UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF A RECOMBINANT GENOTYPE-MATCHED LIVE ATTENUATED VACCINE AGAINST GENOTYPE VII NEWCASTLE DISEASE VIRUS

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By

MUHAMMAD BASHIR BELLO

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

May 2018

DEDICATION

This thesis is dedicated to my beloved parents, Dr. Bello Muhammad Tambuwal and Hajiya Fatima Bello Tambuwal, for their endless love and prayers. May Allah's blessings continue to be with them in this world and the here after.

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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Newcastle disease is a highly contagious viral disease of birds considered as one of the most important militating factors against poultry production all over the world. Current control strategies against the disease rely on the use of vaccines developed more than six decades ago. Although those vaccines are still effective by virtue of the fact that all Newcastle disease viruses (NDV) belong to the same serotype, they neither provide a complete protection in commercial chickens, nor do they block the replication and shedding of the virulent virus following infection with genotype VII NDV. Phylogenetically, the vaccines are classified as either genotype I or II, and are evolutionarily divergent from genotype VII isolates which have been the most predominantly circulating NDV strains in Malaysia and many parts of the world for the past 20 years. Therefore in the present study, reverse genetics technology was applied to genetically attenuate the pathogenicity of a recently isolated virulent Malaysian NDV strain IBS025/13. First and foremost, *in silico* nucleotide substitutions were made on the complete genome sequence of NDV strain IBS025/13, in order to modify the fusion protein (F) cleavage site of the virus from the virulent polybasic (RRQKRF) to avirulent monobasic (GRQGRL) amino acid motifs. Unique *Mlu*I and *Sgr*DI enzyme sites were also created at the P-M junction in order to facilitate future expression of foreign genes using the NDV backbone. The modified full length sequence (15.2 kb) was then entirely synthesised (GenScript, USA), subcloned into pOLTV5 transcription vector under the transcriptional control of T7 promoter and then named pOLTV5-mIBS025. Furthermore, expression plasmids for the NDV's minimum replication machinery (NP, P and L genes) were constructed in pCIneo mammalian expression vector and named pCIneo-NP, pCIneo-P and pCIneo-L respectively. These constructs, collectively referred as helper plasmids, were later tested for functionality in reverse genetics experiments using minigenome rescue system. Next, baby hamster kidney (BHK-21) cells stably expressing T7 RNA

polymerase were co-transfected with the mixture of the helper plasmids and pOLTV5-mIBS025 at an optimized ratio (1:0.5:0.25:0.2µg of POLTV5-mIBS025: pCineo-NP: pCIneo-P: pCIneo-L respectively). Interestingly, a recombinant virus designated NDV mIBS025, was successfully rescued following amplification in specific-pathogen-free (SPF) embryonated eggs, as evidenced by positive hemagglutination reaction. To confirm the identity of the recombinant virus, reverse transcription polymerase chain reaction (RT-PCR) was used to amplify a partial F gene region encompassing the cleavage site. DNA sequencing of the amplicons confirmed the presence of the engineered monobasic F cleavage site. Further biological characterization indicate that the recombinant virus has completely lost its pathogenicity as determined by the mean death time (MDT) in SPF embryonated eggs (150.4 hours) and the intracerebral pathogenicity index (ICPI) (0.0) in one-day old SPF chickens. Interestingly, when the recombinant virus was sequentially passaged in SPF eggs, it was found to not only maintain the monobasic F cleavage site, but also retained its attenuated phenotype after five consecutive passages, indicating the stability of the newly acquired attenuated phenotype. Finally, when one-day old SPF chickens were immunised with either 6log10 EID_{50} LaSota or the recombinant NDV mIBS025, a steadily increasing antibody titer was observed in both groups from 0-21 days post immunization, as determined by hemagglutination inhibition test using either genotype II or genotype VII NDV isolates as the HA antigen. Moreover, birds vaccinated with either LaSota or the recombinant NDVmIBS025 at the dose of 10^6 EID_{50} were completely protected against morbidity and mortality following experimental challenge with a lethal dose of the virulent genotype VII NDV strain IBS002/11. However, while both vaccines reduced the cloacal and oropharyngeal virus shedding compared to the unvaccinated group, the recombinant NDV mIBS025 significantly reduced both the duration and load quantity of the virus shed compared to the LaSota vaccine.

In conclusion, reverse genetics has been used to generate a genotype-matched live attenuated vaccine candidate based on the recently circulating virulent NDV IBS025/13. Immunogenicity assessment indicates that the vaccine induced a high antibody titer capable of protecting chicken against the virulent genotype VII NDV challenge. The vaccine also appears to be more promising in terms of reducing the shedding of the virulent virus post challenge.

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

PEMBANGUNAAN REKOMBINAN VAKSIN HIDUP TERATENUAT PADANAN-GENOTIP TERHADAP VIRUS SAMPAR AYAM GENOTIP VII

Oleh

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Penyakit sampar ayam (ND) adalah wabak virus burung yang mudah berjangkit dan dianggap sebagai salah satu faktor menghadkan yang paling penting terhadap pengeluaran ayam di seluruh dunia. Strategi kawalan semasa terhadap penyakit ini bergantung kepada penggunaan vaksin yang dibangunkan lebih enam dekad yang lalu. Walaupun vaksin tersebut masih dianggap efektif berdasarkan fakta bahawa semua virus penyakit sampar ayam (NDV) tergolong dalam serotip yang sama, namun vaksin ini kerap gagal memberi pelindungan lengkap dalam ayam komersial, menyekat replikasi virus dan pembebasan virus yang virulen selepas dijangkiti dengan virus ND genotip VII. Secara filogenetik, vaksin ini diklasifikasikan sebagai genotip I atau II, dan secara evolusinya berlainan daripada isolat genotip VII, dimana ianya merupakan strain NDV yang paling banyak tersebar di Malaysia dan di pelbagai negara di dunia sejak 20 tahun yang lalu. Oleh itu dalam kajian ini, teknologi genetik songsang telah digunapakai untuk melemahkan kepatogenan secara genetik bagi strain NDV Malaysia (IBS025/13) yay dipencilkan baru-baru ini.

Dengan menggunakan jujukan genom NDV IBS025/13 yang lengkap sebagai templat, penggantian nukleotida secara *in silico* dibuat untuk mengubah suai tempat belahan protein (F) bagi virus dari polibes virulen kepada motif asid amino monobes avirulen. Tempat enzim yang unik bagi MluI dan SgrDl juga dibuat di persimpangan P-M bagi memudahkan pengekspresan gen asing pada masa akan datang. Jujukan yang telah diubahsuai kemudiannya disintesis sepenuhnya (GenScript, USA), disubklon ke dalam pOLTV5 di bawah kawalan transkrip promoter T7 dan seterusnya dinamakan sebagai pOLTV5-mIBS025. Tambahan pula, plasmid ekspresi untuk jentera replikasi minima NDV (gen NP, P dan L) dibina dalam vektor ekspresi mamalia pCIneo dan dinamakan pCIneo-NP, pCIneo-P dan pCIneo-L setiap satunya. Pembinaan ini, secara kolektif merujuk sebagai plasmid penbantu, kemudian diuji untuk fungsi dalam

eksperimen genetik songsang menggunakan sistem penyelamatan minigenom. Selanjutnya, sel BHK 21 secara stabil menghasilkan polimerase RNA T7 telah ditransfeksi bersama dengan campuran plasmid pembantu dan pOLTV5-mIBS025 pada nisbah optimum. Menariknya, virus rekombinan NDV mIBS025, telah berjaya diselamatkan berikutan amplifikasi di dalam telur embrio yang bebas patogen (SPF), seperti yang dibuktikan oleh reaksi hemaglutinasi positif. Untuk mengesahkan identiti virus rekombinan, tindak balas rantai polimerase transkripsi berbalik (RT-PCR) telah digunakan untuk amplifikasi separa gen F yang merangkumi tapak belahan. Penjujukan DNA amplikon mengesahkan kehadiran tapak belahan monobasik F yang direka. Pencirian biologi lebih lanjut menunjukkan bahawa virus rekombinan telah hilang secara keseluruhan patogeniknya seperti yang ditentukan oleh masa kematian purata (MDT) di dalam telur embrio SPF (150.4 jam) dan indeks kepatogenan intracerebral (ICPI) (0.0) dalam ayam SPF berusia satu hari. Menariknya, apabila virus rekombinan secara beransur-ansur dipindahkan dalam telur SPF, ia didapati hanya mengekalkan tapak pemisahan monobasik F, tetapi juga mengekalkan fenotipnya yang dilemahkan selepas lima pemindahan berturut-turut, menunjukkan kestabilan fenotip dilemahkan. Seterusnya, apabila ayam SPF yang sudah berusia satu hari diimunisasi dengan LaSota atau rekombinan NDV mIBS025, titer antibodi yang semakin meningkat diperhatikan dalam kedua-dua kumpulan dari 0-21 hari selepas imunisasi, seperti yang ditentukan oleh ujian penghambatan penghemaglutinatan menggunakan genotip II atau genotip VII NDV asingan sebagai antigen HA. Tambahan lagi, ayam yang divaksin dengan samaada LaSota atau rekombinan NDV mIBS025 dilindungi sepenuhnya terhadap morbiditi dan mortaliti berikutan halangan eksperimen dengan dos virulen genotip VII NDV strain IBS002/11 yang membawa maut. Walau bagaimanapun, semasa kedua-dua vaksin mengurangkan virus kloakal dan orofarinks dibebaskan berbanding dengan kumpulan yang tidak divaksinkan, NDV rekombinan mIBS025 dengan ketara mengurangkan kedua-dua jangkamasa dan jumlah kuantiti virus yang dibebaskan berbanding dengan vaksin LaSota.

Kesimpulannya, genetik songsang telah digunakan untuk menghasilkan calon vaksin hidup yang dilemahkan secara padanan genotip berdasarkan NDV IBS025/13 yang baru tersebar. Penilaian menunjukkan bahawa titer antibodi tinggi yang disebabkan oleh vaksin ini mampu melindungi ayam terhadap ancaman genotip VII NDV yang virulen. Vaksin ini dilihat lebih terjamin dari segi mengurangkan halangan selepas pembebasan virus yang virulen.

