UNIVERSITI PUTRA MALAYSIA

WHOLE GENOME SEQUENCING, CHARACTERISATION AND IMMUNE –RELATED GENE PROFILING OF THE MALAYSIAN GENOTYPE VII NEWCASTLE DISEASE VIRUS IN CHICKENS

DILAN AMILA SATHARASINGHE

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By

DILAN AMILA SATHARASINGHE

**Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree
of Doctor of Philosophy**

May 2016

DEDICATION

This thesis is dedicated

to my parents

for their endless love and support

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Doctor of Philosophy

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Chairman : Abdul Rahman Omar, DVM, PhD
Institute : Institute of Bioscience

Among the different strains of Newcastle disease virus (NDV), genotype VII NDV is the predominant velogenic NDV circulating in South-East Asia including Malaysia causing outbreaks even in well-vaccinated flocks. However, the complete genome sequences of genotype VII Malaysian NDV isolates have never been characterised. Furthermore, the ability of the virus to replicate in different tissues by modulating the host innate immune responses is poorly studied. In this study, we described the application and the establishment of a simple, robust and less resource demanding protocol for whole genome sequencing of NDV by using the Next-Generation Sequencing (NGS) technology on the Illumina MiSeq platform. Five overlapping primer sets were designed to amplify the complete genome of 5 Malaysian NDVs isolated from year 2004 to 2013. Amplified PCR products were subjected to NGS library preparation followed by sequencing in NGS platform. The generated raw sequencing data was imported to genome assembly software to generate consensus by *denovo* assembly and map to reference. This protocol was able to generate accurate consensus-level full genome sequence for 5 virulent NDV isolates (MB128/04, MB076/05, IBS002/11, IBS005/11 and IBS025/13) obtained from outbreaks that occurred from 2004 to 2013. Intracerebral pathogenicity index and cleavage site motif were assessed to determine the pathoypes and virulence of the NDVs. All the isolates exhibited ICPI values more than 1.5 and demonstrated cleavage site motif ^{112}R/K-R-Q-R/K-R↓F^{117} of F gene which is cleavable by a wide range of proteases, confirming the velogenic nature of the isolates. In order to determine the evolution of the isolated Malaysian NDVs from 2004 to 2013, phylogenetic and pairwise comparisons analysis based on complete genome sequence and all proteins were performed. The phylogenetic analysis of partial fusion (F) gene sequences revealed that all the 5 NDV isolates could be grouped under genotype VII, sub-genotype VIIa, or under Lineage 5a viruses. However, the

recent isolates of 2011 (IBS002/2011 and IBS005/2011) have higher genetic distance than NDV isolates of 2004 and 2005 when compared to genotype II vaccine strains. Furthermore, the complete genome sequence of IBS025/13 NDV isolate revealed a natural recombination of genotype II and VII. Further, both phylogenetic and recombination analysis of this isolate confirmed that the nucleoprotein (NP) and the phosphoprotein (P) shared a sequence motif of genotype II, while the rest of the proteins shared genotype VII characters. In addition, the isolate has a genome size of 15,186 nucleotides, which is similar to early genotypes (I-IV) NDVs isolated during the period of 1930 to 1960. The viral copy numbers in infected spleen, lungs and duodenum of Specific-pathogen-free (SPF) chickens with different NDV isolates were quantified by quantitative reverse transcriptase real time PCR. In addition, selected immune-related genes expressions upon infections were assessed by quantitative PCR. Specific-pathogen-free (SPF) chickens infected with the genotype VII isolates MB076/05, IBS002/11 and IBS005/11, as well as the natural recombinant genotype VII, IBS025/13 showed significantly higher viral load ($p < 0.05$) in spleen, lungs and duodenum compared to respective tissues of SPF chickens infected with genotype VIII isolate, AF2240-I. Furthermore, the natural recombinant genotype VII isolate showed the highest virus copy numbers ($p < 0.05$) in infected spleen, lungs and duodenum compared to other genotype VII and VIII isolates suggesting that the recombination event may play a role in virus replication and tissue tropism. The ability of genotype VII viruses to produce high viral load is probably due to the ability of the viruses to inhibit the expression of IFN-α and MDA5 activities at an early phase (12 hpi) of infections. A similar trend for IFN-α and MDA5 expressions was also detected in the infected lungs and duodenum at 12 hpi. A more destructive innate immune responses driven by significant up-regulation of IFN-γ by genotype VII isolates MB076/05, IBS002/11, IBS005/11 and IBS025/13 in spleen and lungs were also detected at later stage of infection at 48 hpi compared to genotype VIII AF2240-1 infected spleen and lungs. In conclusion, we have successfully established a protocol based on NGS technology for complete genome sequencing of NDV, which can be used as part of the diagnostic tools in molecular characterisation and evolution of NDV. Characterisation of NDV isolates in this study has successfully determined the virus tropism and their involvement in influencing the production of important cytokines that associated with innate immune responses.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENJUJUKAN GENOM MENYELURUH, PENCIRIAN DAN PEMPROFILAN GEN BERKAITAN-IMUN VIRUS PENYAKIT SAMPAR GENOTIP VII MALAYSIA PADA AYAM

