Epitope Prediction Of B Cell Gene Encoding F Protein Of Newcastle disease (ND) Virus From Pigeon Isolate (*Columbia livia*)

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Abstract

Newcastle Disease is a highly contagious disease infecting avian animals caused by the ND virus that was previously synonymous with Avian Paramyxovirus type 1 (APMV-1). Even vaccination program management implemented, Indonesia is still an endemic area for ND. B cell epitope is a sequence of peptides (amino acids) found explicitly on specific antigens' surface, which binds to antibodies. Therefore, epitope characterization is very crucial for the development of vaccine making. There were 20 pigeon samples (Columba livia) collected from bird markets in East Java through the necropsy procedure. Three of them were positively infected by Newcastle Disease. Samples were collected in embryonated chicken eggs and identified by the HA test, confirmed by the HI test. PCR tests were performed on the positive samples using forward and reverse primers with a target of 329 bp. The sequencing result was then translated into an amino acid sequence via BioEdit software version 8.0. Prediction of immunogenic epitopes in genes protein F coding was performed using the online Epitope Prediction software Tools / IEDB with the Bepipred Linear Epitope Prediction. Epitope from sample ND / BG / 2019 more likely to be candidates for immunogenic epitopes based on predictions epitope B cell, which has the highest log score of 36.716.

Keyword: Newcastle Disease, F Protein, Amino Acid, B Cell Epitope.

1. Introduction

Newcastle disease (ND) is a kind of disease that highly contagious and fatal if infecting avian species like pigeon. Pigeon itself can be ND virus carrier while still in the wild. ND viruses can affect many species of birds, and chicken is highly susceptible (Shanmuganathan et al., 2017). Newcastle disease can cause severe economic losses also kills more than 55% poultry than usual (Antipas et al., 2012). In Indonesia, ND is an endemic virus, and ND genotype VII can cause 100% morbidity and 80% mortality (Risa Indriani and Dharmayanti, 2016). ND disease caused by the ND virus that was previously synonymous with Avian Paramyxovirus type 1 (APMV-1) that belongs to the Paramyxoviridae family; however, now the ND virus is referred as Asian Avulavirus due to change in taxonomy (Brown & Bevins, 2017).

Based on the clinical signs on ND induced in the chicken and other avian species, there are three significant pathotypes classified: velogenic (highly virulent), mesogenic (intermediate virulent), and lentogenic (non-virulent) strain (Al-Habeeb et al., 2013). During the outbreak, several clinical signs seen in birds include difficulty breathing, loss of appetite, ocular/nasal discharge, blue comb, swelling around the eyes, diarrhea, and death. ND virus can be transmitted through inhalation and ingestion. Also, birds can shed the virus in feces and respiration secretory. ND viruses can infect many species of birds, and chicken is highly susceptible (USDA-APHIS., 2018).

Even vaccination program management implemented, Indonesia is still an endemic area for ND. ND virus spreading in Indonesia today is a part of virulent strains, and some have estimated undergone genetic changes (Etriwati et al., 2017). These genetic changes happened because the ND virus is easily mutated (Indriani and Dharmayanti, 2016). Due to the emergence of novel strains and vaccine failures, the ND virus has evolved into a more significant challenge (Jabbarifakhar et al., 2018).

The RNA genome of ND virus coding six types of protein: Large RNA polymerase (L), fusion protein (F), hemagglutinin-neuraminidase (HN), phosphoprotein (P), nucleocapsid protein (NP), matrix protein (M), in the order 3'-NP-P-M-F-HN-L-5' (Al-Habeeb et al., 2013). F and HN proteins essential in adhesion and fusion of the virion and target cell surface. These two proteins contribute to the characteristic and virulence of the ND virus (Cattoli et al., 2011).

The F protein have 1792 nucleotide long that encodes 533 amino acids long precursor polypeptide. The F gene glycoprotein having important roles to mediate penetration with the host cell. F gene can create pores on plasma membrane through which the viral nucleocapsid is delivered into the host cell cytoplasm. The F protein is type 1 integral membrane protein that synthesized as inactive precursor (F0) that required host cell proteolytic enzyme to cleavage. The cleavage will divide F protein into F1 and F2 sub-unit that connected to each other by disulfide link which is biologically active protein.

B-cells are an important part of the adaptive immune system because they can provide long-term protection against harmful pathogens and molecules. B cells have specific receptors, named immunoglobulins or antibodies, a key component in immune system process. The antibody can recognize their molecular target, called the antigen, between its binding site interactions (paratope) and specific antigen region (epitope).

Epitope characterization is very crucial in the vaccine business. Epitope plays a vital role in antibody function in recognition and binding, which makes epitope analysis essential to understanding the immunological event and for the development of various diagnostic tools for different disease and epitope oriented vaccines (Bi et al., 2019). There are three basic strategies

to identified epitope which is: algorithm, making recombinant DNA protein fractions, and epitope scoring. With epitope scoring, epitopes can be identified using immune epitope database and analysis or IEDB.

