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Emerging of Avian Leukosis Sub-group J (ALV-J) Infections in Broiler Chickens in Malaysia

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Abstract: Recent outbreaks of Newcastle disease (ND) in commercial chickens in Malaysia was associated with infiltration of myeloid cells in the organs of the affected chickens which has not been reported previously and was consistent to those of Avian Leukosis Sub-group J (ALV-J) infection. However, little is known about the disease and it was the objective of the study to determine the sero-prevalence and lesions of ALV-J infection in broiler chickens. Broiler breeders (120) of 66-week-old from two breeder farms in Selangor and Malacca were used in the study. They were divided into 4 groups namely groups A1 (30 hens) or A2 (30 cocks) and B1 (36 hens) or B2 (24 cocks) of chickens from Selangor and Malacca, respectively. The broiler chickens (125) of 5-week-old were sampled from four broiler farms in Johor identified as groups C, D, E and F. The clinical signs were recorded and serum samples were collected for ALV-J antibody detection using enzyme linked immunosorbent assays (ELISA) technique. On necropsy, the gross lesions were recorded. Samples of brain, liver, spleen, kidney, lung, ribs and sciatic nerve, and bursa of Fabricius were collected from the broiler breeders and broilers, respectively for histopathology. The study showed that 10% of the broiler breeder had mild lameness, mainly due to bumble foot (83%). Fatty livers (12%), *Pasteurella multocida* infections (4%) and splenomegaly (2.5%) were also recorded. The chickens in the group C and D had complicated chronic respiratory disease (CCRD). Histologically, infiltrations of myeloid cells were found in the liver of a bird from the group A1. Infiltration of myeloid cells in the follicles of the bursa of Fabricius was also observed in chickens from groups C (10%), D (20%), E (50%) and F (30%). Moderate to severe lesion scoring of the bursa of Fabricius was recorded. The ALV-J antibody was detected in breeder chickens in groups A1 (27%), A2 (27%), B1 (36%) and B2 (42%), but was not detected in the broiler chickens. It was concluded that broiler breeder chickens in Malaysia is not free from ALV-J infections. The overall sero-prevalence of ALV-J in the broiler breeder chickens was 33% with no differences between the hens and cocks. The presence of myeloid cells infiltration in the follicles of the bursa of Fabricius, 10 to 50% of the samples examined suggests that broiler chickens are at high risk of ALV-J infection.

Key words: ALV-J, broiler chickens, myeloid cells, ELISA, histopathology

Introduction

Neoplastic diseases in chickens are of economic importance in poultry industry due to losses resulting from high mortality, poor performance and immunosuppression. Marek's disease (MD) and lymphoid leukosis (LL) are the two commonly known neoplastic diseases of economic importance in Malaysia. Recent detection of high sero-prevalence of avian leukosis virus subgroup-J (ALV-J) antibody positive (97%) in commercial chickens in the country, concurrent with serious Newcastle disease (ND) outbreaks suggests that ALV-J is of a new treat to the industry (Asiah *et al.*, 2001; Nor Faizah *et al.*, 2001).

The avian haemopoietic cells, as in mammals, originate from multi potent progenitor stem cells that differentiate into lymphoid, erythroid, myeloid, and other lineages (Junqueira and Carneiro, 1980). In LL, induce predominantly by ALV subgroups A and B, the target cells for the transformation is primarily the B cells of the lymphoid lineage. In contrast, ALV subgroup J induces late-onset myeloid leukosis targeting mainly the cells of

the myeloid lineage (Venugopal, 1999). An exogenous ALV-J distinct from viruses of subgroups A through E has been isolated from meat-type chickens and is classified as ALV-J (Payne *et al.*, 1991). Unlike exogenous ALV subgroup A, B, C and D which induce LL, that is tumor of the B-cells derived from the bursa of Fabricius, exogenous ALV-J induces myeloid leukosis which is the tumor of the myelomonocytic cell lineage (Arshad, 1998). In addition to causing tumors, ALV-J can reduce productivity and may induce immunosuppression and other production problem in affected flocks (Arshad, 1998).