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This thesis was submitted to the Senate of the 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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µl	Microlitre
Aa	Amino Acid
APMV	Avian *Paramyxovirus*
BEI	Binary ethylenimine
BHK	Baby Hamster kidney
BLAST	Basic Local Alignment Search Tool
bp	Base pair
BSA	Bovine Serum Albumin
CAT	Chloramphenicol acetyl transferase
cDNA	Complementary DNA
CMI	Cell-mediated immunity
CMV	Cytomegalovirus
DAPI	4',6-diamidino-2-phenylindole
DNA	Deoxyribonucleic Acid
dpc	Days post challenge
dpv	Days post vaccination
dsRNA	Double strand deoxyribonucleic acid
E	Glutamic acid
EDTA	Ethylene-diamine-tetraacetic Acid
EGFP	Enhanced green fluorescent protein
ELD_{50}	50% Mean egg lethal dose
ELISA	Enzyme-linked immunosorbent assays

F	Fusion protein
F0	Fusion protein 0
F1	Fusion protein fragment 1
F2	Fusion protein fragment 2
FMDV	Foot and mouth disease virus
GALT	Gastrointestinal associated lymphoid tissue
GE	Gene end
GFP	Green fluorescent protein
GIT	Gastrointestinal tract
GS	Gene start
HA	Hemagglutination
Hamrz	Hammerhead ribozyme
HDVrz	Hepatitis delta virus ribozyme
Hep-2	Human epitheloid carcinoma 2
HI	Hemagglutination inhibition
HIV TAT	Human Immunodeficiency virus trans-activator of transcription
HN	Hemagglutinin-Neuraminidase
HVT	Herpes virus of turkeys
IBD	Infectious bursal disease
ICPI	Intracerebral pathogenicity index
IFAT	Immunofluorescence antibody test
IFN	Interferon
Ig	Immunoglobulin
IGR	Inter genic resions

IRES	internal ribosome entry site
ITU	Independent transcription unit
IVPI	Intravenous pathogenicity index
K	Lysin
L	Large protein
LAMP	Loop mediated isothermal amplification
LB	Luria bertani
LIC	Ligation independent cloning
LND	Lentogenic Newcastle disease
M	Matrix protein
MAB	Monoclonal antibody
MCS	Multiple cloning site
MDT	Mean death time
MEGA	Molecular evolutionary genetics analysis
MHC	Major histocompatibility complex
MLT	Mean lethal dose
MND	Mesogenic Newcastle disease
mRNA	Messenger RNA
MVA	Modified vaccinia Ankara
NCBI	National center for biotechnology information
ND	Newcastle disease
NDV	Newcastle disease virus
NDV mIB025	Genetically modified recombinant Newcastle disease virus IBS025/13

NP	Nucleocapsid protein
NSNS	Negative sense nonsegmented virus
NVND	Neurotropic velogenic Newcastle disease
OE-PCR	Overlap extension polymerase chain reaction
OIE	Office international des epizooties
ORF	Open reading frame
P	Phophoprotein
PAMPs	Pathogen associated molecular patterns
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
Pol-1	RNA polymerase 1
pOLTV5-mIBS025	Full length clone of modified Newcastle disease virus antigenome strain IBS025
Q	Glutamine
R	Arginine
rAF-GFP	Recombinant NDV AF2240 strain expressing GFP
RE	Restriction endonuclease
rHVT	Turkey herpesvirus-based recombinant Vaccine
RIG-1	Retinoic acid inducible gene 1
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
rpm	Revolutions per minute
RT	Reverse transcription

RT-PCR	Reverse transcription-polymerase chain reaction
SEAP	Secreted alkaline phosphatase
SPF	Specific-pathogen-free
STAT	Single transducer and activator of transcription
TAE	Tris-acetate-EDTA
Us	Uracil
UTR	Untranslated regions
VG/GA	Villegas-Glisson/University of Georgia
VLP	Virus-like particle
VVND	Viscerotropic velogenic Newcastle disease

CHAPTER 1

INTRODUCTION

1.1 General background

Newcastle disease is a highly contagious viral disease of birds considered as one of the most important militating factors against poultry production across the globe. It has a worldwide distribution and is associated with significant economic losses in the global poultry industry (Alexander, 2001). It is also included in the World Organisation of Animal Health (OIE) List A diseases. The aetiology of the disease is Newcastle disease virus (NDV), an avian paramyxovirus type-1 (APMV-1) which is a member of the genus Avulavirus in the family *Paramyxoviridae*. The virus is enveloped and has a nearly 15.2 kb non-segmented genome, made up of six genes in the order 3' NP-P-M-F-HN-L 5'. With the exception of the P gene which can undergo RNA editing to give rise to two additional non-structural proteins, V and W (Steward *et al*., 1993), all the genes are transcribed into a single mRNA that encodes a single structural protein namely the Nucleocapsid protein (NP), Phosphoprotein (P), Matrix protein (M), Fusion protein (F), Hemagglutinin-neuraminidase protein (HN) and Large protein (L) (Yusoff and Tan, 2001).

To date, all strains of NDV are grouped into one serotype. However, their genetic diversity is enormous. Information from the available literature reveals two major schemes used to classify NDV isolates on the basis of their genomic characteristics. The first system, proposed by the Aldous group, classifies the NDV isolates into lineages (I-VI) and their respective sublineages (Aldous *et al*, 2003). The other classification scheme proposed by Ballagi-Pordány *et al*. (1996), divides the NDV isolates broadly into class I and class II with several genotypes and subgenotypes in each class. All class I isolates uniquely have a genome length of 15,198 bp and are distributed worldwide in wild birds. More so, apart from a single isolate that caused devastating outbreak in Ireland around the early 1990s (Alexander *et al*., 1992), all class I strains are usually avirulent in chicken (Kim *et al*., 2007). On the other hand, the class II viruses which have been extensively studied constitute both the virulent and avirulent strains. Usually, their total genome length is either 15,186 bp for genotypes isolated before 1960 (early genotypes) or 15,192 bp in the case of late genotypes that were isolated after 1960 (Czeglédi *et al*., 2006) although some recently isolated strains were shown to have a total genome size of 15,186 bp (Satharasinghe *et al*., 2016). Interestingly, a more comprehensive criteria for NDV classification was recently adopted in order to bring an end to the confusion created by simultaneous usage of the two schemes of NDV taxonomy (Diel *et al*., 2012). Based on this unified system of classification, NDV isolates are grouped into class I, with only one genotype and class II having up to 18 genotypes (Snoeck *et al*., 2013). Importantly, all members of class II genotypes I and II, with the exception of a neurotropic virulent chicken strain, isolated in 1948 in the United States, are of low virulence in chicken (Miller *et al*. 2010). Indeed most of the popular commercially available ND vaccines such as

LaSota, B1 and VGGA are derived from these genotype II isolates (Dimitrov *et al*. 2016). Genotypes III to XVIII are however mostly composed of strains that are highly pathogenic in chicken.

Traditionally, the NDV isolates are classified into lentogenic, mesogenic and velogenic pathotypes using indices such as the mean death time (MDT) in 9 to 10-days old embryonated chicken eggs, intracerebral pathogenicity index (ICPI) in 1 day old chicks and intravenous pathogenicity index (IVPI) in 6 weeks old chicken (OIE, 2008). Usually, the lentogenic isolates are considered non virulent because they neither cause any clinical disease in adult birds nor do they kill chicken embryo even after 90 hours of propagation. In contrast, the velogenic strains are associated with high mortality in chicken and can kill chicken embryo in less than 60 hours post inoculation (Alexander and Parsons, 1986). The mesogens are of intermediate virulence usually causing respiratory disease of moderate severity in chicken. In addition to these *in vivo* pathogenicity tests, the amino acid composition of the F protein cleavage site is used as a molecular marker for NDV virulence (Ganar *et al*., 2014; Panda *et al*., 2004). The F protein is normally synthesised in an inactive form, F_0, and is activated via cleavage by certain host cell proteases into two fragments, F1 and F2 linked by disulphide bonds (de Leeuw *et al*., 2005). Virulence of NDV strains is defined by the presence of a multiple basic amino acid residues (lysine or arginine) between positions 112 to 116 and a phenylalanine residue at position 117 on the fusion protein. Isolates with this type of cleavage site are easily cleavable by intracellular furin-like proteases ubiquitously distributed in many chicken organs, explaining the widespread multisystemic lesions associated with the virulent strains of NDV. On the contrary, avirulent NDV strains have a monobasic amino acid residue at the fusion protein cleavage site and can only be activated by trypsin like extracellular proteases mostly present in the respiratory and gastrointestinal systems (Wang *et al*., 2017).

1.2 Statement of research problem

Although, the conventional commercially available vaccines such as LaSota and B1 developed more than 60 years ago, have been offering a promising level of protection against ND for decades, several studies indicate that their protective efficacy against the current wave of ND outbreaks is suboptimal (Miller *et al*., 2013; Miller *et al*., 2007). Evidences from molecular epidemiological studies indicate that the most predominantly circulating strains of NDV in Southeastern Asian countries belong to genotype VII (Liu *et al*., 2007; Tan *et al*., 2010), and they are known to be responsible for the recently recorded outbreaks of ND including those occurring among the farms that vaccinated their birds using the conventional genotype II vaccines. One of the major driving forces for the occurrence of ND outbreaks among the vaccinated birds is the genotype mismatch between the conventional vaccine strains and the circulating field strains. Based on nucleotide and amino acid sequence alignment, the surface glycoproteins (F and HN) of genotype VII NDV are highly divergent from those of the conventional genotype II vaccine strains (Xiao *et al*., 2012; Zhang *et al*., 2014). Thus the sequence variation between the field and vaccine strains might be responsible

for the suboptimal protection of genotype II vaccinated chicken against genotype VII NDV challenge.

No doubt, genotype VII isolates are currently the most disastrous NDV isolates. They are responsible for the on-going fourth ND panzootic and have manifested the potentials to become the fifth panzootic viruses by continuously emerging in new hosts and geographical areas (Miller *et al*., 2015). They have also been shown to defy the current control strategies using the conventional genotype II vaccines. According to the records of the Malaysian Department of Veterinary Services, genotype VII NDV outbreaks (reported cases) are increasing exponentially, from 5 in 2009 to 153 in 2011. Even more recently, genotype VII NDV outbreaks have been recorded in different parts of Malaysia (Satharasinghe *et al*., 2016; Aljumaili *et al*., 2017) where they were associated with devastating economic losses in the national poultry industry. This therefore, highlights the need to improve the current vaccination strategy.

The fact remains the conventional genotype II vaccines when correctly admininstred at the right dose could still protect against clinical disease and mortality due to ND. However, they often fail to completely block virus shedding post challenge among the vaccinated birds (Miller *et al*., 2007). This means that the profile of humoral immune response induced by those vaccines might only protect against clinical signs but not virus replication in the vaccinated birds, presumably due to amino acid substitutions in some neutralising epitopes in the vaccine strain. Consequently, when the vaccinated bird is infected with genotypically distinct NDV isolate, the birds replicate and shed the virulent virus into the environment, posing a potential risk to naive birds in the vicinity. Indeed, live viruses have been reported in cloacal and nasopharyngeal swabs obtained from birds vaccinated with those conventional vaccines (Choi *et al*., 2013). Moreover, depending on the environmental factors and the presence of other concurrent infections, these conventional vaccines may cause disease symptoms in vaccinated birds. These problems, to say the least, underscore the need for the development of genotype-matched ND vaccines which are closely related to the prevailing genotypes and therefore offer a better protection with greater safety by significantly reducing virus shedding post challenge among the vaccinated birds.

