Introducing B Cell Epitopes of Newcastle Disease Virus Obtained from Domestic Pigeons (Columba livia domestica) as Sub-Unit Vaccine Candidate to Eradicate Newcastle Disease Virusin Poultry

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Submission date: 08-Jun-2022 12:43PM (UTC+0800)
Submission ID: 1852730348
File name: Introducing_B_Cell_Epitopes_of_Newcastle.pdf (302.17K)
Word count: 3748
Character count: 21420

Introducing B Cell Epitopes of Newcastle Disease Virus Obtained from Domestic Pigeons (*Columba livia domestica)* as Sub-Unit Vaccine Candidate to Eradicate Newcastle Disease Virus in Poultry

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ABSTRACT:

Poultry farm is important commodity in Indonesia. It provides protein source as Indonesian consume varies kind of its product such as meats (chicken, duck and quail) and eggs. In Indonesia, rearing activities were differentiated into three types such as extensive traditional system, semi-intensive system, and intensive system. All these systems have same problem relate to outbreak of viral disease. One of viral disease causes annual outbreak is Newcastle Disease. It is caused by infection of Avian Paramyxovirus serotype 1. It infects varies avian species such as pigeons, ostrich, water fowl, chicken, and cockatoo. Control such as vaccination has been conducted but it could not protect the poultry from Newcastle Disease Virus (NDV) infection. It is noted that the protectivity of seed vaccine is influenced by the epitopes generates various protectivity level of the vaccination program. Sub-unit vaccine could become the best choice to protect NDV infection. Molecular analyses were conducted to obtain B cell epitopes which could induce immune system safely. Sample of pigeons (*Columba livia*) were collected from live bird market in Surabaya. The collected sample showed clinical signs such as respiratory

disturbance, limping, loss of appetite and subclinical enteric disturbance/diarrhea. Two out of four samples were serologically confirmed to be infected with NDV (Pigeon/Surabaya/2019/01 and Pigeon/Surabaya/2019/03). Molecular approach was conducted to obtain the nucleotide sequence of the samples. The sequence was employed to epitope analyses by using Kolaskar-Tongaonkar antigenicity and Emini surface accessibility softwares. Obtained epitopes were analyzed using Vaxijen, Allertop, and ToxinPred to confirm that the epitopes are safely to be applied. Peptides were obtained from NDV infecting pigeons were noted has possibility to become seed vaccine candidate. Several peptides were obtained from Pigeon/Surabaya/2019/01 and Pigeon/Surabaya/2019/03; SWVYIHLLSTF, CTNVCLSEIQLLHSFA, VRPCMVIVRL, NLTGRKRRTVG and SDREYSQAIAR passed the in-silico screenings. These epitopes are possibly to be used as sub-unit vaccine to eradicate Newcastle Disease Virus.

KEYWORDS: Subunit vaccine, NDV, Pigeon, Peptides.

INTRODUCTION:

Poultry industry is important sector in Indonesia as it serves 65% of protein supply and provides job vacancy for 12 million people. This sector is made up of six sub-sectors (feed, breeding, broiler and layer farms, slaughterhouse and processing) with estimated worth more than 34 billion USD. Indonesian primarily consume broiler and layer chickens but duck and quails form the whole poultry product consumption rate too. In Indonesia, rearing activities are differentiated into three types such as extensive traditional system, semi-intensive system, and intensive system[1,2]. They are classified according to the amount of the company capital. All these farming systems have similar problem relate to outbreak of viral disease. Common viral disease emerge in poultry (both broiler and layer) need to be concerned are caused by Toravirus, Parvovirus, Entero-like virus, Adenovirus, Calici virus, Avian Influenza virus, Newcastle Disease virus, Avian Reovirus, Avian Enchepalomyelitis, and Marek's disease virus[3]. Moreover, World Organization for Animal Health (OIE) has published some viral disease circulate in poultry such as Avian infectious bronchitis[2], Duck virus hepatitis, Gumboro disease and Turkey rhinotracheitis[4]. These viral diseases cause annual lost due to the decrease of production generates stunting and mortality[3,5,6,7]. This condition become the problem for companies to expand the business abroad, because it does not meet standard for biosecurity for poultry production.