Since the first discovery of ALV-J infections in England in 1988, the disease has become a major problem in meat-type birds worldwide (Payne, 1998). ALV-J is transmitted by conventional means through vertical and horizontal transmission. It spreads congenitally through the egg from infected hens to their chicks and to uninfected birds by contact from infected chickens or from the infected environment (Arshad, 1998). Little is known on the ALV-J infection in chickens in Malaysia. It

was the objective of the study to determine the seroprevalence and lesions of ALV-J infections in broiler breeders and broiler chickens in the country.

Materials and Methods

Broiler breeder chickens: One hundred and twenty broiler breeder chickens of 66-week-old were obtained from two breeder farms located in Selangor and Malacca. They were divided into 2 batches namely; A and B. The birds were further divided according to the gender namely; groups A1 (30 hens), or A2 (30 cocks) and B1 (36 hens) or B2 (24 cocks) from farm in Selangor and Malacca, respectively. The chickens were examined for any abnormal clinical signs. Blood samples were collected from the wing vein and the serum samples were analyzed for the antibody to ALV-J using enzyme linked immunosorbent assay (ELISA) techniques (IDEXX Laboratories Incorporation, USA). The chickens were sacrificed and examined for gross lesions. Tissue samples of brain, liver, spleen, kidney, lung, ribs and sciatic nerve were fixed in 10% buffered formalin for histological examination. Samples of liver with generalized pinpoint areas of necrosis were sent for bacteria isolation and identification (Bacteriology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia).

Broiler chickens: One hundred and twenty, five weeks old broiler chickens were sampled randomly from four broiler farms in Johor, identified as the groups C, D, E and F. The chickens were vaccinated against infectious bursal disease (IBD) once at 14-day-old. ND vaccination was carried out at day 4, days 6 and 20 and days 1, 8 and 19 of age in groups C, D, and E and F, respectively. The clinical signs were observed and recorded. Thirty blood samples were collected from each group for detection of antibody against ALV-J using ELISA kit (IDEXX Laboratories Incorporation, USA). Ten chickens from each group were sacrificed and the bursa of Fabricius were collected and fixed in 10% buffered formalin for histological examination.

Histopathology: The tissues were fixed in 10% buffered formalin for 24 hours, trimmed to the thickness of 0.5cm in size, dehydrated in a series of alcohol concentration, and embedded in paraffin wax using an automatic tissue processor. The tissues were sectioned and mounted on glass slide, dewaxed and stained with hematoxylin and eosin (HE) for histological examination. The lesions of the bursa of Fabricius were scored: 0 (normal), 1 (mild), mild to moderate (2), moderate (3), moderate to severe (4) and severe (5) using the method described previously (Hair-Bejo *et al.*, 2000).

ALV-J antibody detection (ELISA): The ELISA

techniques for detection of ALV-J antibody in both the broiler breeder and broiler chickens were carried out according to the methods described by IDEXX Laboratories Incorporation, USA. Briefly, the antigen coated plates and the ELISA kit reagents were adjusted at room temperature prior to the test. The test sample was diluted five hundred folds (1:500) with sample diluent prior to the assay. A 100 μ l of diluted sample was then put into each well of the plate. This was followed by 100 μ l of undiluted negative control into well A1 and A2, 100 μ l of undiluted positive control into well A3 and A4. The plate was incubated for 30 minutes at room temperature. Each well was then washed with approximately 350 μ l of distilled water for 3 times. Goat anti-chicken conjugate (100 μ l) was dispensed into each well. The plate was incubated in room temperature for 30 minutes, followed by washing each well with 350 μ l distilled water for 3 times. TMB solution (100 μ l) was dispensed into each well. The plate was then incubated at room temperature for 15 minutes. Finally, 100 μ l of stop solution was dispensed into each well to stop the reaction. The absorbance values were measured and recorded at 650nm.

