NEWCASTLE DISEASE VIRUS

A Journey from Poultry to Cancer

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11 Januari 2008

Dewan Taklimat
Bangunan Pentadbiran
Universiti Putra Malaysia

Penerbit Universiti Putra Malaysia
Serdang • 2008
http://www.penerbit.upm.edu.my

First Print 2008

UPM Press is a member of the Malaysian Book Publishers Association (MABOPA)
Membership No.: 9802

Perpustakaan Negara Malaysia Cataloguing-in-Publication Data

Khatijah Mohd. Yusoff
Newcastle disease virus : a journey from poultry to cancer / Datin Paduka Dr. Khatijah Yusoff.
Inaugural lecture series
ISBN 978-967-5026-40-9
1. Newcastle disease virus. 2. Paramyxoviruses. 3. Speeches, address, etc., Malay. I. Title
616.91

Design, layout and printed by
Penerbit Universiti Putra Malaysia
43400 UPM Serdang
Selangor Darul Ehsan
Tel: 03-8946 8855 / 8854
Fax: 03-8941 6172
http://www.penerbit.upm.edu.my

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ABSTRACT

Infectious diseases have had a great impact on human lives and civilizations since time immemorial. Success in controlling and eradicating these diseases, on the other hand, has not only overcome human misery but expedited human progress, both individually and collectively. The eradication of smallpox and the successful control of poliomyelitis and hepatitis B can even be measured in terms of the improvement in the world's GDP. However, new and re-emerging infections, including those connected to livestock such as the Nipah virus, SARS and avian influenza, remind us to be ever vigilant and responsive to new threats. The Newcastle disease virus (NDV) causes a highly contagious and fatal respiratory disease in chickens and other types of birds and affects the poultry industry worldwide. Although this virus is effectively controlled by vaccination, it is still endemic in Malaysia and outbreaks of the disease have contributed to major losses for the poultry industry in the country in terms of mortality and loss in egg and meat production. By delineating the genome of this enveloped negative single-stranded RNA virus, various genes have been sequenced, cloned and expressed in plasmid vectors in various organisms. Knowledge of the structural and biological characterization of NDV has provided insights into various applications of this virus in the prevention and treatment of NDV and other diseases as well. Although the virus replicates poorly in normal human cells, causing mild respiratory disease and conjunctivitis, it replicates very well in human cancer cells. This provides a potential for its use in oncology. Cancer is an omnipresent threat to human lives, especially with, but not limited to, ageing, a worldwide modern phenomenon. Its control and eradication remain largely elusive, requiring considerable concerted efforts from everyone. The oncolytic activities of the virus against human malignancies lead to tumor apoptosis and cell death. In this respect, work is in progress to leverage NDV as a viral vector to express and deliver foreign genes for vaccination and gene therapy purposes for specific malignancies in humans. This of course opens up a whole new vista

in research and industry, stretching imaginations and human potentials farther, with the cherished hope of improving the lives of human beings further.

INTRODUCTION

"We have the choice to use the gift of our lives to make the world a better place
– Warner Arber, Nobel Laureate

Over the last millennium, man has seen remarkable contributions from scientists to make the world what it is today. Perhaps one of the greatest achievements is the improvement of our living standards, eradication of infectious viral diseases such as smallpox and control of poliomyelitis, measles and hepatitis B. Whilst we revel in these marvelous milestones, we need to take note of menacing and damaging developments, in particular emerging or re-emerging diseases, such as the Nipah virus, SARS, haemorrhagic dengue fever and avian influenza, within Asia. Joshua Lederburg once commented that viruses are humanity's only real competitors for dominion of our planet, serving both as parasites and genetic elements in their hosts (Lederburg, 1994). They have considerable plasticity in their genetic make-up and in metabolic interactions in their hosts and are capable of rapid evolution. Together with the increase in air transport and the clearing of our jungles to make room for an ever increasing population, the threat of an outbreak of these diseases and even new ones seem to be even greater than ever before! These viruses are potential and powerful destroyers of our livestock and ultimately our lives, through their impact on the economy and health of nations.

Malaysian consumers today demand safe and high quality food at reasonable prices from the industry. Chicken meat is the most popular and cheapest source of meat protein among Malaysians, largely because there are no dietary prohibitions or religious restrictions against the consumption of chicken **(Table 1)**. Over the years, fast food restaurants (QSR) such as Kentucky Fried Chicken (KFC), McDonald's, A&W, Kenny Rogers and Nando's Chickenland have significantly increased the consumption of chicken in Malaysia. The poultry industry in Malaysia has an approximate total revenue of RM 5 billion

annually. Nevertheless, the industry is often hampered by outbreaks of various viral diseases such the avian influenza virus, infectious bronchitis virus, chicken anemia virus and the Newcastle disease virus (NDV).

Table 1 Sources of protein for human consumption

	Poultry	Fish	Lamb	Beef	Pork
Expensive	✗	✗	✓	✓	✗
Seasonal	✗	✓	✗	✗	✗
Acceptance	✓	✓	✗	✗	✗

Note: ✓ = agree; ✗ = do not agree

NDV is an economically important avian paramyxovirus that causes a highly contagious respiratory and neurological disease which is often fatal to many bird species (Alexander, 1999). It belongs to the same family as the Nipah virus (NiV), measles virus, mumps virus, human parainfluenza virus and the respiratory syncytial virus (**Figure 1**).

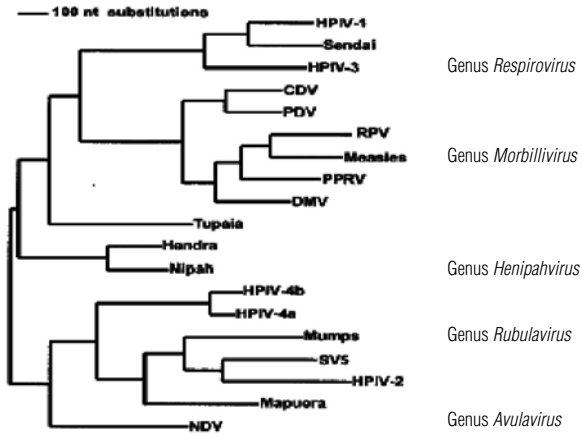

Figure 1 Phylogenetic tree showing the relationship between NDV and other paramyxoviruses.

Reports of recent outbreaks in many countries such as England, France and Malaysia (OIE homepage; http://www.oie.int), often resulting in mass culling, prove that NDV is a continuing threat to the worldwide poultry industry. In Malaysia alone, the disease caused by NDV can amount to over RM 100 million in costs annually. Hence, a constant surveillance and vaccination programme, especially in countries such as Malaysia where the disease appears to be endemic, is needed.

NDV can infect many species of birds. The virus is spread primarily through direct contact, by ingestion and inhalation and can be categorized into three main pathotypes based on the severity of the disease. Lentogenic isolates do not usually cause any disease in adult birds and are considered to be mildly virulent or avirulent. Viruses of intermediate virulence that cause respiratory disease are termed mesogenic, while virulent strains that cause high mortality are termed velogenic. Neurotropic and viscerotropic velogenic strains cause severe intestinal lesions with neurological disease, resulting in high mortality (up to 100% in chickens). We have been working on the molecular biology of a velogenic neutropic-viscerotropic local isolate known as strain AF2240. This virus which was isolated in the 1960's (Lai, 1985) can be grown and purified simply and efficiently (Mohd. Esa *et al*., 1996). The genome of strain AF2240 has been fully sequenced (Yusoff and Tan, 2001) and individual genes were cloned and expressed in various plasmid vectors to understand their interactions between each other as well as with the host proteins.

Based on years of research dedicated to avian viruses, our research team has developed, patented and commercialised several diagnostic assays and vaccines against many of these viruses (**Table 2**). One of the outstanding discoveries made while studying these viral proteins is that the nucleocapsid (NP) of the virus can form ring-like structures which have the ability to carry foreign proteins on its outer structure (Kho *et al*., 2001b). Various proteins have been attached to the NP, and it was shown that these proteins are immunogenic

and can illicit immune responses against various other pathogens (Rabu *et al*., 2002; Sivasamugham *et al*., 2003; 2006). Thus, NDV can serve as a vector to express and deliver foreign genes for vaccination and gene therapy purposes.

Table 2a Products commercialised, prototypes and trademarks, patents and activities on avian research by the Avian Virus Research Group in UPM

A. Products Commercialised

1. Newcastle disease vaccine
2. Fowl pox vaccine
3. Infectious bursal disease vaccine

B. Prototypes

1. IBDReal check, a diagnostic kit for the detection of infectious bursal disease virus subtypes.
2. FluReal H9N2 check, a diagnostic kit for the detection of type A avian influenza virus and subtyping of H9N2 virus.
3. NDReal check, a diagnostic kit for the detection of Newcastle disease virus pathotypes.
4. FluReal H5N1 check, a diagnostic kit for the detection of type A avian influenza virus and subtyping of H5N1 virus.
5. NDPath, a diagnostic kit for the detection of Newcastle disease virus
6. ViroRapid, a nested RT-PCR detection for Newcastle disease virus
7. BioCarrier, a novel drug delivery system

C. Malaysian Trademarks

1. NDPath™ in Class 5; Application No: 05007201; Filing Date: 9 May 2005
2. BioCarrier™ in Class 5; Application No: 05007202; Filing Date: 9 May 2005
3. Real check™, Rapid, Economical and Leader in Animal Diagnostics in Class 5; Application No: 06015350; Filing Date: 29 Aug 2006

