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Research Article

Characterization of Newcastle Disease Virus in Southeast Asia and East Asia: Fusion Protein Gene

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Abstract: The aim of this study was to generate the bioinformatics analysis of the circulating NDVs in Southeast Asia, East Asia, and the vaccine virus strains through a database of isolates stored in GenBank® (National Center for Biotechnology Information, USA). The used isolates were AJ629062.1 (La Sota), AF309418.1 (B1), EF201805.1 (Mukteswar), KT445901.1 (Komarov), JX524203.1 (V4), AY935499.2 (I-2), KC987036.1 (F), KF767104.1 (Indonesia), KF767105.1 (Indonesia), KF767106.1 (Indonesia), HQ697255.1 (Indonesia), HQ697256.1 (Indonesia), HQ697261.1 (Indonesia), JX012096.2 (Malaysia), GU332646.1 (Vietnam), AF358786.1 (Taiwan), AF358788.1 (China), KT380032.1 (Republic of Korea), and KC503416.1 (Japan). As the results, this study have revealed the data of homology, pathotype, genetic distance, B cell epitope prediction, and molecular phylogenetic analysis of circulating NDVs in Southeast Asia and East Asia and vaccine virus strains. Thus, the results of this study can be used as a reference for vaccine design studies in the applications of poultry vaccine industry.

Keywords: bioinformatics analysis, newcastle disease, poultry, vaccine design

Introduction

Newcastle disease (ND) is caused by virulent strains of APMV-1 or Newcastle disease virus (NDV). Infection by a virulent NDV is associated with high mortality in poultry [1]. ND causes economic losses due to morbidity, decreased egg production, death, and post-vaccination reactions [2]. ND has caused significant losses in the poultry industry in various parts of the world [3-7] including in Indonesia [8, 9].

ND is categorized by OIE as a notifiable disease. Virulent NDV infection can cause the death rate to reach 100%, causing limitations on international trade in poultry products and embargoes in countries with ND outbreaks [2]. ND is transmitted via direct contact with the infected birds. In addition, transmission can also be through secretion and excretion of infected birds. Another important transmission route is by air [10]. In addition to infecting poultry, ND virus infections naturally are also found in pigs [11], sheep [12], and even humans [13].

NDV is a virus with a diameter of around 200-300 nanometers. NDV has a genome of around 15 kb that is not segmented, single-stranded which encodes matrix protein (M), hemagglutinin-neuraminidase protein (HN), nucleocapsid protein (NP), phosphoprotein (P), fusion protein (F), RdRps (L), and non-structural proteins, such as V and W from the P gene [14, 15]. F protein has high immunogenic properties [15].

NDV vaccine is divided into two, active vaccine (live) and inactivated vaccine. Live vaccines are vaccines containing live viruses or contain viruses that are weakened, including B1, V4, F, I-2, Komarov, Mukteswar, and La Sota. The inactive vaccine does not have the ability to reproduce in the body of a

vaccinated animals, but is able to stimulate antibody formation. There are many vaccination programs that have been implemented in the farm environment, but there are still many failures. One indication of a failure is a high viral mutation, especially RNA virus.

In this study, we applied bioinformatics analysis to reveal the data of homology, pathotype, genetic distance, prediction of epitope against B cells, and molecular phylogenetic of circulating NDVs in Southeast Asia and East Asia also vaccine virus strains through a database of isolates available in GenBank®. Henceforth, the results of this study can be used as a reference for vaccine virus design studies in the applications of poultry vaccine industry.

Materials

NDV Isolates

All isolates were retrieved from GenBank® (National Center of Biotechnology Information, USA) (see Table 1).

