

DETEKSI DAN KARAKTERISASI MOLEKULER GEN MATRIKS DAN HEMAGLUTININ VIRUS AVIAN INFLUENZA H5N1 DARI BEBERAPA PETERNAKAN YANG MENERAPKAN SISTEM VAKSINASI BERKALA DI YOGYAKARTA

Oleh:
Dito Anggoro
13/350475/PKH/0483

Intisari

Penyakit *Avian Influenza* (AI) adalah salah satu penyakit yang termasuk dalam daftar lima besar Penyakit Hewan Menular Strategis (PHMS) di Indonesia. Penyakit ini disebabkan oleh virus *Avian Influenza* tipe A yang termasuk dalam keluarga Orthomyxoviridae.

Penelitian ini dilakukan untuk mendeteksi keberadaan virus AI di 8 peternakan ayam layer sektor 3 di wilayah Yogyakarta, yang telah menerapkan sistem vaksinasi berkala. Setiap peternakan diambil sebanyak 25 sampel *swab cloaca*. Pengambilan sampel dilakukan selama 6 kali dalam rentang waktu pengambilan setiap 10 minggu sekali. Sampel tersebut kemudian dikelompokkan dengan sistem *pooling*. Hasil *pooling* tersebut dibagi menjadi dua, yaitu untuk isolasi virus dan ekstraksi *ribonucleic acid* (RNA). Hasil ekstraksi kemudian dilanjutkan dengan proses amplifikasi *deoxyribonucleic acid* (DNA) dengan teknik *reverse transcriptase polymerase chain reaction* (RT-PCR). Tahap awal deteksi dilakukan dengan primer matriks (MA) dan hemagglutinin (HA) dari Australian Animal Health Laboratory (AAHL). Hasil sampel positif kemudian dikonfirmasi dengan primer MA peneliti. Sampel yang menunjukkan hasil positif dari ketiga primer tersebut dilakukan karakterisasi lebih lanjut dengan primer *full gen* HA AAHL. Pada tahap akhir dilakukan *subtyping* dengan primer neuraminidase (NA).

Pada penelitian ini diperoleh 2 isolat virus AI subtipe H5N1 pada pengambilan sampel minggu ketiga dan keempat, yang berasal dari peternakan Adi (SL.7). Analisis hasil sekuensing dilakukan menggunakan *software* DNASTar Lasergene 7.0 dan MEGA 6. Hasil karakterisasi gen MA menunjukkan variasi substitusi pada tingkat nukleotida. Berdasarkan analisis susunan asam amino penyusun *cleavage site* sampel SL.7/C/3 dan SL.7/C/4 termasuk kategori HPAI dengan pola sekuens PQRESRRKKR//G. Berdasarkan analisis pohon kekerabatan dapat disimpulkan bahwa sampel SL.7/C/3 dan SL.7/C/4 termasuk *sub sub clade* 2.1.3. Hasil perbedaan jarak genetik sampel SL.7/C/3 dan SL.7/C/4 dengan isolat virus AI Indonesia dari *Gene Bank* tahun 2003-2014 berkisar 0,02 – 0,12 %. Nilai homologi sampel SL.7/C/3 dan SL.7/C/4 dengan isolat 2003-2012 berkisar 92,5 – 97,9%, sedangkan dengan isolat 2012-2014 berkisar 89,1 – 89,7%. Variasi genetik ditemukan dengan adanya substitusi asam amino di tapak antigenik (L138Q, R189M, dan A86T) dan tapak perlekatan reseptor (R189M).

Kata kunci: *Avian Influenza* virus, sekuensing, substitusi, *clade*

DETECTION AND CHARACTERIZATION OF MATRIX AND HEMAGGLUTININ GENE AVIAN INFLUENZA VIRUSES SUBTYPE H5N1 FROM WELL VACCINATED FARMS IN YOGYAKARTA

By:

Dito Anggoro

13/350475/PKH/0483

Abstract

Avian Influenza (AI) is one of the diseases included in the list of top five Strategic Spreading Animal Diseases (PHMS) in Indonesia. The disease is caused by type A Avian Influenza Virus (AIV) in the family of Orthomyxoviridae.

This study was conducted to detect the presence of AI virus from 8 well vaccinated layer farms sector 3 in Yogyakarta. Each of the farms were collected for 25 cloacal swab samples. Cloacal swab samples were collected for 6 times in a time span of every 10 weeks. Samples were grouped by pooling system. The pooled samples were separated for virus isolation and ribonucleic acid (RNA) extraction. Amplification process of deoxyribonucleic acid (DNA) with reverse transcriptase polymerase chain reaction (RT-PCR) was followed then. The first step of detection was done by matrix (MA) and hemagglutinin (HA) primer from Australian Animal Health Laboratory (AAHL). Positive results were confirmed by personal design MA primer. Samples which showed positive results from the primer above then were further characterized by using full HA gene primer. Subtyping was done by neuraminidase (NA) primer in the final stage.

Avian influenza virus H5N1 was isolated from Adi farm (SL.7) at 38 and 48 weeks of age. Sequencing results were analyzed by DNASTAR Lasergene 7.0 and MEGA 6 software. MA gene characterization results showed substitutions variation in the level of nucleotide. Based on the analysis of composition amino acid sequence cleavage site samples SL.7/C/3 and SL.7/C/4 was classified to HPAI. The pattern of sequences were PQRESRRKCR//G. Based on phylogenetic tree, it can be concluded that samples SL.7/C/3 and SL.7/C/4 belong to sub sub clade 2.1.3. The genetic distance differences between SL.7/C/3 and SL.7/C/4 with the Indonesia AI virus isolates Gene Bank from 2003 to 2014 showed genetic differences among 0,02 to 0,12%. Sample SL.7/C/3 and SL.7/C/4 showed homology ranged from 92,5 – 97,9% with AIV isolates from 2003-2011 while with the AIV isolates from 2012 to 2014 ranged from 89,1 – 89,7%. Genetic variation was found with amino acid substitutions in antigenic site (L138Q, R189M, and A86T) and receptors binding site (R189M).

Key words: Avian Influenza virus, sequencing, substitution, clade