Table 2b Patent deposits by the Avian Virus Research Group in UPM

Overseas Patents

1. Invention: Nucleocapsid (NP) and phosphoprotein (P) genes of a Malaysian velogenic Newcastle disease virus strain AF2240; **USA Patent No: 6,939,957**; Date granted: 6 September 2005; Country: USA
2. Invention: Methods for detection and differentiation of infectious bursal disease virus; Application No: 11/268,341; Filing Date: 17 November 2005; Country: USA
3. Invention : Methods for detection and differentiation of infectious bursal virus; Application No: 05256888.8; Filing Date: 7 November 2007; Country: Europe

Malaysian Patents

1. Invention: Nucleotide sequences of the nucleocapsid (NP) and phosphoprotein (P) genes of a Malaysian velogenic Newcastle disease virus strain AF2240 and the production of the NP and P proteins in *Escherichia coli*; **Malaysian Patent No: MY-125227-A;** Date granted: 31 July 2006
2. Invention: Detection of Newcastle disease virus; Application No: PI 20005526; Filing Date: 27 Nov 2000
3. Invention: Peptides that inhibit the propagation of Newcastle disease virus ; Application No: PI 20013687; Filing Date: 6 August 2001
4. Invention: Nucleocapsid protein of Newcastle disease virus as a carrier for immunogens; Application No: PI 20005526; Filing Date: 11 May 2002
6. Invention: Methods for detection and differentiation of infectious bursal disease virus; Application No: PI 20062120; Filing Date: 5 November 2004
5. Invention : Detection and subtyping of H9N2 influenza virus type A by molecular biology method; Application No: PI 20062120; Filing Date: 9 May 2006
7. Invention: Rapid detection and differentiation of Newcastle disease virus pathotypes; Application No: PI 20070884; Filing Date: 5 June 2007

Table 2c Research activities by the Avian Virus Research Group in UPM

Newcastle disease virus

1. Genome and functional analyses of Newcastle disease virus
2. Development of novel therapeutic and diagnostic reagents from Newcastle disease virus
3. Characterisation and pathogenicity of paramyxovirus
4. Molecular characterisation of Newcastle disease and infectious bronchitis viruses
5. Molecular characterisation of Newcastle disease virus
6. Molecular biology of viruses of veterinary importance
7. Identification of Newcastle disease virus by RNA- polymerase chain reaction
8. Location of antibody neutralisation sites in Newcastle disease virus
9. Cloning and rapid sequence analysis of cDNA
10. Development and improvement of Newcastle disease vaccine – strain F
11. Vaccination of village chickens against Newcastle disease
12. Establishment of improved methods for the diagnosis of livestock diseases in Southeast Asia using ELISA
13. Upscaling of food based Newcastle disease vaccine
14. Effect of recombinant glycoproteins of Newcastle disease virus on cancer cells
15. Macromolecular interactions between different proteins of the Newcastle disease virus
16. Development of vaccine delivery platforms for tropical infectious diseases (To design and develop a Newcastle disease virus nucleocapsid-fusion sub-unit vaccine)
17. Determination of the L gene sequence and intergenic regions of Newcastle disease virus strain AF2240

Avian influenza virus

1. Characterisation and pathogenicity of avian influenza virus
2. Development of novel polymerase chain reaction based diagnostic tools for rapid detection of avian influenza

3. Development of prototype vaccines against H5N1 and characterization of the vaccine induced responses
4. Genomic characterization of H5N1 viruses isolated from poultry farms
5. Development and clinical trials of prototype kits for laboratory detection of avian influenza viruses

Infectious bronchitis virus

1. Molecular characterisation of Newcastle disease and infectious bronchitis viruses

Fowlpox virus

1. Cloning of poultry viruses and development of improved fowl pox vaccine

Chicken anemia virus

1. Molecular cloning and pathogenesis of chicken anaemia virus
2. Identification of chicken anemia virus genetic determinants associated with virus attenuation and pathogenicity

Infectious bursal disease virus

1. Development of an effective diagnosis tool and novel vaccine against infectious bursal disease virus infection
2. The dynamics of chicken T lymphocyte and cytokine responses following infection with very virulent infectious bursal disease virus

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Although the virus is very pathogenic to many species of birds, it replicates poorly in humans, causing mild respiratory disease and conjunctivitis (Alexander and Jones, 2001). Nevertheless, once inside a susceptible cell, the virus replicates rapidly and selectively kills human tumors through apoptosis leading to cell death (Sinkovics and Horvath, 2000). The interest in using NDV as an anti-cancer agent has increased steadily in recent years. For the past few years,

our team has also been working on the activities of the virus as an anti-cancer agent.

These vast advances in the structural and biological characterization of NDV in both normal and cancer cells have provided research opportunities to address various applications of this virus in the prevention and treatment of diseases. Let me relate how the lowly common domesticated chicken may help solve this puzzle.

VIRUS CLASSIFICATION AND GENOME

NDV is the prototype paramyxovirus belonging to the genus *Avulavirus* (de Leeuw and Peeters, 1999; Mayo, 2002) of the order *Mononegavirales*, family *Paramyxoviridae*, and subfamily *Paramyxovirinae*. This enveloped virus is also called avian paramyxovirus type-1 (APMV-1) (Alexander, 1997). Its negative-sense single stranded nonsegmented RNA genome of NDV contains 15186 bases (Krishnamurthy and Samal, 1998; Phillips *et al*., 1998) which contains six structural genes that encode the nucleocapsid protein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), haemagglutinin-neuraminidase protein (HN) and the large protein (L) in the order of 3'-leader-NP-P-M-F-HN-L-trailer5' (**Figure 2**) as well as two non-structural proteins, the W and V proteins (de Leeuw and Peeters, 1999; reviewed in Yusoff and Tan, 2001). These non-structural proteins result from transcriptional editing of the mRNA of the *P* gene, by insertion of one or two G residues to result in frameshifts (Steward *et al*., 1993; Locke *et al*., 2000). RNA sequencing studies revealed that the 3' and 5'ends of the genomic RNA respectively, contain a leader sequence and trailer sequence, which have been thought to be cis-regulatory elements in replication, transcription and packaging of the genomic and anti-genomic RNA (Blumberg *et al*., 1981; Chambers *et al*., 1986a; Lamb and Kolakofsky, 2001). Separating these structural genes are intergenic regions of variable lengths (1 to 47 nucleotides) which are probably involved in terminating the mRNA transcription from the

preceding gene, before initiating transcription of the subsequent gene (Isihida *et al*., 1986; Millar *et al*., 1986; Yusoff *et al*., 1987; Philips *et al*., 1998).

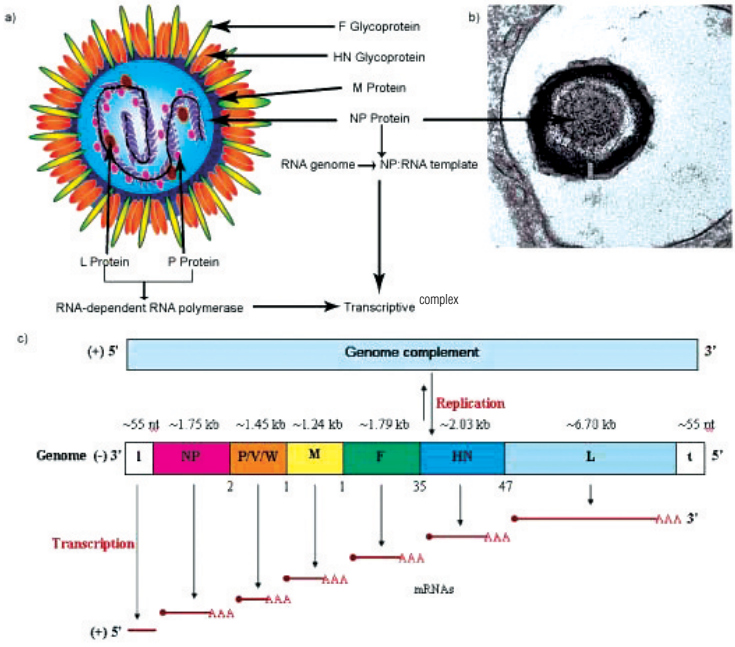

Figure 2 Schematic diagram of the virion structure of NDV showing the proteins involved in the transcriptive complex (Modified from Lamb and Kolakofsky, 2001; Yusoff and Tan, 2001; Fauziah *et al*., 2002).

The viral RNA genome is encapsidated by the nucleocapsid protein (NP) (Mellon and Emmerson, 1978) to form an active ribonucleoprotein (NP:RNA) template core for its transcription and replication (Jahanshiri, unpublished data; 2006). This template core together with the functional viral RNA dependent RNA polymerase, which comprises several copies of the P and L proteins (Yusoff and Tan, 2001), form a transcriptive complex. These three key components of

the transcriptive complex of the virus have been the subject of studies for years because of their important roles at different stages of the virus life cycle.

PROTEINS IN THE TRANSCRIPTIVE COMPLEX

a. Nucleocapsid Protein (NP)

The NP protein is the most abundant protein in the virus. This protein is encoded by the *NP* gene which contains a single open reading frame (ORF) of 1467 nucleotides (Ishida *et al*., 1986; Krishnamurthy and Samal, 1998; Kho *et al*., 2001a). The NP polypeptide comprises 489 amino acids and its molecular mass is 53 kDa. Comparison of the predicted NP amino acid sequence with the other viruses indicated that it shares very low identity (<35%) with other paramyxoviruses but is highly identical (91 to 98%) to various NDV strains (Kho *et al*., 2001a).

Electron microscopic analyses revealed that the NP protein appears as flexible helical structures (**Figure 3a**) with a diameter of about 18 nm and 1 µm within the pleomorphic virions (**Figure 3b**). The NP structures resemble the classical herringbone morphology (Kho *et al*., 2001b) with spikes protruding from a central channel, surrounded by 2200 to 2600 NP subunits (Choppin and Compans, 1975; Yusoff and Tan, 2001). Several NP monomers expressed from *Escherichia coli* formed ring-like particles assembled to appear like "stacked doughnuts" in encapsidating the RNA genome.

