KARAKTERISASI GEN NEURAMINIDASE (NA) DAN POLYMERASE BASIC 2 (PB2) VIRUS AVIAN INFLUENZA H5N1 SUB-SUB-*CLADE* 2.3.2

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Intisari

Penyakit *Avian influenza* (AI) merupakan penyakit yang disebabkan oleh virus influenza tipe A dari keluarga Orthomyxoviridae. Virus AI bersifat zoonosis dan dapat menyebabkan masalah kesehatan manusia dan veteriner. Penelitian ini bertujuan mengetahui *clade* virus AI yang bersirkulasi di wilayah Provinsi Jawa Barat yang menyebabkan kematian pada unggas. Penelitian ini juga bertujuan mengetahui peningkatan virulensi, sensitivitas terhadap *oseltamivir* dan potensi penularan ke manusia serta melihat hubungan kekerabatan gen NA virus AI subtipe H5N1 sub-sub-*clade* 2.3.2. Primer yang digunakan untuk deteksi gen NA10, NA20, NA30 adalah primer dari *Australian Animal Health Laboratory* (AAHL) 2016 sedangkan primer untuk mendeteksi posisi asam amino E627 didesain berdasarkan sekuen gen PB2 yang berasal dari GenBank dengan menggunakan aplikasi *amplifX* pada posisi 1.429-2.163 dengan panjang amplikon 758 bp. Penelitian dilakukan dengan menguji secara serologis semua isolat dengan serum sub-sub-*clade* 2.3.2. Sampel yang positif secara serologis dilanjutkan dengan uji *real time reverse trancriptase polymerase chain reaction* (*real time* RT-PCR) dengan menggunakan primer H5 sub-sub-*clade* 2.3.2.1 dari AAHL 2016. Isolat yang positif sub-sub-*clade* 2.3.2.1 dilakukan amplifikasi dengan metode RT-PCR dan menggunakan primer NA10, NA20, NA 30 dan PB2. Analisis sekuen dilakukan menggunakan *software* UGENE dan MEGA 6. Karakterisasi molekuler fragmen gen NA menunjukkan adanya delesi pada posisi asam amino 49-68 di daerah *stalk region* sedangkan glikosilasi posisi asam amino 88NSS, 146NGS dan 235NGS masih tetap. Hal tersebut menunjukkan peningkatan virulensi virus hanya dipengaruhi oleh hilangnya posisi glikosilasi di daerah *stalk* gen NA. Data analisis molekuler gen NA posisi asam amino E119, R152, D199, H275 dan R293 menunjukkan bahwa virus yang diteliti masih mempunyai sensitivitas terhadap penggunaan *oseltamivir*. Analisis molekuler gen PB2 E627 yang berperan dalam efektifitas replikasi pada suhu tubuh mamalia masih konsisten ditempati oleh asam glutamat yang berarti virus belum memiliki kemampuan adaptasi pada manusia. Berdasarkan pohon filogenetik dengan menggunakan data pembanding dari sekuen gen NA sub-sub-*clade* 2.1.1, sub-sub-*clade* 2.1.3 dan sub-sub-*clade* 2.3.2, isolat penelitian yang digunakan termasuk dalam kelompok virus AI H5N1 sub-sub-*clade* 2.3.2.

Kata kunci : Avian influenza, NA, PB2, PCR, *clade*

THE CHARACTERIZATION OF NEURAMINIDASE (NA) GENE AND POLYMERASE BASIC 2 (PB2) OF AVIAN INFLUENCA H5N1 VIRUS SUB-SUB-CLADE 2.3.2

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Abstract

Avian Influenza (AI) is a disease caused by influenza A virus that belongs to the Orthomyxoviridae family. AI virus is zoonotic and is a potential threat to the health of humans and veterinaries. This study was conducted to determine the clade of AIV circulating in the Province of West Java which has caused deaths in poultry. This study also seeks to investigate the increased virulence and sensitivity to oseltamivir, to understand the potential human infection, as well as to investigate the linkage of NA gene AI virus sub-type H5N1 sub-sub-clade 2.3.2. In order to detect NA10, NA20, NA30 genes, the Australian Animal Health Laboratory (AAHL) 2016's primer was used. Meanwhile, to detect the position of amino acid E627, the primer was designed based on PB2 gene sequence from GenBank using *amplifX* application at positions 1,429-2,163 with amplicon length of 758 bp. The Study was conducted by serologically testing all isolates using the sub-sub-clade 2.3.2 serum. Study continued by conducting real time reverse transcriptase polymerase chain reaction (real time RT-PCR) using H5 sub-sub-clade 2.3.2.1 primer from the AAHL 2016 for samples which were serologically positive. Isolates with positive sub-sub-clade 2.3.2.1 were amplified using RT-PCR method using NA10, NA20, NA 30 and PB2 primers. Sequencing results were anaylized by UGENE and MEGA 6 software. The characterization of NA gen molecular fragment indicates amino-acid deletion at positions 49 to 68 in stalk region. Meanwhile, glycosylation of the amino acid position at 88NSS, 146NGS and 235NGS remains the same. Results show that only deletion of glycosylation position in NA gene stalk area increases virus virulence. Molecular analysis of NA gene, amino acid positions at E119, R152, D199, H275 and R293 indicate sensitivity of the viruses being examined to oseltamivir. Glutamic acid has been consistent in molecular analysis of PB2 E627 gene which take parts in replication effectiveness in mammal's body temperature. In short, viruses have not developed abilities to adapt to humans. Based on phylogenetic trees using comparisons from NA gene sequence of sub-sub-clade 2.1.1, sub-sub-clade 2.1.3 and sub-sub-clade 2.3.2 data, the isolates of this study are categorized as AI H5N1 virus sub-sub-clade 2.3.2 group.

Keywords: Avian influenza, NA, PB2, PCR, clade