

Fusion Protein of Aminoacid Mutations in Newcastle Disease Isolated from Swan Goose Caused Resistance to Infection

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Fusion Protein of Aminoacid Mutations in Newcastle Disease Isolated from Swan Goose Caused Resistance to Infection

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Abstract

Newcastle disease virus (NDV) cloacal swab were collected from unvaccinated swan goose and isolated by inoculation in Specific Pathogen Free embryonated eggs. Haemagglutination and Haemagglutination inhibition tests were done to identify and the, samples were processed to one step Reverse Transcriptase –Polymerase Chain Reaction using primer that amplified sequences of fusion (F) gene. Deduced amino acid sequence of the cleavage site of fusion (F) protein revealed that all isolates had avirulent motifs. Amino acid mutations that occurred were analyzed to the same reference sequence. The result showed that amino acid mutations was not capable of changing the virulence of the virus.

Key words : Newcastle disease virus (NDV), amino acid, mutations and virulence

Newcastle disease RNA virus (NDV) has ability to infect over 240 species of birds (Umali *et al.*, 2014) which possesses approximately 15.000 nucleotides and causes explosive outbreaks among poultry species (Bogoyavlenskij *et al.*, 2005).

NDV viruses belong to one serotype and there are two classes. The genome of class I viruses consist of 15.198 nucleotides (nt) and the genome of class II viruses consist of 15.186 or 15.192 nt (Czegledi *et al.*, 2006) The genome contains six open reading frames (ORF) which encode the nucleoprotein (NP), the phospho-protein (P), the matrix protein (M), the fusion protein (F), the haemagglutinin-neuraminidase (HN) and the large protein (L). At least one additional, non-structural protein (V) and possibly a second one (W), are generated by RNA

editing during P gene transcription (Steward *et al.*, 1993).

Error that may occur during its replication causes RNA virus mutate easily (Jenkins *et al.*, 2002). The mutation of amino acid in cleavage site of fusion protein of Newcastle disease virus leading to changes in their virulence. The analyze amino acid mutations of Newcastle disease virus were analysed in swan goose.

Materials and Methods

The samples from healthy waterfowls in East Java were collected and placed in 1.5 mL centrifuge tubes containing 1.0 mL of transport medium. All samples were isolated by standard virus isolation methods in embryonated chicken eggs (OIE, 2012).

The HA assay was carried as per the World Organization for Animal Health (OIE, *loc cit*). HA positive samples were tested by Haemagglutination Inhibition (HI) test to determine the haemagglutinating agents.

RNA of the viruses was extracted from allantoic fluids using Trizol LS reagen (Invitrogen, Carlsbad, CA) as per the manufacture's instructions, and is amplified for partial gene F. The primers flanked the region on cleavage site into protein coding gene of NDV.

The PCR products and the target band was purified and sequenced using an automatic ABI 3730 XL DNA Analyzer (Applied Biosystem, Foster City, CA, USA).

Results and Discussion

The samples were collected as per standart collection methods and were inoculated in 9-10 days old specific-pathogen-free (chicken

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Fusion Protein of Aminoacid Mutations ...

REFSEQ (JF950510.1)	DRRPRGYLYYAGEGAHL	CQIQSFL	EISCVLLD	CAPPTHPE	HDTKNLSV	LIISVFT	CLS				
LaSota K+				
ND/SW1/2018				
ND/SW2/2018				
ND/SW3/2018				
ND/SW4/2018				
		70	80	90	100	110	120				
REFSEQ (JF950510.1)	MGSRPSTKNP	APMMLTIR	VALVLS	CICPANSI	DGRPLAA	AGIVVTG	DKAVNIYT	SSQTGS			
LaSota K+			
ND/SW1/2018			
ND/SW2/2018			
ND/SW3/2018			
ND/SW4/2018			
		130	140	150	160	170	180				
REFSEQ (JF950510.1)	IIVKLLPNLP	KDKKEACA	KAPLDA	YNRTLLT	LLPLGDS	SIRRIQES	VTTS	GGGRQ	RLIGA		
LaSota K+		
ND/SW1/2018		
ND/SW2/2018		
ND/SW3/2018		
ND/SW4/2018		
		190	200	210	220	230					
REFSEQ (JF950510.1)	GYSKSLCL	HLEGGDR	NALLAPL	LAVWLLG	LQLLHKK	QRPQLK	YKPNK	MLPTSSD			
LaSota K+			
ND/SW1/2018			
ND/SW2/2018			
ND/SW3/2018			
ND/SW4/2018			

Fig 1 Amino acid mutation of gene coding F protein of Newcastle disease virus (ND)

embryos) for 5 days and Four samples shown positive results of HA, ND/SW1/2018, ND/SW2/2018, ND/SW3/2018 and ND/SW4/2018. Samples with positive HA were confirmed by HI (haemagglutinin inhibition). Growth of NDV in allantoic fluid is known by HA test to see the ability of haemagglutination of erythrocytes in viral envelope capable of agglutating erythrocytes of chickens (Alexander *et al.*, 2004) by binding specifically to sialic acid on sensitive cell surface receptors and facilitate the infection process.