Newcastle Disease Virus (NDV) or Avian Paramyxovirus serotype-1 (APMV 1) is the causative agent for Newcastle Disease[8]. It is a contagious disease causes annual outbreak in poultry industry which has six structural proteins and a single stranded negative sense RNA genome[9,10]. NDV's structural protein consists of Nucleoprotein (N), Phospoprotein (P), Matrix protein, Fusion protein (F), Haemagglutinin-Neuraminidase protein (HN), and Polymerase protein (L)[11]. Among these proteins, Fusion protein has the important role in molecular-genotyping of NDV as the virulence virus confirmed by the existence of multiple-basic amino acid sequence located on cleavage site of Fusion protein[12]. Fusion protein is type I integral protein located on the surface of the virion which leads to specific antibody synthesis (immunogenic)[12,13]. Since Avian Paramyxoviruses differ in many serotypes, particularly, APMV 1 has antigenic variation which commonly isolated from pigeon so-called Pigeon Paramyxovirus serotype 1 (PPMV1)[14].

NDV firstly reported in Indonesia on 1927 by Kraneveld[14]. Since then, it becomes major problem in poultry industry even though annual vaccination as prevention has been conducted[15]. Many researches had been conducted to analyze the possibility of seed vaccine candidate isolated from the field NDVs[10,16]. Up till now, conventional seed vaccines were isolated from chicken (i.e.; La Sota, Komarov, Hitchner). In Indonesia, research related to NDV obtained from pigeons has never been conducted before, because NDV outbreak, all of them, occurred in poultry farm. This research is a pilot project to analyze the possibility of NDV obtained from pigeons as sub-unit vaccine candidate using molecular in-silico methods.

MATERIAL AND METHODS:

Ethical approve:

This research has been approved by the ethics commission of experimental animals of Faculty of Veterinary Medicine, Airlangga University.

Sample isolation and identification:

Samples were collected from domestic pigeons (*Columba livia domestica)* suspected to be infected with low virulent NDV and showed several symptoms such as respiratory disturbance, limping, loss of appetite and subclinical enteric disturbance/diarrhea sold in live bird market in Surabaya. The samples were euthanized through cervical dislocation method. Organs from each sample, such brain, caeca tonsil, proventriculus, intestine, lien, lungs, and liver were obtained. Each of organs were homogenized then inoculated into 9-11 old days Specific Antigen Free (SAN) embryonated eggs. They were incubated in 37°C cabinet for 5 days. Observation was conducted once in 24 hours post infection (hpi) and the mortality of embryonated eggs were recorded. Death embryo were stored in -97°C. After incubation period, all living embryonated eggs were stored in -97°C then on the following day, all inoculated embryonated eggs were processed into Hemagglutination assay (HA assay). Before HA assay was conducted, the allantoic fluid of each embryonated eggs was obtained.

HA assay was employed to detect the presence of Hemagglutinin protein in the samples. Positive control used was NDV vaccine containing La Sota. Then positive HA assay samples were processed into Hemagglutination Inhibition assay (HI assay) using Newcastle Disease specific serum (titer of 2^6). Samples showed positive in both assays were processed into molecular identification.

Molecular identification:

The allantoic fluid of positive samples were extracted according to RNeasy Mini Kit (QIAGEN, Cat No./ID: 74104). Obtained RNA then employed to Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) amplified partial gene of fusion protein (768 bp) (Table 1). The RT-PCR was set up as Lid on 95°C; pre-denaturation 94°C for 10 minutes; denaturation 94°C for a minute; annealing 56°C for a minute; extension 72°C for 2 minutes; prolong extension 72°C for 10 minutes. The cycle was repeated for 40 times. In the end of amplification, the temperature was remained as 4°C. The amplicon was visualized on 1% agarose gel electrophoresis. The electrophoresis machine was set as 100V for 1 minutes and used marker 100 bp cDNA ladder (Invitrogen). Samples indicated the present of band were processed into sequencing according to QIAquick® PCR Purification Kit (Qiagen, 28104). After purification, the amplicon was labelled according to BigDye® Terminator Sequencing RR-100. After labelling the amplicon, the sequence the nucleotide was read by ABI 310 xL GENETIC ANALYZER (Applied Biosystem). The obtained sequence was analyzed using Biological Sequence Alignment Editor (BioEdit) ver. 7.2.5.

