



UNIVERSITI PUTRA MALAYSIA

**NUCLEOTIDE SEQUENCE ANALYSIS OF THE HN GENE
OF THE HEAT RESISTANT NDV STRAIN AF2240
AND SUBSTRAIN V4-UPM**

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By

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**Thesis Submitted in Fulfilment of the Requirements for
the Degree of Master of Science in the Faculty of
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**DEDICATED TO MY WIFE
AND OUR PARENTS**



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LIST OF ABBREVIATIONS

A	-	adenine
Ala (A)	-	alanine
Arg (R)	-	arginine
Asn (N)	-	asparagine
Asp (D)	-	aspartic acid
AUS/32	-	NDV strain Australia-Victoria
B1/47	-	NDV strain B1 Hitchner
bp	-	basepair
BEA/45	-	NDV strain Beaudette
C	-	cytosine
cDNA	-	complementary deoxyribonucleic acid
CHI/85	-	NDV strain Chiba
Ci	-	curies
Cys (C)	-	cysteine
°C	-	degrees centigrade
D26/76	-	NDV strain D26
DNA	-	deoxyribonucleic acid
dNTP	-	deoxynucleotide triphosphates
ddNTP	-	dideoxynucleotide triphosphates
dsDNA	-	double-stranded DNA
F	-	fusion protein
G	-	guanine
Gln (Q)	-	glutamine
Glu (E)	-	glutamic acid



Gly (G)	-	glycine
h	-	hour
HA	-	haemagglutinin
HER/33	-	NDV strain Herts
His (H)	-	histidine
HN	-	haemagglutinin-neuraminidase
IBA/85	-	NDV strain Ibaragi
Ile (I)	-	isoleucine
ITA/45	-	NDV strain Italien
kb	-	kilobase
kDa	-	kilodalton
kg	-	kilogram
kPa	-	kilopascal
LAS/46	-	NDV strain La Sota
Leu (L)	-	leucine
Lys (K)	-	lysine
M	-	Molar
MDT	-	mean death time
Met (M)	-	Methionine
min	-	minute
ml	-	mililitre
MLD	-	minimum lethal dose
mmol	-	milimol
mM	-	milimolar
\underline{M}_r	-	molecular weight
mRNA	-	messenger RNA



NA	-	neuraminidase
ND	-	Newcastle disease
NDV	-	Newcastle disease virus
ng	-	nanogram
nm	-	nanometre
NTE	-	Natrium-Tris-EDTA
ORF	-	open reading frame
PBS	-	phosphate saline buffer
PCR	-	Polymerase Chain Reaction
phe (F)	-	phenylalanine
pH	-	<u>Puissance hydrogene</u>
pmol	-	picomol
Pro (P)	-	proline
QUE/66	-	NDV strain Queensland
RBC	-	red blood cells
RNA	-	ribonucleic acid
RNasin	-	RNase inhibitor
RNA-PCR	-	reverse transcription of RNA followed by the Polymerase Chain Reaction
S	-	second
Ser (S)	-	Serine
ssDNA	-	single-stranded DNA
T	-	Thymine
T _m	-	melting temperature
Taq	-	<u>Thermus aquaticus</u>
TBE	-	Tris-Boric-EDTA buffer



TE	-	Tris-EDTA buffer
TEX/48	-	NDV strain Texas, G.B.
Thr (T)	-	threonine
Trp (W)	-	tryptophan
Tyr (Y)	-	tyrosine
UPM	-	Universiti Pertanian Malaysia
U	-	unit
ULS/67	-	NDV strain Ulster
μ l	-	microlitre
μ M	-	micromolar
μ g	-	microgram
V	-	volt
v	-	volume
vRNA	-	viral ribonucleic acid
Val (V)	-	valine
W	-	Watt
w	-	weight
xg	-	centrifugal force



Abstract of the thesis submitted to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirements for the degree of Master of Science.

