Karakterisasi Gen Pengkode Fusion Protein Virus *Newcastle Disease* pada Ayam Kampung di Surabaya

Characterization of Gene Coding Fusion Protein of Newcastle Disease Virus Infected in Native Chicken in Surabaya

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Abstrak

Penelitian ini bertujuan untuk mengetahui homologi nukleotida, persentase homologi, dan hubungan filogenetik gen pengkode Fusion protein Virus *Newcastle diesease* pada ayam kampung (*Gallus gallus domesticus*) di Surabaya menggunakan beberapa perbandingan isolat dari *GenBank*. Sampel penelitian diambil dari organ pencernaan ayam kampung yang dikumpulkan dari pasar tradisional Wonokromo, Surabaya. Sampel diuji menggunakan RT-PCR dengan *primer Forward* dan *Reverse* dengan target 976bp, sampel positif dilanjutkan dengan sekuensing kemudian analisis homologi dan analisis nukletida yang kemudian ditranslasi ke dalam asam amino. Hasil sampel homologi chc/SBY/2018 memiliki kesamaan 88% dengan referensi *sequens*, sedangkan *reference sequence* dengan vaksin lasota memiliki kesamaan 87%, dan hasil homologi tertinggi ditunjukkan oleh perbandingan dengan berbagai isolat dari indonesia 90-95%. Hasil translasi nukleotida menjadi asam amino menunjukkan pergeseran pada struktur asam amino, pergeseran asam amino dapat dipengaruhi oleh banyak hal seperti nutrisi, cuaca, lingkungan, dll. Kesimpulannya adalah sampel chc/SBY/2018 memiliki kemiripan yang cukup tinggi dengan isolat dari Indonesia dan isolat mengalami mutasi pada struktur nukleotida pada asam amino dan analisis filogenetik dari isolat dalam penelitian ini berhubungan dengan beberapa isolat vaksin dan beberapa isolat di Indonesia.

Kata kunci: *Newcastle Disease*, asam amino, homologi

Abstract

This study aimed to discover the homology of nucleotide sequence, homology percentage, and those relations phylogenetic of protein Fusion (F) gene coding of Newcastle disease in domestic chicken (Gallus gallus domesticus) in Surabaya using some comparison isolate from GenBank. Samples were scoured of digestive organs from native chicken, that was collected from a traditional market in Wonokromo, Surabaya. Samples were tested using RT-PCR with primer forward and reverse with target 976bp, a positive sample which is continued with sequencing then homology and nucleotide analysis which is done and which is translated into amino acid. The result of homology chc/SBY/2018 sample has a similarity of 88% with references sequence, while with Lasota vaccine has a similarity of 87%, and the highest result of homology showed by the comparison with various isolates in Indonesia 90-95%. Translation results from nucleotide alignment into amino acid showed shifts in amino acid structure, which is amino acid shifts could be affected by many things like nutrition, wheater, environment, etc. The conclusion was chc/SBY/2018 sample has a quite high similarity with Indonesian isolates and undergoes mutation on nucleotide structure on amino acid and phylogenetic analysis. This study related to some isolates of vaccine and some isolates in Indonesia.

Keywords: Newcastle Disease, amino acid, homology

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INTRODUCTION

Newcastle Disease is a viral disease that is very easily transmitted which attacks many avian species, one of them is domestic poultry (Suarez, 2020). Newcastle Disease is a very important disease in livestock, in the list of infectious animal diseases in the OIE, Newcastle

Disease is categorized as Notifable disease, due to their economic losses impact (OIE, 2012).

To control the Newcastle Disease outbreak, in addition to the culling of infected poultry, billions of vaccine doses (based on strains such as La Sota and B1) have been administered worldwide. This vaccination minimizes economic losses and disease outbreaks, but does not completely prevent viral replication and shedding (Xiang *et al.,* 2017). Until now there has not been one area in Indonesia that is free from ND, although the use of vaccines to combat Newcastle Disease (Bahri and Kusumaningsih, 2005).