Table 1. Sequence of primer amplify partial gene of fusion protein of Newcastle disease virus.

Sequence 5'-3'		Product (bp)
Forward	TTC CTG CTT GAG CGG CAA TA	768
Reverse	AAG CGG TAG AAC GGA GGT TG	

B cell epitopes prediction:

The sequence were processed into B cell epitope prediction according to Kolaskar-Tongaonkar antigenicity and Emini surface accessibility[17,18,19]. Selected epitopes were processed into further immunogenicity analyses using Vaxijen[16]. The immunogenic peptides sequences were processed into Allertop and ToxinPred to pick the one which does not cause allergic and intoxication toward the poultry[20,21].

RESULT:

Sample collection and isolation:

Four pigeons showed subclinical signs of NDV infection was collected from Surabaya. They were euthanized using cervical dislocation method then employed to virus isolation. To confirm the present of NDV, the samples were processed to HA assay. It was conducted to detect the present of Hemagglutinin protein[22]. Positive HA assay was processed to HI assay to confirm that isolated samples was NDV (Table 2). Two out of four samples were serologically confirmed as NDV. After that, molecular identification was conducted to affirmed that isolated samples were NDV.

Molecular identification:

Before processing the samples into RT-PCR, their RNA was extracted then processed to RT-PCR. The amplicons were visualized through agarose gel electrophoresis (Figure 1). The sequences of the samples were analyzed using Biological Sequence Alignment Editor (BioEdit) ver. 7.2.5.

B cell epitope prediction:

Amino acids from each samples were analyzed using Kolaskar-Tongaonkar antigenicity and Emini surface accessibility to obtain the B cell epitopes[18,19]. The epitopes were processed into further immunogenicity analysis using Vaxijen, then Allertop and ToxinPred to pick the peptides sequence which does not cause allergy and intoxication reaction respectively[16,20,21].

DISCUSSION:

Newcastle disease virus infects many birds such as chickens, cockatoo, water fowl species, and pigeons[23,24,25]. Among these host systems, the record showed that NDV causes panzootic in poultry (Miller et al., 2015) and pigeons[7,14,26]. According to the HA and HI assays, two samples were confirmed as Newcastle Disease Virus; Pigeon/Surabaya/2019/01 and Pigeon/Surabaya/2019/03. The possibility of cross reaction is existed among NDV from different genotypes, which means HI assays will generate positive result even though the specific antiserum origin belongs to different genotype compared to isolated NDV. This founding revealed the possibility to design vaccine from various genotype to protect poultry from other NDV outbreak. The positive samples were processed into RNA extraction then processed into Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR). According to visualization, they were molecularly confirmed to be NDV (Figure 1). Further analyses were conducted to reveal peptides which has potency to become subunit vaccine candidate, because it is very important to preserve vaccine which is not only capable to trigger specific immune response, but also safe, and not causing allergy and poisoning. According to the analyses, both samples had possibility to become subunit vaccine candidate. Peptides were possible to cause no harmful for the poultry are Swvyihllstf, Ctnvclseiqllhsfa, and Vrpcmvivrl obtained from Pigeon/Surabaya/2019/01 (Table 3). Whilst Pigeon/Surabaya/2019/03 generated two peptides which possible to become subunit vaccine candidate; NLTGRKRRTVG and SDREYSQAIAR (Table 4).

Table 2. Result of NDV isolation and identification.