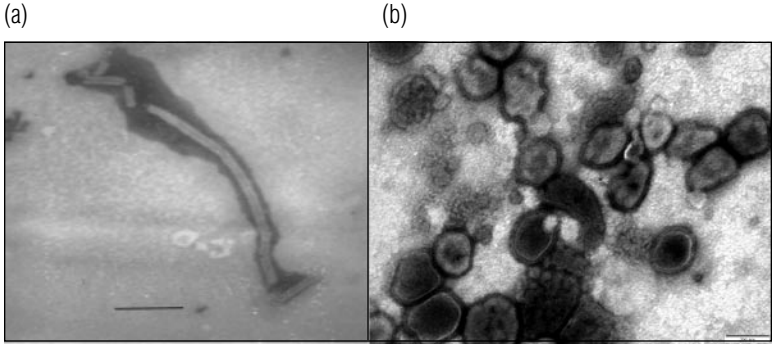

Figure 3 Electron micrographs of (a) the herringbone-like nucleocapsid and (b) pleomorphic NDV particles. Bar represents 100 nm. (From Yusoff and Tan, 2001; Courtesy of Fauziah and Zolkapli; unpublished data)

The NP protein interacts with itself to form a full length herringbone nucleocapsid. A deletion mutagenesis approach was employed to define the contiguous sequence of the NP protein which is involved in self-assembly (Kho *et al*., 2003). A total of 11 deletion mutants with successive deletions from either the N- or the C-end of NP gene were constructed. C-terminal deletion constructs $NP_{\Delta C464}$, $NP_{\Delta C440}$, $NP_{\Delta C405}$, $NP_{\Delta C391}$, $NP_{\Delta C380}$, $NP_{\Delta C375}$, $NP_{\Delta C366}$ and $NP_{\Delta C245}$ differed from the full-length NP by deletion of 25, 49, 84, 98, 109, 114, 123 and 244 amino acids from the C terminus of the NP protein, respectively. N-terminal deletion mutants $NP_{\Delta N26}$, $NP_{\Delta N122}$ and $N_{\Delta PN245}$ were the result of 25, 121 and 244 amino acid deletions from the N terminus of the NP protein, respectively. All of the truncated proteins were successfully expressed in *E. coli* TOP 10 cells and purified using ammonium sulphate and sucrose gradient methods. Electron microscopic examination of the truncated NP fragments revealed that the region of the NP protein required for proper folding to form a herringbone-like particle is located in the NP N-terminal end, encompassing amino acids 1 to 375 (**Figure 4**). Additionally, the first 26 amino acids within this N-terminal region are also involved in the interaction with the P protein (Kho *et al*., 2004).

In contrast, the C-terminal end covering amino acids 376 to 489 is dispensable for the formation of herringbone-like particles (Kho *et al*., 2003). In addition, the C-terminal portion of the NP protein was shown to be exposed on the surface of the ring-like particles as observed by immunogold labeling analysis under electron microscopy (**Figure 5a;** Kho *et al*., 2001b).

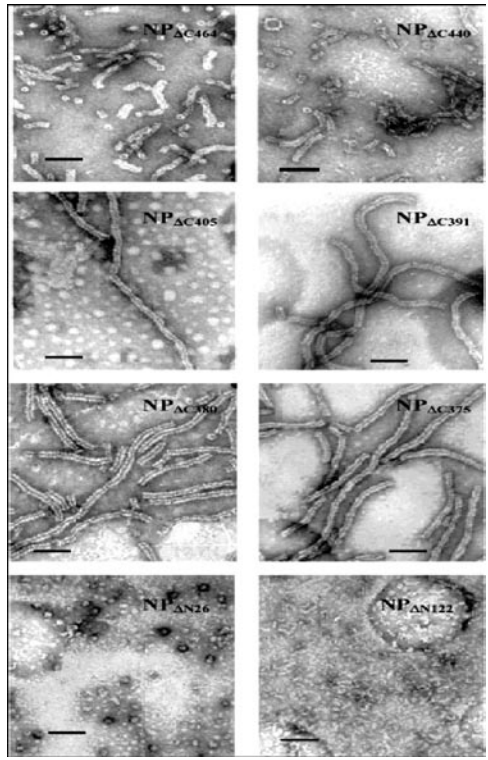

Figure 4 Electron micrographs of N- and C-terminally truncated NP proteins. $NP_{\Delta C464}$ showed the presence of ring and short herringbone-like particles. Similar structures were observed in $NP_{\Delta C440}$. Relatively longer herringbone-like particles were observed for $NP_{\Delta C405}$, $NP_{\Delta C391}$, $NP_{\Delta C380}$ and $NP_{\Delta C375}$. $NP_{\Delta N26}$ protein formed nearly homogeneous aggregates. No regular, ordered structures were seen in $NP_{\Delta N122}$, indicating complete loss of NP–NP interaction in this mutant. Bars represent 100 nm. (From Kho *et al*., 2003).

Kho *et al*. (2003) demonstrated that C-terminal truncated proteins ($NP_{\Delta C405}$, $NP_{\Delta C391}$, $NP_{\Delta C380}$ and $NP_{\Delta C375}$) are self-assembled *in vitro* to form long herringbone-like particles. A construct designated as $NP_{\Delta C391fus}$ which contained the first 391 amino acids of NP fused to 29 amino acids of a foreign peptide harbouring two kinds of epitopes (the *myc* tag and six His residues) was generated and expressed in *E. coli*. Subsequent analysis of the purified recombinant protein indicated that the original long herringbone-like particles was remarkably shorter that that of non-fusion. This observation suggests the possible role of the C-terminal end as a length determination region, and the C-terminus of NP protein seems to be flexible that the length of the herringbone-like particles could be varied by simply deleting or inserting some amino acids! It was thus possible to construct various recombinant NP fusion proteins to deliver immunogenic regions such as hepatitis B core antigen (Kho, 2003), the Nipah virus N protein (Kho, 2003), the immunodominant regions of the VP1 of human enterovirus 71 (Sivasamugham *et al*., 2003) as well as NDV glycoproteins (Rabu *et al*., 2002) without impairing the formation of the ring-like structure. The potential use of such constructs as a molecular carrier in the development of diagnostic kits and vaccines will be discussed later.

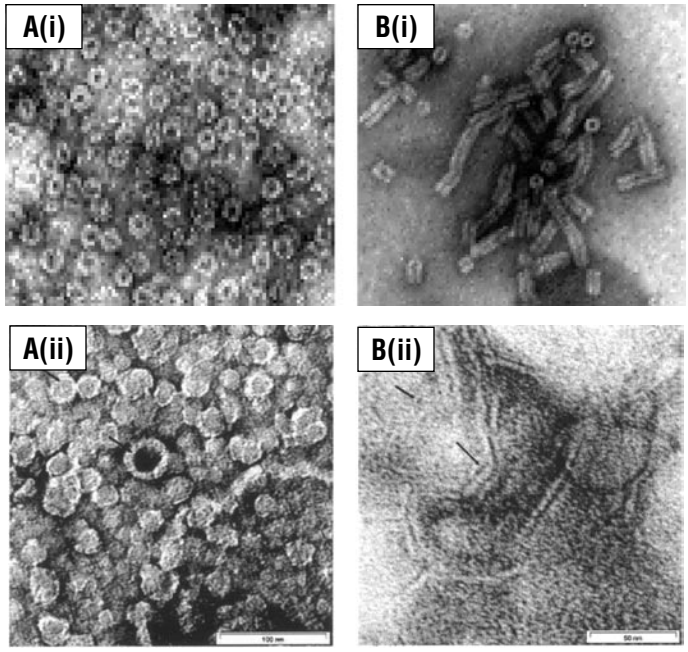

Figure 5 Transmission electron micrographs showing formation of (A) ring- and (B) herringbone-like nucleocapsid structures of (i) NDV and (ii) NiV. (Modified from Kho *et al*., 2001b; Eshaghi *et al*., 2005a)

Similarly, we have studied the genome of the local isolate of another paramyxovirus, the NiV (Abu Bakar *et al*., 2004) and showed that the C-terminal region of the nucleocapsid (N) protein in the Nipah virus (NiV) contains the major antigenic region when probed with antisera from humans and pigs infected naturally (Tan *et al*., 2004b). Solubility analysis of N- and C-terminally deleted mutants in several *E. coli* strains revealed that the full-length N protein has the highest solubility (Ong *et al*., 2002). To further study the N protein of NiV, a recombinant baculovirus was generated and transfected into *Sf9* insect cells. Electron microscopic analysis of the purified NiV N protein showed the presence of spherical and ring- and herringbone-like structures similar to

those of NDV (**Figure 5ii**). The purified nucleocapsids were polydispersed, and polymodal regression analysis indicated that they contained 96% small particles and 4% large particles. The buoyant density of the sample was found to be 1.08 ± 0.02 g/ml (Eshaghi *et al*., 2005a). Furthermore, a phage clone bearing the heptapeptide, SNRTQGE which closely resembled the sequence at the C-terminal of the N protein was selected through biopanning experiments against antibodies in pooled sera of naturally NiV-infected swine. This phage clone can be used as a novel diagnostic reagent in discriminating human and swine NiV sera-positive samples from sera-negative samples (Eshaghi *et al*., 2005c).

b. Phosphoprotein (P)

The polycistronic phosphoprotein (P) gene is located in second proximal to the 3' end of the genome. The *P* gene is approximately 1450 nucleotides and encodes the P protein of 395 amino acids with a calculated molecular weight 42 kDa (McGinnes *et al*., 1988). However, by SDS-PAGE analysis, the P protein has a relative mobility of 53-56 kDa (Kho *et al*., 2002). The acidic N-terminal domain of the P protein may account for this discrepancy (Curran *et al*., 1995a). As was mentioned earlier, two additional proteins, V and W, are expressed from mRNAs derived from transcriptional editing of the P gene (Samson *et al*., 1991; Steward *et al*., 1993). These two proteins share their amino (N) terminal domains with the P protein but vary at the carboxy (C) termini. The V protein has a cysteine-rich C-terminal domain which binds to zinc (Steward *et al*., 1995). This domain also inhibits interferon response and plays a role in the inhibition of apoptosis in certain hosts (Park *et al*., 2003).