The use of vaccines that are not homologous to the viral strains circulating in the outbreak can cause these birds to function as reservoirs and increase the excretion of the virus into the environment by infected birds (Rehmani *et al.,* 2015).Vaccination using homologous vaccines can increase the immune response that is formed and reduce the occurrence of virus shedding compared to heterologous vaccines (Miller *et al.,* 2013). Shedding the virus which allows the recirculation of the virus to the environment can cause the virus mutation and resulting in adaptive changes on immune system (Perozo *et al.,* 2012).

Newcastle Disease is a single-stranded, negative-sense RNA genome, encodes nucleocapsid protein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase protein (HN), RNA polymerase RNA (L) dependent, and two additional nonstructural proteins, V and W, are produced by RNA editing of the P gene (Alexander, 2000). Based on the amino acid sequences inferred from the protein F cleavage site and clinical signs in chickens, NDV can be classified into three Main pathotypes: velogenic, mesogenic, and lentogenic (Xiang *et al.,* 2017). Therefore analyzing protein F homology, phylogenetics and evolutionary distance can assist in the identification of what will become more sophisticated and effective vaccine seeds. Based on the description above, further study was carried out regarding the homology analysis, evolutionary distance and phylogenetics of the Newcastle Disease Fusion protein coding gene in native chickens.

MATERIALS AND METHODS

Sample Colections

The samples from native chicken in traditional market in east java were collected and place in 1,5 ml centrifuge tube containing 1.0 mL of transport medium. All samples were isolated by standard virus isolation methods in embryonated chicken eggs, the sample was isolated on the TAB and identified againts the NDV by the HA test then confirmed by the HI test using Newcastle Disease antiserum. (OIE, 2012).

Nucleic Acid Extraction and RT-PCR

RNA of the viruses was extracted from allantoic fluids using Trizol LS reagen (Invitrogen, Carlsbad,CA) as per manufacture's instructions, and is amplified for parrtial gene F. After the process of RNA extraction, the next stage is amplification for partian gene F. Primer of specific oligonucleotide for RT-PCR amplification were purcahased from PT. Genetika Indonesia. This primer were designed by Rantam, the sequence of oligonucleotide primers were presented in Table 1.

Molecular Analysis

The Molecular analysis is a method to combine and comparing nucleotide sequence 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.

RESULTS AND DISCUSSIONS

Electrophoresis

Based on the results of the electrophoresis in the Figure 1, the sample shows that the amplification of the Fusion protein coding gene in Newcastle Disease virus gives positive results in the form of a DNA band of 976 bp RT-PCR results seen in the well. This is in accordance with what Viljoen (Viljoen *et al.,* 2005).

Table 1. Sequence of oligonucleotide primer RT-PCR

Primer F1	Sekuens 5'-3'	Product	Position
Forward	GTA GAG CTC AAC CTG TAC CT	976 bp	667-687
Reverese	GTT GCG CTT TTT GCT TAT AC		1634-1643

Table 2. Sequence of oligonucleotide primer RT-PCR

No	Isolat Pembanding	1	2	3	4	5	6	7	8	9	10	11	12
1	Chc/SBY1/018	100%											
2	JN872154.1	88%	100%										
3	AY845400.2	87%	98%	100%									
4	HQ697257.1	95%	87%	86%	100%								
5	HQ697258.1	95%	87%	87%	100%	100%							
6	HQ697259.1	95%	87%	86%	100%	100%	100%						
7	HQ697260.1	95%	54%	86%	100%	100%	100%	100%					
8	HQ697254.1	95%	87%	85%	100%	100%	100%	100%	100%				
9	JX393313.1	91%	88%	86%	94%	94%	94%	94%	94%	100%			
10	HQ697261.1	90%	87%	86%	94%	94%	94%	94%	94%	93%	100%		
11	HQ697256.1	90%	87%	86%	94%	94%	94%	94%	94%	93%	100%	100%	
12	HQ697255.1	90%	87%	86%	94%	94%	94%	94%	94%	93%	100%	100%	100%