Table 1. NDV isolates were used in this study

NCBI Accession Number	Strain or Origin of Isolates
AJ629062.1	La Sota (vaccine)
AF309418.1	B1 (vaccine)
EF201805.1	Mukteswar (vaccine)
KT445901.1	Komarov (vaccine)
JX524203.1	V4 (vaccine)
AY935499.2	I-2 (vaccine)
KC987036.1	F (vaccine)
KF767104.1	Indonesia (South East Asia)
KF767105.1	Indonesia (South East Asia)
KF767106.1	Indonesia (South East Asia)
HQ697255.1	Indonesia (South East Asia)
HQ697256.1	Indonesia (South East Asia)
HQ697261.1	Indonesia (South East Asia)
JX012096.2	Malaysia (South East Asia)
GU332646.1	Vietnam (South East Asia)
AF358786.1	Taiwan (East Asia)
AF358788.1	China (East Asia)
KT380032.1	Republic of Korea (East Asia)
KC503416.1	Japan (East Asia)

Nucleotide Sequence Preparation

NDV nucleotide sequences (fusion protein gene; 1662 kb) from all isolates were retrieved from GenBank® (National Center of Biotechnology Information, USA). Multiple sequence alignment of nucleotide sequences was carried out with BioEdit vers. 7.

Sequence Homology

An analysis of nucleotide homology between all NDV isolates carried out using the platform from NCBI, Needleman-Wunsch Global Align Nucleotide Sequences.

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Prediction of B Cell Epitop and Protective Antigens

In this study, we predict the epitope of B cells and their immunogenicity. Epitope and immunogenicity predictions are analyzed through facilities on the IEDB online webserver (www.iedb.org) and VaxiJen v2.0.

Pathotyping of NDV

Analysis of the determination of the type of pathogenicity or pathotype. This analysis used the cleavage site motif of NDV that appears at the 112 to 117 amino acid [9]. Analysis of the type of pathogenicity using BioEdit vers. 7.

Genetic Distance Analysis (*p*-distance)

We analyzed the genetic distance with MEGA X. Analysis of the genetic distance from the NDV isolates used the Kimura two-parameter method [16].

Molecular Phylogenetic Analysis

The data was carried out using the maximum likelihood to obtain a phylogenetic tree. Then, the phylogenetic tree was tested using a bootstrap test on 1000 replications, and the Tamura-Nei substitution model, referring to Tamura *et al*. [17].

Results and Discussions

The results of homology analysis between NDV isolates using Global Align Nucleotide Sequences Needleman-Wunsch are presented in Table 2. As for B cell epitope prediction analysis using the platform from the Immune Epitope Database and Analysis Resource (IEDB) presented in Table 3. In addition, the analysis of the type of pathogenicity based on the motif of the cleavage site is presented in Table 4. Then, for the results of molecular phylogenetic analysis is presented in Figure 1.

The homology analysis data based on the nucleotide sequence of the fusion protein gene (1662 bp) was obtained, the result showed that the homology percentage between NDV isolates from various countries in Southeast Asia and East Asia against NDV isolates (several vaccine strains) on the market ranged from 83 % to 94% (Table 2). In addition, the results of the highest homology analysis were KT380032.1 (Republic of Korea) and KC503416.1 (Japan) to JX524203.1 (V4, NDV vaccine strain) with the score of 94%. The higher nucleotide or amino acid homology value, the genetic relationship is also likely to be closer.

Table 2. The results of B cell epitope prediction analysis and antigenicity of NDV isolates using the platform from the Immune Epitope Database and Analysis Resource (IEDB) and VaxiJen.

B Cell Epitope (BepiPred)	BepiPred Scores	Immunogenicity (VaxiJen)
SVTTSGGGRQG	12.81	Antigen
LPKDKEACAKAPLDA	10.72	Non
NMPKDKEACAKA	10.47	Non
MGPRPSTKNPTP	19.83	Antigen
SVTTSGGGKQG	11.08	Antigen
SVTTSGGRKQ	11.08	Antigen
SVTTSGGGRQG	12.81	Antigen
GSVSTSGGRR	10.62	Antigen
MGSKPSTRIP	11.62	Non
MPKEKEACAKA	10.47	Antigen
MGSKSSTRIPTPPM	15.04	Non
MGSKSSTRIPTP	13.05	Non

MGSKSSTRIPTP	13.05	Non
NMPKDKEACAKTPLEA	13.26	Non
GSVSTSGGRR	10.62	Antigen
GSVSTSGGRR	10.62	Antigen
GSVSTSGGRR	10.62	Antigen
SVTTSGGGKQG	13.61	Antigen
SVTTSGGGKQG	13.61	Antigen