NUCLEOTIDE SEQUENCE ANALYSIS OF THE HN GENE
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AF2240 AND SUBSTRAIN V4-UPM

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Newcastle disease virus (NDV) is the prototype avian Paramyxovirus which causes a major disease in poultry. The highly virulent velogenic NDV strain AF2240 and avirulent substrain V4-UPM possess thermostable haemagglutination and neuraminidase activities compared to other strains. Therefore, the purpose of this study is to sequence the haemagglutinin-neuraminidase (HN) genes of AF2240 and V4-UPM.

The virus was propagated in the allantoic fluid of 9-10 day-old embryonated eggs. The allantoic fluid was harvested after 72 hours incubation at 37°C. Viral purification was carried out on 30% to 60% (W/V) sucrose density gradient centrifugation at 275,000 xg, for 4 hours at 4°C. The viral RNA was extracted using



the hot phenol extraction method. RNA sequencing was performed directly on the genomic RNA using the dideoxy chain termination method. The nucleotide sequence was then confirmed by cycle sequencing and analysed using the computer programs of HIBIO DNASIS and MicroGenie which were linked to the Data Bank.

Nucleotide sequences of the HN genes of AF2240 and V4-UPM have been determined. The HN gene of AF2240 is 1998 nucleotides long with a single open reading frame that encodes a putative protein of 581 amino acids with an estimated M_r of 63.8 kDa. Five potential glycosylation sites Asn-X-Ser/Thr have been found. Comparisons of the nucleotide and amino acid sequences revealed more than 80% homology between this strain and 13 other strains of NDV. The length of AF2240 HN protein is different from other NDV strains. The HN protein of V4-UPM is one amino acid shorter than its parental strain V4(QUE). Eight amino acid substitutions were found in this mutant compared to its parental strain V4(QUE). A mutation at codon 119 has eliminated a potential asparagine glycosylation site on V4-UPM. The deletion of Arg(403) in AF2240 and V4-UPM which is highly conserved among NDV strains is thought to be significant to the thermostability of these viruses.



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**ANALISIS JUJUKAN NUCLEOTIDA GEN HN NDV
STRAIN AF2240 DAN SUBSTRAIN V4-UPM
YANG RINTANG KEPADA SUHU TINGGI**

Oleh

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Virus penyakit Newcastle (NDV) merupakan prototaip Paramyxovirus avian yang menyebabkan penyakit utama dalam industri penternakan ayam. NDV strain AF2240 yang virulen dan substrain V4-UPM yang tidak virulen menunjukkan aktiviti hemaglutinasi dan neuraminidase yang stabil terhadap suhu tinggi. Oleh yang demikian projek ini dijalankan dengan tujuan untuk menjujuk gen hemaglutinin-neuraminidase (HN) AF2240 dan V4-UPM.

Virus ini ditumbuhkan dalam cecair alantoik telur ayam berembrio 9-10 hari. Cecair alantoik dikumpul selepas pengeraman selama 72 jam pada suhu 37°C. Penulenan virus dijalankan dengan pengemparan kecerunan sukrosa 30 % dan 60 % (w/v) pada kelajuan 275,000 xg selama 4 jam pada suhu 4°C. Genom RNA kemudian diekstrak dengan kaedah pengekstrakan fenol panas.



Penjujukan RNA dijalankan secara terus ke atas genom RNA dengan kaedah 'dideoxy chain termination'. Jujukan nukleotida kemudian dipastikan dengan penjujukan kitaran. Jujukan nukleotida dan asid amino kemudian dianalisis dengan program HIBIO DNASIS dan MicroGenie yang disambung kepada Bank Data.