Oleh

DILAN AMILA SATHARASINGHE

Mei 2016

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Di antara pelbagai strain virus penyakit sampar (NDV), NDV genotip VII adalah merupakan jenis NDV velogenik utama yang beredar di Asia Tenggara termasuk Malaysia menyebabkan wabak biarpun dalam kelompok yang telah divaksinasi dengan baik. Walau bagaimanapun, penjujukan genom NDV genotip VII Malaysia yang lengkap belum pernah dicirikan. Tambahan pula, keupayaan virus tersebut untuk mereplikasi di dalam tisu yang berbeza dengan cara mengubah tindakbalas keimunan semula jadi perumah kurang dikaji. Di dalam kajian ini, kami menerangkan perkembangan dan penggunaan satu protokol yang mudah, tahan lasak dan kurang kebergantungan pada sumber bagi penjujukan genom menyeluruh NDV menggunakan teknologi penjujukan generasi mendatang (NGS) pada landasan Illumina MiSeq. Lima set primer bertindih telah direka bagi mengamplifikasi genom keseluruhan lima NDV Malaysia yang dipencilkan dari tahun 2004 hingga 2013. Produk-produk PCR yang telah diamplifikasi kemudiannya menjalani penyediaan bagi perpustakaan NGS diikuti dengan penjujukan pada landasan NGS. Data penjujukan mentah yang terhasil telah diimport ke perisian himpunan genom bagi menjana kesepakatan secara himpunan *de novo* dan pemetaan kepada rujukan. Protokol ini berupaya menjana jujukan genom penuh mengikut tahap persetujuan yang tepat bagi 5 isolat NDV virulen (MB128/04, MB076/05, IBS002/11, IBS005/11 dan IBS025/13) yang didapati dari wabak semasa 2004 hingga 2013. Indeks kepatogenan intraserebrum (ICPI) dan motif pembelahan tapak telah ditaksir bagi menentukan patotip dan kevirulenan NDV tersebut. Kesemua isolat ini mempamerkan nilai ICPI yang lebih daripada 1.5 dan menunjukkan motif pembelahan tapak ^{112}R/K-R-Q-R/K-R↓F^{117} pada gen F yang berupaya dibelah oleh pelbagai jenis protease, dengan itu mengesahkan sifat velogenik isolat tersebut. Bagi menentukan evolusi NDV Malaysia yang telah dipencilkan dari 2004 hingga 2013, filogenetik dan analisis perbandingan

