

INTISARI

Newcastle Disease (ND) merupakan penyakit unggas yang disebabkan oleh virus ND dari keluarga *Paramyxoviridae* genus *Avulavirus* spesies *Avian orthoavulavirus*. Patotipe dan genotipe virus ND diklasifikasikan melalui karakterisasi biologis dan molekuler pada gen *Fusion* (F) dan *Hemagglutinin-Neuraminidase* (HN). Penyakit ND masih ditemukan pada ayam komersial, buras serta burung liar, sehingga berpotensi terjadi penularan ND pada ayam komersial dan burung liar. Penelitian ini bertujuan untuk mengetahui karakter virus ND yang diisolasi dari beberapa ayam dan burung liar, secara molekuler, biologis dan berdasarkan gambaran histopatologis. Sebanyak 3 isolat dan 9 sampel organ/swab kloaka dari ayam dan burung liar baik yang menunjukkan gejala klinis penyakit ND maupun tidak, digunakan dalam penelitian ini. Sampel tersebut diuji dengan qRT-PCR ND dan AI gen matriks sebagai konfirmasi bahwa isolat ND tanpa AI. Sekuensing dilakukan untuk karakterisasi molekuler gen F dan HN dari virus ND. Determinan molekuler penentu patogenisitas berdasarkan pola *cleavage site* pada gen F. Karakterisasi biologis virus ND dengan inokulasi pada Telur Ayam Bertunas-*Specific Antibody Negative* (TAB-SAN) umur 9-11 hari dengan virus ND virulen, non-virulen, Lasota dan kontrol negatif. Pengamatan pada embrio berupa perubahan makroskopis, mikroskopis dan distribusi virus pada jaringan dengan pewarnaan Imunohistokimia. Analisis sekuen berupa *multiple alignment*, variasi nukleotida dan asam amino, jarak genetik dan pohon kekerabatan menggunakan *software* MEGA-X. Analisis hasil pengamatan makroskopis, mikroskopis dan distribusi virus pada jaringan secara deskriptif. Hasil penelitian didapatkan virus yang menyebabkan gejala klinis adalah virus ND dengan *cleavage site* ¹¹²KRQKRF¹¹⁷ pada elang, ¹¹²RRRKR¹¹⁷ pada ayam, dan ¹¹²RRQKRF¹¹⁷ pada ayam dan merak serta secara uji biologis MDT<60 jam, sedangkan pada ayam broiler dan buras yang tidak menunjukkan gejala klinis masing-masing ¹¹²GKQGRL¹¹⁷ dan ¹¹²GRQGRL¹¹⁷ serta MDT>120jam. Mutasi D170N terjadi di situs epitop netralisasi gen F virus dari Broiler_A032009017. Variasi asam amino gen HN di epitop netralisasi dan situs antigenik masing-masing pada posisi ke 263 dan 494 yang terjadi pada isolat strain lentogenik. Virus ND virulen menyebabkan perubahan embrio berupa perdarahan, kerdil dan pertumbuhan bulu sedikit, namun pada infeksi ND non-virulen tidak menunjukkan perubahan tersebut. Virus virulen menyebabkan perdarahan, nekrosis dan infiltrasi sel radang yang lebih dominan dibandingkan dengan infeksi virus non-virulen. Berdasarkan karakterisasi molekuler dan uji biologis, disimpulkan bahwa virus ND yang menyebabkan gejala klinis adalah virulen, genotipe VII subgenotipe VII.2 dan strain velogenik. Virus yang diisolasi dari ayam yang tidak bergejala adalah virus ND non-virulent, genotipe I dan II serta strain lentogenik. Variasi asam amino terjadi pada virus ND non-virulen yaitu di epitop netralisasi gen F dan HN, serta di situs antigenik gen HN, sedangkan pada virus virulen tetap lestari. Virus ND virulen dan non-virulen terdistribusi di berbagai jaringan embrio tetapi pada virus non-virulen tidak menyebabkan kerusakan seperti pada virus virulen serta tidak menyebabkan kematian embrio.

Kata kunci: Virus *Newcastle Disease*, gen F, gen HN, Genotipe, Patogenisitas.

ABSTRACT

Newcastle Disease (ND) is a poultry disease caused by the ND virus (NDV) from Paramyxoviridae family, Avulavirus genus, Avian orthoavulavirus species. NDV pathotypes and genotypes are classified through biological and molecular characterization of F and HN gene. ND is still found in commercial and native chickens also wild birds, so there is a potential for ND transmission to commercial chickens and wild birds. This study aims to determine characteristics of the NDV that isolated from chickens and wild birds regarding the molecular, biological and histopathological features. Three isolates and nine samples of cloacal organs/swabs from chickens and wild birds, both showing clinical symptoms of ND and not, were used in this study. The isolates were tested by qRT-PCR ND and AI matrix gene as confirmation that the isolates were NDV without AIV. Sequencing was performed for molecular characterization of F and HN genes from NDV. Molecular determinants of pathogenicity based on cleavage sites in the F gene. Biological characterization of the NDV by inoculation of 9-11 days Embryonic Chicken Egg-Specific Antibody Negative (ECE-SAN) with virulent, nonvirulent, Lasota strain and negative control. Observations on embryos included macroscopic, microscopic changes and virus distribution in tissues used immunohistochemistry staining. Sequence analysis of multiple alignments, nucleotide and amino acid variations, genetic distances and phylogenetic trees using MEGA-X software. Analysis of macroscopic, microscopic and distribution of viruses is descriptive. The results showed that the virus caused clinical symptoms were NDV with cleavage site of ¹¹²KRQKRF¹¹⁷ in eagles, ¹¹²RRRKRKRF¹¹⁷ in chickens, and ¹¹²RRQKRF¹¹⁷ in chickens and peacock and biologically tested MDT <60 hours whereas broiler and native chicken that did not show clinical symptoms had ¹¹²GKQGRL¹¹⁷ and ¹¹²GRQGRL¹¹⁷ respectively, and MDT > 120 hours. The amino acid mutation (D170N) occur at the neutralization epitope site of the F gene from Broiler_A032009017. The variations of the HN gene in the neutralization epitope and antigenic sites at positions 263 and 494 respectively, occur in lentogenic strain isolates. Virulent NDV viruses cause embryonic lesions such as hemorrhage, stunting and slight feather growth, but non-virulent NDV infections do not show these changes. Virulent viruses cause hemorrhage, necrosis, and inflammatory cell infiltration which are more dominant than non-virulent viral infections. Based on molecular characterization and biological tests, it was concluded that the NDV causing clinical symptoms was virulent, genotype VII subgenotype VII.2 and velogenic strain. Viruses isolated from asymptomatic chickens were non-virulent NDV, lentogenic strains, genotypes I and II. Amino acid variations occur in nonvirulent ND viruses, at the neutralization epitopes of the F and HN genes, as well as at the antigenic sites of the HN genes, whereas in virulent viruses they remain conserved. Virulent and nonvirulent NDV are distributed in various embryonic tissues, but nonvirulent viruses do not cause damage as virulent viruses and do not cause embryonic death.

Keywords: Newcastle Disease Virus, F gene, HN gene, Genotype, Pathogenicity, Virus distribution, Chicken embryo