1.3 Justification

With the advent of reverse genetics system for NDV, numerous genetic manipulations ranging from individual genes mutations to complete swapping of some genes with other identical homologues have been accomplished (Peeters *et al*., 2001). Indeed, rationally designed vaccines with greatly enhanced safety and protective efficacy have been generated within a relatively short period of time. Hu *et al*. (2011) used reverse genetics technique and attenuated a highly virulent genotype VII NDV by mutating the F protein cleavage site of the virus. The resulting recombinant virus, when used to vaccinate birds, was found to effectively protect them against virulent NDV challenge 3-4 weeks post-immunization. More so, compared to the birds vaccinated with LaSota vaccine, virus shedding among the birds vaccinated with this recombinant vaccine was

significantly lower. Similarly, Xiao *et al*. (2012) recovered a stably attenuated virus from a highly virulent isolate by changing the virulent F protein cleavage site motif ‘‘^{112}RRQKRF^{117}’’ into an avirulent motif ‘‘^{112}GRQGRL^{117}’’. The recombinant virus also reduced the challenged virus shedding in the vaccinated animals compared to the LaSota vaccine. This therefore confirms the speculation by Miller *et al*., (2007) that genotype matched vaccines not only effectively prevent mortality and morbidity due to ND challenge, they also substantially reduce virus shedding among the vaccinated animals.

Recently, a highly virulent NDV strain designated IBS025/13, was isolated from a vaccinated commercial broiler flock in Malaysia. The virus, which has the total genome length of 15,186 bp, was shown to be a naturally recombinant NDV with its entire NP and greater parts of its P genes derived from genotype II while the M, F, HN and L genes were all derived from genotype VII. It has the typical velogenic fusion protein cleavage site with multiple basic amino acid residues and high ICPI index of 1.69 (Satharasinghe *et al*., 2016). In addition, tissue tropism studies have shown that the virus equally has high tropism in both respiratory and gastrointestinal systems (Satharasinghe, 2016). It also grows to a high titre in chicken embryonated eggs, making it a good candidate for vaccine development. Using reverse genetics technology, it is possible to attenuate the virulence of this virus without tempering with its unique tissue tropism characteristics. This way, a highly effective genotype-matched vaccine with the potential of enhanced ND control can be developed.

1.4 Research hypothesis

Recombinant genotype-matched live attenuated Newcastle disease vaccine is completely protective in chicken and is more effective in reducing cloacal and oropharyngeal virus shedding compared to the conventional LaSota vaccine, following challenge with lethal dose of genotype VII NDV.

1.5 Main objective

The main objective of this work was to develop an effective genotype matched live attenuated Newcastle disease vaccine using reverse genetics technology.

1.6 Specific objectives

1. To construct and express functional helper plasmids for the recovery of modified NDV strain IBS025/13 (mIBS025).
2. To design, synthesise and rescue a recombinant attenuated NDV mIBS025 entirely from cloned cDNA.

3. To biologically characterize the genetic stability and pathogenicity of the recombinant NDV mIBS025, in specific-pathogen-free embryonated eggs and one-day old chicks
4. To evaluate the protective immunity and safety induced by the recombinant virus (mIBS025) against challenge with virulent genotype VII NDV isolate in specific-pathogen-free chicken.

REFERENCES

Abdul-Aziz, T. A., and Arp, L. H. (1983). Pathology of the trachea in turkeys exposed by aerosol to lentogenic strains of Newcastle disease virus. *Avian Diseases*, *27*(4), 1002–1011.

Afzal, F., Saeed, A., Sharif, M. A., Ayub, N., and Hassan, S. (2012). Pathogenicity of avian influenza virus H5N1 2007 isolates from Pakistan. *Asian Pacific Journal of Tropical Biomedicine*, *2*(1), 380-382.

Al-Garib, S. O., Gielkens, A. L. J., Gruys, D. E., Hartog, L., and Koch, G. (2003). Immunoglobulin class distribution of systemic and mucosal antibody responses to Newcastle disease in chickens. *Avian Diseases*, *47*(1), 32–40.

Aldous, E. W., and Alexander, D. J. (2001). Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1). *Avian Pathology*, *30*, 117–128.

Aldous, E. W., Mynn, J. K., Banks, J., and Alexander, D. J. (2003). A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene. *Avian Pathology*, *32*(3), 239–256.

Alexander, D. J. (2000a). Newcastle disease and other avian paramyxoviruses. *Revue Scientifique et Technique (International Office of Epizootics)*, *19*(2), 443–462.

Alexander, D. J. (2000b). Newcastle disease and other avian paramyxoviruses Aetiology. *Diseases of Poultry; Chapt. 19; Pp. 496-519;* Iowa State Univ. Press; Ames, Iowa; 9th Edition; Calnek,B.W., Barnes,H.J., Beard,C.W., Reid,W.M., and Yoder,H.W., Jr. (Eds.), 19(2), 443–462.

Alexander, D. J. (2001). Newcastle disease. *British Poultry Science*, *42*(1), 5–22.

Alexander, D. J. (2009). Ecology and epidemiology of Newcastle disease. *Avian Influenza and Newcastle Disease: A Field and Laboratory Manual*, 19–26.

Alexander, D. J., Aldous, E. W., and Fuller, C. M. (2012). The long view: a selective review of 40 years of Newcastle disease research. *Avian Pathology*, *41*(4), 329–335.

Alexander, D. J., Campbell, G., Manvell, R. J., Collins, M. S., Parsons, G., and McNulty, M. S. (1992). Characterisation of an antigenically unusual virus responsible for two outbreaks of Newcastle disease in the Republic of Ireland in 1990. *Veterinary Record*, *130*(4), 65–68.

Alexander, D. J., and Parsons, G. (1986). Pathogenicity for chickens of avian paramyxovirus type 1 isolates obtained from pigeons in Great Britain during 1983-85. *Avian Pathology*, *15*(3), 487–493.

Alexander, D. J., Parsons, G., and Marshall, R. (1986). Avian paramyxovirus type 1 infections of racing pigeons: 4 laboratory assessment of vaccination. *Veterinary record*, *118*, 262–266.

Andersen, C. R., Nielsen, L. S., Baer, A., Tolstrup, A. B., and Weilguny, D. (2011). Efficient expression from one CMV enhancer controlling two core promoters. *Molecular Biotechnology*, *48*(2), 128–137.

Awan, M. A., Otte, M. J., and James, A. D. (1994). The epidemiology of Newcastle disease in rural poultry: A review. *Avian Pathology*, 23(3), 405-423

Ballagi-Pordány, A, Wehmann, E., Herczeg, J., Belák, S., and Lomniczi, B. (1996). Identification and grouping of Newcastle disease virus strains by restriction site analysis of a region from the F gene. *Archives of Virology*, *141*, 243–261.

Banerjee, M., Reed, W. M., Fitzgerald, S. D., and Panigraphy, B. (1994). Neurotropic velogenic Newcastle disease in cormorants in Michigan: pathology and virus characterization. *Avian Diseases*, *38*(4), 873–878.

Baron, M. D., and Barrett, T. (1997). Rescue of rinderpest virus from cloned cDNA. *Journal of Virology*, *71*(2), 1265–1271.

Bishop, G. A., and Hostager, B. S. (2001). B lymphocyte activation by contact-mediated interactions with T lymphocytes. *Current Opinion in Immunology*, 13(3), 278-285

Blumberg, B. M., Leppert, M., and Kolakofsky, D. (1981). Interaction of VSV leader RNA and nucleocapsid protein may control VSV genome replication. *Cell*, *23*(3), 837–45.

Brian, D. A., and Baric, R. S. (2005). Coronavirus genome structure and replication. *Current Topics in Microbiology and Immunology*, *287*, 1–30.

Brown, C. C., King, D. J., and Seal, B. S. (1999). Pathogenesis of Newcastle disease in chickens experimentally infected with viruses of different virulence. *Veterinary Pathology*, *36*(2), 125–132.

Bryksin, A. V., and Matsumura, I. (2010). Overlap extension PCR cloning: A simple and reliable way to create recombinant plasmids. *BioTechniques*, *48*(6), 463–465.

Buchholz, U. J., Finke, S., and Conzelmann, K. K. (1999). Generation of bovine respiratory syncytial virus (BRSV) from cDNA: BRSV NS2 is not essential for virus replication in tissue culture, and the human RSV leader region acts as a functional BRSV genome promoter. *Journal of Virology*, *73*(1), 251–259.

Camenisch, G., Bandli, R., and Hoop, R. (2008). Monitoring of wild birds for Newcastle disease virus in Switzerland using real time RT-PCR. *Journal of Wildlife Diseases*, *44*(3), 772–6.

Cattoli, G., Susta, L., Terregino, C., and Brown, C. (2011). Newcastle disease: a review of field recognition and current methods of laboratory detection. *Journal of Veterinary Diagnostic Investigation*, *23*(4), 637–656.

Cha-Aim, K., Hoshida, H., Fukunaga, T., and Akada, R. (2012). Fusion PCR via novel overlap sequences. *Methods in Molecular Biology*, *852*, 97–110.

Chaplin, P., Howley, P., Meisinger-Henschel, C., Rathe, I., Felder, E., and Heller, K. (2011). MVA (Modified Vaccinia Ankara) virus variant cultivation method and vaccine uses. *Berl Munch Tierarztl Wochenschr.*, 128(11-12), 464-72

Cheng, X., Wang, W., Xu, Q., Harper, J., and Carroll, D. (2016). Genetic modification of oncolytic Newcastle disease virus for cancer therapy. *Journal of Virology*, *90*(1), 5343–5352.

Choi, K. S., Kye, S. J., Kim, J. Y., and Lee, H. S. (2013). Genetic and antigenic variation of shedding viruses from vaccinated chickens after challenge with virulent Newcastle disease virus. *Avian Diseases*, *57*(2), 303–306.

Choi, K. S., Lee, E. K., Jeon, W. J., and Kwon, J. H. (2010). Antigenic and immunogenic investigation of the virulence motif of the Newcastle disease virus fusion protein. *Journal of Veterinary Science*, *11*(3), 205–211.

Chulan, U., Ibrahim, A. L., Mustaffa Babjee, A., and Sheikh-Omar, A. R. (1982). Vaccination against Newcastle disease. *Tropical Animal Health and Production*, *14*(3), 177–184.

Coleman, N. A., and Peeples, M. E. (1993). The matrix protein of Newcastle disease virus localizes to the nucleus via a bipartite nuclear localization signal. *Virology*, *195*(2), 596–607.

Conan, A., Goutard, F. L., Sorn, S., and Vong, S. (2012). Biosecurity measures for backyard poultry in developing countries: A systematic review. *BMC Veterinary Research*, 8, 240.

Conzelmann, K.-K. (1998). Nonsegmented negative-strand rna viruses: Genetics and Manipulation of Viral Genomes. *Annual Review of Genetics*, *32*(1), 123–162.

Conzelmann, K. K. (2004). Reverse genetics of mononegavirales. *Current Topics in Microbiology and Immunology*, *283*, 1–41.

Conzelmann, K. K., and Schnell, M. (1994). Rescue of synthetic genomic RNA analogs of rabies virus by plasmid-encoded proteins. *Journal of Virology*, *68*(2), 713–719.