Jujukan nukleotida gen HN NDV strain AF2240 dan substrain V4-UPM telah dikenalpasti. Gen HN AF2240 terdiri daripada 1998 nukleotida dan mengandungi satu rangka bacaan terbuka yang mengkodkan satu protein yang terdiri daripada 581 asid amino, dengan anggaran berat molekul 63.8 kDa. Sementara itu lima tapak glikosilasi asparagina Asn-X-Ser/Thr telah ditemui. AF2240 menunjukkan lebih daripada 80% homologi dalam jujukan nukleotida dan asid amino berbanding dengan 13 strain NDV yang lain. Protein HN AF2240 mengandungi bilangan asid amino yang berbeza dengan strain-strain NDV yang lain. Protein HN V4-UPM didapati satu nukleotida lebih pendek daripada strain induk V4(QUE). Lapan asid amino telah berubah dalam mutan ini berbanding dengan strain induknya V4(QUE). Mutan ini kehilangan satu tapak glikosilasi asparagina disebabkan oleh mutasi yang berlaku pada kodon 119. Delesi asid amino argina(403) pada AF2240 dan V4-UPM dianggap terlibat dalam kestabilan suhu kedua-dua virus ini.



CHAPTER I

INTRODUCTION

Newcastle disease (ND) is an economically important disease in poultry which often resulted in high mortality, loss in body weight and a drop in egg production. The causative agent for this highly infectious disease is the Newcastle disease virus (NDV) which belongs to the genus Paramyxovirus, under the family of Paramyxoviridae.

Due to its economic importance and its use as a laboratory model, major advances in science have been channeled to study the virus. As a result, a much complete understanding, not only in biochemistry and basic virology of the virus but also the ecology, epizootiology, antigenicity, immunology and other important aspects in the control of the disease have been achieved (Alexander, 1988).

In Malaysia, strict regulations are imposed upon the handling of NDV and the use of ND vaccines. UPM has been carrying out research on NDV since 20 years



ago. In 1977, UPM was requested by the Australian poultry industry to evaluate the stability and effectiveness of the V4 NDV as vaccine against NDV. This was followed up by the production of NDV clones UPM-AC/1 and UPM-AC/2 as vaccine (Ibrahim, 1992).

The poultry industry in Malaysia has developed from a small holder to a modern and most scientifically advanced primary industry. Poultry eggs and meat provide the cheapest source of protein for the Malaysian. According to ^{an} estimation, Malaysia produces 2.7 billion eggs annually with a per capita consumption of 212 eggs per annum, while the production of meat has increased to over 162,000 tons annually with a per capita consumption of 12-44 kg per annum (Ibrahim, 1992).

The main methods available for the control of ND are quarantine, slaughter or vaccination. In many countries vaccination is the main method of control. Vaccination against ND can be performed using either live or inactivated vaccines (Meulemans, 1988). Although the vaccination programmes have been introduced 40 years ago, ND continues to be a major threat to the Malaysian poultry industry. This is because the control of ND by conventional vaccination is feasible only for chickens reared under commercial conditions (Ideris, 1989).



The practice of backyard poultry keeping in Malaysia and other countries of South East Asia in which small numbers of scavenging chickens are kept by rural households under free range conditions, makes it very difficult for conventional vaccination. For this reason, an attempt was made to isolate a heat resistant clone from the avirulent V4 strain (Simmons, 1967) of NDV. As a result a heat-resistant variant designated V4-UPM was isolated and was used for the preparation of an oral food-based vaccine, thus overcoming the problem of village chicken vaccination (Ideris et al., 1990). This variant poses thermostable haemagglutination and neuraminidase activities compared with parental virions (Ideris et al., 1990; Idris, 1992). Therefore it is important to sequence the haemagglutinin-neuraminidase (HN) gene of this variant and to locate the genetic lesion(s) which may be significant to the thermostability feature(s) of this variant.

With the advent of the 'biotechnology revolution', novel approaches have been used in the design and development of engineered vaccines. They include recombinant subunit , synthetic peptide, antiidiotype, novel immunoadjuvantation and recombinant live vaccines (Kong, 1992). These genetically engineered vaccines are potentially purer, safer, cheaper and possess

