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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 economis losses to this sector. Aim of this research was conducted to epitope prediction from genes encoding F protein of Newcastle Disease Virus (NDV) isolated from waterfowl. Samples of this research collected from cloacal swabs of seventy one waterfowls. Consisting of twenty four domestic swan, thirty three Muscovy ducks, and fourteen domestic ducks form waterfowls 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 699 bp. The sequencing results were analyzed by forward and reverse primer to generate the complete gene. Candidate epitopes were analysed by predictions tools from Immune Epitope Database online program. One epitope was predicted as a peptide vaccine for B cell (HGKILDPGWRPPGARWAPDLLP) from ND/AG3/2018 sample and more likely to be immunogen vaccine candidate based on epitope prediction immunogenic epitope candidates have hisghest 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 infecti 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 Kraneveld 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-str ural proteins composing the genome. The six major proteins are nucleocapsid protein (N), phosphoprotein protein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuramidaseprotein (HN), large polymerase protein (L) and other two non-structural proteins which are V and W protein. Each of protein ha 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 has been their great variation in pathogenicity. Strains of NDV have been classified into five patotypes on the basis of clinical signs, namely: (i) Viscerotropic velogenic which is a highly pathogenic for in which haemorragic intestinal lesions, (ii) Neurotropic velogenic which presents with high mortality, usually following respiratory and nervous sign, (iii) Mesogenic: which presents with respiratory sign, occasional nervous sign, but low mortality, (iv) Lentogenic or respiratory that presents with mild or subclinical respiratory infection, (v) Asymtomatic 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 desi 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 immunogenic and minimal allergenic effect.

II. MATERIALS AND METHODS

A. *Samples collection, virus isolations, and identification*

Cloacal 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 Javs Province (Table 1.)

TABLE 1 SAMPLES COLLECTED FROM FIELD ISOLATED

No	Location	Species of Waterfowl			Total
		Domestic swan	*Muscovy ducks*	*Domestic ducks*	
1	Mulyorejo		1		1
		4			4
				5	5
2	Rowokangkung, Lumajang		22		22
		4			4
3	Pacar Kembang, Surabaya	10			10
				9	9
4	Kenjeran, Surabaya		10		10
		6			6
Total		24	33	14	71

All samples were inoculated into the allantoic cavities of 9-to-11-day-old specific-pathogen-free (chicken embryos) and incubated for 4-7 days with the temperature of 37^0 C [8].Observations were made every 12 hours and the ad embryonic chicken egss was stored in the refrigerator. The presence of NDV was confirmated by hemagglutination test, as well as hemagglutination inhibition test using LaSota specific polyclonal sera.

B. *Nucleic acid extraction, RT-PCR, and sequencing*

For genetic characterization of ND otal RNA was extracted from allantoic fluid using Trizol LS reagen (Invitrogen, Carlsbad, CA) according to the manufacturer's intruction.

After the process of RNA extraction, the next stage is amplification for partian gene F. Primer of specific oligonucleotide for RT-PCR amplification were purchased from PT. Genetika 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 complate F genes was follow: 95^{o}C lid on se ng, 94^{o}C pre-denaturation for 10 min, 94^{o}C denaturation for 1 min, 57^{o}C annealing for 1 min,with a final extension step at 72 ^{o}C for 10 min.

TABLE II SEQUENCE OF OLIGONUCLEOTIDE PRIMER RT-PCR FOR AMPLIFICATION OF F GENE NDV

Primer	Sekuen 5'-3'	Product	Position
Forward	GAC CGC TGA CCA CGA GGT TA	699bp	4306-4326
Reverse	AGT CGG AGG ATG TTG GCA GC		4981-5005

C. *Molecular analysis*

Molecular analysis is a method to combine and comparing nucleotide sequence nucleotide that had been read by sequencer mechine. 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 is accessible and antigenic [9]. To predict the B cell epitope, we used the methods provided by immune epitope database (http://toolseidb.ofg/bcell/) with default threshold BepiPred value of 0,35 [10].

III. RESULTS

All samples were then inoculated in 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: ND/SW1/2018, ND/SW2/2018/, ND/SW3/2018, ND/SW4/2018, and MK4. Samples with positive HA test was then continued to be identified with HI test. The HI test based on the principle that the hemagglutiation the viral envelope and this can be inhibited by specific antibodies. Agglutination resistance occurs perfectly against the 4 HA unit antigen [11]. HI identification utilizes Newcastle disease antiserum. HA and HI test results from harvested alantoic fluid shown in table 3.

TABLE III HA AND HI TEST RESULTS OF ALLANTOIC FLUID INOCULATED WITH NDV

Sample Forward	Mean death time (MDT)	Identification test	
		HA	HI
ND/SW1/2018	96 h	2^8	2^7
ND/SW2/2018	100h	2^7	2^7
ND/SW3/2018	96h	2^7	2^7
ND/SW3/2018	110h	2^8	2^7
MK4	48 h	2^7	2^0
Lasota positive isolate	120h	2^7	2^7

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 seen in the presence of DNA band 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 1.5% agarose gel. Lanes: M =Molecular size marker; SW1, SW2, SW3, SW4 K+ (La Sota Isolate).

The B cell epitope is a specific antigen region that has a high interaction with B cell lymphocyte. As a result, B cell could produce the antigen-specific antibody and memory cell [6]. 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 cloaca swab samples from waterfowl were inoculated onspecific-pathogen-free (chicken embryos). Waterfowl cloacal swabs is used because NDV is known to be transmitted through digestive and respiratory devices [8].