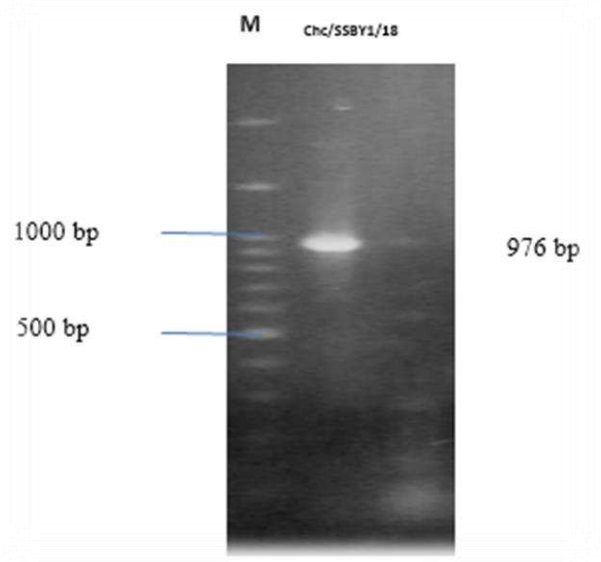

Figure 1. The results of the Newcastle Disease virus F gene amplification with 1% Agarose. The number to the left of the image represents the DNA leadder marker value of 100 bp. M is a 100 bp ledder DNA marker, Chc/SBY1/2018 is a positive sample of native chickens showing a band length of 976 bp.

	1	2	3	4	5	6	7	8	9	10	11	12
1. JN872154.1-BEA(REF)												
2. Chc/SBY1/2018	0.070											
3. Chc/seragen/2012	0.080	0.030										
4. Chc/banjarmasin/2012	0.080	0.031	0.001									
5. Chc/kudus/2012	0.080	0.030	0.000	0.001								
6. Chc/bali/2012	0.080	0.057	0.034	0.034	0.034							
7. Chc/gianyar/2012	0.080	0.030	0.000	0.001	0.000	0.034						
8. Chc/makasar/2012	0.081	0.056	0.035	0.035	0.035	0.002	0.035					
9. Chc/sukorejo/2012	0.080	0.056	0.034	0.035	0.034	0.002	0.034	0.002				
10. Chc/jakarta/2013	0.073	0.052	0.034	0.035	0.034	0.041	0.034	0.041	0.041			
11. Chc/kudus/2010	0.080	0.030	0.000	0.001	0.000	0.034	0.000	0.035	0.034	0.034		
12. vaksinlasota/2016	0.012	0.081	0.088	0.089	0.088	0.086	0.088	0.085	0.087	0.083	0.088	

Figure 2. The results of the analysis of the evolutionary distance based on the nucleotide arrangement of the Fusion gene coding for the Newcastle Disease virus protein which was compared with the nucleotide arrangement of the Fusion protein coding gene for Newcastle Disease vaccine isolates used in Indonesia and scattered in several regions of Indonesia from the 2010-2016 GenBank data.

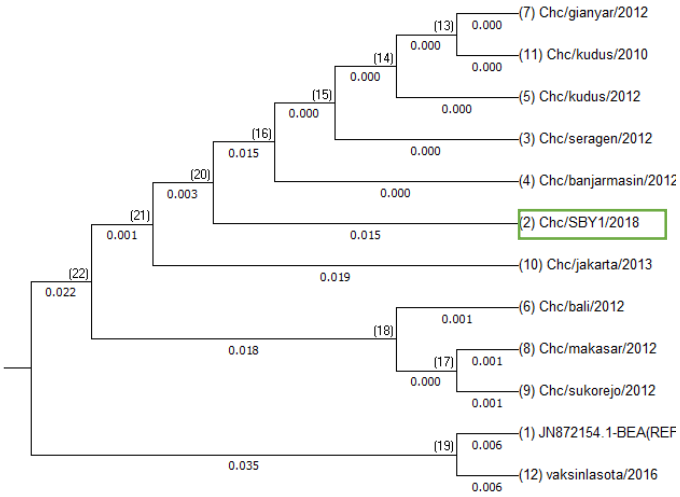

Figure 3. Phylogenetic tree based on the nucleotide arrangement of the Fusion protein Newcastle Disease virus coding gene nucleotide which is compared with the nucleotide arrangement of the Fusion protein coding gene for Newcastle Disease vaccine isolates used in Indonesia and scattered in several regions of Indonesia from GenBank 2010-2016.