2. Material and Methods

2.1.Research Design

This research was a descriptive laboratory - exploratory research because there was no treatment done to the samples. The samples were gathered using the convenience sampling method, a type of non-probability sampling method that cost-effective are the samples taken from groups of individuals that are easier to search.

20 Samples were collected using a purposive sampling method. In this study, several non-vaccinated pigeon organ samples (Columba livia) were used which were taken from the bird market in East Java.

2.2.Variable Operational Definition

In this study, Newcastle Disease virus were collected from various bird market in East Java. Necropsy was used to collect the samples which is brain, proventriculus, pulmo, thrachea, hepar, intestine which later inoculated into embryonated chicken eggs. Protein F is the main target of the immune response that having important role in the fusion between virus and the main target cell. Epitope is structure of three dimensional folded immunoglobulin molecules forming a surface that fulfils the structure of specific surface antigens. B cell epitope is an epitope which can bind to antibody.

The research were conducted at the Stem Cell Research Center Lab (Institute of Tropical Disease) and Veterinary Medicine Virology Lab of Universitas Airlangga.

2.3.Research Procedure

2.3.1. Samples Collection

Samples were gathered using the convenience sampling method. The samples then processed into a 10% suspension and then inoculated into the aged nine days TAB for 120 hours, with each sample using 3 or 4 TAB. Eggs are candled once a day. If the TAB is off during the incubation period, place the TAB in the refrigerator at four °C, and on the last day of the incubation period, all the TABs are placed in the refrigerator.

2.3.2. Samples Preparation

The allantoic fluid from the TAB was collected, then checked through the Hemagglutination (HA) and Hemagglutination Inhibition (HI) test using the micro technical method. After the positive samples of ND collected, the RNA then extracted and processed into RT-PCR test with specific primer product of 329 bp.

The whole sequence of PCR products was trimmed, and the sequence was used for homology analysis of fusion protein which the number of products 329 bp. The sequence editing was done using Biological Alignment Editor (BioEdit) version 7.0.5.3. Both forward and reverse sequences are combined using BioEdit version 7.5.0.3. The reference of the sequence used to edit the samples was Newcastle Disease Virus LaSota.

2.4.Data Analysis

Bioedit software is used to analyze proteins present in nucleotide sequences. The nucleotide sequence is then translated into amino acids using the BioEdit ver software. 8.0 will produce a protein translation of each Newcastle disease. One amino acid is encoded by three nucleotides. The translational amino acid sequence will be aligned using the ClustalW software integrated with the Bioedit ver software. 8.0.

Prediction of immunogenic epitopes in genes protein F coding was performed using the online Epitope Prediction software Tools / IEDB with the Bepipred Linear Epitope Prediction.

3. Research Result

There were 20 pigeon samples (Columba livia) collected from bird markets in East Java through the necropsy procedure. Organs taken in this study were the brain, respiratory organs (trachea and Pulmo), liver, and digestive organs (proventriculus, ventriculus, and intestine).

Table 1. Pigeon isolates (Columba livia) collected from East Java.

No	Location	Samples Code
1	Bratang Bird Market	BD1 / 2019 BD2 / 2019 BD3 / 2019 Bh1 / 2019
2	Candi, Sidoarjo	CnS1 / 2019 CnS2 / 2019 CnS3 / 2019 CnS4 / 2019 CnS5 / 2019
3	Sidoarjo City Central	SK1 / 2019 SK2 / 2019 SK3 / 2019
4	Gresik	BG1 / 2019 BG2 / 2019 BG3 / 2019
5	Krian	BK1 / 2019 BK2 / 2019 BK3 / 2019 BK4 / 2019
6	Pasuruan	BP1 / 2019
Total		20

From the market 20 samples of Newcastle disease virus was collected. The samples collected then inoculated into embryonated chicken eggs and tested using HA and HI test.

Table 2. Positive Result of HA and HI test.

Isolate		Organ	HA Titer	HI Titer
Bratang Bird Market	ND/BD1/2019	Brain	2^8	2^5
		Intestine	2^8	2^5
	ND/BD2/2019	Brain	2^{10}	2^6
		Proventrikulus	2^{10}	2^6
		Intestine	2^{10}	2^6
Gresik	ND/BG1/2019	Brain	2^{10}	2^6
		Proventrikulus	2^{10}	2^6
		Intestine	2^9	2^6
Lasota Vaccine	K^+	-	2^6	2^6

The HA and HI titer was tested positive if the titer was near 2^6. There were 3 samples that tested for Newcastle disease virus positife which is ND/BD1/2019, ND/BD2/2019, and ND/BG1/2019.