The growth of NDV in allantoic fluid by HA test aimed to identify the ability of erythrocytes hemagglutination of viral envelope agglutinate 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 hemagglutinin). 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. Hemaglutinin 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.

Haemagglutination 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-PCRby using specific primers in the F-gene region, F-for 5 GAC CGC TGA CCA CGA GGT TA 3' and F-rev 5 'AGT CGG AGG ATG TGG GCA GC 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 was used to predict specific areas in proteins that bind to the B cell receptor, and must be on the surface and immunogenic. BepiPred linear epitope prediction tools is the programme based on a Hidden Markov model. The best single method for predicting linear epitope and a trend scale 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 47–58 (HGKILDPGWRPPGARWAPDLLP) 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 or inactivated microorganism contains a few hundred of unnecessary proteins for the induction of immunity, that may cause allergenic 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 allergenic effect [18,19]. 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 (HGKILDPGWRPPGARWAPDLLP) 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.

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REFERENCES

[1] I. Capua, and J. D. Alexander, "Avian Influenza and Newcastle disease," Springer, pp. 27-30, 2009.

[2] G. A. Y. Kencana, K. I Made, and N. K. M. I Gusti, " Confirm the diagnosis of Newcastle disease in chicken field isolate in Bali using

RT-PCR. Faculty of Veterinary Medicine Udayana University. Denpasar. Bali

[3] S. Kumar, N. Baibaswatsa, L. C. Peter, and S. K. Samal, "Using of the Newcastle disease virus F and HN Protein in Protective Immunity by using a Recombinant avian paramyxovirus type 3 vector in chicken," J Virol. Vol 85, pp. 6521-6534. 2011.

[4] A. Kusumaningsih and S. A. Bahri, "Potential, opportunities and strategies for animal vaccine development in Indonesia," J. Litbang Pertanian, Vol 24, pp. 113-121. 2005.

[5] D. A. Hewajuli and N. L. P. I. Dharmayanti, "Pathogenicity of the Newcastle disease Virus in chicken," Wartazoa, pp. 72-80. 2011.

[6] J. C. F. M. Dortmans, P. J. N. Rottier, G. Koch, and B. P. H. Peetersi, "The Replication complex is associated with the virulence of Newcastle disease virus," J. Virol, Vol 19, pp. 10113-10120. 2010.

[7] D. J. Alexander and D. A. Senna, "Newcastle disease and other avian paramyxovirus. In: L. Dufour-Zavala, J.R. Glisson et al, "A laboratory manual for the isolation, identification and characterization of avian pathogens," AAAP, Vol 4, pp. 135-141. 2008.

[8] OIE (Office International des Epizooties), "Newcastle Disease. Manual of Diagnostic Test and Vaccines for Terrestrial Animals," Chapter 2.3.14. http://www.oie.int/international-standart-setting/terrestrial-manual/access online. 2012.

[9] R. Vita, J. A. Overton, J. A. Greenbaum, J. Ponomarenko, J. D. Clark, J. R. Cantrell, D. K. Wheeler, J. L. Gabbard, D. Hix, A. Sette, and B. Peters, "The imune epitope database (IEDB) 3.0," Nucleic Acids Research, Vol. 43, pp. 405–412. 2015.

[10] H. Anayet, H. Mehjabeen, and A. Jibran, "A computational assay to design an epitope-based peptide vaccine against Saint Louis Encephalitis virus," Bioinformatics and Biology Insights, Vol. 7, pp. 347–355. 2013.

[11] FAO, "A technology Review: Newcastle disease with Special Emphasis on its Effect on Village Chicken," http://www.fao.org. 2004.

[12] D. J. Alexander, "A Technology Review: Newcastle disease with special Emphasis on Its Effect on Village Chicken," In: FAO Animal Production and Health. 2004.

[13] H. Aris, D. Kristiawan, S. H. Irianingsih, and D. W. Yudianingtyas, "Ampification of Fusin Protein Encoding Gene of Newcastle disease Virus from Field Specimens by OneStep RT-PCR Method," Jurnal Veteriner, Vol. 3, pp. 387-393. 2013.

[14] J. Erik, P. Larsen, O. Lund, and M. Nielsen, "Improved method for predicting linear B-cell epitopes," Immunome Research, Vol. **7**, pp. 1–7. 2006.

[15] L. S. Sitompul, N. Widodo, M. S. Djati, and D. H. Utomo, "Epitope mapping of gp350/220 conserved domain of epstein barr virus to develop nesopharyngeal carcinoma (Npc) vaccine." Bioinformation, Vol. 8, pp. 8–11. 2012.

[16] W. Li, M. D. Joshi, S. Singhania, K. H. Ramsey, and A. K. Murthy, "Peptide vaccine: Progress and challenges," Vaccines, Vol. 2, pp. 515–536. 2014.

[17] Y. T. Lo, T. W. Pai, W. K. Wu, and H. T. Chang, "Prediction of conformational epitopes with the use of a knowledge-based energy function and geometrically related neighbouring residue characteristics," BioMed Central Bioinformatics, Vol. **14**, pp. 1–10. 2013.

[18] A. Purcell, W., J. McCluskey, and J. Rossjohn, "More than one reason to rethink the use of peptides in vaccine design," Nature Reviews. Drug Discovery, Vol. 6, pp. 404–414. 2007.

[19] P. A. Reche, R. F. Darren, F. H. Masha, and H. Yoshihiko, "Peptide-based immunotherapeutics and vaccine," Journal of Immunology Research, Vol. 2014, pp. 1–2. 2014.

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