UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF LIVE ATTENUATED FOWL ADENOVIRUS ISOLATE OF MALAYSIA FOR VACCINE PRODUCTION

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By

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November 2017

Chairman: Professor Mohd Hair Bin Bejo, PhD
Faculty: Veterinary Medicine

Fowl adenovirus (FAdV) infection is one of the major threats among viral diseases in poultry industry worldwide. The disease caused severe economic losses due to high mortality and poor production. Malaysian`s FAdV isolate are highly pathogenic in chickens and has been identified as primary pathogen of inclusion body hepatitis (IBH) and gizzard erosion. It is indeed an urgent need to develop FAdV vaccines to prevent and control FAdV infection. It was objective of the study to develop live attenuated FAdV isolate of Malaysia for future production of vaccine against the disease.

FAdV isolate (UPM1137) was obtained from liver sample of chickens during outbreak of IBH and gizzard erosion. The isolate was successfully isolated and identified in specific pathogen free (SPF) embryonated chicken eggs and Vero cells. Thickening and cloudy of chorioallantoic membrane (CAM) with pale, petechial haemorrhages and multifocal area of necrosis of the liver and hydropericardium of embryo were recorded at day 9 post-inoculation (pi). The cytopathic effect (CPE) in Vero cells revealed cytoplasmic extensions and formation of plaque at day 6pi. It was confirmed that the isolate belong to FAdV by PCR with expected PCR size product and through sequence analysis. Partial hexon gene region with 1166 base pair (bp) of nucleotide (nt) sequence from SPF embryos and Vero cells samples were assigned with Genbank accession number, KF866370 (UPM1137E2) and KF866371 (UPM1137V1), respectively. For fiber gene, 1094bp of nucleotide sequence were deposited into GenBank with accession number KY305950 (UPM1137E2) and KY364903 (UPM1137V1). The isolate is highly related to group E species under serotype 8b.

The isolate is highly pathogenic in SPF embryonated chicken eggs with 100% mortality and typical gross and histological lesions associated with FAdV. The embryonic mortality pattern was delayed from 17th consecutive passages (E17) to E20. This is consistent with the molecular changes in both L1 loop and knob regions of the nucleotide bases and amino acid in hexon and fiber gene and may indicate virus

attenuation. Molecular changes were also prominent at 5th consecutive passages of the virus in Vero cells (V5). However, the FAdV isolate was highly susceptible to be adapted and attenuated in primary chicken embryo liver (CEL) cells. Early CPE was observed at 2nd consecutive passages (CEL2) to CEL19 within 24 to 48 hours post inoculation (pi) in the form of cells rounding, refractile and clumping. The CPE formation was delayed from CEL20 to CEL35 within 48 to 72 hours pi. Nucleotide sequences of hexon and fiber gene from all propagated isolates were deposited to GenBank and assigned with the accession numbers KY305943 to KY305949 (hexon) and KY305951 to KY305957 (fiber). Molecular changes in L1 loop of hexon gene are minimal in nucleotide bases of CEL5 to CEL20 without caused any amino acid changes. All changes mainly occur in other part of hexon gene. However, changes noticeable from CEL25 to CEL35 propagated FAdV isolates. Sequence analysis in fiber gene revealed both nucleotide and amino acid changes in knob region are prominent at high passage level from CEL20 to CEL35. It was demonstrated that the marker gene for adaptation in CEL cells and SPF embryonated chicken eggs were identified in nucleotide of L1 loop hexon gene at position 90(T-C) and in knob of fiber gene at both nucleotide and amino acid at position 1078(G-C) and 360(A-P), respectively. Additionally, the marker gene for attenuation in CEL cells was detected in knob region of fiber gene at position 1062(A-C). Sequence analysis of hexon and fiber gene product of all the samples either from the SPF eggs, Vero cells or CEL cells passages revealed 1166bp and 1094bp, respectively belongs to FAdV group E serotype 8b. Phylogenetic tree constructions revealed that isolates were clustered and closed to each other.