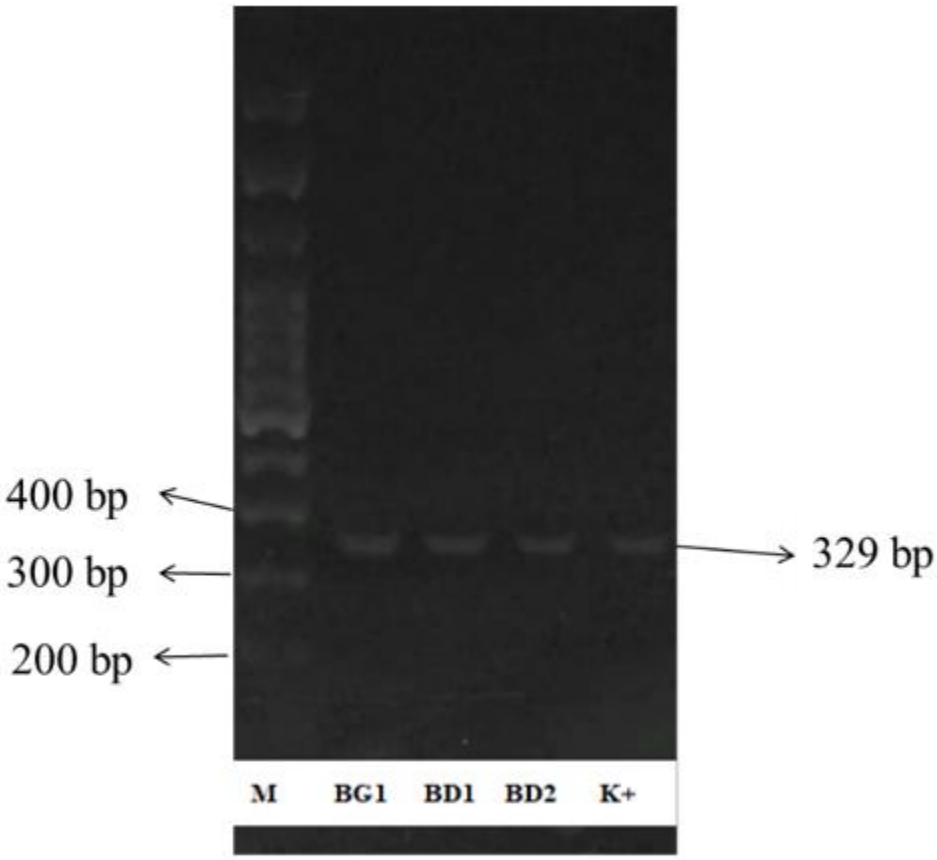

Figure 1. Result of PCR electrophoresis.

Electrophoresis of PCR Products with 1.5% Agarose. The number to the left of the image shows a DNA ladder marker value of 100 bp. M is a DNA ladder marker 100 bp, K+ is a positive control. BG1, BD1, BD2, and positive control (LaSota) shows a tape length of 329 bp.

The whole sequence of PCR products was trimmed, and the sequence was used for homology analysis of fusion protein which the number of products 329 bp. The sequence editing was done using Biological Alignment Editor (BioEdit) version 7.0.5.3. Both forward and reverse sequences are combined using BioEdit version 7.5.0.3. The reference of the sequence used to edit the samples is Newcastle Disease Virus LaSota.