The results of multiple alignment the amino acid sequence of the protein coding gene F Newcastle disease (ND) virus showed mutation in several positions (Fig 1). The point shows the similarity of the amino acid sequence and the letters that appear indicate differences in amino acid sequences as the variable sites, which can mutate, leading to multiple alignment. Region that do not changed was called conserved sites.

Amino acid sequences were analyzed in

the cleavage site. The lentogenic virus has the motif of single basic amino acid on the F cleavage site 112G/EK/RGG/E-R116 and L (Leucine) at residue 117 and can be cleaved by protease enzymes such as trypsin which can be found in the digestive and respiratory tract (Choi *et al.*, 2010). Based on the amino acid in cleavage site the samples ND/SW1/2018, ND/SW2/2018, ND/SW3/2018, ND/SW4/2018, and LaSota positive isolates have similar cleavage site structure in lentogenic strains. The motif is single basic amino acid 112G/EK/RGG/E-R116 and L (Leucine) at residue 117.

The smallest mutations are point mutations, in which only a single base pair is changed into another base pair. Another kind of mutations called nonsynonymous mutation, in which an amino acid sequence is changed (Alam, 2013). RNA viruses mutate faster than DNA viruses, single-stranded viruses mutate faster than double-strand virus, and genome size appears to correlate negatively with mutation

rate. Viral mutation rates are not merely caused by polymerase errors, but also by the ability of a virus to correct DNA mismatches by proof reading and/or post-replicative repair (Sanjuan and Domingo-Calap, 2016). Polymerase enzyme of RNA virus is characteristically low fidelity leading to easy to mutation. Each mutant in quasispecies is genetically linked (Lauring and Andino, 2010).

Summary

Newcastle disease virus (NDV) isolated from swan goose have some amino acid mutations. These mutation in the virus cannot change their virulence.

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Newcastle disease RNA virus (NDV) has ability to infect over 240 species of birds (Umali *et al.*, 2014) which possesses approximately 15.000 nucleotides and causes explosive outbreaks among poultry species (Bogoyavlenskij *et al.*, 2005).

NDV viruses belong to one serotype and there are two classes. The genome of class I viruses consist of 15.198 nucleotides (nt) and the genome of class II viruses consist of 15.186 or 15.192 nt (Czegledi *et al.*, 2006) The genome contains six open reading frames (ORF) which encode the nucleoprotein (NP), the phosphoprotein (P), the matrix protein (M), the fusion protein (F), the haemagglutinin-neuraminidase (HN) and the large protein (L). At least one additional, non-structural protein (V) and possibly a second one (W), are generated by RNA

editing during P gene transcription (Steward *et al.*, 1993).

Error that may occur during its replication causes RNA virus mutate easily (Jenkins *et al.*, 2002). The mutation of amino acid in cleavage site of fusion protein of Newcastle disease virus leading to changes in their virulence. The analyze amino acid mutations of Newcastle disease virus were analysed in swan goose.

Materials and Methods

The samples from healthy waterfowls in East Java were collected and placed in 1.5 mL centrifuge tubes containing 1.0 mL of transport medium. All samples were isolated by standard virus isolation methods in embryonated chicken eggs (OIE, 2012).

The HA assay was carried as per the World Organization for Animal Health (OIE, *loc cit*). HA positive samples were tested by Haemagglutination Inhibition (HI) test to determine the haemagglutinating agents.