It has been shown that the P proteins of many paramyxoviruses including NDV are oligomerized (Curran *et al*., 1995b; Jahanshiri *et al.,* 2005). The oligomerized P protein interacts with the L protein and NP-RNA template to form the minimal transcription unit of paramyxoviruses (Curran, 1998; Tabouriech *et*

al., 2000). In the P-L complex, P acts as a cofactor that serves both to stabilize the L protein (Smallwood *et al*., 1994) and to place the polymerase complex (P-L) on the NP-RNA template because the L protein by itself is unable to interact directly with the NP-RNA (Mellon and Emmerson, 1978; Horikami and Moyer, 1995). Jahanshiri (unpublished data; 2006) showed that the C-terminal half of the NP protein (amino acids 292 to 338) is involved in the stabilization of the L protein.

In addition, the P protein inhibits NP self-assembly. The unassembled NP (NP°) is the active form of soluble NP protein used to assemble the nascent RNA chain during genome replication. When the P protein is in this NP°-P complex, it inhibits NP self assembly, acting as a chaperon to prevent uncontrolled encapsidation of non-viral RNA by NP protein (Errington and Emmerson, 1997). To identify the domain(s) involved in the NDV P protein self-association and interaction with the NP, a yeast two-hybrid system was employed using full length P and its deletion derivatives (Jahanshiri *et al*., 2005). The 45 amino acids located at the C-terminal (amino acids 247 to 291), which is next to the L interactive region, seemed to be involved in both P:P as well as P:NP interactions (**Table 3**).

Table 3 P-P and NP-P interactions in the yeast two-hybrid system

Full length and deletion mutants of P[a]	ß-gal activity (%)[b]	
	P:P	P:NP
P_{1-395}	100	100
P_{51-395}	67	464
$P_{110-395}$	57	172
$P_{147-395}$	43	256
$P_{197-395}$	27	292
$P_{247-395}$	17	40
$P_{292-395}$	1	4
P_{1-352}	40	ND[c]
P_{1-291}	50	352
P_{1-246}	1	8
P_{1-196}	1	12
$P_{197-291}$	40	16

[a] The numbers in subscript indicate the amino acid residues cloned

[b] ß-gal activity of full length P clone was defined as 100%

[c] Not determined

(From Jahanshiri *et al*., 2005)

c. Large Protein (L)

The L protein, the largest but least abundant structural protein of NDV is coded by a 6700 bp gene and comprises 2204 amino acids with calculated molecular weight of approximately 249 kDa (Yusoff *et al*., 1987). The L protein possesses the RNA-dependent RNA polymerase activity and also encodes post-translational modification activities, such as capping, methylation and poly-adenylation of mRNAs (Yusoff and Tan, 2001). Sequence alignment studies of all the L

proteins of non-segmented negative-strand RNA viruses revealed the presence of six highly conserved domains, I to VI, which are joined by variable non-conserved regions (Poch *et al*., 1990; Sidhu *et al*., 1993). In a comparative sequence analysis of the L genes, we suggested that the L proteins could exist in two different forms due to a compensatory mutation in the highly conserved Domain V (Kusumaningtyas *et al*., 2004). This mutation significantly changed the hydrophobicity and total charge of the L protein. It is possible that this change in the protein structure could be related to the role of the protein in replication and that this compensatory mutation is an adaptation process of the virus to restore fitness in the environment. In a detailed analysis of these six domains within the transcriptive complex, Adzahar (unpublished data; 2006) showed that Domains III, IV and V interacted with both the P and NP proteins, but their levels of interaction were shown to be different. Although these domains share extensive sequence similarity with corresponding domains in other L proteins, the precise roles of these conserved domains in the L protein have not been determined.

MATRIX PROTEIN

The matrix protein is the smallest of the six structural proteins of NDV. It forms the internal viral envelope and contains 364 amino acids with a calculated molecular weight of approximately 40 kDa (Chambers *et al*., 1986c; Seal *et al*., 2000; unpublished data by Jemain, 1999). This protein is believed to play an important role in the assembly of the virus, by interacting with the nucleocapsid of NDV, the lipid bilayer of the host cell and also the glycoproteins which are exposed on the inner surface of the membrane. Although the M protein is not exposed to the significant immunological selection pressure, this protein exhibited antigenic variations which probably resulted from spontaneous mutations (Panshin *et al*., 1997).

HN AND F GLYCOPROTEINS

The HN and F glycoproteins are located on the envelope of the virus. They are responsible for virus entry and fusion into the host cell membrane; the HN protein mediates attachment of the virus to the cell, and the F protein mediates fusion of the viral envelope with the cell membranes (Yusoff and Tan, 2001). They appear to be essential for the viral replication, and as such could represent the primary target for neutralizing antibodies as well as a potential target for antiviral agents (Ali *et al*., 1996). We have shown that recombinant plasmids carrying the genes for both proteins were able to protect chickens against the virus (Loke *et al*., 2005).

The HN glycoprotein of NDV is a multifunctional protein. It possesses receptor recognition and neuraminidase (NA) activities. It recognizes sialic-acid containing receptors on cell surfaces. The NA removes the sialic acid in the virus to prevent it from self-agglutinating as well as promotes fusion activity of the F protein, thereby allowing the virus to penetrate the cell membrane (Yusoff and Tan, 2001). Nucleotide sequence analysis of the HN gene among 15 strains of NDV isolated in the past 50 years revealed that the whole HN gene comprises around 2000 nucleotides and carries an open reading frame encoding 571, 577, 581 or 616 amino acids (Sakaguchi *et al*., 1989; Tan *et al*., 1995; Yusoff *et al*., 1996). The largest of all, HN_{0616} can be converted to a biologically active HN protein by proteolytic cleavage of 45 residues from the C-terminus of the HN_0 precursor. However, the other three translation products of 571, 577 and 581 amino acids are already in their active form and are usually found in virulent strains (Yusoff *et al*., 1997; Romer-Oberdorfer *et al*., 2003). Thus, in principle, the length of the HN protein could be a pathogenic determinant of NDV. However, its contribution to the virulence of the virus is still inconclusive (Estevez *et al*., 2007).

Various monoclonal antibodies resistant mutants were used to delineate the antigenic sites (Chambers *et al*., 1988; Yusoff *et al*., 1988a,b; 1989a) as well

as locate the region involved in neuraminidase activity (Hughes *et al*., 1991). The HN glycoprotein is anchored in the viral envelope near its amino terminus (Lamb and Kolakofsky, 2001). Analysis of the HN gene showed that a stretch of amino acids ranging from 27 to 48 is highly hydrophobic, providing evidence for the amino terminal attachment of HN to the viral membrane (Yusoff and Tan, 2001). In general, the HN protein contains six potential glycosylation sites, five of which are conserved among the paramyxoviruses (Tan *et al*., 1995; Yusoff *et al*., 1996). Two of these sites, at residues 341 and 481, were confirmed to be glycosylated from point mutation analysis and X-ray structural analyses (Yusoff *et al*., 1988b; Crennel *et al*., 2000). The HN protein has been expressed in the *Autographa californica* unclear polyhedrosis virus (AcMNPV) derived vector under the control of the polyhedron promoter in infected *Sf9* insect cells and shown to retain its biological, functional and immunological activities (Ong *et al*., 1999a;b).

The F glycoprotein is the major virulence factor for NDV (Romer-Oberdorfer *et al*., 2003). It mediates fusion of the viral and cellular membranes synthesized as an inactive precursor, F_0 containing 553 amino acids with a calculated molecular weight 55 kDa (Chambers *et al*., 1986b; Salih *et al*., 2000). This F_0 precursor is proteolytically cleaved at the peptide bond of residues 116 and 117 at a region known as the F_0 cleavage site (GKQGR↓L), to generate two disulfide linked polypeptides, F_1 (48 to 54 kDa) and F_2 (10 to 16 kDa) by specific cellular proteases. This cleavage site on the F protein has been widely used for the identification of NDV (Yusoff *et al*., 1993; Kho *et al*., 2000; Khianizadeh *et al*., 2002). Unlike the HN, the transmembrane domain of the F glycoprotein, which anchors the protein in the membrane, is located near the C-terminus (residues 501 to 527). Therefore it is not surprising that the major neutralizing epitopes are located on the N-terminal domain of the F protein based on results from reciprocal competitive binding assay between anti-F monoclonal antibodies and cross-resistance plaque assays of escaped mutants raised against these

antibodies (Yusoff *et al*., 1989b). There are five to six potential glycosylation sites within the F glycoprotein. A conserved hydrophobic region of some 20 residues known as fusion peptide is located at the amino terminus of the F_1 fragment. This region is thought to participate directly in the fusion of viral and host cell membranes (Lamb and Kolakofsky, 2001). Like the HN protein, the recombinant F protein has also been expressed in *Sf9* insect cells using the AcMNPV (Salih *et al*., 2001). The recombinant virus was morphology distinct from the wild-type virus. The expressed recombinant F protein was uncleaved but glycosylated and could be recognized by NDV polyclonal antibodies. Therefore, it will be possible to produce the recombinant F protein in large quantities through this expression system.