The pathogenicity, immunogenicity and efficacy of an attenuated FAdV isolate CEL35 ($10^{6.7}TCID_{50}$/ml) was determined in 1-day-old SPF chickens via oral (0.1ml) and subcutaneous (SQ) (0.1ml) route of inoculation. On day 14 post-inoculation (pi), chickens in challenged groups were inoculated with 0.2ml pathogenic FAdV isolate, UPM11134 ($10^{8.3}TCID_{50}$/ml) via intraperitoneal (IP) route. The study shown that the attenuated FAdV isolate is non-pathogenic and safe in SPF chickens without exhibit clinical signs associated with FAdV infection throughout the trial. The FAdV inoculated chickens in challenged group were normal without exhibit abnormal clinical signs, gross and histological lesions. However, chickens in non-inoculated group showed clinical signs of weakness, depression and recumbency at day 4 post-challenge (pc) prior recovery on day 8pc. The body weight was significantly lower ($p<0.05$) and the liver was pale at 14 day pc as compared to non-challenged group. FAdV antibody titer was not detected in control group throughout the trial. In group of chicken inoculated via SQ route, the antibody titer was 648 ± 188 and 324 ± 85 at day 14 and 28pi, respectively. The antibody titer in chicken inoculated via oral route was 316 ± 118 and 163 ± 17 at day 14 and 28pi, respectively, was not significantly difference ($p>0.05$) when compared to the SQ and control groups. In challenged groups, the antibody titer was significantly increased ($p<0.05$) mainly in control (6276 ± 1983) and oral (5496 ± 1688) groups with exception in SQ (3895 ± 1858) group at day 14pc. It was demonstrated that attenuated FAdV is safe, non-pathogenic, immunogenic and able to protect chickens from FAdV infection. The subcutaneous is an effective route of inoculation as compared to oral route.

The pathogenicity and immunogenicity of FAdV attenuated in CEL cells, CEL35 ($10^{6.7}TCID_{50}$/ml), and in SPF embryonated chicken eggs, E20 ($10^{8.7}TCID_{50}$/ml) were

determined in 1-day-old commercial broiler chickens via oral (0.5ml) or intraperitoneal (IP) (0.5ml) route. The study revealed that the attenuated isolates were non-pathogenic in commercial broiler chickens. Neither clinical signs nor gross and histological lesions in the liver was recorded in all groups of chicken throughout 21 days trial. The mean body weight of chickens was significantly low ($p<0.05$) in inoculated groups as compared to control group at days 7 and 14pi in both routes of inoculation. At day 21pi, body weight in inoculated chickens was significantly high ($p<0.05$) as compared to control group. The FAdV antibody titer was high in day old chicks due to presence of maternal derived antibody (MDA) and was significantly declined ($p<0.05$) from day 0 (7795 ± 1414) to day 21pi (69 ± 34). The FAdV inoculated chickens using CEL35 attenuated isolate revealed oral (3985 ± 776) and IP (3734 ± 809) route had identical pattern of antibody titer and were not significant difference ($p>0.05$) between route of inoculation throughout the trial. Similarly, in chickens inoculated with E20 FAdV, the antibody titer in oral (4119 ± 792) and IP (4598 ± 871) group were equally distributed among the routes. There were no differences in antibody titer between attenuated isolates either through oral or IP routes ($p>0.05$). However, FAdV inoculated chickens with CEL35 (2348 ± 1800) and E20 (1833 ± 792) attenuated isolates via IP route have high antibody titer significantly ($p<0.05$) compared to control group (69 ± 34) at day 21pi. It was concluded that the attenuated FAdV isolate are non-pathogenic and immunogenic in commercial broiler chickens. The attenuated FAdV isolate in CEL cells and SPF embryonated chicken eggs induced similar antibody response either through oral or IP routes.

