks. Avian Dis. 29:1140-1154.

Witter, R. L., J. M. Sharma and A. M. Fadly. 1986. Nonbursal lymphomas induced by nondefectivereticuloendotheliosis virus. Avian Pathol. 15: 467-486.

Witter, R. L., E. J. Smith and L. B. Crittenden.

1981. Tolerance, viral shedding, and neoplasia in chickens infected with non-defective reticuloendotheliosis viruses. Avian Dis. 25:374-394.