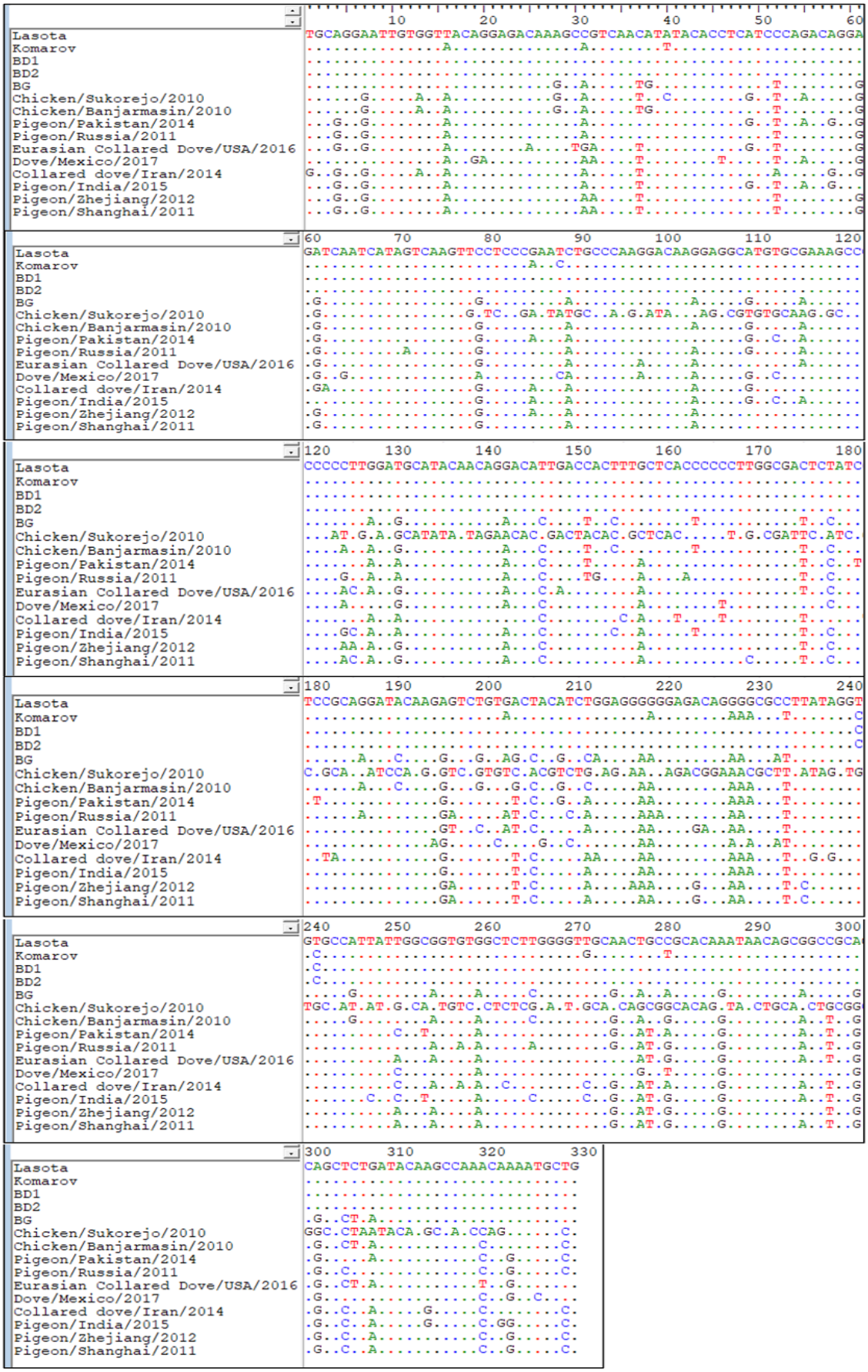

Figure 2. Nucleotide Sequence of Domestic Pigeon (Columbia Livia) Isolate and The Comparison

One amino acid is encoded by three nucleotides, so that from 329 bp of nucleotide length when translated, it will be about 110 bp of amino acids. The translational amino acid sequence will be aligned using the ClustalW software integrated with the Bioedit ver software. 8.