Results and Discussion

Clinical signs and gross lesions: Neither significant clinical signs nor typical gross lesions of ALV-J infections observed in the present study. The broiler breeders in all groups were normal, except for chickens in groups A1 (1), A2 (1) and A3 (10) showed abnormal gait. Some of the broiler chickens in group D showed respiratory problems upon presentation. The mortality of the chickens in groups C, D, E and F were about 3.5%, 8, 3 and 3%, respectively. Chickens in the group C and D had complicated chronic respiratory disease (CCRD). Pinpoint hepatic necrosis was observed in groups A1 (2) and A2 (3). *Pasteurella multocida* was isolated from the liver samples. Fatty liver (9) and bumble foot (10) were recorded in groups B1 and B2, respectively. Splenomegaly was observed in groups A1 (1) and A2 (2). It was reported that the gross lesions for ALV-J can be observed in the rib cage bones, keel bone and the visceral organs. Nodules and protrusions commonly observed on the surface of the sternum, ribs, vertebrae, and synsacrum (Nakamura *et al.*, 2000).

Histopathology: Infiltrations of myeloid cells were found in the liver of a chicken from the group A1 (Fig. 1). The liver, spleen, sciatic nerves, brain, ribs, kidney and lungs of the other chickens from groups A1, A2, B1 and B2 were no significant findings. The lesion scoring of bursa of Fabricius were 3.2 ± 0.13 , 3.6 ± 0.22 , 3.4 ± 0.31 and 4.3 ± 0.15 in groups C, D, E and F, respectively. Infiltration of myeloid cells in the bursal follicles were observed in chickens in groups C (1/10), D (2/10), E (5/10) and F (3/10). The eosinophilic granulocytic myeloid cells

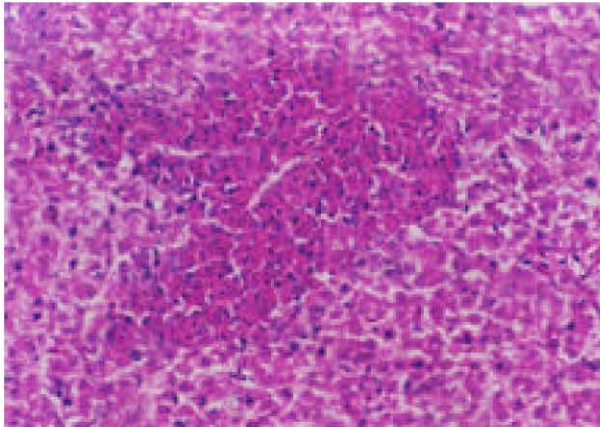


Fig.1: Myeloid cells infiltrations in the liver of broiler breeder chicken. HE, x200.

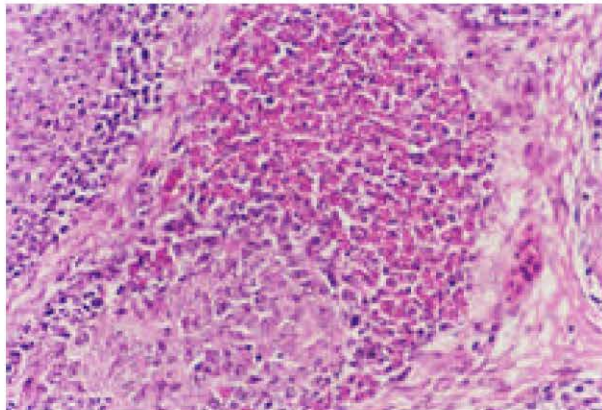


Fig. 2: Myeloid cells infiltrations in the bursa of Fabricius at the cortical region of the follicle in the broiler chicken. HE, x100.