secara berpasangan ke atas jujukan keseluruhan genom dan kesemua protein-protein telah dijalankan. Analisis filogenetik penjujukan gabungan separa gen F memperlihatkan bahawa kelima-lima isolat NDV tersebut boleh digolongkan di bawah genotip VII, sub-genotip VIIa, atau di bawah salasilah virus 5a. Walau bagaimanapun, isolat terkini dari 2011 (IBS002/2011 dan IBS0052011) mempunyai jarak genetik yang lebih tinggi berbanding isolat NDV dari 2004 hingga 2005 apabila dibandingkan dengan strain vaksin genotip II. Tambahan lagi, penjujukan genom isolat NDV IBS025/13 yang lengkap menunjukkan rekombinasi semula jadi genotip II dan VII. Analisis filogenetik dan rekombinasi isolat ini mengesahkan bahawa protein nukleokapsid (NP) dan fosfoprotein (P) berkongsi jujukan motif genotip II dan protein selebihnya berkongsi ciri genotip VII. Sebagai tambahan, isolat tersebut mempunyai saiz genom sebesar 15,186 nukleotida, di mana ia adalah sama dengan genotip NDV terdahulu (I-IV) yang dipencilkan sekitar tahun 1930 hingga 1960. Jumlah salinan virus pada limpa, paru-paru dan duodenum ayam bebas patogen khusus (SPF) yang dijangkiti dengan isolat-isolat NDV berbeza telah ditentukan secara PCR masa nyata transkriptase berbalik kuantitatif. Sebagai tambahan, penzahiran gen-gen berkaitan imun terpilih akibat jangkitan telah ditaksirkan secara PCR kuantitatif. Ayam bebas patogen khusus (SPF) yang dijangkiti isolat genotip VII MB076/05, IBS002/11, IBS005/11 dan rekombinan semula jadi genotip VII menunjukkan replikasi virus yang lebih tinggi secara signifikan ($p < 0.05$) dalam limpa, paru-paru dan duodenum berbanding tisu-tisu yang berkaitan pada ayam SPF yang dijangkiti isolat genotip VIII, AF2240-1. Sebagai tambahan, isolat rekombinan semula jadi genotip VII IBS025/13 menunjukkan replikasi virus tertinggi ($p < 0.05$) dalam limpa, paru-paru dan duodenum yang dijangkiti berbanding isolat genotip VII dan VIII yang lain memberi gambaran bahawa rekombinasi mungkin memainkan peranan dalam replikasi virus dan tropism tisu. Keupayaan virus genotip VII untuk menghasil replikasi virus tinggi mungkin disebabkan keupayaan virus tersebut untuk merencat ekspresi IFN-α dan MDA5 pada fasa awal (12 hpi) jangkitan. Kecenderungan ekspresi yang sama IFN-α dan MDA5 juga telah dikesan dalam paru-paru dan duodenum yang dijangkiti pada 12 hpi. Suatu tindakbalas keimunan semula jadi yang lebih membinasakan dipacu oleh pengawalaturan meningkat yang signifikan ke atas IFN-γ oleh isolat genotip VII MB076/05, IBS002/11, IBS005/11 dan IBS025/13 pada limpa dan paru-paru juga telah dikesan di peringkat akhir jangkitan pada 48 hpi berbanding pada limpa dan paru-paru yang dijangkiti genotip VIII AF2240-1. Sebagai kesimpulannya, kami telah berjaya membangunkan suatu protokol berasaskan teknologi NGS bagi penjujukan genom NDV yang lengkap yang boleh digunakan sebagai sebahagian daripada alat diagnostik dalam pencirian molekular dan evolusi NDV. Pencirian isolat NDV dalam kajian ini telah berjaya dalam mencirikan tropism virus dan peranan virus dalam menganggu penghasilan sitokin penting yang terlibat dengan sistem keimunan inat.

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I certify that a Thesis Examination Committee has met on 11 May 2016 to conduct the final examination of Dilan Amila Satharasinghe on his thesis entitled "Whole Genome Sequencing, Characterisation and Immune-Related Gene Profiling of the Malaysian Genotype Vii Newcastle Disease Virus in Chickens" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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aa	Amino acid
APC	Antigen presenting cells
APMV-1	Avian *Paramyxovirus* -1
ATM	Amplicon tagment mix
AVMA	American Veterinary Medical Association
BIC	Lowest bayesian information criterion
BLAST	Basic local alignment search tool
BLASTN	Basic local alignment search tool for nucleotide databases
bp	Base pairs
cDNA	Compliment deoxyribonucleic acid
CDS	coding strand
CMI	Cell-mediate immunity
Cq	Quantitative cycle value
CT	Threshold cycle
CTL	Cytotoxic t lymphocyte
DDT	Dithiothreitol
DNA	Deoxyribonucleic acid
DNTP	Deoxynucleotide
DSDNA	Double stranded deoxyribonucleic acid
ELD_{50}	Mean Egg Lethal Dose
ELISA	Enzyme linked immunosorbent assay