Corbanie, E. A., Remon, J. P., Van Reeth, K., Landman, W. J. M., van Eck, J. H. H., and Vervaet, C. (2007). Spray drying of an attenuated live Newcastle disease vaccine virus intended for respiratory mass vaccination of poultry. *Vaccine*, *25*(49), 8306–8317.

Cornax, I., Miller, P. J., and Afonso, C. L. (2012). Characterization of Live LaSota Vaccine Strain–Induced Protection in Chickens upon Early Challenge with a Virulent Newcastle Disease Virus of Heterologous Genotype. *Avian Diseases Digest*, *7*(3), e7–e8.

Cserep, T. (2008). Vaccines and vaccination. *Poultry Diseases*, Elsevier Ltd. http://doi.org/10.1016/B978-0-7020-2862-5.50010-6

Curran, J., and Kolakofsky, D. (1999). Replication of paramyxoviruses. *Advances in Virus Research*, *54*, 403–22.

Czeglédi, A., Ujvári, D., Somogyi, E., Wehmann, E., Werner, O., and Lomniczi, B. (2006). Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications. *Virus Research*, *120*(1–2), 36–48.

Darzacq, X., Singer, R. H., and Shav-Tal, Y. (2005). Dynamics of transcription and mRNA export. *Current Opinion in Cell Biology*, 17(3): 332–339.

de Geus, E. D., Rebel, J. M. J., and Vervelde, L. (2012). Induction of respiratory immune responses in the chicken; implications for development of mucosal avian influenza virus vaccines. *Veterinary Quarterly*, 32(2):75-86

de Leeuw, O. S., Koch, G., Hartog, L., Ravenshorst, N., and Peeters, B. P. H. (2005). Virulence of Newcastle disease virus is determined by the cleavage site of the fusion protein and by both the stem region and globular head of the haemagglutinin-neuraminidase protein. *Journal of General Virology*, *86*(6), 1759–1769.

Diel, D. G., da Silva, L. H. A., Liu, H., Wang, Z., Miller, P. J., and Afonso, C. L. (2012). Genetic diversity of avian paramyxovirus type 1: Proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes. *Infection, Genetics and Evolution*, *12*(8), 1770–1779.

Dimitrov, K. M., Afonso, C. L., Yu, Q., and Miller, P. J. (2016). Newcastle disease vaccines—A solved problem or a continuous challenge? *Veterinary Microbiology*, 206:126-136

Dimitrov, K. M., Ramey, A. M., Qiu, X., Bahl, J., and Afonso, C. L. (2016). Temporal, geographic, and host distribution of avian paramyxovirus 1 (Newcastle disease virus). *Infection , Genetics and Evolution*, *39*, 22–34.

Djikeng, A., Halpin, R., Kuzmickas, R., DePasse, J., Feldblyum, J., Sengamalay, N., Afonso, C., Zhang, X., Anderson, N. G., Ghedin, E. and Spiro, D. J. (2008). Viral genome sequencing by random priming methods. *BMC Genomics*, *9*(1), 5.

Doria-Rose, N. A., and Haigwood, N. L. (2003). DNA vaccine strategies: Candidates for immune modulation and immunization regimens. *Methods*, 31(3), 207-16.

Dortmans, J. C. F. M., Rottier, P. J. M., Koch, G., and Peeters, B. P. H. (2010). The viral replication complex is associated with the virulence of Newcastle disease virus. *Journal of Virology*, *84*(19), 10113–10120.

Dortmans, J. C., Koch, G., Rottier, P. J., and Peeters, B. P. (2011). Virulence of newcastle disease virus: What is known so far? *Veterinary Research*, 42,122.

Duan, Z., Song, Q., Wang, Y., He, L., Chen, J., Zhu, Y., Hu,S. and Liu, X. (2013). Characterization of signal sequences determining the nuclear export of Newcastle disease virus matrix protein. *Archives of Virology*, *158*(12), 2589–2595.

Durbin, A. P., Hall, S. L., Siew, J. W., Whitehead, S. S., Collins, P. L., and Murphy, B. R. (1997). Recovery of infectious human parainfluenza virus type 3 from cDNA. *Virology*, *235*(2), 323–32.

Ecco, R., Susta, L., Afonso, C. L., Miller, P. J., and Brown, C. (2011). Neurological lesions in chickens experimentally infected with virulent Newcastle disease virus isolates. *Avian Pathology*, *40*(2), 145–152.

El Najjar, F., Schmitt, A. P., and Dutch, R. E. (2014). Paramyxovirus glycoprotein incorporation, assembly and budding: A three way dance for infectious particle production. *Viruses*, 6(8), 3019-54

Esaki, M., Godoy, A., Rosenberger, J. K., Rosenberger, S. C., Gardin, Y., Yasuda, A., and Dorsey, K. M. (2013). Protection and antibody response caused by turkey herpesvirus vector Newcastle disease vaccine. *Avian Diseases*, *57*(4), 750–755.

Ewer, K. J., Lambe, T., Rollier, C. S., Spencer, A. J., Hill, A. V. S., and Dorrell, L. (2016). Viral vectors as vaccine platforms: From immunogenicity to impact. *Current Opinion in Immunology*, 41, 47-54.

Falcon, M. D. (2004). Exotic Newcastle disease. *Seminars in Avian and Exotic Pet Medicine*, *13*(2), 79–85.

Feizi, A., and Nazeri, M. (2011). Comparative Study of Antibody Titers Obtained from Avinew , Lasota , and Clone30 Vaccines in Broiler Chicks with Hi Test. *Australian Journal of Basic and Applied Sciences*, 5(8), 554-558

Felsenstein, J. (1985). Confidence-Limits on phylogenies - an approach using the bootstrap. *Evolution*, *39*(4), 783–791.

Feng, H., Wei, D., Nan, G., Cui, S. J., Chen, Z. N., and Bian, H. (2011). Construction of a minigenome rescue system for Newcastle disease virus strain Italien. *Archives of Virology*, *156*(4), 611–616.

Firouzamandi, M., Moeini, H., Hosseini, D., Hair Bejo, M., Omar, A. R., Mehrbod, P., and Ideris, A. (2016). Improved immunogenicity of Newcastle disease virus fusion protein genes. *Journal of Veterinary Science*, *17*(1), 21–26.

Firouzamandi, M., Moeini, H., Hosseini, S. D., Bejo, M. H., Omar, A. R., Mehrbod, P., El Zowalaty, M. E., Webster, T. J and Ideris, A. (2016). Preparation, characterization, and in ovo vaccination of dextran-spermine nanoparticle dna vaccine coexpressing the fusion and hemagglutinin genes against newcastle disease. *International Journal of Nanomedicine*, *11*, 259–267.

Fukanoki, S., Iwakura, T., Iwaki, S., Matsumoto, K., Takeda, R., Ikeda, K., Shi, Z and Mori, H. (2001). Safety and efficacy of water-in-oil-in-water emulsion vaccines containing Newcastle disease virus haemagglutinin-neuraminidase glycoprotein. *Avian Pathology*, *30*(5), 509–516.

Ganar, K., Das, M., Sinha, S., and Kumar, S. (2014). Newcastle disease virus: Current status and our understanding. *Virus Research*, *184*, 71–81.

Garcin, D., Pelet, T., Calain, P., Roux, L., Curran, J., and Kolakofsky, D. (1995). A highly recombinogenic system for the recovery of infectious Sendai paramyxovirus from cDNA: generation of a novel copy-back nondefective interfering virus. *The EMBO Journal*, *14*(24), 6087–6094.

Ge, J., Liu, Y., Jin, L., Gao, D., Bai, C., and Ping, W. (2016). Construction of recombinant baculovirus vaccines for newcastle disease virus and an assessment of their immunogenicity. *Journal of biotechnology*, 231:201-211

Ge, J., Wang, X., Tao, L., Wen, Z., Feng, N., Yang, S., Xia, X., Yang, C., Chen, H and Bu, Z. (2011). Newcastle disease virus-vectored rabies vaccine is safe, highly immunogenic, and provides long-lasting protection in dogs and cats. *Journal of Virology*, *85*(16), 8241–8252.

Gharaibeh, S., and Mahmoud, K. (2013). Decay of maternal antibodies in broiler chickens. *Poultry Science*, *92*(9), 2333–6.

Grass, E. E. (1972). Exotic Newcastle disease in the United States. *Proceedings of the 21st Western Poultry Disease Conference and 6th Poultry Health Symposium*, March 11-14,1972, 26–30.

Gupta, K. C., and Kingsbury, D. W. (1984). Complete sequences of the intergenic and mRNA start signals in the sendai virus genome: Homologies with the genome of vesicular stomatitis virus. *Nucleic Acids Research*, *12*(9), 3829–3841.

Gururaj, K., Kirubaharan, J. J., Gupta, V. K., and Pawaiya, R. S. (2014). Review Article Past and Present of Reverse Genetics in Animal Virology with Special Reference to Non – Segmented Negative Stranded RNA Viruses : a Review. *Advances in Animal and Veterinary Sciences*, *2*, 40–48.

Habjan, M., Penski, N., Spiegel, M., and Weber, F. (2008). T7 RNA polymerase-dependent and -independent systems for cDNA-based rescue of Rift Valley fever virus. *Journal of General Virology*, *89*(9), 2157–2166.

Hall, T. A. (1999). BioEdit: A user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98.

Hamid, H., Campbell, R. S. F., and Lamichhane, C. (1990). The Pathology of Infection of Chickens With the Lentogenic V4 Strain of Newcastle Disease Virus. *Avian Pathology*, *19*(4), 687–696.

Han, Q., Gao, X., Wu, P., Xiao, S., Wang, X., Liu, P., Tong, L., Hao, H., Zhang, S., Dang, R. and Yang, Z. (2017). Re-evaluation the immune efficacy of Newcastle disease virus vaccine in commercial laying chickens. *Research in Veterinary Science*, *111*, 63–66.

Hanson, R. P., and Brandly, C. A. (1958). Newcastle disease. *Annals of the New York Academy of Sciences*, *70*(3), 585–597.

Haryanto, A., Purwaningrum, M., Verawati, S., Irianingsih, S. H., and Wijayanti, N. (2015). Pathotyping of Local Isolates Newcastle Disease Virus from Field Specimens by RT-PCR and Restriction Endonuclease Analysis. *Procedia Chemistry*, *14*, 85–90.

He, J., Pan, Z., Tian, G., Liu, X., Liu, Y., Guo, X., An, Y., Song, L., Wu, H., Cao, H., Yu, D., Che, R., Xu, P., Rasoul, L. M., Li, D. and Yin, J. (2016). Newcastle disease virus chimeras expressing the Hemagglutinin- Neuraminidase protein of mesogenic strain exhibits an enhanced anti-hepatoma efficacy. *Virus Research*, *221*, 23–29.

Heiden, S., Grund, C., Röder, A., Granzow, H., Kühnel, D., Mettenleiter, T. C., and Römer-Oberdörfer, A. (2014). Different regions of the newcastle disease virus fusion protein modulate pathogenicity. *PloS One*, *9*(12), e113344.