were only found at the cortex of one or two follicles for each positive bursa of Fabricius (Fig. 2). Focal to diffuse myeloid cells infiltration in the lungs, liver, spleen and kidneys was reported previously (Asiah *et al.*, 2001). Nakamura *et al.* (2000) reported that grulated myelocytes proliferation observed in the bone marrow of various bones and in the periosteum of the sternum, ribs, vertebrae, and synsacrum of chickens with ALV-J infections. The first proliferation of tumor cell was suggested to occur in the bone marrow of epiphysis (Nakamura *et al.*, 2000). The myelocytes invaded through Haversian and Volkmann's canal from the bone marrow to periosteal areas. Hematopoiesis was suppressed by marked proliferation of tumor cell in the bone marrow of the whole bone. Marked proliferation of myelocyte can also be seen in the bone marrow and periosteum of ossified cartilaginous rings of the trachea and larynx (Nakamura *et al.*, 2000). However, till to date, there is still lacking of typical gross and histopathology

lesions that can be used to confirm the present of ALV-J. Serology tests such as ELISA and virus neutralizing techniques can be useful to diagnose and confirm the present of ALV-J infections.

ALV-J antibody detection (ELISA): Eight (8/30) or 27% of serum samples were positive for ALV-J in groups A1 and A2, whilst thirteen (13/36 or 36%) and ten (10/24 or 42%) of the serum were positive for ALV-J in groups B1 and B2, respectively which is lower than findings reported previously, up to 97% (Asiah *et al.*, 2001). In contrast, all the serum samples of broiler chickens from the groups C, D, E and F were negative for ALV-J. The positive ALV-J antibody results only indicate exposure to the gp85 protein of ALV-J (Fuchs *et al.*, 2000). Recently, polymerase chain reaction (PCR) assays and RT-PCR test have been developed for the detection of ALV-J proviral DNA and viral RNA in infected materials. However, the sensitive of the PCR is still lacking (Smith *et al.*, 1998), and further development and evaluation of PCR tests as a method for detecting ALV-J infections are required (Payne, 1998).

It is important to note that the present of ALV-J in the broiler breeders can lead to high risk of ALV-J infection in their progeny. It was reported that over 90% of the virus positive embryos were produce from hens infected with ALV-J between 29 and 34 weeks of age under experimental condition (Witter *et al.*, 2000). Although, the age of the broiler chickens are short, at about 5-7 weeks and thus not enough time for the development significant neoplastic lesions, the disease can cause reduction in productivity and may induce immunosuppression. Munger and Barners (1999) reported that cases of myeloid leukosis or ALV-J infections in broiler breeders diagnosed at Rollins Animal Disease Diagnostic Laboratory (RADDL), North Carolina, US when compared to the number of neoplastic cases submitted to the laboratory had increased from 25% in 1993 to 61% in 1998. Neoplastic diseases namely MD, LL and ALV-J infection of chickens throughout the previous ten years (1990-1999) diagnosed at the Faculty of Veterinary Medicine, Universiti Putra Malaysia were about 1-2% (Ooi, 2001). Of this, the present of LL (63%) was the highest followed by MD (31%) and ALV-J (6%). Among these neoplastic diseases, only Marek's is successfully prevented through vaccination, whilst both LL and ALV-J only can be control or eradicated through screening and elimination of infected flock (Zavala, 2001). Thus, efforts are directed at eliminating of the virus at the breeder level by the primary breeding companies (Venugopal, 1999). This involve testing pedigree birds at 20 weeks for group specific antigen (GSA) in the cloacal/vaginal swabs, at 22 weeks for viraemia and serum antibodies, at 23 weeks for GSA in albumen of the first two eggs, at 26 weeks for GSA in the meconium of the hatched

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chicks and at 40 weeks for GSA in the egg albumen (Payne, 1998). However, the widespread antigenic diversity between ALV-J and the lack of knowledge on the immunogenic properties of different viral antigens, make the eradication programme not so successful. Effective vaccine may be an alternative method to eliminate the disease.