It was concluded that FAdV is highly susceptible in SPF embryonated chicken eggs and primary CEL cells rather than in Vero cells with nucleotide and amino acid changes in hexon at L1 loop region and in fiber gene at knob region. The attenuated isolates are non-pathogenic in SPF and commercial broiler chickens with capability to induce FAdV antibody. It appears that parenteral route is an effective route of FAdV inoculation when compared to the oral route and the attenuated isolates are suitable to be used for future development of FAdV vaccine against the disease.

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

PEMBANGUNAN VIRUS ADENO UNGGAS ISOLAT MALAYSIA HIDUP YANG DILEMAHKAN UNTUK PRODUKSI VAKSIN

Oleh

NORFITRIAH BT MOHAMED SOHAIMI

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Infeksi virus adeno unggas (FAdV) adalah satu ancaman besar di kalangan penyakit virus dalam industri ternakan poultri di seluruh dunia. Penyakit ini menyebabkan kerugian ekonomi yang besar kerana kematian yang tinggi dan kejatuhan pengeluaran. FAdV isolat Malaysia sangat patogenik pada ayam dan telah dikenal pasti sebagai patogen utama `Inclusion body hepatitis (IBH)` dan `gizzard erosion`. Ia sememangnya merupakan keperluan mendesak untuk membangunkan vaksin FAdV untuk mencegah dan mengawal jangkitan FAdV. Adalah menjadi objektif bagi kajian ini untuk membangunkan FAdV isolat Malaysia hidup yang dilemahkan untuk produksi vaksin masa depan terhadap penyakit ini.

FAdV isolat Malaysia (UPM1137) telah diperolehi dari sampel hati ayam semasa wabak IBH dan gizzard erosion. Isolat tersebut telah berjaya dipencilkan dan dikenal pasti dalam telur ayam berembrio bebas patogen khusus (SPF) dan sel Vero. Penebalan dan kekelaman pada membran korioalantoik dengan hati yang pucat, pendarahan berbintik dan nekrosis multifokal disertai dengan hidroperikardium dalam embrio telah dicatatkan pada hari ke-9 pasca-infeksi (pi). Kesan sitopatik (CPE) dalam sel Vero menunjukkan penyambungan sitoplasma dan pembentukan plak pada hari ke-6pi. Isolat telah disahkan sebagai FAdV melalui PCR dengan saiz PCR produk dalam jangkaan dan juga melalui analisis jujukan. Sebahagian gen hexon dengan 1166 pasangan bes (bp) pada jujukan nukleotida dari sampel embrio SPF dan sel Vero masing-masing diberikan nombor akses GenBank, KF866370 (UPM1137E2) dan KF866371 (UPM1137V1). Bagi gen fiber, 1094bp pada jujukan nukleotida telah dimasukkan ke GenBank dengan nombor akses KY305950 (UPM1137E2) dan KY364903 (UPM1137V1). Isolat FAdV ini sangat berkait rapat dengan spesies kumpulan E di bawah serotip 8b.

Isolat ini sangat patogenik dalam telur ayam berembrio SPF dengan 100% kematian dan tipikal lesi mata kasar dan histologi berkaitan dengan FAdV. Corak kematian

