Epitope Prediction from Genes Encoding F Protein of Newcastle Disease Virus (NDV) Isolates Swan (Cygnus Olor) for Vaccine Development to Prevent Infectious Disease

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Abstract—Currently, the poultry industry is threatened by virulent viruses of endemic disease that can cause major economic losses to this sector. Aim of this research was conducted on epitope prediction from genes encoding F protein of Newcastle Disease Virus (NDV) isolated from waterfowl. Samples of this research collected from clausal swabs of seventy-one waterfowl. Consisting of twenty-four domestic swan, thirty-three Muscovy ducks, and fourteen domestic ducks form waterfows in East Java Province, and also positive LaSota isolates (ATCC). Samples were isolated in embryonated chicken eggs (ECEs) with Specific Pathogen Free (SPF) and identified by HA test and confirmed as NDV by HI test. Positive samples were performed by RT-PCR using forward and reverse primer with target of 690 bp. The sequencing results were analyzed by forward and reverse primer to generate the complete gene. Candidate epitopes were analysed by prediction tools from Immune Epitope Database online program. One epitope was predicted as a peptide vaccine for B cell (HGKILDPGRWPGARVAPDLP) from NDAG2/2018 sample and more likely to be immunogenic vaccine candidate based on epitope prediction immunogenic epitope candidates have highest log score.

Keywords—Epitope, F Protein, Homology, Newcastle disease virus, Phylogenetic tree, RT-PCR.

I. INTRODUCTION

Newcastle disease is a crucial disease in the world of animal husbandry. According to OIE, this disease is included in the list of infectious animal diseases [1]. Newcastle disease is caused by a virus belonging to the family Paramyxoviridae and genus Paramyxovirus. Its shape is usually circle with a diameter of 100-500 nm, or filament [2].

In 1926, Newcastle disease was first reported by Kraneyeld in Jakarta [3]. Since then, Newcastle disease has been reported everywhere. Until now, there is no single area in Indonesia free from this disease despite the vaccination program, as part of prevention, that has been done since 1950 [4].

Newcastle disease virus (NDV) has six major proteins and two non-structural proteins composing the genome. The six major proteins are microcapsid protein (N), phosphoprotein protein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase protein (HN), large polymerase protein (L) and other two non-structural proteins which are V and W protein. Each of protein has roles in determining the virulence of NDV [5]. Proteins M, F, and HN are the proteins associated with viral envelopes, while F and HN proteins are functioned to set viral entry and release [6].
Different strains of NDV have been their great variation in pathogenicity. Strains of NDV have been classified into five pathotypes on the basis of clinical signs, namely: (i) Viscerotopic velogenic which is a highly pathogenic for in which haemorrhagic intestinal lesions, (ii) Neurotropic velogenic which presents with high mortality, usually following respiratory and nervous signs, (iii) Mesogenic which presents with respiratory signs, occasional nervous sign, but low mortality, (iv) Lecenic or respiratory that presents with mild or subclinical respiratory infection, (v) Asymptomatic that usually consists of a subclinical enteric infection [7]. Wild fowls, especially waterfowls, can be reservoirs for lentogenic strains [8].

The aim of this study was to design a vaccine for NDV using peptide of its fusion protein as an immunogen to stimulate protective immune response. Epitope prediction appropriate protein residues would help in production of peptide vaccine with powerful immunoresponse and minimal allergic effect.

II. MATERIALS AND METHODS

A. Samples collection, virus isolation, and identification

Clinical swabs were collected from seventy one waterfowl consisting of twenty four Domestic swan, thirty three Muscovy ducks, and fourteen Domestic ducks from waterfowl in East Java Province (Table 1).

| No | Location  | Species of Waterfowl | Total |
|----|-----------|----------------------|-------|
|    |           | Domestic swan | Muscovy ducks | Domestic ducks |
| 1  | Malang    | 4               | 1              | 5               |
| 2  | Rewakanger | 4               | 22             | 26              |
| 3  | Ponoromang | 9               | 10             | 19              |
| 4  | Tarakan   | 6               | 10             | 16              |
| Total | 24         | 33             | 14             | 71              |

All samples were inoculated into the allantonic cavities of 9-to-11-day-old specific-pathogen-free (chicken embryos) allantoic fluid aged 9-10 days for 5 days and were observed every 24 hours. A total of 71 samples were tested. There were 5 samples showing results of HA: ND5/SW1/2018, ND5/SW2/2018, ND5/SW3/2018, ND5/SW4/2018, and MK. Samples with positive HA test was then continued to be identified with HI test. The HI test based on the principle that the hemagglutination the viral envelope and this can be inhibited by specific antibodies. Agglutination resistance occurs perfectly against the 4 HA unit antigen [1]. HI identification utilizes Newcastle disease antisera. HA and HI test results from harvested allantoic fluid shown in table 3.

C. Molecular analysis

Molecular analysis is a method to combine and comparing nucleotide sequence nucleotide that had been read by sequencer machine. The sequence of nucleotides were analyzed using Biological Sequence Alignment Editor (BioEdit) version 7.0.5.3 and Molecular Evolutionary Genetics Analysis (MEGA) version 6. B cell epitope was accessible and antigenic [19]. To predict the B cell epitope, we used the methods provided by immune epitope database (http://tools.iedb.org/bcell/) with default threshold BepiPred value of 0.33 [10].

