

**KLONING DAN KARAKTERISASI GEN HA VIRUS *HIGHLY PATHOGENIC AVIAN INFLUENZA* (HPAI) SUBTIPE H5 ISOLAT PUYUH ASAL ACEH**

**Oleh**  
**Teuku Zahrial Helmi**

**INTISARI**

Penelitian ini bertujuan untuk mengkarakterisasi gen hemagglutinin (HA) virus HPAI sub tipe H5N1 isolat puyuh asal Aceh dan kloning gen HA virus *highly pathogenic avian influenza* (HPAI) sub tipe H5N1 isolat puyuh untuk mendapatkan sekuen (*full genome*) HA sebagai dasar dalam mempelajari tingkat virulensi dan patogenesis virus AI, dan kekerabatan dengan virus HPAI yang pernah bersirkulasi di Aceh atau daerah lain di Indonesia berdasarkan data yang telah ada di *genebank*. Sampel penelitian berupa swab dan organ dari unggas yang diduga terinfeksi virus AI, yang dikoleksi dari 6 kabupaten di Provinsi Aceh. Isolasi dan propagasi virus AI dilakukan secara *in ovo* menggunakan telur ayam berembrio SPF umur 9-11 hari, dan dilanjutkan dengan uji hambatan hemaglutinasi/hemagglutination *inhibition* (HI) menggunakan serum spesifik antivirus AI-H5N1. Pemeriksaan molekuler dilakukan melalui ekstraksi RNA Virus, amplifikasi gen H5 dengan metode *Reverse Transcriptase–Polymerase Chain Reaction* (RT-PCR) dan analisis produk hasil PCR dengan elektroforesis. Produk hasil PCR selanjutnya dilakukan kloning gen HA menggunakan *pET SUMO TA Cloning® vector*, kemudian plasmid ditransformasi ke sel kompeten. Dilakukan juga skreening *E.Coli* yang membawa Plasmid dan *Deoxyribonucleic Acid* (DNA) rekombinan, kemudian dilakukan isolasi dan uji PCR DNA Plasmid dan DNA rekombinan. Berdasarkan pemeriksaan serologis dan molekuler, virus AI yang diisolasi dari berbagai jenis unggas (ayam, itik, dan puyuh) di Provinsi Aceh dapat diidentifikasi sebagai virus AI tipe A dan sub tipe H5. Pola pengulangan asam amino pada daerah *cleavage site* pada isolat virus AI sub tipe H5 asal puyuh dari Provinsi Aceh adalah PQRESRRKKR//GLF. Berdasarkan pola asam amino pada daerah *cleavage site* (CS) tersebut dapat dipastikan bahwa virus yang diteliti dapat diklasifikasikan ke dalam kelompok virus AI dengan patogenesisitas tinggi. Berdasarkan analisis jarak genetik dan filogenetik, virus AI sub tipe H5 asal puyuh dari Provinsi Aceh, termasuk ke dalam kelompok *sub clade* 2.1.3 dengan homologi berkisar antara 99,9%-91,9% dengan isolat pembandingan dari *genebank*. Kloning gen HA virus AI sub tipe H5 dalam penelitian ini setelah dilakukan pensejajaran basa nukleotida, mempunyai homologi dengan gen HA virus AI adalah sebesar 280 bp, dan tidak ada ekspresi gen H5 yang teramati, hal ini kemungkinan disebabkan oleh besarnya gen yang diinsersikan, atau produk PCR gen target yang tidak spesifik.

Kata kunci: Virus AI; sub tipe H5N1; kloning; ekspresi gen; Puyuh; Isolat Aceh

**CLONING AND CHARACTERIZATION HA GENE HIGHLY  
PATHOGENIC AVIAN INFLUENZA VIRUS (HPAI)  
SUBTYPE H5 ISOLATE FROM QUAIL IN ACEH**

**By:**

**Teuku Zahrial Helmi**

**ABSTRACT**

This experiment was designed to characterize the genes hemagglutinin (HA) genes of avian influenza virus subtype H5N1 isolated from quail of Aceh and HA gene cloning of HPAI virus subtype H5N1 quail isolates quail to sequence full genome of HA as the basis in studying the level of virulence and circulated pathogenicity of AI virus, and to see kinship with HPAI viruses that has ever in Aceh or elsewhere in Indonesia based on the existing data in the gene bank. The research sample consisted of swabs and organs of suspected AI virus infected poultry, that were collected from 6 districts in Aceh province. Isolation and virus AI propagation was conducted in ovo using SPF embryonated chicken eggs 9-11 days old, and continued with the hemagglutination/hemagglutination inhibition (HI) tests using specific serum anti AI-H5NI virus. Molecular examination was conducted by virus DNA extraction, amplification of genes H5 with RT-PCR method and analysis of PCR products by electrophoresis. PCR products is then performed using the HA gene cloning in pET SUMO TA Cloning® vector, the plasmid was further transformed into competent cells. Screening of E. coli that carry Plasmid and recombinant DNA was done, then continued with Plasmid DNA isolation and PCR test and recombinant DNA. Based on serological and molecular, it is appeared that AI virus isolated from various species of poultry (chicken, duck, and quail) in the province of Aceh can be identified as AI virus type A and subtype H5. The pattern of amino acids at the cleavage site of AI virus subtype H5 isolated from quails of Aceh province is PQRESRRKKR//GLF. Based on the amino acid pattern in the CS area it can be confirmed that the viruses in this experiment were classified into highly pathogenicity AI (HPAI). Based on genetic distance and phylogenetic analysis, AI virus subtype H5 quail origin of Aceh province can be grouped into sub-clade 2.1.3 with homology ranged between 99.9% -91.9% with the reference isolates from Genbank. Cloning HA gene avian influenza virus subtype H5 in this study after the alignment of the nucleotide bases, has homology with the HA gene of AI virus is of 280 bp, and no expression of genes H5 observed, this is likely due to the large genes diinsarkan, or PCR products no specific target genes.

**Keywords:** AI Virus; Subtype H5N1; cloning; gene expression; quail; isolates Aceh