F	Fusion protein
FAO	Food and Agriculture Organisation
FFPE	Formalin-fixed-paraffin-embedded
GAPDH	Glyceraldehyde-3-phosphate-dehydrogenase
GDNA	Genomic DNA
HA	Haemagglutination
HAU	Haemagglutinating unit
HI	Haemagglutinin inhibition
HIV	Human immunodeficiency virus
HN	Haemagglutinin and neuraminidase
hpi	Hours after post inoculation
HS	High sensitivity
HVT	Herpesvirus of turkeys
IACUC	Institutional Animal Care and Use Committee
IB	Infectious bronchitis
IBD	Infectious bursal disease
IBS	Institute of Bioscience
ICPI	Intracerebral pathogenicity index
IFIT-5	Interferon-induced protein with tetratricopeptide repeats 5
IFN	Interferon
Ig	Immunoglobulin
IHC	Immunohistochemistry

IL	Interleukin
iNOS	Inducible nitric oxide synthase
ISCOMS	Immune stimulatory complex
ISG	Interferon-stimulated gene
ISRGS	Interferon-stimulated response genes
IVPI	Intravenous pathogenicity index
kbp	Kilo base pairs
kDa	Kilodalton
L	Large polymerase protein
M	Matrix protein
MDA5	Melanoma differentiation associated protein-5
MDT	Mean death time
MHC I	Major histocompatibility complex class I
MIP-3A	Macrophage inflammatory protein-3 alpha
mRNA	messenger Ribonucleic acid
Mx	Myxovirus resistance gene
NA	Neuraminidase
NCBI	National centre for biotechnology information
NCR	Non-coding region
ND	Newcastle Disease
NDV	Newcastle Disease virus
ng/µL	Nanogram/microlitre
NGS	Next-Generation Sequencing

NK	Natural killer
nM	Nanomolar
NO	Nitric oxide
NP	Nucleoprotein
NPM	Nextera PCR Master mix
NT	Neutralise tagment buffer
nts	Nucleotides
NVNDV	Neurotropic Velogenic Newcastle Disease virus
OIE	World Organisation of Animal Health
ORF	Open reading frame
P	Phosphoprotein
PCR	Polymerase Chain Reaction
PAMP	Pathogen associated molecular pattern
PASC	Pairwise sequence comparisons
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffer saline
pg	Picogram
PI	Percentage identity
pM	Picomolar
PRR	Pattern recognition receptor
qPCR	Quantitative polymerase chain reaction
qRT-PCR	Quantitative reverse transcriptase –Polymerase Chain Reaction

RACE	Rapid amplification of cDNA ends
RDP	Recombinant detection program
RIG- I	Retinoic-acid-inducible gene I
RNA	Ribonucleic acid
rRNA	Ribosomal Ribonucleic acid
RRT-PCR	Real time reverse transcription Polymerase Chain Reaction
RSB	Resuspension buffer
RT	Reverse transcription
RT-PCR	Reverse transcription Polymerase Chain Reaction
SIMPLOT	A standard similarity plot
SNPS	Single nucleotide polymorphisms
SPF	Specific-pathogen–free
TAE	Tris-acetate buffer
Taq	*Thermus aquaticus*
TD	Tagment DNA buffer
Th 1	T helper cell type 1
Th2	T helper cell type 2
TLR	Toll-like receptors
Treg	T regulatory cells
UTR	Un-translated regions
V	V protein
VG/GA	Villegas-Glisson/University of Georgia

VNND	Velogenic Neurotropic Newcastle Disease
VRI	Veterinary Research Institute
v/v	Volume/ volume
VVND	Velogenic visceroptropic Newcastle Disease
W	W protein
w/v	weight/volume
WHO	World Health Organisation
µg	Microgram
µL	Microlitre
µM	Micromolar

CHAPTER 1

GENERAL INTRODUCTION

1.1. Background

Newcastle Disease (ND) is one of the most important infectious diseases of poultry owing to its' global distribution and potential for devastating losses. The disease is caused by the Newcastle Disease virus (NDV), which potentially infects most species of birds, and for susceptible poultry it is highly infectious and usually lethal (Alexander, 1988). The ND is reported across at least six of the seven continents of the world and endemic in many countries. Because of its worldwide occurrence and geographical distribution, the World Organisation of Animal Health (OIE) has defined ND as a "notifiable disease" if the virus has an intracerebral pathogenicity index (ICPI) equal to or higher than 0.7 and/or consists multiple basic amino acids at the C-terminus of the fusion protein cleavage site (OIE, 2015).

