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Phylogenetic Analysis And Prediction Of Epitope Immunogen Gene Encoding Fusion Protein Newcastle Disease Virus Of Blitar Isolate

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5 ABSTRACT

The aims of this research are to analyze nucleotides sequence, phylogenetic prediction and structure epitope of F protein from Blitar isolate ND virus. Samples of lungs, proventriculus, and feces were collected from chicken infected ND virus in Blitar. Samples were isolated in embryonated eggs and identified by HA test, HI test, and PCR to confirm of ND virus. The result from PCR were sequenced then analysed used GENETYX program ver. 10. Epitope of F protein was analyzed using free access online software IEDB program. DNA fragment has been obtained 699 bp lengths. Base on phylogenetic analysis showed that Blitar ND samples were part of genotype VII, that was genotypically different if compare with isolate strain in GenBank. Thus, it could be concluded that epitope prediction of the F protein of ND virus were isolated from Blitar samples has a chance as immunogen candidate, which can be developed as seed vaccine.

Key words: Newcastle disease, *Fusion protein*, *Blitar*

Introduction

² *Newcastle Disease* (ND) is a respiratory disease and systematic which has acute and easy infects caused by the virus (Tabbu, 2000). ND is a significant disease in the world of animal husbandry, in the list of infectious animal disease contained in OIE, ND is categorized as a *Notifiable disease*, because the disease is economically very detrimental. ND virus infection other than can cause death toll reaches 100%, it can also give a great impact to the economic zone where the trading restriction can occur and the embargo on the area or country where the ND outbreak happened (Capua I and Alexander j. d., 2009). The efforts of breeders in preventing the onset of this disease is with a good biosecurity, sanitation and the implementation maximization of the vaccination program.

Newcastle disease (ND) caused by a virus that is included in the family of *Paramyxoviridae*, genus *Paramyxovirus*, shaped pleomorphic are usually spherical with a diameter of 100-500 nm, but there are also have filaments shaped, and envelope (Kencana, 2012). The vaccination program at different levels

of age as well as supported by optimal management practices are a ND disease prevention efforts, but undeniable that until recently the case of ND is still widely found in the field. Such ND disease case can happens on a farm which has been implementing vaccination properly even on broiler chicken farm which has not been yet implementing ND vaccination.

ND infection on vaccinated chicken also caused by the low response of poultry toward vaccine therefore can easily infected. It related to the ND vaccine that is unable to protect vaccinated poultry from the virus infection and growtg, besides that the maternal anti body also take a role on the spring poultry vaccination (Kapezynski and King, 2005). This study is purposed to analyze nucleotide structure, phylogenetic structure of ND case in Blitar, and predict the epitope of immunogenic gen decoder of protein F of ND virus. Where protein F is the main protein that define the virulence level of ND virus.

Material and method

Samples of lungs, proventriculus and chicken feces which is infected by ND virus of a farm on Blitar that later being isolated on *chicken embrioned egg* (TAB) and identification toward ND virus through hemaglutinasi test (HA) which is confirmed with hemaglutinasi resistance (HI) using positive ND serum. Then, isolation RNA is implemented by using kit, afterward being *reverse transcriptase-polymerase chain reaction* (RT-PCR) checked using primer forward (MSF1), reverse (NDVR). Positive result of PCR examination then being sequenced, therefore nucleotide sequence is detected and being *alignment* using program, GENETYX version 10. Epitope prediction of B cell analysis on protein F is using IEDB *software online*.

Result

The result of this study is detected the DNA fragment with 699 bp length of the PCR amplification result sample. On phylogenetic tree result shows that the four samples are include in genotype VII. the results of epitope immunogen prediction of F protein ND virus showed sample of SN has better chance as a candidates immunogen which has 6 candidates B-cell epitopes on the F protein, compared to samples KP, KV 1 and KV 2, which has 5 candidates of B cell epitopes immunogen on the F protein.

Discussion

On this study can be concluded that circulated virus in Blitar according to genotype is different with vaccine isolate being used. It shows that epidemic disease happens in Blitar is not caused by ND virus comes from vaccine isolate, but field virus genotype VII which is the cause of ND disease in Indonesia as reported in Indonesia before (Adi *et al.*, 2010), therefore epitope prediction of B cell protein F ND virus gained from Blitar sample can be as immunogenic candidate that can be used as vaccine candidate.

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