embrionik mengalami kelewatan dari passage konsekutif ke-17 (E17) hingga ke E20. Ia konsisten dengan perubahan molekular di kedua-dua bahagian `loop L1` dan knob daripada bes nukleotida dan asid amino dalam gen hexon dan fiber dan mungkin menandakan pelemahan virus. Perubahan molekular juga ketara pada virus di passage konsekutif yg ke-5 dalam sel Vero (V5). Walaubagaimanapun, isolat FAdV sangat sensitif untuk disesuaikan dan dilemahkan di dalam sel primer hati embrio ayam (CEL). CPE awal telah dicatatkan pada konsekutif passage yang ke-2 (CEL2) hingga ke CEL19 dalam masa 24 hingga 48 jam pi dalam bentuk sel yang membulat, bersinar dan berkumpul-kumpul. Pembentukan CPE mengalami kelewatan dari CEL20 ke CEL35 dalam masa 48 hingga 72 jam pi. Jujukan nukleotida pada gen hexon dan fiber daripada kesemua isolat yang dibiakkan berulang-kali telah dimasukkan ke GenBank dan diberikan nombor akses KY305943 hingga KY305949 (hexon) dan KY305951 hingga KY305957 (fiber). Perubahan molekular pada `loop L1` dalam gen hexon adalah minimum dalam jujukan nukleotida bes dari CEL5 ke CEL20 tanpa sebarang perubahan pada asid amino. Kesemua perubahan berlaku kebanyakannya di kawasan gen hexon yang lain. Namun, perubahan ketara pada isolat FAdV yang dibiakkan berulang kali daripada CEL25 hingga ke CEL35. Analysis jujukan dalam gen fiber mendapati kedua-dua perubahan pada nukleotida dan asid amino di bahagian knob ketara pada passage yang tinggi dari CEL20 hingga ke CEL35. Kajian ini menunjukkan bahawa gen penanda untuk penyesuaian di dalam sel CEL and telur ayam berembrio SPF telah dikenalpasti pada nukleotida gen hexon `loop L1` di posisi 90(T-C) dan pada gen fiber di bahagian knob di kedua-dua nukleotida dan asid amino masing-masing di posisi 1078(G-C) dan 360(A-P). Di samping itu, gen penanda untuk pelemahan di dalam sel CEL telah dikesan pada bahagian knob di gen fiber di posisi 1062(A-C). Analysis jujukan bagi produk gen hexon dan fiber daripada kesemua sampel sama ada dari telur SPF, sel Vero dan sel CEL mendapati 1166bp dan 1094bp masing-masing tergolong dalam kumpulan E FAdV serotip 8b. Pembinaan pokok filogenetik menunjukkan kesemua isolat dikumpulkan dan saling berkait rapat antara satu sama lain.

Patogenisiti, imunogenisiti dan efikasi isolat FAdV CEL35 ($10^{6.7}TCID_{50}$/ml) yang dilemahkan telah ditentukan di dalam ayam SPF berumur satu hari melalui laluan inokulasi oral (0.1ml) dan subkutaneus (SQ) (0.1ml). Pada hari ke-14pi, ayam dalam kumpulan dicabar telah disuntik dengan 0.2ml isolat FAdV yang patogenik, UPM11134($10^{8.3}TCID_{50}$/ml) melalui laluan intraperitoneal (IP). Kajian ini menunjukkan isolat FAdV yang dilemahkan tidak patogenik dan selamat di dalam ayam SPF tanpa menunjukkan tanda-tanda klinikal berkaitan dengan infeksi FAdV sepanjang tempoh ujikaji. Ayam yang telah diinokulat dengan FAdV dalam kumpulan dicabar adalah normal tanpa menunjukkan tanda-tanda klinikal dan lesi mata kasar dan histologi. Walaubagaimanapun, bagi ayam dalam kumpulan kawalan yang tidak diinokulat menunjukkan tanda-tanda lemah, murung dan terbaring pada hari ke-4 pasca dicabar (pc) sebelum pulih pada hari ke-8pc. Berat badan ayam adalah signifikan rendah ($p<0.05$) dan hati yang pucat pada hari ke-14pc berbanding dengan kumpulan ayam yang tidak dicabar. Titer antibodi FAdV tidak dikesan dalam kumpulan kawalan sepanjang ujikaji. Dalam kumpulan ayam yang diinokulat melalui SQ, titer antibodi adalah 648 ± 188 dan 324 ± 85 masing-masing pada hari yang ke-14 dan 28pi. Titer antibodi dalam ayam yang diinokulat melalui oral adalah 316 ± 118 dan 163 ± 17 masing-masing pada hari yang ke-14 dan 28pi dan tidak berubah secara signifikan ($p>0.05$) apabila dibandingkan dengan kumpulan kawalan dan kumpulan SQ. Dalam kumpulan dicabar, titer antibodi meningkat secara signifikan terutama dalam kumpulan

kawalan (6276 ± 1983) dan oral (5496 ± 1688) kecuali kumpulan SQ (3895 ± 1858) pada hari ke-14pc. Kajian ini telah menunjukkan bahawa isolat FAdV yang dilemahkan adalah selamat, tidak patogenik, imunogenik dan berkebolehan melindungi ayam dari infeksi FAdV. Inokulasi laluan subkutaneus adalah efektif berbanding dengan laluan oral.