NDV is an enveloped RNA virus which is also referred to as avian paramyxovirus-1 (APMV-1) which belongs to the genus Avulavirus of the family Paramyxoviridae (Fauquet & Fargette, 2005; Mayo, 2002). The virus has a negative sense, single-stranded, non-segmented RNA genome of approximately 15.2 kbp (kilo base pairs) (Alexander & Senne, 2008a). The genome consists of 6 genes in the order of 3'-NP-P-M-F-HN-L-5', respectively. Three of the proteins, namely nucleoprotein (NP), phospoprotein (P) and large polymerase protein (L) are constituents of nucleocapsid of the virion whilst the inner layer of the virion is made out from the matrix (M) protein. Meanwhile, two of the external glycoproteins are the fusion protein (F) and haemagglutinin-neuraminidase protein (HN) present on the surface of the virus. In addition, two non-structural proteins V and W are produced by the RNA editing process of P gene transcription (Steward *et al.*, 1993).

Based on the severity of the disease, NDV strains are classified into three pathotypes namely velogenic, mesogenic and lentogenic (Brown *et al.*, 1999). Velogenic viruses are further classified into viscerotropic and neurotropic based on their subsequent behaviour once inoculated to adult birds (Alexander & Senne, 2008b). Both types of velogenic viruses cause severe clinical disease, but based on the clinical manifestation they are distinguishable. Severe systemic illness, with mark depression and mortality within five days of infection, are the clinical symptoms of birds infected with velogenic viscerotropic NDV (VVND) (Kommers *et al.*, 2001; Brown *et al.*, 1999). In

contrast, velogenic neurotropic strains (VNND) infected birds have extended duration of infection and neurological signs such as paresis and paralysis as the predominant clinical symptoms (Brown *et al*., 1999). Chickens infected with mesogenic strains may show respiratory and neurological signs (Bhaiyat *et al*., 1995) or no obvious clinical signs in the infected chickens (Brown *et al*., 1999). Lentogenic NDV does not generally cause apparent clinical signs in adult chickens (Brown *et al*., 1999).

Since different strains of NDV demonstrated different pathogenicity and virulence, numerous studies have conducted to assess the role of HN, F and a non-structural V protein on the virulence of the respected pathotypes. The F protein mediates the fusion of the viral envelope with the host cell membrane (Lamb *et al*., 2006). Cleavage of the F protein in virulent NDV strains take place intracellular by pervasive subtilisin-like proteases such as furin, PC6 and PACE 4, whereas cleavage in avirulent strains occurs by trypsin-like enzymes only found in certain tissues (Fujii *et al*., 1999). The HN protein of NDV is a multifunctional protein that involved in both receptor identification and neuraminidase (NA) activities linked with the virus. Recognition of sialic acid-containing receptors on host cell surface, promotion of fusion activity of F protein and assisting to release the sialic acid from progeny virus particles by NA, which eventually prevents self-agglutination of progeny viruses are the functions steered by HN protein (Lamb & Kolakofsky, 1996). NDV, V protein functions as an IFN antagonist by preventing the increase in type I IFNs by NDV infection (Jang *et al*., 2010).

Although NDV strains are grouped as APMV-1, the viruses showed vast genetic diversity (Alexander & Senne 2008b; Kim *et al*., 2007a; Aldous *et al*., 2003). With no incongruity there are two methods which are currently been used to classify the NDV strains according to genomic information in which both methods use the partial F gene sequence of NDV genome classification (Courtney *et al*., 2013; Snoeck *et al*., 2013; Miller *et al*., 2010). Aldous et al. (2003) grouped NDV into 6 lineages and 13 sub-lineages which were later included with 3 sub-lineages (Snoeck *et al*., 2008). According to the second system, NDVs are classified into two major groups, represented by class I and class II. Class I viruses were further subdivided into nine genotypes and class II into ten genotypes (Kim *et al*., 2007b; Czeglédi *et al*., 2006; Ballagi-Pordany *et al*., 1996). Generally, there are only minor differences between these two classification systems (Miller *et al*., 2007).