Sample	Organ	HA titer	HI titer
Pigeon/Surabaya/2019/01	brain	2^8	+
	caeca tonsil	-	-

	proventriculus	-	-
	intestine	2^8	+
	lien	-	-
	lungs	-	-
	liver	-	-
Pigeon/Surabaya/2019/02	brain	-	-
	caeca tonsil	-	-
	proventriculus	-	-
	intestine	-	-
	lien	-	-
	lungs	-	-
	liver	-	-
Pigeon/Surabaya/2019/03	brain	2^{10}	+
	caeca tonsil	-	-
	proventriculus	2^{10}	+
	intestine	2^{10}	+
	lien	-	-
	lungs	-	-
	liver	-	-
Pigeon/Surabaya/2019/04	brain	-	-
	caeca tonsil	-	-
	proventriculus	-	-
	Intestine	-	-
	lien	-	-
	lungs	-	-
	liver	-	-
Control (vaccine)		2^6	+

Figure 1. Agarose gel electrophoresis confirmed that isolated samples were NDV. M: Marker; A: positive control; B: Pigeon/Surabaya/2019/01; C: Pigeon/Surabaya/2019/03.

Figure 2. Graphic of B cell epitopes prediction using Kolaskar-Tongaonkar antigenicity obtained from Pigeon/Surabaya/2019/01.

Figure 3. Graphic of B cell epitopes prediction using Kolaskar-Tongaonkar antigenicity obtained from Pigeon/Surabaya/2019/03.

Figure 4. Graphic of B cell epitopes prediction using Emini surface accessibility obtained from Pigeon/Surabaya/2019/01.

Table 3. B cell epitopes which could be used as subunit vaccine candidate (marked as green highlight) obtained from Pigeon/Surabaya/2019/01.

S. No.	Peptide	Length	Vaxijen	Allertop	ToxinPred
1	FSRLYPAAFSVF	12	Probable non-antigen		
2	SWVYIHLLSTF	11	Probable antigen	Probable non allergen	Non-toxin
3	RSFVVPSA	8	Probable non-antigen		
4	WLVHLCLACCCFLPTCPRHCLNCQYQAQL	29	Probable antigen	Probable non allergen	Toxin
5	NLVLRCSLD	9	Probable non-antigen		
6	CTNVCLSEIQLLHSFA	16	Probable antigen	Probable non allergen	Non toxin
7	VRPCMVIVRL	10	Probable antigen	Probable non allergen	Non toxin

Table 4. B cell epitopes which could be used as subunit vaccine candidate (marked as green highlight) obtained from Pigeon/Surabaya/2019/03.

S. No.	Peptide	Length	Vaxijen	Allertop	ToxinPred
1	CEAWLPHHAGGSSDRSTRASLANLYP	26	Probable antigen	Probable allergen	
2	NLTGRKRRTVG	11	Probable antigen	Probable non-allergen	Non-toxin
3	SDREYSQAIAR	11	Probable antigen	Probable non-allergen	Non toxin
4	V	1	Can not be predicted		
5	SACTSGNMLNPEVHRRQEKQLERYRE	26	Probable non-antigen		
6	CPRHCLNCQYQAQL	14	Probable antigen	Probable non-allergen	Probable toxin
7	DKLKQIPHASE	11	Probable non-antigen		
8	T	1	Can not be predicted		

Table 5. B cell epitopes obtained from Pigeon/Surabaya/2019/01 could not pass the screening.

S. No.	Peptide	Length	Vaxijen	Allertop	ToxinPred
1	SSDRST	6	Probable non-antigen		
2	TGRKRRT	7	Probable antigen	Probable non-allergen	The peptides were too short
3	SSDREYS	7	Probable antigen	Probable allergen	
4	EVHRRQEKQ LERYRERM	17	Probable non-antigen		

Table 6. B cell epitopes obtained from Pigeon/Surabaya/2019/03 could not pass the screening.

No.	Peptide	Length	Vaxijen	Allertop	ToxinPred
1	SSDRST	6	Probable non-antigen		
2	TGRKRRT	7	Probable antigen	Probable non allergen	The peptides were too short
3	SSDREYS	7	Probable antigen	Probable allergen	
4	EVHRRQEKQLERYRERM	17	Probable non-antigen		

Figure 5. Graphic of B cell epitopes prediction using Emini surface accessibility obtained from Pigeon/Surabaya/2019/03.