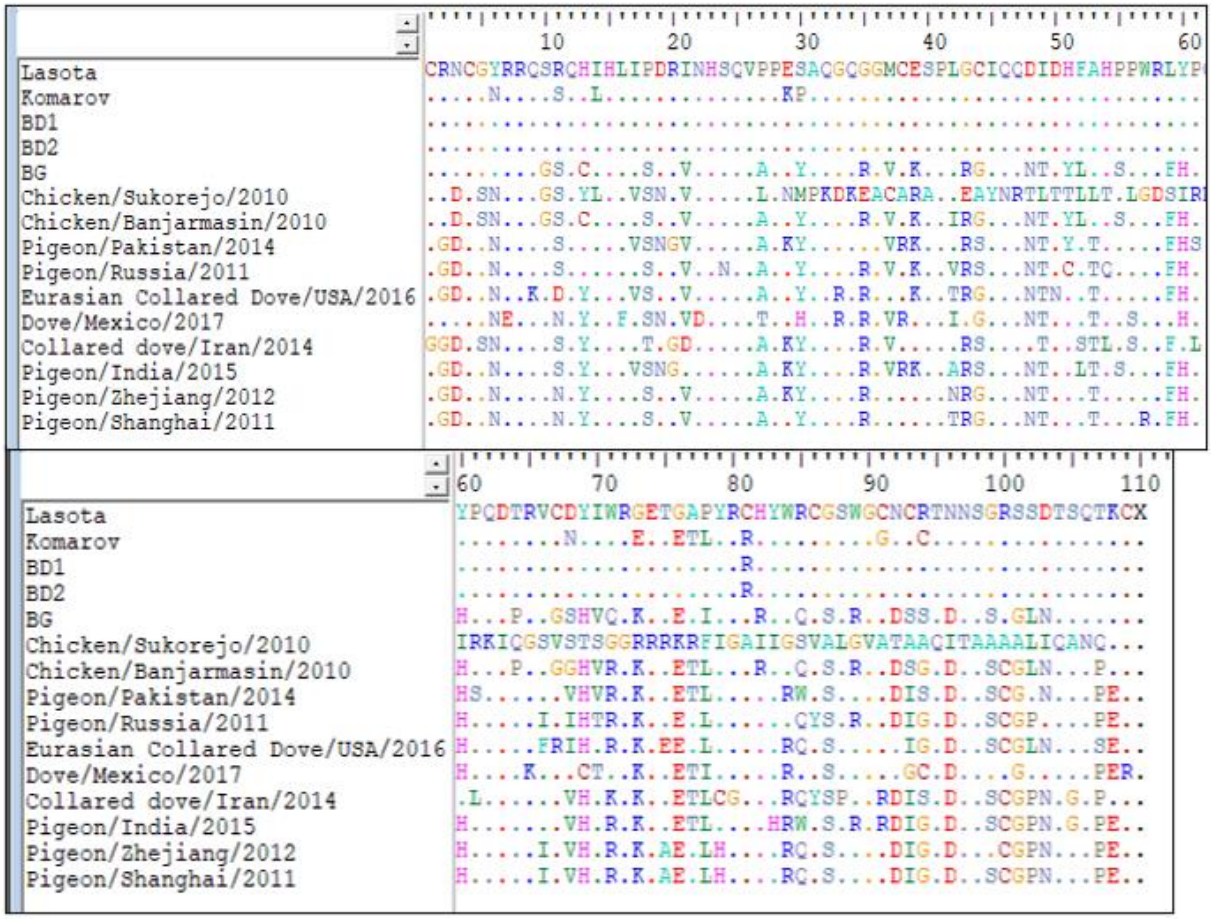

Figure 3. Amino Acid Sequence of Domestic Pigeon (Columbia Livia) Isolate and The Comparison.

The result above shown the amino acid sequence of the samples compared to sequences from GenBank and LaSota was used from the reference sequence. If the sequence below reference sequence symbolized by dot it means that the sequence below having same sequence with the reference sequence. If sequence below symbolized by alphabet means that the sequence having diferrence residues from the reference sequence. The translation results show that there are differences in samples of BD1 and BD2 with Refseq of 1 residue. BG1 sample shows a difference with Refseq of 40 residues.

The nucleotide sequence of protein F coding gene from Newcastle disease virus (ND) that has been obtained is then translated into an acid sequence amino via BioEdit software version 8.0. Prediction of immunogenic epitopes in genes protein F coding was performed using the online Epitope Prediction software Tools / IEDB with the Bepipred Linear Epitope Prediction.

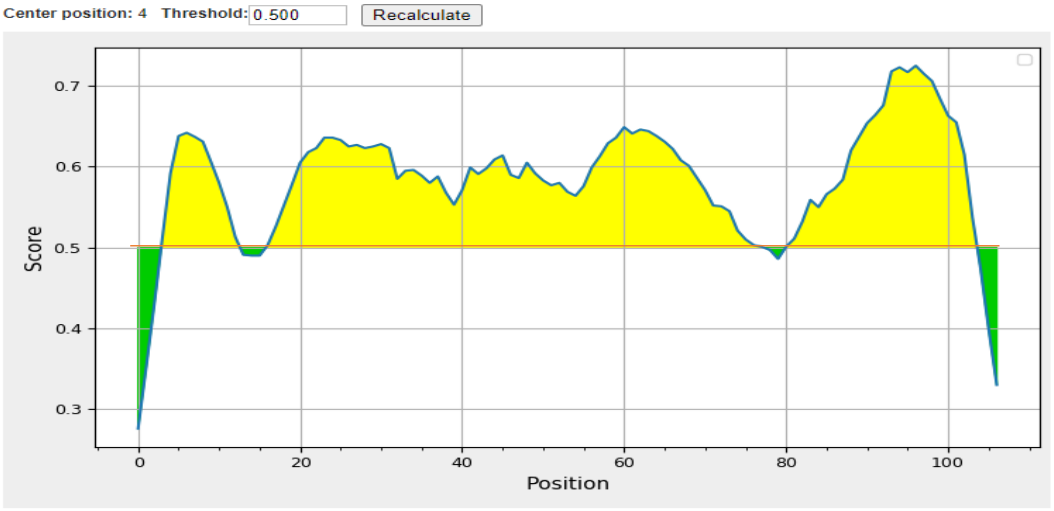

Figure 4. Prediction of B-cell epitopes from amino acid sequences of isolates ND / BG1 / 2019

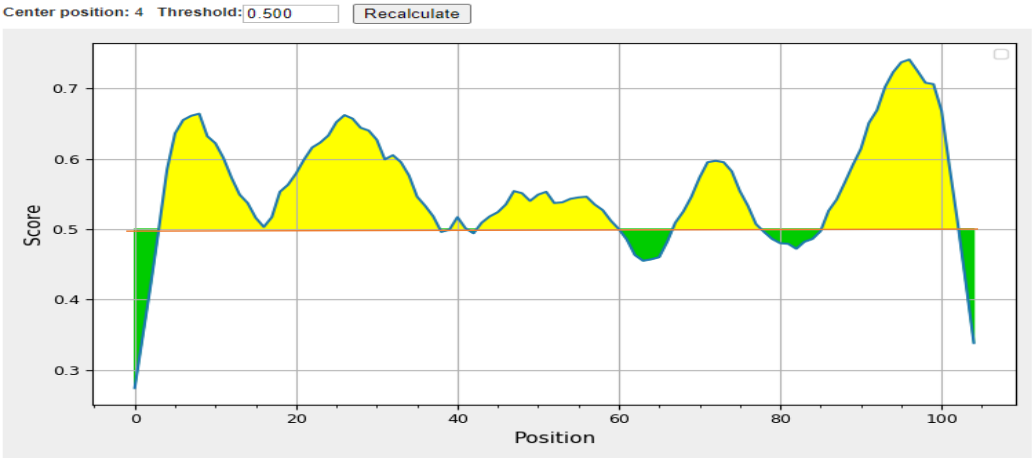

Figure 5. Prediction of B-cell epitopes from amino acid sequences of isolates ND / BD1 / 2019