MALAYSIAN ISOLATES OF NDV

NDV has a large economic impact on the Malaysian poultry industry, ranging from losses due to disease and the expense of vaccination to the huge cost of diagnostic laboratory investigations. For this reason, a heat-resistant NDV variant, V4(UPM), was isolated following a heat selection process of the V4(Que) strain (Simmons, 1967) and was developed as a thermostable feed pellet vaccine (Aini *et al*., 1990). This is now commercially available to local farmers (**Table 2; Figure 6**). Most of the vaccine trials carried out in Malaysia use the strain AF2240 for challenge. This viscerotropic-velogenic NDV strain was first isolated from a local form field outbreak in the 1960s and was reported to cause high mortality and morbidity in poultry. This isolate differs from the other NDV isolates by having a different HN protein length (Tan *et al*., 1995). The two local isolates, AF2240 and V4 (UPM), show significant higher thermostability in HA, NA and infectivity (Tan *et al*., 1995; Yusoff *et al*., 1996; Tang *et al*., 2000).

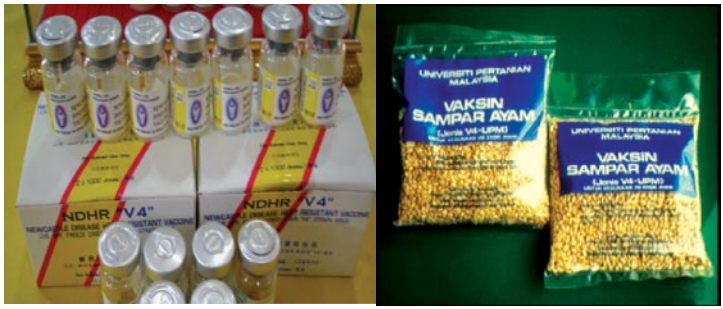

Figure 6 Heat-stable vaccines against Newcastle disease virus (Courtesy of Aini Ideris)

IDENTIFICATION AND DIFFERENTIATION

An early diagnosis of NDV would allow more effective and timely control of the disease. The most widely used methods for NDV detection are routine serological tests such as virus neutralization and haemagglutination-inhibition (HI) assays (Alexander, 1989). Although these tests are relatively inexpensive and simple to prepare, they can be time consuming and lack the required sensitivity when sera from other species are tested. Indirect enzyme-linked immunosorbent assays (ELISAs) have been frequently used to identify NDV. However it must be remembered that serological diagnosis is dependent on the expected immune status of the bird involved. Standardization of the results is often difficult to achieve since they depend on reference strains as well as reference antibodies. NDV specific antibodies are often not readily available to all laboratories. To address these problems, we have developed a reverse transcriptase-polymerase chain reaction (RT-PCR) method to detect the virus directly from infected allantoic fluids (Yusoff *et al*., 1993). Later, a one-tube nested RT-PCR technique that is coupled with an ELISA-based colorimetric detection system was developed (Kho *et al*., 2000). The single tube used in this test not only saves time and cost but also reduces the chance of contamination, especially when large numbers

of samples are being analysed. Sensitivity and specificity is enhanced by subjecting the PCR products to a second round of PCR with an internal set of primers (nested PCR) based on the consensus sequence of the F gene sequence. Instead of detection by agarose gel electrophoresis, which is simple but is often used specially for high sample throughput, an alternative colorimetric detection method involving the use of biotin-avidin peroxidase was used. Samples can be readily analysed visually or by using an ELISA reader (**Figure 7**). It has been shown that the technique was more sensitive than the gel electrophoresis or the HI test. No cross-reactions with other avian viruses such as infectious bronchitis virus, infectious bursal disease virus, avian influenza virus and fowl pox virus were observed.

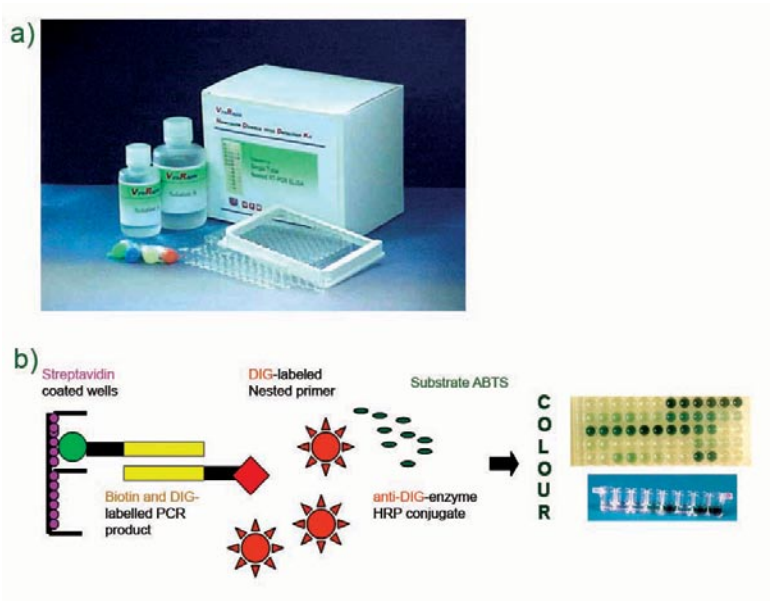

Figure 7 ELISA test for NDV based nested RT-PCR. Using nested primers, PCR products which have been labeled with biotin and digoxigenin could be captured onto stretavidin ELISA plates for colorimetric assay. Samples which were positive with NDV produced a dark green colour (From Kho *et al.*, 2000).

The efficacy of this nested PCR-ELISA technique was also compared with the conventional diagnostic tests for NDV (haemagglutination assay and non-nested RT-PCR) by testing against tissue specimens collected from chickens with signs of NDV infection. Results indicated that 60% of the tissue specimens were positive for NDV based on the nested RT-PCR whilst only 23% and 6% of them were positive by both the non-nested PCR and conventional haemagglutination assay, respectively.

As was mentioned earlier, extra amino acids can be attached to the C-terminus of the ring-like NP protein to become novel molecular carriers (Table 2a) for the presentation of epitopes in the development of diagnostics and sub-unit vaccines (Kho *et al*., 2001b; Rabu *et al*., 2002; Kho, 2003; Sivasamugham *et al*., 2006). Taking advantage of the fact that the recombinant NP protein can be readily synthesised in *E. coli* without impairment of the overall morphology, a large scale purification technique using expanded bed adsorption chromatography was established (Tan *et al*., 2005, 2006a; b). The protein can be purified directly from unclarified *E. coli* cells expressing the recombinant NP. By comparing this new technique with the conventional multistep centrifugation method, we showed that not only was the processing time reduced from 56 hours to 7.5 hours, but that a protein yield of 9.6% with a purification factor of 6.6 was achieved.

Similarly, various purification techniques and detection assays have been developed in our laboratory to grow and identify the avian poxvirus avian (Yusoff *et al*., 1992), influenza virus subtype H9N2 (Chaharaein *et al*., 2006) and NiV (Eshaghi *et al*., 2004a;b; 2005a;b;c; Ong *et al*., 2005; Tan *et al*., 2004b). There are however some limitations, such as its inability to distinguish the various NDV pathotypes. Again, an extra sequencing step is required for phatotyping. Whilst the velogenic and mesogenic strains are pathogeneic, the lentogenic strains are avirulent and have been used as live vaccines to control the disease (Yusoff and Tan, 2001). However, current diagnosis of NDV is unable to differentiate virulent NDV in a vaccinated flock of chicken. The ability to distinguish virulent NDV

strains in flocks is important because international veterinary regulatory bodies require a definitive diagnosis of virulent NDV to enable effective prevention of an outbreak by strict control measures and trade embargo restrictions (Aldous and Alexander, 2001).

Thus far, pathotyping of NDV strains involves the use of inoculated embryonated chicken eggs or chicks from which parameters such as mean death time (MDT), intracerebral pathogenicity index (ICPI) and intravenous pathogenicity index (IVPI) are determined (Alexander, 2001). These methods are laborious and time consuming. Over the past decade, numerous molecular techniques have been employed to identify and delineate NDV pathotypes: PCR coupled to restriction enzyme digestion (Stauber *et al*., 1995); PCR-sequencing (Seal *et al*., 1995) and real-time PCR (Aldous and Alexander, 2001). Many of these techniques use the sequence of the F protein cleavage site as the basis to distinguish the virulent viruses from the avirulent ones. The former contains a di-basic amino acid motif followed by a phenylalanine (F) residue within this site (RRQRR↓F) (Yusoff and Tan, 2001).

Tan *et al*. (2004a; 2007) developed a SYBR green I real time PCR technique to detect and distinguish the NDV pathotypes. Pathotype-specific primers were designed to detect specific sequence of velogenic strains and lentogenic/vaccine strains, respectively. After establishing the optimum condition of the real time PCR, the assay was performed on 20 previously characterized NDV strains which included 8 velogenic field isolates, 4 lentogenic field isolates and 8 vaccine strains. When the velogenic-specific primer was used against the velogenic strains, it showed early amplification (Ct<24) with melting temperature at 86.0 ± 0.283°C. On the other hand, when the lentogenic-specific primer was tested against the lentogenic/vaccine strains, it showed early amplification (Ct<24) with melting temperature at 87.4 ± 0.207°C.