It was concluded that broiler breeder chickens in Malaysia is not free from ALV-J infections. The overall sero-prevalence of ALV-J in the broiler breeder chickens was 33% with no differences between the hens and cocks. The presence of myeloid cells infiltration in the follicles of the bursa of Fabricius, 10 to 50% of the samples examined suggest that broiler chickens are at high risk of ALV-J infection.

References

- Arshad, S.S., 1998. Avian leukosis virus subgroup J: disease and diagnosis. Proceedings of 21st Symposium of the Malaysian Society for Microbiology, 15-16 Dec. 1998, pp: 5-9.
- Asiah, N.M., M. Hair-Bejo, Y.S.L. Karen, L.W.S. Amanda, M.A. Masawati, A.H. Nor Faziah, C.K. Mah and U. Chulan, 2001. Avian leukosis virus subgroup J infections in chickens in Peninsular Malaysia. Proceedings of 2nd International Congress/13th VAM congress and CVA-Australasia/Oceania Regional Symposium, 27-30 August, 2001, Kuala Lumpur, pp: 90-92.
- Fuchs, A., B. Myrick, T. Jackson and V. Leathers, 2000. Development of avian leukosis virus subgroup-J specific antibody test kit. IDEXX Laboratories, One IDEXX Drive, Westbrook, Maine.
- Hair-Bejo, M., S. Salina, H. Hafiza and S. Julaida, 2000. *In ovo* vaccination against infectious bursal disease in broiler chickens. J. Vet. Malaysia, 12: 63-69.
- Junqueira, L.C. and J. Carneiro, 1980. Basic Histology. Third Edition, Maruzen Asian dition.
- Munger, L.L. and H.J. Barners, 1999. Tumors associated with avian leukosis serogroup J virus. North Carolina Rollins Animal Disease Diagnostic Laboratory (RADDL). <http://www.cvm.ncsu.edu/info/departs/fae/PHM/jvirus/intro/html>.
- Nakamura, K., M. Ogiso, K. Naoki, N. Kamazaki, H. Hihara and N. Yuasa, 2000. Lesions of bone and bone marrow in Myeloid leukosis occurring naturally in adult broiler breeders. Avian Disease, 44: 215-221.
- Nor Faizah, A. H., M.A. Maswati, N.M. Asiah, M. Rosala, A. Zabidah and A.A. Rahim, 2001. Re-emergence of velogenic Newcastle disease virus in Malaysia. Proceedings of 2nd International Congress/13th VAM Congress and CAV-Australasia/Oceania Regional Symposium., 27-30 Aug, 2001. Kuala Lumpur, pp: 86-87.
- Ooi, P.T., 2001. Prevalence of neoplastic diseases in broiler chickens. Final Year Project, DVM, Faculty of Veterinary Medicine, Universiti Putra Malaysia.
- Payne, L.N., S.R. Brown, N. Bumstaed, K. Howes, A. Judith, Frazier and E. Margaret, 1991. A novel subgroup of exogenous Avian leukosis virus in chickens. J. General Virol., 72: 801-807.
- Payne, L.N., 1998. HPRS-103: Retrovirus strikes back: the emergence of subgroup J avian leukosis virus. Avian Path., 27: S36-S45.
- Smith, L.M., S.R. Brown, K. Howes, S. Mcleod, S.S. Arshad, G.S. Barron, K. Venugopal, J.C. McKay and L.N. Payne, 1998. Development and application of polymerase chain reaction (PCR) tests for the detection of subgroup J avian leukosis virus. Virus Res., 54: 87-98.
- Venugopal, K., 1999. Avian leukosis virus subgroup J: a rapidly evolving group of oncogenic retroviruses. Res. Vet. Sci., 67: 113-119.
- Witter, R.L., L.D. Bacon, H.D. Hunt, R.E. Silva and A.M. Fadly, 2000. Avian leukosis virus subgroup J infection profiles in broiler breeder chickens: association with virus transmission to progeny. Avian Disease, 44: 913-931.
- Zavala, G., 2001. Myeloid leukosis. Vineland Labs publication (<http://www.vinelandlabs.com>).