Table 3. The analysis of the type of pathogenicity based on the cleavage site of NDV isolates

Cleavage Site Motif	Pathotype
^{112}GRQGRL^{117}	Avirulent
^{112}GRQGRL^{117}	Avirulent
^{112}RRQRRF^{117}	Virulent
^{112}RRQKRF^{117}	Virulent
^{112}GKQGRL^{117}	Avirulent
^{112}GKQGRL^{117}	Avirulent
^{112}GRQGRL^{117}	Avirulent
^{112}RRQKRF^{117}	Virulent
^{112}RRQKRF^{117}	Virulent
^{112}RRQKRF^{117}	Virulent
^{112}RRRKRF^{117}	Virulent
^{112}RRRKRF^{117}	Virulent
^{112}RRRKRF^{117}	Virulent
^{112}RRQKRF^{117}	Virulent
^{112}RRQKRF^{117}	Virulent
^{112}RRQKRF^{117}	Virulent
^{112}RRQKRF^{117}	Virulent
^{112}GKQGRL^{117}	Avirulent
^{112}GKQGRL^{117}	Avirulent

Epitopes from all samples of NDV isolates are predicted to use BepiPred on the tools.iedb.org/bcell to determine the potential for B cell recognition with an accuracy of around 75%, this prediction works based on a specific algorithm with a combination of Markov hidden statistical methods and trend scale, bigger scores indicate the potential for high B cell recognition so that it can be used for further analysis [18]. After being tested using BepiPred, peptides are tested using the VaxiJen v2.0 to determine the characteristics of immunogenicity so that they can be distinguished including non-antigens or antigens. The performance of this server uses the alignment principle alignment-independent prediction, but now it is developed based on the physicochemical properties of the target protein without alignment. So that, the prediction has an accuracy of around 70% to 89% [19, 20].

Based on Table 3, the results of the BepiPred and VaxiJen prediction analysis conducted on all NDV isolates obtained from NCBI (see Table 1), Komarov (KT445901.1) has the highest predictive score of 19.83 of the seven isolates with the 'MGPRPSTKNPTP' peptide as the antigenic. The Indonesia/Southeast Asia

isolate virus (KF767104.1) with a score of 10.62 with 'GSVSTSGGRR' peptide is antigenic due to Indonesia/Southeast Asia isolates (HQ697256.1 and HQ697261.1) despite having high scores but not antigenic, Vietnam/Southeast Asia isolate (GU332646.1) with a score of 10.62 with 'GSVSTSGGRR' peptide is antigenic compared to Malaysia isolate that are not antigenic, and isolates of Republic of Korea and Japan with a score of 13.61 with 'SVTTSGGGKQG' peptide and 'SVTTSGGGKQG' peptide both of which are East Asia have the same score and are antigenic.

B cell epitope prediction is a method used to predict protein regions that can be recognized as an epitope response to cell B. Epitope is part or part of an antigen molecule that binds to antibodies [15]. This area is very important which can be used as a basis for designing certain types of vaccines or specific antibodies. To design a vaccine, the epitope must be considered to determine the active side of the antigen which will later be used to bind to the antibodies. A good epitope usually has a length of more than 9 peptides.

Analysis of the cleveage site motif of NDV isolates for the determination of pathotypes obtained that 12 isolates classified as virulent (velogenic) and 7 isolates classified as lentogenic (see Table 4). Most virulent strains of the NDV have motif ^{112}RRQKRF^{117} on cleavage site protein F_0 [15]. In addition, other motifs for virulent strains are ^{112}KRQKRF^{117}, ^{112}RRQRRF^{117}, and ^{112}RRRKRF^{117} [9]. Whereas for avirulent (lentogenic) strain, it has motif of ^{112}GKQGRL^{117} [21] and ^{112}GRQGRL^{117} [9]. In general, molecular pathotyping of NDVs was carried out on Fusion protein gene. The main reason is because there are cleavage sites in this gene that play a role in the pathogenicity of the NDV [9].