III. RESULTS

The process of RNA extraction, the next stage is amplification for partial gene F. Primer of specific oligonucleotide for RT-PCR amplification were purchased from PT. Generika Indonesia. This primer were designed by Rantam. The primers flanked of the region on cleavage site into protein coding gene of NDV. The sequence of oligonucleotide primers were presented in Table 2. Conditions for amplification RT-PCR of complete F genes was followed: 95°C lid on 1 min, 94°C de-naturation for 10 min, 94°C denaturation for 1 min, 57°C annealing for 1 min, with a final extension step at 72°C for 10 min.

After RNA extraction and RT-PCR test using one pair of primer, PCR product was obtained and viewed by
electrophoresis. The electrophoresis results showed isolates tested by forward and reverse primer found in DNA fragment size of 699 bp as shown in Fig. 1.

![Fig. 1. F gene amplification results. Polymerase chain reaction product size of 699 bp. The amplicons were electrophoresed in 1% agarose gel. Lane M: Molecular size marker; SW1, SW2, SW3, SW4 Lk (La Sota isolate).](image)

The B cell epitope specific antigen region that has a high binding with B cell lymphocytes. As a result, B cell could produce the antigen-specific antibody and memory cell. The BepiPred linear epitope prediction in this study with the average binders score of fusion protein to B cell was 0.173 with a minimum -0.000 and maximum 1.538.

IV. DISCUSSION

A total of seventy one cloacal swab samples from waterfowl were inoculated on specific-pathogen-free (SPF) chickens embryos. Waterfowl cloacal swabs were used because NDV is known to be transmitted through digestive and respiratory tracts [9].

The growth of NDV in allantoic fluid by HA test aimed to identify the ability of erythrocytes hemagglutination of viral envelope agglutinates the erythrocytes of chickens [12]. There were five samples (ND/SW1/2018, ND/SW2/2018, ND/SW3/2018, ND/SW4/2018) of seventy one field samples tested showing positive HA test. Samples with positive HA test were confirmed by HI test (inhibitory hemagglutination). The HI test is based on the principle that hemagglutinin in a viral envelope can agglutinate chicken erythrocytes, and this can be inhibited by specific antibodies. Agglutination inhibition occurs perfectly against 4 HA Unit antigen. Hemagglutinin of NDV can bind specifically to sialic acid on sensitive cell surface of the receptors and facilitate the infection process. According to Aris et al. [13], this delicate receptor is also owned by red blood cells (erythrocytes) of chickens.

Hemagglutination is shown in chicken erythrocytes mixed with ND virus in equal proportions. The phenomenon of hemagglutination can be inhibited with specific antibodies against hemagglutinin of the ND virus. This ability is used as the basis for the identification of the ND virus commonly called as the HI test (hemagglutination inhibition). The samples that were continued to the RT-PCR stage were the samples of ND/SW1/2018, ND/SW2/2018, ND/SW3/2018, ND/SW4/2018 and positive isolates of La Sota. The electrophoresis results of Newcastle disease virus RT-PCR using specific primers in the F-gene region, F-forward GAG CAC CCG TGA GCA CCA GGA GTT TAT and F-reverse SAGT CGG AGG ATG TGG GCA GCC have shown good results with proven detection of DNA fragments in the sample with a length of 699 bp.

In our study, we predicted the B cell epitopes of the NDV fusion protein based on the IEDB. These methods were used to predict specific areas in proteins that bind to the B cell receptor, and must be on the surface and immunogenic. Equipped linear epitope prediction tools in the programme based on a Hidden Markov model. The best single method for predicting linear epitope and a trend which the positive predictions are characterised by E as B cell linear epitope [14].

The results of the epitope predictions indicated that there were potential peptide sequences as the B cell epitopes. We predicted that the peptide sequences from 47-88 (HKGKLDPQWRPGAPWWADLLP) amino acids are capable of inducing the desired immune response as B cell epitopes. The epitope prediction tools could facilitate the development of vaccines and predict the epitopes [15]. Vaccine production that depends on biochemical experiments can be expensive, time consuming and not always work, although this vaccine formulation of attenuated inactivated microorganism contains a few hundred of unnecessary proteins for the induction of immunity, that may cause allergic or reactogenic responses [16,17]. Therefore, prediction of epitopes of appropriate protein residues would help in production of peptide vaccine with powerful immunogenic and minimal allergic effect [16,18]. Further work is required to clone the gene for these peptides on some carrier vectors in order to collect data on the extent of elicitation of immune response and to ensure that this vaccine will provide long-term protection.

In conclusion, our experiment showed one epitope (HKGKLDPQWRPGAPWWADLLP) proposed to be a peptide vaccine against NDV. Further study is also needed to identify the interaction between these peptides with T cells and antibodies.

ACKNOWLEDGMENT

The authors would like to thank the Ministry of Research, Technology and Higher Education of the Republic of Indonesia through Master Degree to Doctorate for Superior Scholarship Program (PMSIU), grant number 2146/D3/PG/2017.

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