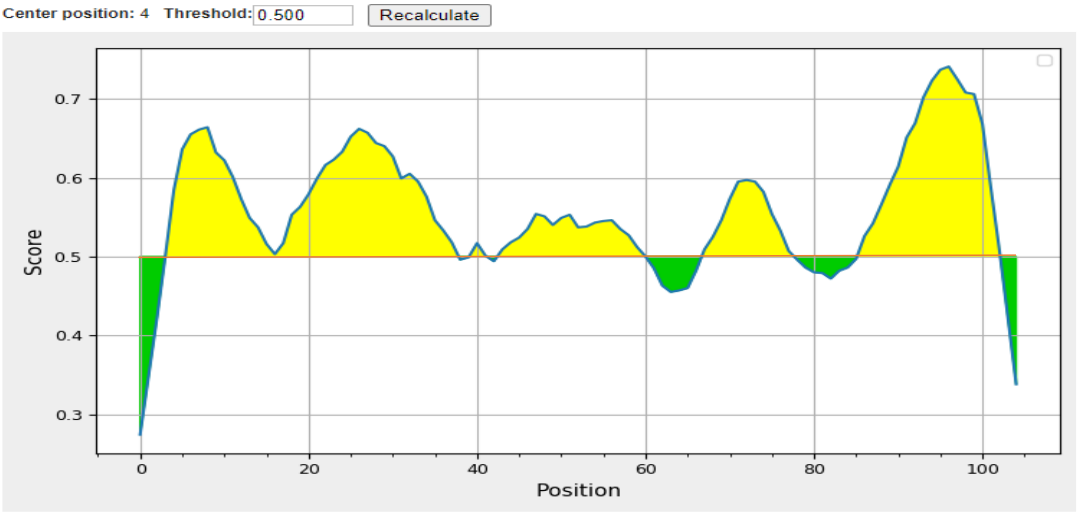

Figure 6. Prediction of B-cell epitopes from amino acid sequences of isolates ND / BD2 / 2019

Explanation: A yellow area above the threshold (red line) is proposed to be part of the epitope immunogenic against B cells. Area green is not proposed.

Table 3. Prediction of B-cell epitopes from amino acid sequences of ND / BG1 / 2019 isolates.

No	Start	End	Peptide	Length	Log score
1	4	13	CGYRRQGSQC	10	5.901
2	17	78	ISDRVNHSQVAPEYAGQRGVCKS PLRGIQQNTDYLAHSPWRFHPDP RVGSHVQRKETEAIYR	62	36.716
3	81	104	YWQCSSRGCDSSTDNSSRGLNTSQ	24	15.085
TOTAL			3		

Figure 4 and Table 3 show the predicted immunogenic epitopes against B cells from the amino acid sequence ND / BG1 / 2019. There are three epitopes of the acidic arrangement amino isolate ND / BG1 / 2019 based on the epitope's affinity to B cells.

Table 4. Prediction of B-cell epitopes from amino acid sequences of ND / BD1 / 2019 isolates.

No	Start	End	Peptide	Length	Log score
1	4	38	CGYRRQSRQHIHLIPDRINHSAPPE SAQGGGMCES	35	20.771
2	41	42	GC	2	1.018
3	44	60	QQDIDHFAHPPWLYPDT	17	9.116
4	68	78	RGETGAPYRRH	11	6.116
5	87	103	CNCRTNNSGRSSDTSQT	17	10.965
TOTAL			5		

Figure 5 and Table 4 show the predicted immunogenic epitopes against B cells from the amino acid sequence ND / BD1 / 2019. There are five epitopes of the acidic arrangement amino isolate ND / BD1 / 2019 based on the epitope's affinity to B cells.

Table 5. Prediction of B-cell epitopes from amino acid sequences of ND / BD2 / 2019 isolates.

No	Start	End	Peptide	Length	Log score
1	4	38	CGYRRQSRQHIHLIPDRINHSAPPE SAQGGGMCES	35	20.771
2	41	42	GC	2	1.018
3	44	60	QQDIDHFAHPPWLYPDT	17	9.116
4	68	78	RGETGAPYRRH	11	6.116
5	87	103	CNCRTNNSGRSSDTSQT	17	10.965
TOTAL			5		

Figure 6 and Table 5 show the predicted immunogenic epitopes against B cells from the amino acid sequence ND / BD2 / 2019. There are five epitopes of the acidic arrangement amino isolate ND / BD2 / 2019 based on the epitope's affinity to B cells.

