AOP 8

EPIDEMIOLOGY STUDY OF AVIAN INFLUENZA (H5N1) VIRUS : DISTRIBUTION IN DAY OLD CHICK (DOC)

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INTRODUCTION

Influenza is recognized as a zoonotic disease; with the most commonly affect animals being humans, pigs, horses and species of aquatic birds (McCauley *et al.*, 1995). Avian influenza viruses belong to genus Influenza A virus family *Orthomyxoviridae*, are divided into 16 hemagglutinin (HA) and 9 neuraminidase (NA) subtypes on the basis of their antigenic specificity (Fouchier *et al.*, 2005). Avian Influenza (AI) or bird flu caused by virus subtype H5N1 is still present in Indonesia. The Department of Agriculture of Indonesia has banned poultry distribution from endemic area to non-endemic area to prevent and control spreading of AI virus, except for distribution of day old chick (DOC). The aim of the present study is to detect the possibility of the avian influenza virus infection in DOC that will be distributed from endemic areas to some non-endemic areas. Epidemiological data were collected by doing interviews.

MATERIALS AND METHODS

Two hundred and fourty day old chicks from several farms in Kabupaten Subang, Cianjur, Tanggerang, Bogor and Sukabumi were taken from Soekarno-Hatta Airport before distributed. Antibody titers were measured by using *Haemaglutination Inhibition* (HI) test and then performed necropsy procedures. Trachea, lung, kidney, liver and intestine of each necropsied birds were subjected to pathological examination. Tissues were routinely processed, and stained with hematoxylin and eosin (HE) for histopathology. Additional serial sections were prepared for immunohistochemistry by the streptavidin-biotin complex (SAB) immunoperoxidase method. The primary antibody employed was mouse-anti H5N1 (Astawa *et al.*, 2007).

Fresh tissues of egg yolk, trachea and lung were tested by using *Reverse Transcriptase-Polymerase Chains Reactions* (RT-PCR) with matrix primer pairs (FAI; RAI). Positive samples were then further tested with H5 primer pairs (FH5; RH5) (Lee *et al.* 2004). Laboratory result and questioners were then analyzed using logistic analysis.

RESULTS AND DISCUSSION

Haemaglutination Inhibition* (HI) and *Immunohistochemistry

Evaluation of antibody titers by using HI test demonstrated that 146 birds (60,83 %) have protective titers. This result indicated that condition of DOCs immune system were predominantly protective. Previous study indicated that adult chickens with antibody titer lower than 2^3 were not protective for infection; but did not potentially in viral shedding. Chickens with antibody titer higher than 2^5 titer were potential in viral shedding and protected from infection (Kumar *et al.*, 2007. Histologically, the lesion of internal organ (trachea, lung, heart, kidney, liver and intestine) were mildly found, including lymphocyte infiltrations and congestion. Examination by using immunohistochemistry method demonstrated that 158 samples (65.8%) were positive for subtype H5N1 virus in tissues. Sixty-five point eight percent of samples showed presence of antigen only in trachea, lung, intestine; and 34.2% of samples showed presence of antigen in all tissues examined (trachea, lung, intestine, liver and kidney). Avian influenza antigen is rarely found in the liver and kidney probably due to the AI virus infection is still at an early stage so that has not spread to other visceral organs. According to Mo *et al.* (1997) strongly suspected HPAI virus attacks the respiratory system and then replicates here and spread to all visceral organs.

Reverse Transcryptase - Polymerase Chain Reaction (RT-PCR)

Egg yolk samples examination by performing RT-PCR showed that 44 egg yolk samples (55%) were positive of Influenza A. Nineteen (43.2%) samples were positive for virus subtype H5 and 25 samples (56.8%) were positive to another subtype (Hx). In addition, evaluation of pooled samples of trachea and lung showed 3 samples were positive matrix (Influenza A) but negative H5. Those results showed that avian influenza were able to spread by vertical transmission (presumptive); because viral concentrations were highest in the egg yolk than within tracheas and lungs. Handayani (2009) noted that the AI virus can be detected in the duck ovary using immunohistochemistry staining method so that a large possibility of the virus in eggs.

Epidemiology

Laboratory results and questioners were then analyzed by using *Logistic Analysis*. The highest prevalence of Avian Influenza distribution in DOC occurred in Bogor (91.7%) and the lowest prevalence was Sukabumi (77.6%). The highest AI infection case in broiler DOCs was found in Bogor district during rainy season. Transportation using private vehicle could minimize the risk of AI infection. The result showed that DOCs were infected with AI virus in subclinical symptoms and DOC is one of the potential causes of the rapid spreading of AI in Indonesia, so cautious distribution to free areas need to be taken.

CONCLUSION

1. Avian influenza viruses are found in DOCs tissues even though they have protective maternal antibodies. Antibody titer of parents are able to protect the DOCs from acute infection and cause only subclinical symptoms.
2. The distribution of day old chicks are potential to play an important role in rapid spreading of AI, therefore its distribution to the free areas should be got more attention.

REFERENCES

Astawa NM *et al.* 2007. Immunological detection of avian influenza virus in infected ducks by monoclonal antibodies against AIV-H5N1. *Microbiology Indonesia* 2007: 119-124.

Fouchier RAH *et al.* 2005. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. *HJ Virol.*H 79(5):2814-22.

Lee CW and Suarez DL. 2004. Application of real-time RT-PCR for the quantitation and competitive replication study of H5 and H7 subtype Avian Influenza virus. J. *Virol Methods,* 119: 151–158.

Kumar M *et al.* 2007. Assosiation of serologic and protective response of avian Influenza vaccines in chickens. *Avian Dis,* 51:481-483.

Handayani KS. 2009. Distribusi Virus Avian Influenza (H5N1) pada Jaringan Tubuh Itik Dengan Metode Imunohistokimia. *Tesis.* Sekolah Pasca Sarjana IPB.

McCauley *et al.* 1995. 4-Guanidino-Neu5AC2en fails to protect chickens from infection with highly pathogenic avian influenza virus. *Antiviral Res,* 27: 179-186.

Mo IP *et al.* 1997. Comparative pathology of chicken experimentally inoculated with avian influenza viruses of low and high pathogenic. *Avian Dis,* 41 : 125-136.