In the genetic distance analysis (*p*-distance) the Kimura two-parameter method is used. NDV isolates (see Table 1) have values between 0.000 and 0.182. On the other hand, the genetic distance will be correlated to the level of effectiveness of the vaccination results and become the cause of the outbreak of ND even though a complete and repeated vaccination program has been carried out. The difference in genetic distance that is quite far between field viruses and vaccine viruses will affect the success of vaccination. The success of vaccination is largely determined by the introduction of antibodies to the field virus after vaccination. In addition, the RNA virus has a high level of mutation and there is an opportunity for viral evolution due to gene recombination between genotypes.

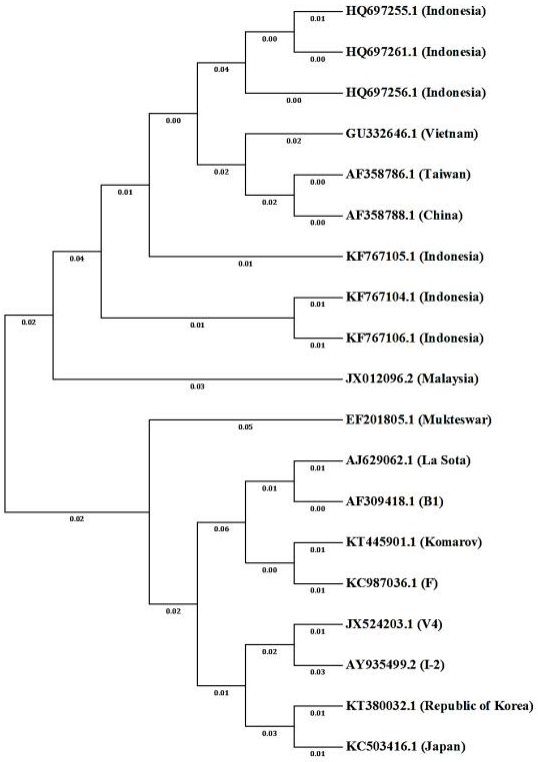

Figure 1. The results of molecular phylogenetic analysis from NDV isolates.

Molecular phylogenetic analysis in this study revealed the genetic relationship between NDV isolates (see Figure 1). The number of countries included in the Southeast Asia and East Asia region reached 18 countries, but in the GenBank® (NCBI) database only NDV isolates were available from seven countries based on the F protein gene of NDV. Isolates from Indonesia such as KF767104.1, KF767105.1, KF767106.1, have close genetic relationship, as well as other isolates from Indonesia HQ697255.1, HQ697256.1, and HQ697261.1. In addition, the same result was found in isolates from Vietnam (GU332646.1), Taiwan (AF358786.1), and China (AF358788.1), as well as isolates from the Republic of Korea (KT380032.1) and Japan (KC503416.1). Geographical proximity of the region is a factor in the emergence of close genetic relationship between NDVs in various countries [22].

So far, vaccination is an effective approach to controlling disease in animal health. Vaccines are agents that enhance the adaptive immune response. Vaccination can reduce the effects of infection and disease. Thus, the immune system recognizes the vaccine agent as a foreign object, then destroys and remembers it [19].

In addition, genomics has revolutionized the study of vaccine development. The ability to sequence the whole genome from virulent organism has led some to screen *in silico* for the most protective antigens before conducting confirmatory experiments (*in vitro* and *in vivo*). Apart from advantages, such as low cost and speed, the success of bioinformatics approach depends on the accuracy of predictions, and many tools are available to facilitate this process [19]. Basically, diversity of NDV (antigenic, pathogenicity, and genetic) needs to be studied to answer why ND outbreaks always appear or endemic. So far, vaccinations against NDVs have been carried out regularly. If the vaccine-induced immunity is unable to neutralize the NDVs in the field, it is necessary for new efforts to develop novel vaccines.

Conclusion

In this study, we revealed the data of homology, pathotype, genetic distance, B cell epitope prediction, and molecular phylogenetic analysis of circulating NDVs in Southeast Asia and East Asia and vaccine virus strains. In sum, the results of this study can be used as a reference for vaccine virus design studies in the applications of poultry vaccine industry.

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