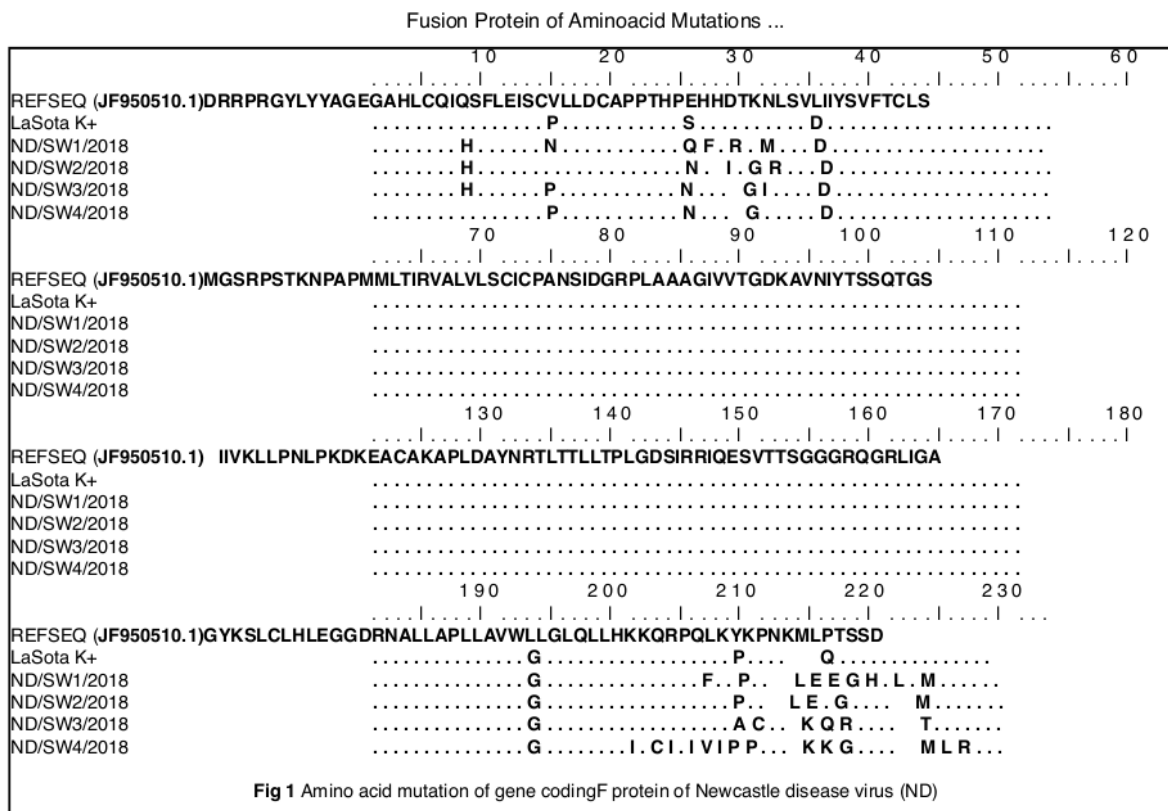
RNA of the viruses was extracted from allantoic fluids using Trizol LS reagen (Invitrogen, Carlsbad, CA) as per the manufacture's instructions, and is amplified for partial gene F. The primers flanked the region on cleavage site into protein coding gene of NDV.

The PCR products and the target band was purified and sequenced using an automatic ABI 3730 XL DNA Analyzer (Applied Biosystem, Foster City, CA, USA).

Results and Discussion

The samples were collected as per standart collection methods and were inoculated in 9-10 days old specific-pathogen-free (chicken

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embryos) for 5 days and Four samples shown positive results of HA, ND/SW1/2018, ND/SW2/2018, ND/SW3/2018 and ND/SW4/2018. Samples with positive HA were confirmed by HI (haemagglutinin inhibition). Growth of NDV in allantoic fluid is known by HA test to see the ability of haemagglutination of erythrocytes in viral envelope capable of agglutating erythrocytes of chickens (Alexander *et al.*, 2004) by binding specifically to sialic acid on sensitive cell surface receptors and facilitate the infection process.

The results of multiple alignment the amino acid sequence of the protein coding gene F Newcastle disease (ND) virus showed mutation in several positions (Fig 1). The point shows the similarity of the amino acid sequence and the letters that appear indicate differences in amino acid sequences as the variable sites, which can mutate, leading to multiple alignment. Region that do not changed was called conserved sites.

Amino acid sequences were analyzed in

the cleavage site. The lentogenic virus has the motif of single basic amino acid on the F cleavage site 112G/EK/RGG/E-R116 and L (Leucine) at residue 117 and can be cleaved by protease enzymes such as trypsin which can be found in the digestive and respiratory tract (Choi *et al.*, 2010). Based on the amino acid in cleavage site the samples ND/SW1/2018, ND/SW2/2018, ND/SW3/2018, ND/SW4/2018, and LaSota positive isolates have similar cleavage site structure in lentogenic strains. The motif is single basic amino acid 112G/EK/RGG/E-R116 and L (Leucine) at residue 117.

The smallest mutations are point mutations, in which only a single base pair is changed into another base pair. Another kind of mutations called nonsynonymous mutation, in which an amino acid sequence is changed (Alam, 2013). RNA viruses mutate faster than DNA viruses, single-stranded viruses mutate faster than double-strand virus, and genome size appears to correlate negatively with mutation

rate. Viral mutation rates are not merely caused by polymerase errors, but also by the ability of a virus to correct DNA mismatches by proof reading and/or post-replicative repair (Sanjuan and Domingo-Calap, 2016). Polymerase enzyme of RNA virus is characteristically low fidelity leading to easy to mutation. Each mutant in quasispecies is genetically linked (Lauring and Andino, 2010).

Summary

Newcastle disease virus (NDV) isolated from swan goose have some amino acid mutations. These mutation in the virus cannot change their virulence.

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