Herczeg, J., Wehmann, E., Bragg, R. R., Travassos Dias, P. M., Hadjiev, G., Werner, O., and Lomniczi, B. (1999). Two novel genetic groups (VIIb and VIII) responsible for recent Newcastle disease outbreaks in Southern Africa, one (VIIb) of which reached Southern Europe. *Archives of Virology*, *144*(11), 2087–2099.

Hitchner, S. B., Reising, G., and Van Roekel, H. (1951). Characteristics of the B1 strain of Newcastle disease virus. *American Journal of Veterinary Research*, *12*(44), 246–249.

Ho, S. C. L., Bardor, M., Feng, H., Mariati, Tong, Y. W., Song, Z., Yap, M. G., and Yang, Y. (2012). IRES-mediated tricistronic vectors for enhancing generation of high monoclonal antibody expressing CHO cell lines. *Journal of Biotechnology*, *157*(1), 130–139.

Hoenen, T., and Feldmann, H. (2014). Reverse genetics systems as tools for the development of novel therapies against filoviruses. *Expert Review of Anti-Infective Therapy*, *12*(10), 1253–63.

Hofstad, M. S. (1953). A method of evaluating immunity following vaccination of chickens with inactivated Newcastle disease vaccine. *American Journal of Veterinary Research*, *14*(53), 590–593.

Hooper, P. T., Hansson, E., Young, J. G., Russell, G. M., and Della-Porta, A. J. (1999). Lesions in the Upper Respiratory Tract in Chickens Experimentally Infected With Newcastle Disease Viruses Isolated in Australia. *Australian Veterinary Journal*, *77*(1), 50–51.

Hu, Z., Hu, S., Meng, C., Wang, X., Zhu, J., and Liu, X. (2011). Generation of a genotype VII Newcastle disease virus vaccine candidate with high yield in embryonated chicken eggs. *Avian Diseases*, *55*(3), 391–7.

Huang, Z., Elankumaran, S., Panda, A., and Samal, S. K. (2003). Recombinant Newcastle disease virus as a vaccine vector. *Poultry Science*, *82*(6), 899–906.

Huang, Z., Elankumaran, S., Yunus, A. S., and Samal, S. K. (2004). A recombinant Newcastle disease virus (NDV) expressing VP2 protein of infectious bursal disease virus (IBDV) protects against NDV and IBDV. *Journal of Virology*, *78*(18), 10054–10063.

Huang, Z., Krishnamurthy, S., Panda, A., and Samal, S. K. (2001). High-level expression of a foreign gene from the most 3'-proximal locus of a recombinant Newcastle disease virus. *The Journal of General Virology*, *82*(7), 1729–1736.

Huang, Z., Krishnamurthy, S., Panda, A., and Samal, S. K. (2003). Newcastle disease virus V protein is associated with viral pathogenesis and functions as an alpha interferon antagonist. *Journal of Virology*, *77*(16), 8676–8685.

Huang, Z., Panda, A., Elankumaran, S., Govindarajan, D., Rockemann, D. D., and Samal, S. K. (2004). The hemagglutinin-neuraminidase protein of Newcastle disease virus determines tropism and virulence. *Journal of Virology*, *78*(8), 4176–4184.

Hugh-Jones, M., Allan, W. H., Dark, F. A, and Harper, G. J. (1973). The evidence for the airborne spread of Newcastle disease. *The Journal of Hygiene*, *71*(2), 325–339.

Iannello, A., Debbeche, O., Martin, E., Attalah, L. H., Samarani, S., and Ahmad, A. (2006). Viral strategies for evading antiviral cellular immune responses of the host. *Journal of Leukocyte Biology*, *79*, 16–35.

Iorio, R. M., Field, G. M., Sauvron, J. M., Mirza, A. M., Deng, R., Mahon, P. J., and Langedijk, J. P. (2001). Structural and functional relationship between the receptor recognition and neuraminidase activities of the Newcastle disease virus hemagglutinin-neuraminidase protein: receptor recognition is dependent on neuraminidase activity. *Journal of Virology*, *75*(4), 1918–1927.

Izadi, A., Moslemi, E., and Shahhosseiny, M. H. (2012). Comparison of SYBR Green and turbidimetry methods for loop mediated isothermal amplification (LAMP) product detection in diagnosis of hepatitis B virus (HBV). *African Journal of Microbiology Research*, *6*(42), 7003–7007.

Jahanshiri, F., Eshaghi, M., and Yusoff, K. (2005). Identification of phosphoprotein : phosphoprotein and phosphoprotein : nucleocapsid protein interaction domains of the Newcastle disease virus. *Archives of Virology*, *150*(3), 611–618.

Janke, M., Peeters, B., de Leeuw, O., Moorman, R., Arnold, A., Fournier, P., and Schirrmacher, V. (2007). Recombinant Newcastle disease virus (NDV) with inserted gene coding for GM-CSF as a new vector for cancer immunogene therapy. *Gene Therapy*, *14*(23), 1639–1649.

Jiang, Y., Liu, H., Liu, P., and Kong, X. (2009). Plasmids driven minigenome rescue system for Newcastle disease virus V4 strain. *Molecular Biology Reports*, *36*(7), 1909–1914.

Jin, J., Zhao, J., Ren, Y., Zhong, Q., and Zhang, G. (2016). Contribution of HN protein length diversity to Newcastle disease virus virulence, replication and biological activities. *Scientific Reports*, *6*, 36890.

Kano, R., Konnai, S., Onuma, M., and Ohashi, K. (2009). Cytokine profiles in chickens infected with virulent and avirulent Marek's disease viruses: Interferon-gamma is a key factor in the protection of Marek's disease by vaccination. *Microbiology and Immunology*, *53*(4), 224–232.

Kapczynski, D. R., Afonso, C. L., and Miller, P. J. (2013). Immune responses of poultry to Newcastle disease virus. *Developmental and Comparative Immunology*, *41*(3), 447–453.

Kapczynski, D. R., and King, D. J. (2005). Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastl. *Vaccine*, *23*(26), 3424–3433.

Karaca, K., Sharma, J. M., Winslow, B. J., Junker, D. E., Reddy, S., Cochran, M., and McMillen, J. (1998). Recombinant fowlpox viruses coexpressing chicken type I IFN and newcastle disease virus HN and F genes: Influence of IFN on protective efficacy and humoral responses of chickens following in ovo or post-hatch administration of recombinant viruses. *Vaccine*, *16*(16), 1496–1503.

Karpala, A. J., Doran, T. J., and Bean, A. G. D. (2005). Immune responses to dsRNA: Implications for gene silencing technologies. *Immunology and Cell Biology*, *83*(3), 211–216.

Kartoglu, U., and Milstien, J. (2014). Tools and approaches to ensure quality of vaccines throughout the cold chain. *Expert Review of Vaccines*, *13*(7), 843–854.

Karunakaran, D., Halvorson, D. A, Sivanandan, V., and Newman, J. a. (2009). Pathogenicity of avian influenza viruses isolated from wild mallard ducks and domestic turkeys. *Avian Diseases*, *32*(2), 319–323.

Kato, A., Sakai, Y., Shioda, T., Kondo, T., Nakanishi, M., and Nagai, Y. (1996). Initiation of Sendai virus multiplication from transfected cDNA or RNA with negative or positive sense. *Genes to Cells : Devoted to Molecular and Cellular Mechanisms*, *1*(6), 569–579.

Kho, C. L., Tan, W. S., Tey, B. T., and Yusoff, K. (2003). Newcastle disease virus nucleocapsid protein: self-assembly and length-determination domains. *The Journal of General Virology*, *84 (8)*, 2163–2168.

Kho, C. L., Tan, W. S., and Yusoff, K. (2002). Cloning and expression of the phosphoprotein gene of Newcastle disease virus in Escherichia coli. *Journal of Biochemistry, Molecular Biology, and Biophysics*, 6(2), 117-21.

Kieft, J. S. (2008). Viral IRES RNA structures and ribosome interactions. *Trends in Biochemical Sciences*, *33*(6), 274–283.

Kim, B. Y., Lee, D. H., Kim, M. S., Jang, J. H., Lee, Y. N., Park, J. K., Yuk, S. S., Lee, J. B., Park, S. Y., Choi, I. S. and Song, C. S. (2012). Exchange of Newcastle disease viruses in Korea: The relatedness of isolates between wild birds, live bird markets, poultry farms and neighboring countries. *Infection, Genetics and Evolution*, *12*(2), 478–482.

Kim, L. M., King, D. J., Curry, P. E., Suarez, D. L., Swayne, D. E., Stallknecht, D. E., Slemons, R. D., Pedersen, J .C., Senne, D. A., Winker, K. and Afonso, C. L. (2007). Phylogenetic Diversity among Low-Virulence Newcastle Disease Viruses from Waterfowl and Shorebirds and Comparison of Genotype Distributions to Those of Poultry-Origin Isolates. *Journal of Virology*, *81*(22), 12641–12653.

Kim, L. M., King, D. J., Guzman, H., Tesh, R. B., Travassos Da Rosa, A. P. A., Bueno, R., Dennet, J. A. and Afonso, C. L. (2008). Biological and phylogenetic characterization of pigeon paramyxovirus serotype 1 circulating in wild North American pigeons and doves. *Journal of Clinical Microbiology*, *46*(10), 3303–3310.

Kim, L. M., Suarez, D. L., and Afonso, C. L. (2008). Detection of a broad range of class I and II Newcastle disease viruses using multiplex real-time reverse transcription polymerase chain reaction assay. *Journal of Veterinary Diagnostic Investigation*, *20*(4), 414–425.

Kim, S.-H., Paldurai, A., Xiao, S., Collins, P. L., and Samal, S. K. (2014). Modified Newcastle disease virus vectors expressing the H5 hemagglutinin induce enhanced protection against highly pathogenic H5N1 avian influenza virus in chickens. *Vaccine*, *32*(35), 4428–4435.

Kim, S.-H., and Samal, S. K. (2010). Role of untranslated regions in regulation of gene expression, replication, and pathogenicity of Newcastle disease virus expressing green fluorescent protein. *Journal of Virology*, *84*(5), 2629–34.

Kim, S.-H., Wanasen, N., Paldurai, A., Xiao, S., Collins, P. L., and Samal, S. K. (2013). Newcastle disease virus fusion protein is the major contributor to protective immunity of genotype-matched vaccine. *PloS One*, *8*(8), e74022.

Kim, S., Paldurai, A., and Samal, S. K. (2017). A novel chimeric Newcastle disease virus vectored vaccine against highly pathogenic avian in fl uenza virus. *Virology*, *503*, 31–36.

Krishnamurthy, S., Huang, Z., and Samal, S. K. (2000). Recovery of a virulent strain of newcastle disease virus from cloned cDNA: expression of a foreign gene results in growth retardation and attenuation. *Virology*, *278*(1), 168–182.

Krishnamurthy, S., and Samal, S. K. (1998). Nucleotide sequences of the trailer, nucleocapsid protein gene and intergenic regions of Newcastle disease virus strain Beaudette C and completion of the entire genome sequence. *Journal of General Virology*, *79*, 2419–2424.