Ramanujam *et al.* (2002; 2004) and Tan *et al.* (2004c) suggested an alternative approach for the NDV pathotyping. It was shown that a filamentous

M13 bacteriophage displaying a peptide bearing the TLTTKLY sequence, isolated from a phage displayed peptide library against the velogenic NDV strain AF2240, could be used to differentiate velogenic NDV strains from mesogenic and lentogenic strains via a newly established indirect phage ELISA. The drawback of this method is that this phage could not be used as a capturing reagent in a dot blot assay or ELISA for detecting unpurified NDV samples, because the phage bound nonspecifically to host proteins in allantoic fluid. Since purifying the samples is often time consuming and laborious, this method is not feasible for screening large flocks of birds during an outbreak where fast and reliable results are required. To overcome this limitation, Lee *et al*. (2006) recently performed biopanning experiments by using a disulfide constrained phage displayed heptapeptide library against three pathotypes of NDV strains: velogenic (highly virulent), mesogenic (moderately virulent) and lentogenic (avirulent). A phage clone bearing the peptide sequence SWGEYDM capable of distinguishing virulent from avirulent NDV strains was isolated. This phage clone was employed as a diagnostic reagent in a dot blot assay and it successfully detected only virulent NDV strains.

VIROTHERAPY AS CANCER MEDICINE

It has been known since early last century that NDV has the ability to infect and kill cancer cells (reviewed by Sinkovics and Horvath, 2000). The virus exhibits selective growth in and killing of a variety of cancer cells. This is because like similar to the life cycle of many other RNA viruses, the formation of double-stranded RNA (dsRNA) is integral in the replication and transcription of NDV. It is well known that dsRNA activates a spectrum of cellular defense mechanisms including the activation of the RNA binding protein kinase (PKR) and the release of interferon α and β (Williams, 1999). It is thus not surprising that tumour cells, which are usually defective in PKR activation and interferon response pathways, tend to be permissive to NDV infection. Moreover, its

replication occurs in the cytoplasm of host cells (**Figure 8**) and thus avoids the possibility of integration into the host genome which may result in deleterious complications.

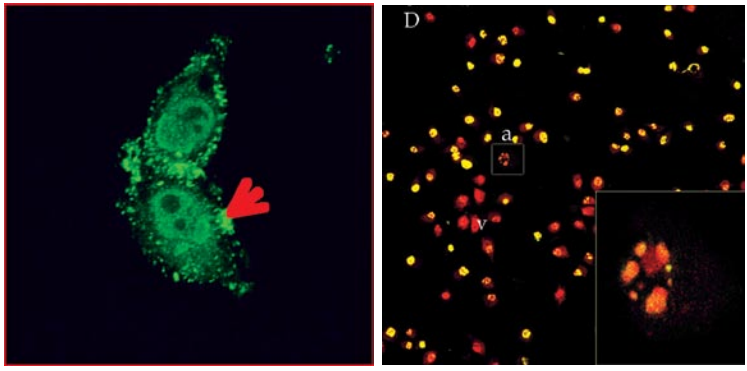

Figure 8 Effect of NDV infection in MCF-7 cancer cells. (A) Confocal micrograph of NDV in the cytoplasm of the infected cells; (B) Apoptotic bodies observed in the infected cells (From Fauziah *et al*., 2004; Zolkapli *et al*., 2004)

Advancements in reverse genetics has permitted the construction of recombinant NDV carrying transgenes such as the enhanced green fluorescent protein (EGFP) which can be retargeted to specific cancer cells (Kirn *et al*., 2002; Zhao and Peeters, 2003; Bian *et al*., 2005). It is therefore not surprising that the idea of NDV being used as an anti-cancer vaccine has been revived and its oncolytic properties studied in human clinical trials (Omar *et al.,* 2003). In many instances there were favorable results of partial to complete regression of tumors in various types of tumors including those in advanced stages that were not responsive to standard therapy (**Table 5**). The ability of NDV to selectively replicate in cancer cells is one of the most important features in the effectiveness of the virotherapy (**Figure 9**). Treatment with ATV in combination with a low-dose of recombinant interleukin-2 (IL-2) and interferon-alpha 2

(IFN-2a) was able to improve relapse-free and overall survival of patients with locally advanced renal cancer cells (Kirchner *et al*., 1995).

Table 5 Application of NDV as virotherapy against advanced tumours in clinical trials

Form and strain of NDV	Clinical Phase	Tumour targets
Live virus – PV701	I	Advanced solid tumours
Live virus – MTH68/H	-	Advanced tumours of the renal, colon and breast
VO - Cassel	II	Stage II malignant melanoma
VO- Ulster	II	Advanced tumours of the renal, colon and breast
ATV - Cassel	I	Colorectal carcinoma (Dukes stage B2, C, D)
ATV - Cassel	-	Renal carcinoma
ATV – La Sota IV	-	Advanced tumours of digestive tract
ATV – PV701	III	Breast carcinoma

ATV = autologous live cell NDV modified tumour vaccine; VO = viral oncolysate (Modified from Omar *et al*. (2003)

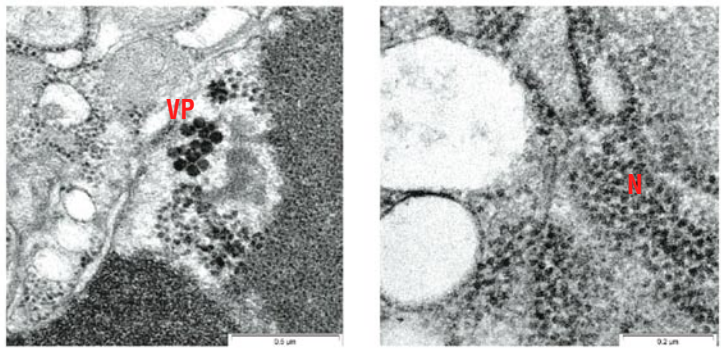

Figure 9 TEM micrograph of NDV-infected MCF-7 breast cancer cells at 12 hours post-infection. Note: virus particles (VP) and accumulation of nucleocapsids (N) in the cytoplasm. (Courtesy of Fauziah *et al*., 2004).

ONCOLYTIC NDV-INDUCES APOPTOSIS

Some but not all NDV strains are known to evoke cellular apoptosis in cancer cells during infection (**Figure 8**). Apoptosis which is an energy-dependent process of cell suicide is also known as programmed cell death. It is a natural response of the cells when exposed to a variety of stimuli. Apoptotic cells have a characteristic morphology (**Figure 10**) and show distinct biochemical processes that can be detected using transmission electron microscope (**Figures 11 and 12**) and expression of apoptotic gene markers (**Figure 8**) (Zolkapli *et al*., 2003; Fauziah *et al*., 2004). In general, the mechanisms associated with virus-induced apoptosis are associated with one or more of the host regulatory genes that function as an oncogene and/or tumour suppressor factor**.** Recently it has been shown that NDV cytotoxicity against tumor cells is due to multiple caspase-dependent pathways of apoptosis independent of interferon signaling competence. It is believed that NDV triggers apoptosis by activating the mitochondrial/intrinsic pathway and that it acts independently of the death receptor/extrinsic pathway (Elankumaran *et al*., 2006).

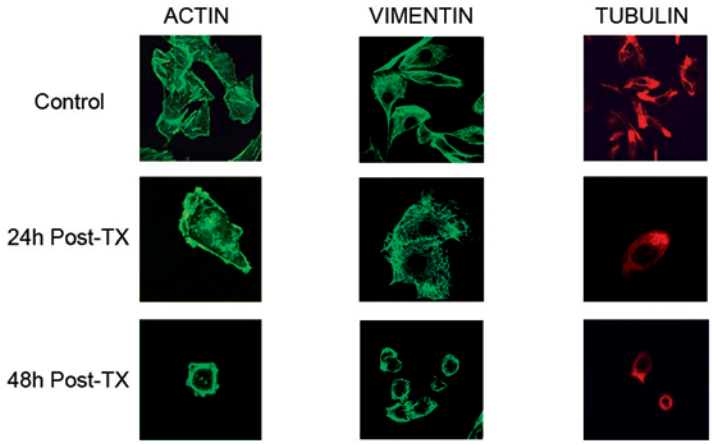

Figure 10 Confocal micrograph of cytoskeletal proteins of breast cancer cells treated with NDV (Courtesy of Zolkapli *et al.*, 2003).

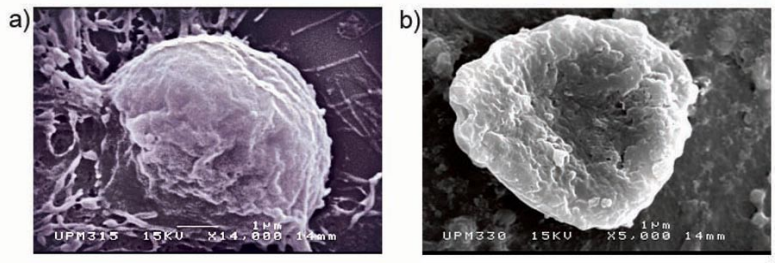

Figure 11 Scanning electron micrograph of MCF-7 breast cancer cell line untreated and treated (B) with AF2240 strain of NDV for 72 hours. Notice the shrunken with blebbing plasma membrane of the treated cells (B). (Courtesy of Fauziah *et al.*, 2004).

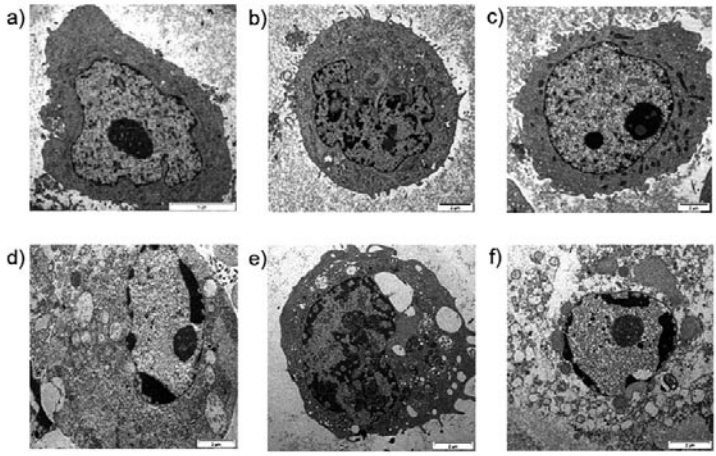

Figure12 TEM pictures of control (A) and treated (C-F) MCF-7 for 6, 12, 24, 48 and 72 hours respectively. Note the fragmented nucleus. (Courtesy of Fauziah *et al*., 2004).

