MOLECULAR CHARACTERIZATION ON HEMAGGLUTININ-NEURAMINIDASE (HN) FRAGMENT GENE OF NEWCASTLE DISEASE (ND) VIRUS ISOLATED FROM PERIODICALLY-VACCINATED FARMS

Abstract

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Newcastle Disease (ND) is a bird-infecting disease that is caused by Avian paramyxovirus virus type 1 (ND virus). ND is known as being contagious, acute and infecting various bird species, including poultry. It has been acknowledged as causing some disadvantages, including up to 100% morbidity and mortality, and reducing meat and egg productions. Furthermore, Hemagglutinin Neuraminidase (HN) in ND virus is a surfacial glycoprotein functioning during attachment, assembly and maturation of ND virus. In fact, Indonesia has been recognized as an endemic country, in which ND has been continuously reported to occur in commercial chicken farms despite having implemented vaccination programs periodically. This study aims at characterizing ND virus isolated from periodically-vaccinated commercial farms, comparing its genetic kinship with archived ND virus originated from Indonesia as well as vaccine strains based on their HN gene fragment. This study gathers archived samples from well-vaccinated chicken farms maintained by Microbiology Laboratory at the Faculty of Veterinary Medicine, Gadjah Mada University, Indonesia. The HN gene fragment of ND virus is amplified according to primer *forward* 5' GTGAGTGCAACCCCTTTAGGTTGT 5' and *reverse* 3' TAGACCCCAGTGATGCATGAGTTG 3' with a 694bp product length. The amplified result is visualized by using electrophoresis gel for being taken into sequencing phase afterwards. Next, the sequence is compared with the sequence of ND virus' HN gene nucleotide in the NCBI Genbank database. The analyses on nucleotide and multiple alignment sequence is conducted by using Mega7. Phylogenetic sequence to discover genotype is conducted through a bootstrap analysis by using Kimura method in Mega7. Looking at the result of sequence analyses, the structure of amino acid residue at position 345-353 for all isolates is PDEQDYQIR. The structure for the samples are the same as for archived samples from Indonesia and either LaSota or B1 vaccine strains. Sample isolates are genetically close to those from Sragen, Gianyar, Banjarmasin, Kudus and Pakistan in particular. According to nucleotide, the genetic distance among isolates under investigation is 0-1.2 %, while their homology value is 98.8-100%. The distance among the isolates and achived Indonesian isolates is 0-7.7% at homology 92.3-99.8%. Then, the distance between observed isolates and LaSota vaccine strain is 16.5-16.7% with a homology value at 83.3-83.5%.

Keywords: Newcastle Disease Virus, Hemaglutinin Neuraminidase protein, RT-PCR, sequencing, vaccination.

KARAKTERISASI MOLEKULER FRAGMEN GEN *HEMAGLUTININ NEURAMINIDASE* (HN) PADA VIRUS *NEWCASTLE DISEASE* (ND) YANG DIISOLASI DARI PETERNAKAN YANG MENERAPKAN VAKSINASI ND BERKALA

Intisari

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Newcastle Disease (ND) merupakan penyakit pada unggas yang disebabkan oleh virus *Avian Paramyxovirus* tipe 1 (virus ND). Penyakit ND bersifat menular, akut dan menyerang berbagai spesies unggas. Kerugian yang ditimbulkan oleh penyakit tersebut antara lain morbiditas dan mortalitas hingga 100% serta menyebabkan penurunan potensi produksi daging dan telur. Protein *Hemaglutinin Neuraminidase* (HN) pada virus ND merupakan salah satu glikoprotein permukaan virus yang berfungsi dalam proses penempelan, penggabungan, maupun dalam proses pematangan virus ND. Indonesia merupakan negara endemis ND, di mana penyakit ND masih terus dilaporkan terjadi pada peternakan ayam komersial meskipun telah menerapkan program vaksinasi secara berkala. Penelitian ini bertujuan untuk mengkarakterisasi virus ND yang diisolasi dari peternakan ayam komersial yang sudah menerapkan program vaksinasi ND berkala, membandingkan hubungan kekerabatan dengan virus ND Indonesia sebelumnya dan strain vaksin berdasarkan fragmen gen HN dari virus ND. Penelitian ini menggunakan sampel dari peternakan ayam *well-vaccinated* koleksi Laboratorium Mikrobiologi Fakultas Kedokteran Hewan UGM, Yogyakarta. Fragmen gen HN virus ND diamplifikasi dengan menggunakan primer *forward* 5' GTGAGTGCAACCCCTTTAGGTTGT 3' dan primer *reverse* 5' TAGACCCCAGTGATGCATGAGTTG 3' dengan panjang produk 694bp. Hasil amplifikasi divisualisasikan dengan menggunakan gel elektroforesis, selanjutnya dilakukan sekuensing. Sekuen fragmen gen HN dibandingkan dengan *database* sekuen nukleotida gen HN ND di *Genbank* NCBI. Analisis nukleotida, *multiple alignment* sekuen dilakukan dengan perangkat lunak Mega versi 7. Analisis filogenetik untuk mengetahui genotipe dilakukan dengan analisis *bootstrap* menggunakan metode Kimura pada Mega 7. Dari hasil analisis sekuen pada fragmen protein HN, susunan residu asam amino pada posisi 345-353 adalah PDEQDYQIR pada seluruh isolat. Pola susunan asam amino sampel sama dengan isolat Indonesia terdahulu dan strain vaksin LaSota dan B1. Isolat sampel memiliki kekerabatan dekat dengan isolat dari Sragen, Gianyar, Banjarmasin, Kudus dan Pakistan. Jarak genetik nukleotida antar isolat yang diteliti adalah 0-1,2 %, nilai homologi antar isolat 98,8-100%. Jarak genetik nukleotida antara isolat dengan isolat Indonesia terdahulu adalah 0-7,7% dan memiliki nilai homologi 92,3-99,8%. Jarak genetik nukleotida isolat yang diteliti dengan strain vaksin LaSota adalah 16,5-16,7% dan memiliki nilai homologi 83,3-83,5%.

Kata kunci: Virus *Newcastle Disease*, protein *Hemaglutinin Neuraminidase*, RT-PCR, sekuensing, vaksinasi.