Several studies revealed that genotype V, VI and VII of class II viruses were the most prevalent viruses currently circulating worldwide. Out of these, genotype VII viruses were predominantly isolated from recent NDV outbreaks in Asia, Africa, Middle East and South America (Zhang *et al*., 2014; Perozo *et al*., 2012a; Khan *et al*., 2010). Several studies have demonstrated that high viral replication

and intense innate immune response are associated with severe pathological lesions of lymphoid tissues following infection with NDV genotype VIId strains (Hu *et al.,* 2015; Hu *et al*., 2012; Ecco *et al*., 2011a). In addition, multiple genotypes associated recombination events have also been reported for NDV strains isolated from Indonesia (Han *et al*., 2008) and China (Qin *et al*., 2008a).

Prophylactic vaccination is practiced to control ND throughout the world (Miller *et al*., 2007). Despite the practise of mass vaccination, NDV outbreaks have been reported throughout the world and led to substantial economic losses to the industry over past years (Miller *et al*., 2010). Recent research has observed incomplete protection and virus shedding following vaccination may contribute to transmission of the virus to susceptible chickens, which eventually lead to outbreaks (Samuel *et al* 2013; Xiao *et al*., 2012). Eight isolates recovered from ND suspected cases from various states of Malaysia during 2004-2005 have been characterised as genotype VII based on the partial F gene sequence (Tan *et al*., 2010). In addition, previous studies revealed that infection with genotype VII shed more viruses than other virulent genotypes following vaccination with currently available commercial vaccines (Kiarash *et al*., 2015; Susta *et al*., 2014; Samual *et al*., 2013).

In order to establish an efficient control strategy and develop effective vaccines, a greater understanding of the mechanism of pathogenesis of the currently circulating virulent NDVs is required (Rue *et al*., 2011). Results from previous independent studies demonstrated that velogenic NDV strains belong to different genotypes induce distinct pathological changes that correlates with strong innate immune responses through up regulating groups of genes associated with innate anti-viral and inflammatory responses (Rasoli *et al*., 2014; Ebrahimi *et al*., 2012; Mase *et al*., 2002). The intense innate immune responses may contribute to the high mortality and severe pathological damage in infected chickens indicating their involvement in the pathogenesis of the virus (Liu *et al*., 2012; Rue *et al*., 2011).

Similar to other RNA viruses, NDV has a high genetic instability and potential for evolution and mutations (Miller *et al*., 2010). A sequence analysis of selected regions of NDV will not be able to identify recombination including the F and HN genes (Song *et al*., 2011). Complete genome sequencing using Sanger sequencing protocol requires large quantities of starting materials (Logan *et al*., 2014) and high resource demanding methods such as cloning to vectors prior to the sequence acquisition (Barzon *et al*., 2013). The advancements in whole genome sequencing technology and bioinformatics analysis, facilitated discovery of several NDV strains with recombination events and evolution diversity of NDV (Chong *et al*., 2010; Qin *et al*., 2008a).

1.2. Problem of Statements

Although intensive vaccination programs have been implemented in Malaysia; ND outbreaks have been reported even in vaccinated farms (Berhanu *et al*., 2010). More recently, an increased in NDV outbreaks have been reported in different states in Malaysia including in well-vaccinated farms (Jaganathan *et al*., 2015; Kiarash *et al*., 2015; OIE, 2015). Fundamental studies in addressing the reasons behind the repeated outbreaks and the interaction between NDV and the host are lacking. Furthermore, most of the molecular characterisation studies of NDV genome focus on partial sequencing of the F and occasionally the HN genes. Therefore, the current study focuses to establish a protocol to determine the whole genome sequence of NDV genotype VII by using next generation sequencing (NGS) technology. Therefore, the hypothesis of this study is that complete genome sequencing and biological characterisation of NDV strains will be able to determine the evolution of NDV involved in outbreaks against current different physiological, environmental or immunological pressures in chickens. Secondly, the analysis of different regulation of genes associated with antiviral and proinflammatory responses in chickens following infection with different genotypes will assist in the understanding of the molecular pathogenesis of ND in poultry. Based on the above hypotheses, the objectives of this study are;

1.3. Objectives

1) To establish a next-generation sequencing protocol for complete genome sequencing of genotype VII NDV.

2) To determine the molecular characteristics and evolution of genotype VII NDV isolated from 2004 to 2013 in Malaysia.

3) To determine the pathotypes and virus tropisms of NDV genotype VII isolates isolated from 2004 to 2013 in Malaysia in comparisons with a genotype VIII NDV isolate.

4) To characterise the expression profiles of selected antiviral and proinflammatory genes following genotype VII and genotype VIII NDV infections in chickens

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