The positive samples were processed into RNA extraction then processed into Reverse-Transcriptase Polymerase Chain

Reaction (RT-PCR). According to visualization, they were molecularly confirmed to be NDV (Figure 1). Further analyses were conducted to reveal peptides which has potency to become subunit vaccine candidate, because it is very important to preserve vaccine which is not only capable to trigger specific immune response, but also safe, and not causing allergy and poisoning. According to the analyses, both samples had possibility to become subunit vaccine candidate. Peptides were possible to cause no harmful for the poultry are SWVYIHLLSTF, CTNVCLSEIQLLHSFA, and VRPCMVIVRL obtained from Pigeon/Surabaya/2019/01 (Table 3). Whilst Pigeon/Surabaya/2019/03 generated two peptides which possible to become subunit vaccine candidate; NLTGRKRRTVG and SDREYSQAIAR (Table 4). Newcastle Disease Virus-infecting pigeon has been discovered since 1981 (Pigeon Paramyxovirus serotype 1/PPMV1). It is firstly reported in Italy infecting racing pigeons[27]. Then it spread to Great Britain, Eurasia, Bangladesh, and China[26,28,29,30]. In Indonesia, NDV-infecting pigeon is not the main concern because most NDV infection relate to outbreak in poultry farm. Primarily, the main symptom is associated with neurological disturbance. In some cases the main clinical signs are drowsiness and enteric disturbance[26]. Newcastle Disease Virus-infecting pigeon commonly belongs to genotype VI of Avian Paramyxovirus 1 (APMV1). These genotypes were suggested to be appeared as the result of multiple passages between chicken and pigeon[27].

Vaccination is the prevention method to protect animals or human from viral infection[31]. Part of antigen which role in specific antibody recognition is called epitopes[32]. It attaches to part of antibody (paratope) which leads to antibody-antigen neutralization[33]. B cell epitopes prediction has purpose to predict peptides originate from the antigen which trigger the host-humoral immune response (specific antibody)[32]. These epitopes can be administrated to the host, hence it is called as subunit vaccine. Subunit vaccine consist of small-purified immunogenic peptides which originate from particular part or product of antigen, such as toxin, fragment or surface molecule. Subunit vaccine works differ according to the type of the particles. Protein antigens usually works on T cell dependent adaptive immunity while polysaccharide antigen works on T cell independent response[34]. Subunit vaccines can induce specific immune response but less warring than whole-antigen vaccine[35]. The use of vaccination to prevent Newcastle Disease Virus Infection is very important since the use of antiviral drugs never been administered in poultry to treat this disease. In human medicine, some antiviral drugs can be combined to prevent secondary effect of particular virus[36]. The arrangement of subunit vaccine, as the way to eradicate NDV, must consider the safety of the poultry. Hence, even though the epitopes are obtained from the particular region of the whole genome, it need to be examined to prevent allergic and toxicity. Further analyses (both in-vivo and in-vitro experiments) toward these peptides must be conducted to know how it works in triggering immunity response in poultry.

ACKNOWLEDGMENTS:

This research was funded by Airlangga University through Credential Grant Program. The authors would express the gratitude toward Faculty of Veterinary Medicine for providing all necessities during the research.

CONFLICT OF INTEREST:

The authors stated that there is no conflict of interest during the research and writing process.