4. Discussion

4.1.HA and HI test of The Samples

From the 20 samples obtained, three samples resulted positive for Newcastle disease infection based on hemagglutination (HA) and hemagglutination inhibition (HI) tests. The samples were Bratang isolates (ND / BD1 / 2019, ND / BD2 / 2019, and Gresik isolates (ND / BG1 / 2019) (Table 4.1). Of the organs that have been removed, not all showed positive HA test results. This can be caused by the absence of an antigen (hemagglutinin) in these organs. CFSPH (2016) stated that Newcastle Disease (ND) virus has hemagglutinin, which can bind to poultry erythrocytes. Bratang isolates (ND / BD1 / 2019) that were positive for the HA-HI test included brain and intestine organs. Bratang (ND / BD2 / 2019), and Gresik (ND / BG1 / 2019) isolate that tested positive for HA-HI included brain, proventriculus, and intestine organs. The results obtained showed varied HA-HI titers, as shown in Table 4.1. The minimum titer for the HA test, which shows Newcastle Disease (ND) virus infection is 22 and the HI test is ≥ 22.

4.2.RNA extraction, One-Step RT-PCR, and Electrophoresis

Some There was some step for RNA extraction. The material used was trizol to lyse the cells, chloroform to separate the liquid phase and organic phase, ethanol 70% as washing solution, and Nuclease Free Water (NFW) as DNA solvent.

After the RNA extraction, samples were amplified using the One-Step RT-PCR method. The samples used for this method were ND/ BD1/ 2019, ND/ BD2/ 2019, ND/ BG1/ 2019, and positive control (LaSota).

PCR electrophoresis result from the ND virus using specified primer on the F gene region shown good result confirmed with DNA fragment detected at 329 bp long. This gene region with 329 bp long nucleotide translated to amino acids then analyzed with B cell epitope prediction.

4.3.Amino Acid Analysis

The sequencing result of nucleotides was then translated into amino acids. one amino acid is encoded by three nucleotides, so a 329 bp long nucleotide, when translated, will be a 110 bp long amino acid. The amino acids are then aligned using the ClustalW software, which is integrated with the BioEdit software. The results of multiple alignments of amino acids in samples BD1, BD2, and BG1 using the ClustalW software showed a difference with the samples obtained from Genbank.

Samples BD1 and BD2, the amino acid on order 81 is R (Arginine) in Refseq is C (Cysteine). For the amino acids in the BG1 sample it has many differences with Refseq, namely on the order 10 BG1 is G (Glycine) and Refseq is S (Serine), the sequence 11 BG1 is S (Serine) and Refseq is R (Arginine), sequence 13 BG1 is C (Cysteine) and Refseq is H (Histidine), sequence 18 BG1 is S (Serine) and Refseq is P (Proline), sequence 21 BG1 is P (Proline) and Refseq is I (Isoleucine), sequence 27 BG1 is A (Alanine) and Refseq is P (Proline), sequence 30 BG1 is Y (Tyrosine) and Refseq is S (Serine), order 35 BG1 is R (Arginine) and Refseq is G (Glycine), order 37 BG1 is V (Valine) and Refseq is M (Methionine), sequence 39 BG1 is K (Lysine) and Refseq is E (Glutamic acid), sequence 43 BG1 is R (Arginine) and Refseq is G (Glycine), sequence 44 BG1 is G (Glycine) and Refseq is C (Cysteine), sequence 48 BG1 is N

(Asparagine) and Refseq is D (Aspartic acid), sequence 49 BG1 is T (Threonine) and Refseq is I (Isoleucine), sequence 51 BG1 is Y (Tyrosine) and Refseq is H (Histidine), order 52 BG1 is L (Leucine) and Refseq is F (Phenylalanine), sequence 55 BG1 is S (Serine) and Refseq is P (Proline), order 59 BG1 is F (Phenylalanine) and Refseq is L (Leucine), order 60 BG1 is H (Histidine) and Refseq is Y (Tyrosine), sequence 64 BG1 is P (Proline) and Refseq is T (Threonine), sequence 67 BG1 is G (Glycine) and Refseq is C (Cysteine), order 68 BG1 is S (Serine) and Refseq is D (Aspartic acid), sequence 69 BG1 is H (Histidine) and Refseq is Y (Tyrosine), sequence 70 BG1 is V (Valine) and Refseq is I (Isoleucine), sequence 71 BG1 is C (Cysteine) and Refseq is K (Lysine), sequence 73 BG1 is K (Lysine) and Refseq is G (Glycine), order 76 BG1 is E (Glutamic acid) and Refseq is G (Glycine), sequence 78 BG1 is I (Isoleucine) and Refseq is P (Proline), sequence 82 BG1 is R (Arginine) and Refseq is H (Histidine), sequence 85 BG1 is C (Cysteine) and Refseq is R (Arginine), the order of 87 BG1 is S (Serine) and Refseq is G (Glycine), sequence 89 BG1 is R (Arginine) and Refseq is W (Tryptophan), sequence 92 BG1 is D (Aspartic acid) and Refseq is N (Asparagine), sequence 93 BG1 is S (Serine) and Refseq is C (Cysteine), sequence 94 BG1 is S (Serine) and Refseq is R (Arginine), sequence 96 BG1 is D (Aspartic acid) and Refseq is N (Asparagine), sequence 99 BG1 is S (Serine) and Refseq is G (Glycine), sequence 101 BG1 is G (Glycine) and Refseq is S (Serine), sequence 102 BG1 is L (Leucine) and Refseq is D (Aspartic acid), sequence 103 BG1 is N (Asparagine) and Refseq is T (Threonine).