ONCLOYTIC MALAYSIAN ISOLATES OF NDV

So far several different isolates of NDV have been isolated and characterized by researchers at UPM and the Veterinary Research Institute. In 2000, a collaborative project between UPM and USM, funded by the National Cancer Council (MAKNA), was launched with the primary target being the development of local NDV vaccines with oncolytic properties. The oncolytic effects of six (AF2240, 01/ C, Ijuk, S, F, V4) strains of NDV were screened on commercially available tumor cell lines, CEM-SS (T-lymphoblastic leukemic cells), MCF-7 and MDA-231 (breast cancer), HT29 (colorectal cancer) and HL60 (acute promelocytic leukemia). Based on the colorimetric microtiter (MTT) cytotoxicity assay, strains AF2240, F and V4 showed significant oncolytic effects on MDA-231 and MCF-7 cells whereas strain Ijuk showed significant killing of MDA-231 cells only. Strain V4 also showed significant killing effect on the CEM-SS, HT29

and HL60 tumor cells. Compared to V4 and F, the strain AF2240 was far more superior in destroying breast cancer cells. In most cases, regardless of NDV strains and cancer cells, the oncolytic effects were demonstrated only on cancer cells but not on normal (3T3) cells. However, inactivation of NDV abrogates the oncolytic activity on cancer cells (Omar *et al.*, 2003). Currently, studies are also underway in characterising the oncolytic effects of NDV strains AF2240. This local strain seems to have the potential to be developed as an anti-cancer agent for the treatment of cancer in humans. In a study by Zulkapli *et al.* (2004) and Fauziah *et al.* (2004) it was observed that strain AF2240 was able to infect MCF-7 cells resulting in apoptosis (**Figures 8 and 9**). All of the three types of cytoskeletal proteins, actin, vimentin and tubulin, showed degradation after 48 hours post-infection (**Figure 10**). The cells also exhibited blebbing and nuclear fragmentation (**Figures 11 and 12**).

INHIBITION OF VIRUS REPLICATION

One of the main drawbacks that limits the use of NDV *in vivo* anticancer treatment is that the virus binds to every cell, whether it is normal or a tumour cell since the sialic acid-containing receptors for the virus is ubiquitously expressed on cell surfaces. As mentioned earlier, the virus shows preferential replication in tumour cells. What happens if the virus mutates and causes systemic infection in humans? Is this going to be another newly re-emerging infectious disease? As a precautionary measure, we have constructed a novel therapeutic agent against NDV infection. Ramanujam *et al.* (2002), using filamentous bacteriophage M13 which displays random heptapeptide library on its PIII protein, showed that a fusion phage carrying the amino acid sequence TLTTKLY selected from the panning procedure, inhibited the propagation of NDV. An antibody competition assay revealed that the selected phage was capable of competing with the polyclonal antibodies raised against NDV for binding sites on the virus. Determination of the binding affinity of this phage

with NDV by an equilibrium binding assay in solution revealed two different dissociation constants, suggesting that there could be two distinct binding sites for the phage on NDV. Synthetic peptides with the sequence CTLTTKLYC, either in linear or cyclic conformations inhibited the binding of phage bearing the same sequence to NDV. These peptides also inhibited the hemolytic activity of the virus as well as its propagation (**Figure 13**) in embryonated chicken eggs (Ramanujam *et al*., 2002) as well as in mammalian cells (Ji *et al*., 2005). Hence the selected peptide may represent a lead anti viral compound for the treatment of viral infections.

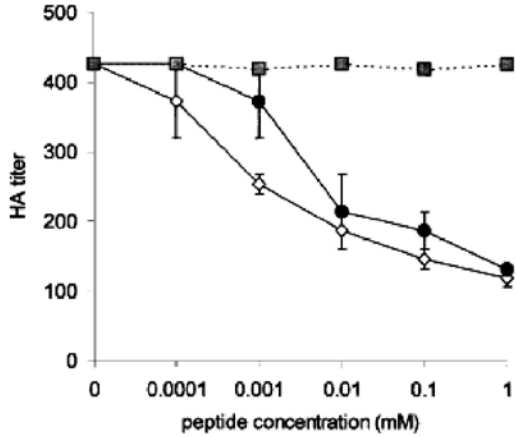

Figure 13 Inhibition of NDV propagation with synthetic peptides. Both the peptides inhibited NDV propagation in embryonated eggs with the cyclic peptide being the better inhibitor. The experiment was performed twice in triplicate and the error bars represent the standard deviation of the mean of the two sets of experiments. ◇ Cyclic peptide, ● linear peptide, ■ negative control (Ramanujam *et al*., 2002)

To delineate the structure-activity relationship of the peptide, CTLTTKLYC, mutational (alanine-substitution mutagenesis) and detailed molecular modeling studies based on NMR data were carried out (Chia *et al*., 2006). The peptide

displayed on the pIII protein of the filamentous M13 bacteriophage was synthesized and mutated in order to identify the amino acid residues involved in the interactions with NDV. Mutations of C1 and K6 to A1 and A6 did not affect the binding significantly, but substitution of Y8 with A8 significantly reduced the interaction suggesting that Y8 plays an important role in the peptide-virus interaction. The three-dimensional structure of the peptide was determined using circular dichroism (CD), nuclear magnetic resonance (NMR), and molecular modeling. We showed that the peptide exhibited two possible conformers; a structure with consecutive β-turns or one with a β-hairpin bend (**Figure 14**). Furthermore, the peptide in its cyclic form showed better activity than the linear peptide. The first β-turn in the peptide may stabilize the overall peptide conformation and contribute to the activity whilst the second β-turn may expose the Y8 residue in proper orientation to the receptor for interaction with the surface proteins of the virus.

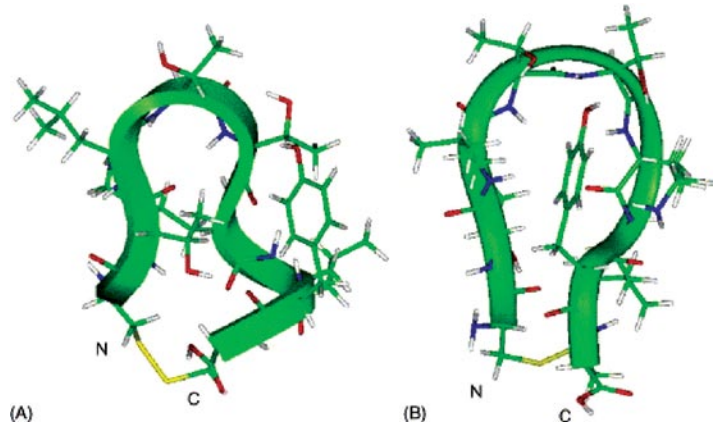

Figure 14. Proposed model for the cyclic peptide CTLTTKLYC. (A) Structure I represents an average structure from a family of structures that exhibits two consecutive β-turns. (B) Structure II represents an average structure of another family, which has β-hairpin bend type of conformation (From Chia *et al*., 2006).

CONCLUSION

In this Professorial Inaugural Lecture I have presented the research on the Newcastle disease virus and its journey from poultry to cancer. There are so many scientists worldwide working on the virus and the route undertaken to reach where we are today has been a long and tedious one. We have had to cross many hurdles along the way and faced many setbacks but we will surely be successful one day. Where do we go from here? The virus indeed exhibits very complex biological functions and interactions, the mechanism of many which are as yet unknown. NDV vaccines are now routinely used to control the disease in poultry, but have they got a new role now as a chemotherapeutic agent in humans? Through reverse genetics could we now construct recombinant NDV vaccines or nanobiomolecules which can deliver specific drugs targeted at the cancer cells? What would be the mode of delivery of the viruses as chemotherapeutic agents? Other issues include biosafety, efficacy, feasibility and production costs required to produce therapeutic grade "human vaccines" as well as comparability and compatibility with other currently used chemotherapeutic agents or adjuvants. The pharmacodynamics and the pharmacokinetics need be worked out as well as efficient modes of delivery identified. It has indeed opened up a whole new area of scientific, clinical and biomedical engineering programmes which will prove to be challenging, but certainly exciting and potentially hugely rewarding.

Another impeding hurdle will be public acceptance of using paramyxoviruses like NDV in virotherapy. The functions and interactions of the paramyxoviral proteins with the cell are associated with severe diseases in humans and animals. In the last two decades, previously unidentified paramyxoviruses have emerged as the cause of serious disease outbreaks in a number of animal species including humans. Despite the considerable progress made, complete understanding of the viral pathogenesis has yet to be achieved. Therefore, more emphasis needs to be placed on analyzing individual viral components and

their interactions with each other and cellular factors to fully understand their biological functions. This information along with advances in biomolecular engineering such as reverse genetic technology, would generate possibilities for new pharmaceutical products that will have impact on the prevention, diagnosis and treatment of diseases.