REFERENCE:

1. Hidayat C. Asmarasari SA. Native chicken production in Indonesia: A Review. Indonesian Journal of Animal Science 2015; 17(1):1–11.
2. Ray A. Pradhan RK. Poultry farming-an overview. Research Journal of Science and Technology 2011; 3(3):129–136.
3. Hartawan R. Luh N. Indi P. Runting-stunting syndrome in Sukabumi and Tangerang. Indonesian Journal of Veterinary Sciences 2017; 11(2):65–69.
4. OIE. Diseases, infections and infestations listed by the OIE. Terrestrial Animal Health Code 2018; 1–4.
5. Cook JKA. Jackwood M. Jones RC. The long view: 40 years of infectious bronchitis research. Avian Pathology 2012; 41(3):239–250. https://doi.org/10.1080/03079457.2012.680432
6. Daniels P. Wiyono A. Sawitri E. Poermadjaja B. Sims LD. H5N1 highly pathogenic avian influenza in Indonesia: Retrospective considerations. Current Topics in Microbiology and Immunology 2013; 365:171–184. https://doi.org/10.1007/82_2012_265
7. Miller PJ. Haddas R. Simanov L. Lublin A. Rehmani SF. Wajid A. Bibi T et al. (2015). Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features. Infection, genetics and evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in infecTious Diseases 2015; 29:216–229. https://doi.org/10.1016/j.meegid.2014.10.032
8. Krishnamurthy S. Samal SK. Nucleotide sequences of the trailer, nucleocapsid protein gene and intergenic regions of Newcastle disease virus strain Beaudette C and completion of the entire genome sequence. The Journal of General Virology 1998; 79(Pt 10): 2419–2424.
9. Hussein MA. Khammas EJ. Isolation and identification of avian Newcastle disease in poultry from Karbala City, Iraq. Research Journal of Pharmacy and Technology 2019; 12(5):2229. https://doi.org/10.5958/0974-360x.2019.00371.8
10. Putri N. Ernawati R. Suwarno. Rahmahani J. Rantam F. Epitope prediction from genes encoding F protein of Newcastle disease virus (NDV) isolates swan (*Cygnus olor*) for vaccine development to prevent infectious disease. Advances in Health Sciences Research 2020; 19:94–97. https://doi.org/10.2991/isessah-19.2019.26
11. Dortmans JCFM. Rottier PJM. Koch G. Peeters BPH. The viral replication complex is associated with the virulence of Newcastle disease virus. Journal of Virology 2010; 84(19):10113–10120. https://doi.org/10.1128/JVI.00097-10
12. Kim S. Wanasen N. Paldurai A. Xiao S. Collins PL. Samal SK. Newcastle disease virus fusion protein is the major contributor to protective immunity of genotype-matched vaccine. PloS one 2013; 8(8):1–10. https://doi.org/10.1371/journal.pone.0074022
13. Smith EC. Popa A. Chang A. Masante C. Dutch RE. Viral entry mechanisms: The increasing diversity of paramyxovirus entry. The FEBS journal 2009; 276(24):7217–7227. https://doi.org/10.1111/j.1742-4658.2009.07401.x
14. Gogoi P. Ganar K. Kumar S. Avian paramyxovirus: A brief review. Transboundary and emerging diseases 2017; 64(1):53–67. https://doi.org/10.1111/tbed.12355
15. Xiao S. Paldurai A. Nayak B. Samuel A. Bharoto EE. Prajitno TY. Collins PL et al. Complete genome sequences of Newcastle disease virus strains circulating in chicken populations of Indonesia. Journal of Virology 2012; 86(10):5969–5970. https://doi.org/10.1128/JVI.00546-12
16. Ansori ANM. Kharisma VD. Characterization of Newcastle disease virus in Southeast Asia and East Asia: Fusion protein gene. EKSAKTA: Journal Ilmu-Ilmu MIPA 2020; 20(1):14–20. https://doi.org/10.20885/eksakta.vol1.iss1.art3
17. Putri DD. Handharyani E. Soejoedono RD. Setiyono A. Mayasari NLPI. Poetri ON. Pathotypic characterization of Newcastle disease virus isolated from vaccinated chicken in West Java, Indonesia. Veterinary World 2017; 10(4):438–444. https://doi.org/10.14202/vetworld.2017.438-444
18. Emini EA. Hughes JV. Perlow DS. Boger J. (1985). Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. Journal of Virology 1985; 55(3):836–839. https://doi.org/10.1128/jvi.55.3.836-839.1985

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