4.4.Prediction of Immunogenic Epitopes Against B-Cells in Amino Acid Protein F Newcastle Disease

Immunogenic epitopes against B-cells can be categorized into two types. They are linear (continuous) and conformational (discontinuous) epitopes. Linear epitopes include continuous side chains in a sequence, whereas a conformational epitope consists of separated, but side chains have adjacent places (Zhang et al., 2011). Based on these B cells, immunogenic epitopes become stimulants that increase the response humoral immune system for antibody production. B cell receptors will then recognize epitopes to these B cells.

The prediction results show that there were five identical immunogenic epitopes between ND / BD1 / 2019 isolates and ND / BD2 / 2019 isolates, which is: CGYRRQSRQHIHLIPDRINHSAPPESAQGGGMCES, GC, QQDIDHFAHPP WLYPDT, RGETGAPYRRH, and CNCRTNNSGRSSDTSQT.

ND / BD1 / 2019 and ND / BD2 / 2019 isolates have a number more immunogenic epitopes from all isolates, with five immunogenic epitope candidates, as shown in Table 4 and 5 CGYRRQSRQHIHLIPDRINHSAPPESAQGGGMCES, GC, QQDIDHFAHPPWLYPDT, RGETGAPYRRH, and CNCRTNNSG RSSDTSQT. Which is also identical. It shown that ND / BD1 / 2019 and ND / BD2 / 2019 isolates are homolog.

Based on the log score figures in Table 3 immunogenic epitopes ISDRVNHSQVAPEYAGQRGVCKSPLRGIQQNTDYLAHSPWRFHPDPRVGSHVQRKE TEAIYR isolate ND / BG1 / 2019 has the highest log score height, which is 36.716.

Based on the log score figures in Table 4 immunogenic epitopes CGYRRQSRQHIHLIPDRINHSAPPESAQGGGMCES isolate ND / BD1 / 2019 has the highest log score height, which is 20.771.

Based on the log score figures in Table 5 immunogenic epitopes CGYRRQSRQHIHLIPDRINHSAPPESAQGGGMCES isolate ND / BD2 / 2019 has the highest log score height, which is 20.771.

The log score figures indicate that the result epitope prediction with a high log score has good immunogenic properties.

Besides finding out the epitope in the responsible protein in triggering the body's immune response, identification, and analysis of the epitopes with highly immunogenic properties can be used for vaccine development epitope-based. Epitope-based vaccines offer various advantages such as high purity, large vaccine production capacity, and production efficiency (Topuzoğullari et al., 2020). Epitope-based vaccines with Conserve epitopes can also be designed to induce a response immunity so that it can become a universal vaccine (Sette & Fikes, 2003).

5. Conclusion

The amino acid residues of samples BD1, BD2, and BG1 showed differences from the amino acid residues obtained from Genbank.

Epitope ISDRVNHSQVAPEYAGQRGVCKSPLRGIQQNTDYLAHSPWRF HPDPRVGSHVQRKETEAIYRHGKILDPGWRPPGARWAPDLLP from sample ND / BG1 / 2019 more likely to be candidate for immunogenic epitopes based on predictions epitope B cell, which has the highest log score of 36.716.

6. Suggestion

Based on the research results, the suggestions are necessary to conduct further research to obtain complete gene protein sequences F to know a complete epitope prediction of viral F protein Newcastle disease (ND) pigeon isolate (*Columbia livia*) as a vaccine candidate.

7. Acknowledgement

Praise the presence of Allah SWT for the gift of favor, abundance of love, and strength in each step of the author, so the author can carry out this research and completed the thesis with the title " Epitope Prediction of B Cell Gene Encoding F Protein of Newcastle Disease (ND) Virus from Pigeon Isolate (*Columbia livia*). On this occasion, the author would like to express his gratitude to author supervisor Prof. Dr. Fedik Abdul Rantam, drh. And co-supervisor Dr. Eka Pramyrtha Hestianah, drh., M.Kes.

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Magelang, January 03th 2021

Author

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