Kumar, R., Tiwari, A. K., Chaturvedi, U., Kumar, G. R., Sahoo, A. P., Kumar, S., and Tiwari, S. (2013). Cloning and expression analysis of multiple proteins encoding P gene of newcastle disease virus. *Indian Journal of Experimental Biology*, *51*(2), 116–123.

Kwon, H.-J., Cho, S.-H., Ahn, Y.-J., Seo, S.-H., Choi, K.-S., and Kim, S.-J. (2003). Molecular epidemiology of Newcastle disease in Republic of Korea. *Veterinary Microbiology*, *95*(1–2), 39–48.

Lambrecht, B., Gonze, M., Meulemans, G., and Van Den Berg, T. P. (2004). Assessment of the cell-mediated immune response in chickens by detection of chicken interferon-γ in response to mitogen and recall Newcastle disease viral antigen stimulation. *Avian Pathology*, *33*(3), 343–350.

Le Mercier, P., Jacob, Y., Tanner, K., and Tordo, N. (2002). A novel expression cassette of lyssavirus shows that the distantly related Mokola virus can rescue a defective rabies virus genome. *Journal of Virology*, *76*(4), 2024–2027.

Lee, E. K., Jeon, W. J., Kwon, J. H., Yang, C. B., and Choi, K. S. (2009). Molecular epidemiological investigation of Newcastle disease virus from domestic ducks in Korea. *Veterinary Microbiology*, *134*(3–4), 241–248.

Lee, Y. J., Sung, H. W., Choi, J. G., Lee, E. K., Yoon, H., Kim, J. H., and Song, C. S. (2008). Protection of chickens from Newcastle disease with a recombinant baculovirus subunit vaccine expressing the fusion and hemagglutinin-neuraminidase proteins. *Journal of Veterinary Science*, *9*(3), 301–308.

Li, B.-Y., Li, X.-R., Lan, X., Yin, X.-P., Li, Z.-Y., Yang, B., and Liu, J.-X. (2011). Rescue of Newcastle disease virus from cloned cDNA using an RNA polymerase II promoter. *Archives of Virology*, *156*(6), 979–86.

Li, X., Chai, T., Wang, Z., Song, C., Cao, H., Liu, J., Zhang, X. X., WeiWang, Yao, M. and Miao, Z. (2009). Occurrence and transmission of Newcastle disease virus aerosol originating from infected chickens under experimental conditions. *Veterinary Microbiology*, *136*(3–4), 226–232.

Li, Y., Reddy, K., Reid, S. M., Cox, W. J., Brown, I. H., Britton, P., Nair, V. and Iqbal, M. (2011). Recombinant herpesvirus of turkeys as a vector-based vaccine against highly pathogenic H7N1 avian influenza and Marek's disease. *Vaccine*, *29*(46), 8257–8266.

Liu, H., Wang, Z., Wang, Y., Sun, C., Zheng, D., and Wu, Y. (2008). Characterization of Newcastle disease virus isolated from waterfowl in China. *Avian Diseases*, *52*(1), 150–5.

Liu, H., Wang, Z., Wu, Y., Zheng, D., Sun, C., Bi, D., Zuo, Y. and Xu, T. (2007). Molecular epidemiological analysis of Newcastle disease virus isolated in China in 2005. *Journal of Virological Methods*, *140*(1–2), 206–211.

Liu, Y. L., Hu, S. L., Zhang, Y. M., Sun, S. J., Romer-Oberdorfer, A., Veits, J., Wu, Y. T., Wan, H. Q. and Liu, X. F. (2007). Generation of a velogenic Newcastle disease virus from cDNA and expression of the green fluorescent protein. *Archives of Virology*, *152*(7), 1241–1249.

Lozano-Dubernard, B., Soto-Priante, E., Sarfati-Mizrahi, D., Castro-Peralta, F., Flores-Castro, R., Loza-Rubio, E., and Gay-Gutierrez, M. (2010). Protection and differentiation of infected from vaccinated animals by an inactivated recombinant Newcastle disease virus/avian influenza H5 vaccine. *Avian Diseases*, *54*(0005–2086 (Print)), 242–245.

Lumeij, J. T., and Stam, J. W. E. (1985). Paramyxovirus disease in racing pigeons. *Veterinary Quarterly*, *7*(1), 60–65.

Maamary, J., Array, F., Gao, Q., García-Sastre, A., Steinman, R. M., Palese, P., and Nchinda, G. (2011). Newcastle disease virus expressing a dendritic cell-targeted HIV gag protein induces a potent gag-specific immune response in mice. *Journal of Virology*, *85*(5), 2235–2246.

Mahon, P. J., Mirza, A. M., Musich, T. A., and Iorio, R. M. (2008). Engineered intermonomeric disulfide bonds in the globular domain of Newcastle disease virus hemagglutinin-neuraminidase protein: implications for the mechanism of fusion promotion. *Journal of Virology*, *82*(21), 10386–10396.

Makkay, A. M., Krell, P. J., and Nagy, É. (1999). Antibody detection-based differential ELISA for NDV-infected or vaccinated chickens versus NDV HN-subunit vaccinated chickens. *Veterinary Microbiology*, *66*(3), 209–222.

Marangon, S., and Busani, L. (2006). The use of vaccination in poultry production. *Revue Scientifique et Technique (International Office of Epizootics)*, *26*(1), 265–274.

Marks, F. S., Rodenbusch, C. R., Okino, C. H., Hein, H. E., Costa, E. F., Machado, G., Canal, C. W. Brentano, L. and Corbellini, L. G. (2014). Targeted survey of Newcastle disease virus in backyard poultry flocks located in wintering site for migratory birds from Southern Brazil. *Preventive Veterinary Medicine*, *116*(1–2), 197–202.

Massin, P., Rodrigues, P., Marasescu, M., van der Werf, S., and Naffakh, N. (2005). Cloning of the chicken RNA polymerase I promoter and use for reverse genetics of influenza A viruses in avian cells. *Journal of Virology*, *79*(21), 13811–13816.

McGinnes, L. W., Pantua, H., Reitter, J., and Morrison, T. G. (2006). Newcastle Disease Virus: Propagation, Quantification, and Storage. In: *Current Protocols in Microbiology* (p. 15F.2.1-15F.2.18).

Melanson, V. R., and Iorio, R. M. (2004). Amino acid substitutions in the F-specific domain in the stalk of the Newcastle disease virus HN protein modulate fusion and interfere with its interaction with the F protein. *Journal of Virology*, *78*(23), 13053–13061.

Metcalf, C. J. E., Ferrari, M., Graham, A. L., and Grenfell, B. T. (2015). Understanding Herd Immunity. *Trends in Immunology*, 36(2), 753–755.

Miller, P. J., Afonso, C. L., El Attrache, J., Dorsey, K. M., Courtney, S. C., Guo, Z., and Kapczynski, D. R. (2013). Effects of Newcastle disease virus vaccine antibodies on the shedding and transmission of challenge viruses. *Developmental and Comparative Immunology*, *41*(4), 505–513.

Miller, P. J., Decanini, E. L., and Afonso, C. L. (2010). Newcastle disease: evolution of genotypes and the related diagnostic challenges. *Infection, Genetics and Evolution* , *10*(1), 26–35.

Miller, P. J., Haddas, R., Simanov, L., Lublin, A., Rehmani, S. F., Wajid, A., Bibi, T., Khan T. A., Yaqub, T., Setiyaningsih and Afonso, C. L. (2015). Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features. *Infection, Genetics and Evolution*, *29*, 216–229.

Miller, P. J., Kim, L. M., Ip, H. S., and Afonso, C. L. (2009). Evolutionary dynamics of Newcastle disease virus. *Virology*, *391*(1), 64–72.

Miller, P. J., King, D. J., Afonso, C. L., and Suarez, D. L. (2007). Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge. *Vaccine*, *25*(41), 7238–7246.

Ming, P. G., Huang, Y., Tang, Q., Du, J. L., Tao, X. Y., Yan, J. X., and Hu, R. L. (2009). Construction and identification of the helper plasmids for reverse genetic system of rabies virus street strain. *Virologica Sinica*, *24*(6), 559–565.

Molouki, A., and Peeters, B. (2016). Rescue of recombinant Newcastle disease virus: current cloning strategies and RNA polymerase provision systems. *Archives of Virology*, 162(1), 1-12

Morgan, R. W., Gelb Jr., J., Pope, C. R., and Sondermeijer, P. J. (1993). Efficacy in chickens of a herpesvirus of turkeys recombinant vaccine containing the fusion gene of Newcastle disease virus: onset of protection and effect of maternal antibodies. *Avian Diseases*, *37*(4), 1032–1040.

Mori, Y., and Notomi, T. (2009). Loop-mediated isothermal amplification (LAMP): A rapid, accurate, and cost-effective diagnostic method for infectious diseases. *Journal of Infection and Chemotherapy*, 15(2), 62-69

Muir, W. I., Bryden, W. L., and Husband, A. J. (2000). Immunity, vaccination and the avian intestinal tract. *Developmental and Comparative Immunology, 24(2-3), 325-42.*

Munir, M., Zohari, S., Abbas, M., and Berg, M. (2012). Sequencing and analysis of the complete genome of Newcastle disease virus isolated from a commercial poultry farm in 2010. *Archives of Virology*, *157*(4), 765–8.

Münz, C., Steinman, R. M., and Fujii, S. (2005). Dendritic cell maturation by innate lymphocytes: coordinated stimulation of innate and adaptive immunity. *The Journal of Experimental Medicine*, *202*(2), 203–207.

Murakami, S., Horimoto, T., Yamada, S., Kakugawa, S., Goto, H., and Kawaoka, Y. (2008). Establishment of canine RNA polymerase I-driven reverse genetics for influenza A virus: its application for H5N1 vaccine production. *Journal of Virology*, *82*(3), 1605–1609.

Murulitharan, K., Yusoff, K., Omar, A. R., and Molouki, A. (2013). Characterization of Malaysian velogenic NDV strain AF2240-I genomic sequence: a comparative study. *Virus Genes*, *46*(3), 431–40.

Nagai, Y., Hamaguchi, M., and Toyoda, T. (1989). Molecular Biology of Newcastle Disease Virus. *Pandey, R. (Ed.). Progress in Veterinary Microbiology and Immunology,* Vol. 5. Nononcogenic Avian Viruses. Vii+136P. S. Karger Ag: Basel, Switzerland; New York, New York, Usa. Illus. ISBN 3-8055-4827-3. 0 (0)., 16–64.

Nanthakumar, T., Kataria, R. S., Tiwari, A. K., Butchaiah, G., and Kataria, J. M. (2000). Pathotyping of Newcastle disease viruses by RT-PCR and restriction rnzyme analysis. *Veterinary Research Communications*, *24*(4), 275–286.

Neumann, G., Zobel, A., and Hobom, G. (1994). RNA polymerase I-mediated expression of influenza viral RNA molecules. *Virology*, *202*(1), 477–9.