Let me end my Lecture by referring to Albert Einstein who made the following observations, "Madness is doing things over and over again, and expecting a different result" and "A discovery is said to be an accident meeting a prepared mind". I would regard these observations as immutable truths in expeditions of scientific enquiry and we may well keep these as reminders along the voyage. My students who continue to push the boundaries of Science, would, as I have, appreciate these immutable truths. John F. Kennedy is famous for his saying "Don't ask what your country can do for you but ask what you can do for your country. But is was his brother, Robert who would embolden us scientists on this voyage into the unknown. He said, "Only those who dare to fail greatly can ever achieve greatly". Furthermore he said, "There are those who look at things the way they are, and ask why….I dream of things that never were, and ask why not?" Despite being enunciated by a politician these can still be accepted as immutable truths by scientists!

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BIOGRAPHY

Khatijah Yusoff was born in Penang. After receiving her early education there, she won a Colombo Plan Scholarship to continue her tertiary education at the La Trobe University, Australia where she graduated with a First Class Honours degree in Microbiology in 1979. She then won a La Trobe University research scholarship to complete her PhD on "Genetic and molecular analysis of plasmid RP1: Interactions with prophage B3 and aspects of conjugal transmission" in 1983 under the supervision of Dr. Vilma A. Stanisich.

She joined the Universiti Putra Malaysia (then known as Universiti Pertanian Malaysia) as a lecturer in 1983. It was then that her research interest progressed from microbial genetics to the molecular biology of the Newcastle disease virus (NDV) and other paramyxoviruses. After working as a post-doctoral Research Associate to Professor Peter Emmerson at the University of Newcastle-upon-Tyne, UK, she began to develop her research on NDV by collaborating with Professor Abdul Latif Ibrahim. She was promoted to Associate Professor in 1994 and became a full Professor in 2001. She served as the Head of the Department of Biochemistry and Microbiology at the Faculty of Science and Environmental Studies in 2000, subsequently becoming the Deputy Dean for Research and Graduate Studies at the Faculty of Biotechnology and Biomolecular Sciences from 2004 till 2006 before she was promoted to Deanship. In 2007, Khatijah became the first woman to be appointed as the Deputy Vice Chancellor for Academic and International Affairs at UPM.

Paramyxoviruses continue to capture her interest and imagination to this day. Some of her work includes the determination of the first complete sequence of the L gene, epitope mapping of the haemagglutinin-neuraminidase and fusion proteins and the molecular biology of local NDV strains including the challenge strain AF2240 and a heat stable vaccine strain V4(UPM). Pursuing vaccine research, she is currently investigating the potential of developing recombinant vaccines in various expression systems and novel therapeutics using reversed

genetics as well as nanobiotechnology. Furthermore, she is studying the genome and functions of NDV through phage display technology and protein-virus interactions in order to develop specific antiviral drugs, diagnostic tests, characterise the virus receptor(s) and study the assembly and infection of NDV in cancer cells. Her other research interests include poultry viruses such as infectious bronchitis, infectious bursal disease and chicken anaemia viruses. Her research has also extended beyond viruses and poultry, to include human viruses such as the Nipah virus and the hepatitis B virus as well as *Escherichia coli, Campylobacter jejuni, Lactococcus lactis* and the genetic diversity of Asian water buffalo and fresh water fish.

In recognition of her teaching and research in microbiology, in particular on the molecular biology of NDV, Khatijah has received many national and international awards. Later this year, she will be receiving the Distinguished Alumni Award from her alma mater, La Trobe University, the ninth person to receive this honour from its over 120,000 alumni. Khatijah was elected as a Fellow of the Academy of Sciences Malaysia last year. She was awarded the UNESCO Carlos J. Finlay Prize for microbiology in 2005, the second Asian scientist to receive such an honour. She also won the National Young Scientist Award by the Ministry of Science, Technology and Environment in 1990. In 2002 in recognition of her contributions to the poultry industry Khatijah was honoured by the Houghton Trust to deliver the Houghton Lecture at the XIIth World Veterinary Poultry Association (WVPA) Congress, the first Asian scientist to be bestowed such an honour. In 2006, HRH the Sultan of Selangor bestowed upon her the Royal Award "Dato' Sultan Sharafuddin Idris Shah" (D.S.I.S.) which carries the title "Datin Paduka".

Khatijah's work is made possible by her receipt of support in the form of grants from various agencies and ministries. These include the La Trobe University Research Grant (Australia) and the UPM Research Grant as well as grants from the North of England Cancer Research Campaign (UK), Agriculture

and Food Research Council (UK), Australian Centre for International Agricultural Research (ACIAR), Third World Academy of Sciences (TWAS), Japan Society for the Promotion of Science (JSPS), Intensification of Research in Priority Areas (IRPA), National Biotechnology Directorate 'Top Down' Fund, Science Fund, Malaysian Genome Institute and the Fundamental Research Grant Scheme (FRGS).

She has published over 100 publications in cited refereed journals and presented over 300 papers at national and international conferences. In addition, she has filed several patents and trade marks, and currently holds a US as well as a Malaysian patent. Some of her achievements include an RT-PCR-ELISA test developed for mass screening of NDV in chickens; the NP protein of NDV genetically modified to act as a molecular carrier for drug and vaccine delivery systems and an anti-NDV peptide developed to inhibit NDV replication *in vivo* as well as to distinguish between chicken infected with the virulent field strains from those which have been vaccinated. These inventions have won her several medals at competitions on inventions and innovations.

Khatijah is also a dedicated lecturer and has been so acknowledged through receiving the Excellent Service Award on several occasions from UPM. She enjoys teaching students and through the interest instilled in them, many of her undergraduate students have continued their studies to postgraduate level and have themselves become academicians. She has served on various committees, particularly in the development and promotion of teaching and learning as well as in scientific research. Together with her colleagues, she has supervised over 100 postgraduate students, 75 of whom have graduated. She is also active in several local and international professional bodies. Khatijah is one of the founding members of the Malaysian Society for Molecular Biology and Biotechnology, and is also active in the Malaysian Society for Molecular Biology and Biochemistry, Malaysian Society for Microbiology and Malaysian Applied Biology Society. She has served as a member of several Editorial

Boards, as a member of the panel of reviewers for several journals and was formerly the Chief Editor for the Asia-Pacific Journal for Molecular Biology and Biotechnology. She is also a member of the Malaysian Genetic Modification Advisory Committee. At the international level, she is a member of the American Society for Microbiology (USA), Society for General Microbiology (UK) and Third World Organisation for Women in Science (TWOWS). Khatijah is an associate member of the International Molecular Biology Network (IMBN) for Asia and the Pacific Rim and she is also involved with the Consultative Group on International Agricultural Research (CGIAR) and the International Islamic Academy for Life Sciences and Biotechnology. Recently, Khatijah was appointed to the International Advisory Committee for Taif University, Saudi Arabia.

Last but not least, Khatijah is married to Professor Dato' Dr. Khalid Yusoff, a prominent cardiologist who is currently the Dean of the Medical Faculty, Universiti Technologi MARA and they are blessed with two wonderful children, Zul and Azzah.

ACKNOWLEDGEMENT

First and foremost, syukur alhamdullilah for His Blessings to allow me to make this journey of mine this far. My research has been supported by the La Trobe University Research Grant (Australia; 1983-1984); UPM Research Grant (1984-1985); the North of England Cancer Research Campaign (UK; 1985-1986), Agriculture and Food Research Council (UK; Project No. 492124; 1987-1988), Third World Academy of Sciences (TWAS; No. 93-074RG/BIO/AS), Japan Society for the Promotion of Science (JSPS; 1992), Intensification of Research in Priority Areas (IRPA No. 1-07-05-027; 01-02-05-27; 01-02-04-0379; 01-02-04-0074; 01-02-04-0107; 01-02-04-0053-EA001; 01-02-04-EA001; 09-02-04-0753-EA001), National Biotechnology Directorate 'Top Down' Fund; Nos. 06-02-09-001 BTK/TD/006; 01-02-0204-T002; 206-02-030-T002(6-02-03-128); 06-02-09-001 BTK/TD/002, Science Fund (02-01-04-SF0004), Malaysian Genome Institute (07-05-16-MGI-GMB13), and the Fundamental Research Grant Scheme (FRGS).

My very sincere thanks to my teachers, mentors, colleagues, friends and students for their continued support and for making the environment conducive and enjoyable.

Last and most important of all, my very special thank you (with lots of hugs and kisses) to my family, especially Khalid, Zul and Azzah for their understanding of how important research is in my life and for taking all those bribes to allow me to work in a really fascinating field!

LIST OF INAUGURAL LECTURES

1. Prof. Dr. Sulaiman M. Yassin
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2. Prof. Ir. Abang Abdullah Abang Ali
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3. Prof. Dr. Abdul Rahman Abdul Razak
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4. Prof. Dr. Mohamed Suleiman
   Numerical Solution of Ordinary Differential Equations: A Historical Perspective
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5. Prof. Dr. Mohd. Ariff Hussein
   Changing Roles of Agricultural Economics
   5 March 1994

6. Prof. Dr. Mohd. Ismail Ahmad
   Marketing Management: Prospects and Challenges for Agriculture
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7. Prof. Dr. Mohamed Mahyuddin Mohd. Dahan
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   20 April 1994

8. Prof. Dr. Ruth Kiew
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9. Prof. Ir. Dr. Mohd. Zohadie Bardaie
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10. Prof. Dr. Shamsuddin Jusop
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11. Prof. Dr. Abdul Salam Abdullah
    Natural Toxicants Affecting Animal Health and Production
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12. Prof. Dr. Mohd. Yusof Hussein
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    9 July 1994

13. Prof. Dr. Kapt. Mohd. Ibrahim Haji Mohamed
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14. Prof. Dr. Hj. Amat Juhari Moain
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15. Prof. Dr. Law Ah Theem
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18. Prof. Dr. Mohamed Shariff Mohamed Din
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20. Prof. Dr. Abdul Hamid Mahmood
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