Homology Analysis

The results of homology analysis of isolates Chc/SBY1/2018 are shown in Table 2. The results of the homology analysis of the F protein sequences of sample Chc/SBY1/2018 with JN872154.1-REFSEQ Beaudette 2012 showed that 88% of these homology results meant that the isolated samples were close to one species, while the Chc/SBY1/2018 sample was compared to AY845400.2-Lasota Vaccine 2016 shows a similarity of 87% of these homology results, meaning that the isolated samples approach one species. The highest homology results were found in the comparison of Chc/SBY1/2018 samples with isolates HQ697257.1-Gianyar 2012, HQ697258.1-Sragen 2012, HQ697259.1-Kudus 017 2010, HQ697260.1-Kudus 018 2012, HQ697254.1-Banjarmasin 2012 successively showed 95% similarity, while the Chc/SBY1/2018 sample with isolate JX393313.1-Jakarta 2013 showed 91% similarity, besides that the results of the homology of the Chc/SBY1/2018 sample with HQ697261.1-Bali 2012, HQ697255.1-Sukorejo 2012 showed a homolog result 90% this homology result means that the isolated sample is one species.

The difference in homology that is quite far between the sample with the control isolate and the vaccine virus will affect vaccination, the success of vaccination is largely determined by the introduction of antibodies to the field virus after vaccination.The introduction of antibodies is influenced by the homology of the vaccine virus with field virus isolates, a homologous vaccine virus with a field virus will stimulate the production of antibodies faster because it recognizes the infecting field virus. The introduction of these antibodies will accelerate the neutralization of the virus in the host body during the initial infection (Perozo *et al.,* 2012). Transmission of Newcastle Disease from one animal to another through contact with sick animals, excretion and also carcasses of Newcastle Disease sufferers. The route of transmission is through the digestive and respiratory organs. Viruses that are mixed with mucus or viruses in feces and urine last up to 2 months, even in a dry state they last much longer. Similarly, viruses that contaminate litter and other cage equipment (Kementrian Pertanian, 2014).

Analysis Evolutionary Distance

Based on Figure 2, the greatest evolutionary distance in the comparison of Chc/SBY1/2018 isolates compared to the Lasota vaccine isolate is 8.1%, while the comparison of the sample with

refseq shows an evolutionary distance of 7.0%, and the evolutionary distance between the sample and the isolate. viruses circulating in various regions of Indonesia range from 3.0% to 5.7%. Zero evolutionary distance means there is no difference, if there is a distance value, it means that there is a difference or mutation. The evolutionary distance that is far enough between the sample with the control isolate and the vaccine virus will affect vaccination (Perozo *et al.,* 2012).

Analysis Phylogenetic

Based on the results of the analysis phylogenetic in the Figure 3, the results of phylogenetic analysis showed that the Chc/SBY/2018 sample had a clade of closeness to the virus isolate HQ697257.1-Gianyar 2012, HQ697258.1-Sragen 2012, HQ697259.1-Kudus 017 2010, HQ697260.1-Kudus 018 2012, HQ697254.1-Banjarmasin 2012, JX393313.1-Jakarta 2013, and different clade with virus isolates HQ697261.1-Bali 2012, HQ697255.1-Sukorejo 2012, HQ697256.1-Makasar 2012, while with Lasota and refseq vaccine isolates it is one cluster. Given the nature of RNA viruses with high mutation rates, and the opportunity for viral evolution due to gene recombination between genotypes II (Qin *et al.,* 2008). So it is interesting to further investigate whether the other gene fragments are also one cluster with the vaccine line or one cluster with isolates scattered from various regions in Indonesia, whether these isolates are actually revertants from the vaccine line.

CONCLUSION

Based on the study conducted, it was concluded that the Chc/SBY1/2018 isolate isolated from the Wonokromo traditional market had characters close to the HQ697257.1-Gianyar 2012 virus isolate, HQ697258.1-Sragen 2012, HQ697259.1-Kudus 017 2010, HQ697260.1-Kudus 018 2012, HQ697254.1-Banjarmasin 2012, JX393313.1-Jakarta 2013, Chc/SBY1/2018 isolates are close to velogenic strains, this can be seen from homology analysis, evolutionary distance analysis, and phylogenetic results.

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