Patogenisiti dan imunogenisiti FAdV yang dilemahkan dalam sel CEL iaitu CEL35 ($10^{6.7}TCID_{50}$/ml) dan di dalam telur ayam berembrio SPF, E20 ($10^{8.7}TCID_{50}$/ml) telah ditentukan dalam ayam pedaging komersial berumur satu hari melalui laluan oral (0.5ml) dan intraperitoneal (IP) (0.5ml). Kajian menunjukkan bahawa isolat yang dilemahkan tidak patogenik dalam ayam pedaging komersial. Tiada tanda-tanda klinikal dan juga lesi mata kasar dan histologi pada hati yang direkodkan pada kesemua kumpulan ayam sepanjang ujikaji selama 21 hari. Purata bagi berat badan ayam adalah signifikan rendah ($p<0.05$) dalam kumpulan yang diinokulat berbanding dengan kumpulan kawalan pada hari ke-7 dan 14pi bagi kedua-dua laluan inokulasi. Pada hari ke-21pi, berat badan ayam yang diinokulat adalah signifikan tinggi ($p<0.05$) berbanding dengan kumpulan kawalan. Titer antibodi FAdV adalah tinggi dalam anak ayam umur sehari kerana kehadiran `maternal derived antibody (MDA)` dan menurun secara signifikan ($p<0.05$) dari hari 0 (7795 ± 1414) ke hari 21 pi (69 ± 34). Ayam yang diinokulat menggunakan isolat yang dilemahkan daripada CEL35 menunjukkan laluan oral (3985 ± 776) dan IP (3734 ± 809) mempunyai corak titer antibodi yang sama dan tiada perbezaan secara signifikan ($p>0.05$) di antara laluan inokulasi sepanjang ujikaji. Seperti juga ayam yang diinokulat dengan E20 FAdV, titer antibodi bagi kumpulan oral (4119 ± 792) dan IP (4598 ± 871) adalah sama rata di kalangan laluan. Tiada perbezaan pada titer antibodi di antara isolat yang dilemahkan sama ada melalui laluan oral atau IP ($p>0.05$). Namun, ayam yang diinokulat dengan isolat FAdV yang lemah CEL35 (2348 ± 1800) dan E20 (1833 ± 792) melalui laluan IP mempunyai titer antibody yang tinggi secara signifikan ($p<0.05$) berbanding dengan kumpulan kawalan (69 ± 34) pada hari ke-21pi. Kajian ini dapat disimpulkan bahawa isolat FAdV yang dilemahkan tidak patogenik dan imunogenik dalam ayam pedaging komersial. Isolat FAdV yang dilemahkan dalam sel CEL dan telur ayam berembrio SPF merangsang tindak balas antibodi yang serupa sama ada melalui laluan oral atau IP.

Ini dapat disimpulkan bahawa FAdV sangat sensitif dalam telur ayam berembrio SPF dan sel primer CEL daripada sel Vero dengan perubahan nukleotida dan asid amino pada bahagian `loop L1` dalam hexon dan pada knob dalam gen fiber. Isolat yang dilemahkan tidak patogenik dalam ayam SPF dan ayam pedaging komersial dengan kebolehan merangsang antibodi FAdV. Ia menunjukkan bahawa laluan parenteral adalah laluan inokulasi FAdV yang efektif berbanding dengan laluan oral, dan isolat yang lemah ini sesuai untuk digunakan dalam pembangunan vaksin FAdV masa hadapan terhadap penyakit ini.