Njagi, L. W., Nyaga, P. N., Bebora, L. C., Mbuthia, P. G., and Minga, U. M. (2012). Effect of immunosuppression on newcastle disease virus persistence in ducks with different immune status. *ISRN Veterinary Science*, *2012*, 253809.

Noton, S. L., and Fearns, R. (2015). Initiation and regulation of paramyxovirus transcription and replication. *Virology*, 479-480:545-54 .

OIE. (2008). Newcastle disease. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees)*. (Vol. 1, pp. 576–589).

Ong, H. K., Ali, A. M., Omar, A. R., and Yusoff, K. (2000). Cloning and expression of the HN gene from the velogenic viscerotropic Newcastle disease virus strain AF2240 in Sf9 insect cells. *Cytotechnology*, *32*(3), 243–51.

Oyebanji, V. O., Emikpe, B. O., Omolade, A. O., Odeniyi, M. O., Salami, A., Osowole, O. I., Kasali, O. B. and Akinboade, O. A. (2017). Evaluation of immune response in challenged chickens vaccinated with Newcastle disease vaccine using gums from Cedrela odorata and Khaya senegalensis as delivery agents. *Journal of Immunoassay and Immunochemistry*, *38*(4), 378–388.

Paldurai, A., Kim, S.-H., Nayak, B., Xiao, S., Shive, H., Collins, P. L., and Samal, S. K. (2014). Evaluation of the contributions of the individual viral genes to Newcastle disease virus virulence and pathogenesis. *Journal of Virology*, *88*(15), 8579–8596.

Palya, V., Kiss, I., Tatár-Kis, T., Mató, T., Felföldi, B., and Gardin, Y. (2012). Advancement in Vaccination Against Newcastle Disease: Recombinant HVT NDV Provides High Clinical Protection and Reduces Challenge Virus Shedding with the Absence of Vaccine Reactions. *Avian Diseases*, *56*(2), 282–287.

Panda, A., Huang, Z., Elankumaran, S., Rockemann, D. D., and Samal, S. K. (2004). Role of fusion protein cleavage site in the virulence of Newcastle disease virus. *Microbial Pathogenesis*, *36*(1), 1–10.

Park, C. S., and Choi, Y. S. (2005). How do follicular dendritic cells interact intimately with B cells in the germinal centre? *Immunology*, 114(1): 2–10.

Patton, J. T., Davis, N. L., and Wertz, G. W. (1984). N protein alone satisfies the requirement for protein synthesis during RNA replication of vesicular stomatitis virus. *J Virol*, *49*(2), 303–309.

Pearson, G. L., and McCann, M. K. (1975). The role of indigenous wild, semidomestic, and exotic birds in the epizootiology of velogenic viscerotropic Newcastle disease in Southern California, 1972-1973. *Journal of American Veterinary Medical Association*, *167*(7), 610–614.

Pedersen, J. C. (2008). Hemagglutination-inhibition test for avian influenza virus subtype identification and the detection and quantitation of serum antibodies to the avian influenza virus. *Methods in Molecular Biology*, 436, 53–66.

Peeters, B. P., de Leeuw, O. S., Koch, G., and Gielkens, A. L. (1999). Rescue of Newcastle disease virus from cloned cDNA: Evidence that cleavability of the fusion protein is a major determinant for virulence. *Journal of Virology*, *73*(6), 5001–5009.

Peeters, B. P. H., De Leeuw, O. S., Verstegen, I., Koch, G., and Gielkens, A. L. J. (2001). Generation of a recombinant chimeric Newcastle disease virus vaccine that allows serological differentiation between vaccinated and infected animals. *Vaccine*, *19*, 1616–1627.

Peeters, B. P. H., Gruijthuijsen, Y. K., De Leeuw, O. S., and Gielkens, A. L. J. (2000). Genome replication of Newcastle disease virus: Involvement of the rule-of-six. *Archives of Virology*, *145*, 1829–1845.

Pfaller, C. K., Cattaneo, R., and Schnell, M. J. (2015). Reverse genetics of Mononegavirales: How they work, new vaccines, and new cancer therapeutics. *Virology*, 1–14.

Pham, H. M., Nakajima, C., Ohashi, K., and Onuma, M. (2005). Loop-Mediated Isothermal Amplification for Rapid Detection of Newcastle Disease Virus. *Journal of Clinical Microbiology*, *43*(4), 1646–1650.

Piacenti, A. M., King, D. J., Seal, B. S., Zhang, J., and Brown, C. C. (2006). Pathogenesis of Newcastle disease in commercial and specific pathogen-free turkeys experimentally infected with isolates of different virulence. *Veterinary Pathology*, *43*(2), 168–78.

Rahman, M. M., Bari, A. S. M., Giasuddin, M., Islam, M. R., Alam, J., Sil, G. C., and Rahman, M. M. (2002). Evaluation of maternal and humoral immunity against Newcastle Disease virus in chicken. *International Journal of Poultry Science*, *1*(5), 161–163.

Rashid, H., Khandaker, G., and Booy, R. (2012). Vaccination and herd immunity: what more do we know? *Current Opinion in Infectious Diseases*, *25*(3), 243–249.

Rasoli, M., Yeap, S. K., Tan, S. W., Moeini, H., Ideris, A., Bejo, M. H., Alitheen N. B, Kaiser, P and Omar, A. R. (2014). Alteration in lymphocyte responses, cytokine and chemokine profiles in chickens infected with genotype VII and VIII velogenic Newcastle disease virus. *Comparative Immunology, Microbiology and Infectious Diseases*, *37*(1), 11–21.

Rauw, F., Gardin, Y., Palya, V., van Borm, S., Gonze, M., Lemaire, S., van den Berg T. and Lambrecht, B. (2009). Humoral, cell-mediated and mucosal immunity induced by oculo-nasal vaccination of one-day-old SPF and conventional layer chicks with two different live Newcastle disease vaccines. *Vaccine*, *27*(27), 3631–3642.

Razmaraii, N., Toroghi, R., Babaei, H., Khalili, I., Sadigh-Eteghad, S., and Froghy, L. (2012). Immunogenicity of commercial, formaldehyde and binary ethylenimine inactivated Newcastle disease virus vaccines in specific pathogen free chickens. *Archives of Razi Institute*, *67*(1), 21–25.

Reguera, J., Cusack, S., and Kolakofsky, D. (2014). Segmented negative strand RNA virus nucleoprotein structure. *Current Opinion in Virology*, *5*(1), 7–15.

Rehwinkel, J., Tan, C. P., Goubau, D., Schulz, O., Pichlmair, A., Bier, K., Robb, N., Vreede, F., Barclay, W., Fodor, E. and Reis e Sousa, C. (2010). RIG-I detects viral genomic RNA during negative-strand RNA virus infection. *Cell*, *140*(3), 397–408.

Reynolds, D. L., and Maraqa, A. D. (2000). Protective immunity against Newcastle disease: the role of cell-mediated immunity. *Avian Diseases*, *44*(1), 138–44.

Römer-Oberdörfer, A., Mundt, E., Mebatsion, T., Buchholz, U. J., and Mettenleiter, T. C. (1999). Generation of recombinant lentogenic Newcastle disease virus from cDNA. *The Journal of General Virology*, *80*(11), 2987–95.

Roohani, K., Tan, S. W., Yeap, S. K., Ideris, A., Bejo, M. H., and Omar, A. R. (2015). Characterisation of genotype VII Newcastle disease virus (NDV) isolated from NDV vaccinated chickens, and the efficacy of LaSota and recombinant genotype VII vaccines against challenge with velogenic NDV. *Journal of Veterinary Science*, *16*(4), 447–457.

Rothenberg, E., Smotkin, D., Baltimore, D., and Weinberg, R. A. (1977). In vitro synthesis of infectious DNA of murine leukaemia virus. *Nature*, *269*(5624), 122–126.

Rout, S. N., and Samal, S. K. (2008). The Large Polymerase Protein Is Associated with the Virulence of Newcastle Disease Virus. *Journal of Virology*, *82*(16), 7828–7836.

Ryan, M. D., and Drew, J. (1994). Foot-and-mouth disease virus 2A oligopeptide mediated cleavage of an artificial polyprotein. *The EMBO Journal*, *13*(4), 928–33.

Saitou, N., and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*(4), 406–425.

Sakai, K., Yada, K., Sakabe, G., Tani, O., Miyaji, K., Nakamura, M., and Takehara, K. (2006). Serological and virological studies of Newcastle disease and avian influenza in slaughter-age ostriches (Struthio camelus) in Japan. *The Journal of Veterinary Medical Science / the Japanese Society of Veterinary Science*, *68*(5), 491–4.

Samal, S., Khattar, S. K., Kumar, S., Collins, P. L., and Samal, S. K. (2012). Coordinate deletion of N-glycans from the heptad repeats of the fusion F protein of Newcastle disease virus yields a hyperfusogenic virus with increased replication, virulence, and immunogenicity. *Journal of Virology*, *86*, 2501–2511.

Samal, S., Kumar, S., Khattar, S. K., and Samal, S. K. (2011). A single amino acid change, Q114R, in the cleavage-site sequence of Newcastle disease virus fusion protein attenuates viral replication and pathogenicity. *Journal of General Virology*, *92*, 2333–2338.

Satharasinghe, D. A., Murulitharan, K., Tan, S. W., Yeap, S. K., Munir, M., Ideris, A., and Omar, A. R. (2016). Detection of Inter-Lineage Natural Recombination in Avian Paramyxovirus Serotype 1 Using Simplified Deep Sequencing Platform. *Frontiers in Microbiology*, *7*, 1–14.

Seal, B. S., King, D. J., and Sellers, H. S. (2000). The avian response to Newcastle disease virus. *Developmental and Comparative Immunology*, *24*, 257–268.

Sharma, J. M. (1999). Introduction to poultry vaccines and immunity. *Advances in Veterinary Medicine*, *41*(C), 481–494.

Shohaimi, S. A., Raus, R. A., Huai, O. G., Asmayatim, B. M., Nayan, N., and Yusuf, A. M. (2015). Sequence and phylogenetic analysis of newcastle disease virus genotype VII isolated in Malaysia during 1999-2012. *Jurnal Teknologi*, *77*(25), 159–164.

Siddique, N., Naeem, K., Abbas, M. A., Ali Malik, A., Rashid, F., Rafique, S., Ghafar, A. and Rehman, A. (2013). Sequence and phylogenetic analysis of virulent Newcastle disease virus isolates from Pakistan during 2009-2013 reveals circulation of new sub genotype. *Virology*, *444*, 37–40.

Siegrist, C. (2008). Vaccine immunology 2. *Most*, *22*, 17–36.

Smith-Garvin, J. E., Koretzky, G. A., and Jordan, M. S. (2009). T Cell Activation. *Annual Review of Immunology*, *27*(1), 591–619.

Snoeck, C. J., Owoade, A. A., Couacy-Hymann, E., Alkali, B. R., Okwen, M. P., Adeyanju, A. T., Komoyo, G. F.., Nakouné, E., Le Faou, A. and Muller, C. P. (2013). High Genetic Diversity of Newcastle Disease Virus in Poultry in West and Central Africa: Cocirculation of Genotype XIV and Newly Defined Genotypes XVII and XVIII. *Journal of Clinical Microbiology*, *51*(7), 2250–2260.

Steward, M., Samson, A. C., Errington, W., and Emmerson, P. T. (1995). The Newcastle disease virus V protein binds zinc. *Archives of Virology*, *140*(7), 1321–1328.

Steward, M., Vipond, I. B., Millar, N. S., and Emmerson, P. T. (1993). RNA editing in Newcastle disease virus. *The Journal of General Virology*, *74 (12)*, 2539–2547.

Stobart, C., and Moore, M. (2014). RNA Virus Reverse Genetics and Vaccine Design. *Viruses*, *6*(7), 2531–2550.

Su, J., Dou, Y., You, Y., and Cai, X. (2015). Application of minigenome technology in virology research of the Paramyxoviridae family. *Journal of Microbiology, Immunology and Infection*, *48*(2), 123–129.

Sun, H. L., Wang, Y. F., Miao, D. Y., Zhang, P. J., Zhi, H. D., Xu, L. L., Wang M., Tong, G. Z and Wang, M. (2006). Construction and characterization of recombinant fowlpox virus co-expressing F and HN genes of newcastle disease virus and gB gene of infectious larygnotracheitis virus. *Chinese Journal of Biotechnology*, *22*(6), 931–938.

Susta, L., Miller, P. J., Afonso, C. L., and Brown, C. C. (2011). Clinicopathological Characterization in Poultry of Three Strains of Newcastle Disease Virus Isolated From Recent Outbreaks. *Veterinary Pathology*, *48*(2), 349–360.

Swayne, D. E., and King, D. J. (2003). Zoonosis Update Avian Influenza and Newcastle disease. *Veterinary Medicine Today*, 222 (11), 1534-1540.

Tan, S. W., Ideris, A., Omar, A. R., Yusoff, K., and Hair-Bejo, M. (2010). Sequence and phylogenetic analysis of Newcastle disease virus genotypes isolated in Malaysia between 2004 and 2005. *Archives of Virology*, *155*(1), 63–70.

Tamura, K., Nei, M., and Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences*, *101*(30), 11030–11035.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725–2729.

Terregino, C., Cattoli, G., Grossele, B., Bertoli, E., Tisato, E., and Capua, I. (2003). Characterization of Newcastle disease virus isolates obtained from Eurasian collared doves (Streptopelia decaocto) in Italy. *Avian Pathology*, *32*(1), 63–68.

Thornton, D. H., Hopkins, I. G., and Nancy Hebert, C. (1980). Potency of live newcastle disease vaccines. *Avian Pathology*, *9*(3), 457–464.

Tlaxca, J. L., Ellis, S., and Remmele, R. L. (2014). Live attenuated and inactivated viral vaccine formulation and nasal delivery: Potential and challenges. *Advanced Drug Delivery Reviews*, *93*, 56–78.

Van Dyken, S. J., and Locksley, R. M. (2013). Interleukin-4- and Interleukin-13-Mediated Alternatively Activated Macrophages: Roles in Homeostasis and Disease. *Annual Review of Immunology*, *31*(1), 317–343.

Victora, G. D. (2014). SnapShot: The germinal center reaction. *Cell*, *159*(3), 700–700.

Vijayarani, K., Muthusamy, S., Tirumurugaan, K. G., Sakthivelan, S. M., and Kumanan, K. (2010). Pathotyping of a Newcastle disease virus isolated from peacock (Pavo cristatus). *Tropical Animal Health and Production*, *42*(3), 415–419.

Vulliémoz, D., and Roux, L. (2001). "Rule of six": How does the Sendai virus RNA polymerase keep count? *Journal of Virology*, *75*(10), 4506–4518.

Wakamatsu, N., King, D. J., Seal, B. S., Samal, S. K., and Brown, C. C. (2006). The pathogenesis of Newcastle disease: A comparison of selected Newcastle disease virus wild-type strains and their infectious clones. *Virology, 353*, 333–343.

Walpita, P., and Flick, R. (2005). Reverse genetics of negative-stranded RNA viruses: A global perspective. *FEMS Microbiology Letters*, 244(1), 9-18.

Wang, J., Cong, Y., Yin, R., Feng, N., Yang, S., Xia, X., Xiao, Y., Wang, W., Liu, X., Hu, S., Ding, C., Yu, S., Wang, C. and Ding, Z. (2015). Generation and evaluation of a recombinant genotype VII Newcastle disease virus expressing VP3 protein of goose parvovirus as a bivalent vaccine in goslings. *Virus Research*, *203*, 77–83.

Wang, J. T., McElvain, L. E., and Whelan, S. P. J. (2007). Vesicular stomatitis virus mRNA capping machinery requires specific cis-acting signals in the RNA. *Journal of Virolology*, *81*(20), 11499–11506.

Wang, J., Wang, C., Feng, N., Wang, H., Zheng, X., Yang, S., Gao, Y., Xia, X., Yin, R., Liu X.., Hu, S., Ding, C., Yu, S., Cong, Y. and Ding, Z. (2015). Development of a reverse genetics system based on RNA polymerase II for Newcastle disease virus genotype VII. *Virus Genes*, *50*(1), 152–155.

Wang, Y., Yu, W., Huo, N., Wang, W., Guo, Y., Wei, Q., Wang, X. Zhang, S. Yang, Z and Xiao, S. (2017). Comprehensive analysis of amino acid sequence diversity at the F protein cleavage site of Newcastle disease virus in fusogenic activity, (M). *PLoS ONE,* 12(9): e0183923

Wang, Z., Vreede, F. T., Mitchell, J. O., and Viljoen, G. J. (2001). Rapid detection and differentiation of Newcastle disease virus isolates by a triple one-step RT-PCR. *The Onderstepoort Journal of Veterinary Research*, *68*, 131–134.

Weli, S. C., and Tryland, M. (2011). Avipoxviruses: infection biology and their use as vaccine vectors. *Virology Journal*, *8*(1), 49.

Whelan, S. P. J., Barr, J. N., and Wertz, G. W. (2004). Transcription and replication of nonsegmented negative-strand RNA viruses. *Current Topics in Microbiology and Immunology*, *283*, 61–119.

Winterfield, R. W., Goldman, C. L., and Seadale, E. H. (1957). Newcastle Disease Immunization Studies: 4. Vaccination of chickens with B1, F and Lasota strains of Newcastle disease dirus administered through the drinking water. *Poultry Science*, *36*(5), 1076–1088.

Wise, M. G., Sellers, H. S., Alvarez, R., and Seal, B. S. (2004). RNA-dependent RNA polymerase gene analysis of worldwide Newcastle disease virus isolates representing different virulence types and their phylogenetic relationship with other members of the paramyxoviridae. *Virus Research*, *104*(1), 71–80.

Xiao, S., Nayak, B., Samuel, A., Paldurai, A., Kanabagattebasavarajappa, M., Prajitno, T. Y., Bharoto, E. E., Peter L. Collins, P. L. and Samal, S. K. (2012). Generation by reverse genetics of an effective, stable, live-attenuated Newcastle disease virus vaccine based on a currently circulating, highly virulent Indonesian strain. *PLoS ONE*, *7*(12): e52751.

Xue, C., Cong, Y., Yin, R., Sun, Y., Ding, C., Yu, S., Liu, X., Hu, S., Qian, J., Yuan, Q., Yang, M., Wang, C. and Ding, Z. (2017). Genetic diversity of the genotype VII Newcastle disease virus: identification of a novel VIIj sub-genotype. *Virus Genes*, *53*(1), 63–70.

Yan, Y., Rout, S. N., Kim, S.-H., and Samal, S. K. (2009). Role of Untranslated Regions of the Hemagglutinin-Neuraminidase gene in replication and pathogenicity of Newcastle disease virus. *Journal of Virology*, *83*(11), 5943–5946.

Yan, Y., and Samal, S. K. (2008). Role of intergenic sequences in newcastle disease virus RNA transcription and pathogenesis. *Journal of Virology*, *82*(3), 1323–1331.

Yu, Y., Qiu, X., Xu, D., Zhan, Y., Meng, C., Wei, N., Chen, H., Tan, L., Yu S., Liu, X., Qin, A. and Ding, C. (2012). Rescue of virulent class I Newcastle disease virus variant 9a5b-D5C1. *Virology Journal*, *9*(1), 120.

Yusoff, K., and Wen Siang Tan. (2001). Newcastle disease virus: Macromolecules and opportunities. *Avian Pathology*, 30(5), 439-55.

Zhai, L., Li, Y., Wang, W., and Hu, S. (2011). Enhancement of humoral immune responses to inactivated Newcastle disease and avian influenza vaccines by oral administration of ginseng stem-and-leaf saponins in chickens. *Poultry Science*, *90*, 1955–1959.

Zhang, X., Liu, H., Liu, P., Peeters, B. P. H., Zhao, C., and Kong, X. (2013). Recovery of avirulent, thermostable Newcastle disease virus strain NDV4-C from cloned cDNA and stable expression of an inserted foreign gene. *Archives of Virology*, *158*(10), 2115–2120.

Zhang, Y. yuan, Shao, M. yu, Yu, X. hui, Zhao, J., and Zhang, G. zhong. (2014). Molecular characterization of chicken-derived genotype VIId Newcastle disease virus isolates in China during 2005-2012 reveals a new length in hemagglutinin-neuraminidase. *Infection, Genetics and Evolution*, *21*, 359–366.

Zhang, Z., Zhao, W., Li, D., Yang, J., Zsak, L., and Yu, Q. (2015). Development of a newcastle disease virus vector expressing a foreign gene through an internal ribosomal entry site provides direct proof for a sequential transcription mechanism. *Journal of General Virology*, *96*(8), 2028–2035.

Zhao, K., Chen, G., Shi, X., Gao, T., Li, W., Zhao, Y., Zhang, F., Wu, J., Cui, X. and Wang, Y.-F. (2012). Preparation and efficacy of a live Newcastle Disease virus vaccine encapsulated in Chitosan nanoparticles. *PLoS ONE*, *7*(12), e53314.

Zhao, K., Zhang, Y., Zhang, X., Li, W., Shi, C., Guo, C., Dai, C., Chen, Q., Jin, Z., Zhao, Y., Cui, H., Wang, Y. and Wang, Y. (2014). Preparation and efficacy of newcastle disease virus dna vaccine encapsulated in chitosan nanoparticles. *International Journal of Nanomedicine*, *9*(1), 389–402.

Zhao, W., Zhang, Z., Zsak, L., and Yu, Q. (2013). Effects of the HN gene C-terminal extensions on the Newcastle disease virus virulence. *Virus Genes*, *47*(3), 498–504.

Zhao, W., Zhang, Z., Zsak, L., and Yu, Q. (2014). P and M gene junction is the optimal insertion site in Newcastle disease virus vaccine vector for foreign gene expression. *The Journal of General Virology*, 96, 40–45.