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I certify that a Thesis Examination Committee has met on 17 November 2017 to conduct the final examination of Norfitriah binti Mohamed Sohaimi on her thesis entitled "Development of Live Attenuated Fowl Adenovirus Isolate of Malaysia for Vaccine Production" 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 fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

aa	amino acid
AAV	Avian Adenovirus
AdV	Adenovirus
ADP	Adenovirus Death Protein
ANOVA	Analysis of variance
APC	Antigen Presenting Cell
BLAST	Basic Local Alignment Search Tool
bp	base pair
CAM	Chorioallantoic membrane
CAR	Coxsackievirus-Adenovirus Receptor
CAV	Chicken Anaemia Virus
CE	Chicken Embryonated
CEL	Chicken embryo liver
CEL5	Chicken embryo liver passage 5
CEF	Chicken Embryo Fibroblast
CEK	Chicken Embryo Kidney
CELO	Chicken embryo lethal orphan
CD	Cluster of Differentiation
CH-SAH	Continuous hepatoma cell line
CK	Chicken Kidney
CMI	Cell mediated immunity
CPE	Cytopathic effect
DAdV	Duck Adenovirus
DBP	DNA Binding Protein
dCMP	deoxycytidine monophosphate
DMEM	Dulbecco`s Modified Eagle Medium
DNA	Deoxyribonucleic Acid
E2	Embryo passage 2
EID_{50}	50% Egg Infective Dose
EDTA	Ethylenediaminetetraacetate
ELISA	Enzyme Linked Immunoabsorbent Assay
FBS	Fetal Bovine Serum
FAdV	Fowl Adenovirus
GALT	Gut Associated Lymphoid Tissue
GM-CSF	Granulocyte macrophage colony-stimulating factor
γ-IFN	Gamma interferon
HAdV	Human Adenovirus
HE	Haematoxylin and eosin
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
HHS	Hepatitis Hydropericardium Syndrome
HPS	Hydropericardium syndrome
HSD	Honest Significant Difference
HVRs	Hypervariable Regions
IACUC	Institutional Animal Care and Use Committee
IBH	Inclusion Body Hepatitis
IBD	Infectious Bursal Disease
IBV	Infectious Bronchitis Virus
ICTV	International Committee on Taxonomy of Viruses

Ig	Immunoglobulin
IM	Intramuscular
INIB	Intranuclear Inclusion Body
IP	Intraperitoneal
ITR	Inverted Terminal Repeat
JTT	Jones-Taylor-Thorton
kDa	kiloDalton
kB	kilobyte
kb	kilobase
LAMP	Loop-Mediated Isothermal Amplification
LB	Luria broth
MDA	Maternal Derived Antibody
MEGA	Molecular Evolutionary Genetics Analysis
MHC	Major Histocompatibility Complex
MLP	Major Late Promoter
mRNA	messenger RNA
$NaHCO_3$	Sodium Hydrogen Carbonate
NCBI	National Center for Biotechnology Information
NDV	Newcastle Disease Virus
nm	nanometer
nt	nucleotide
ORF	Open Reading Frame
OD	Optical Density
PBS	Phosphate Buffer Saline
pi	Post-inoculation
pv	Post-vaccination
pc	Post-challenge
PCR	Polymerase Chain Reaction
PES	Polyethersulfone
pfu	Plaque forming unit
pH	Potential of hydrogen
QT35	Quail fibroblast cell line
RE	Restriction Enzyme
RFLP	Restriction Fragment Length Polymorphism
RGD	Arginine-Glycine-Aspartic Acid
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	revolutions per minute
SAdV	Simian Adenovirus
SRBCs	Sheep Red Blood cells
SEM	Standard Error Mean
SPF	Specific Pathogen Free
SPSS	Statistical Package for the Social Sciences
SQ	Subcutaneous
TAdV	Turkey Adenovirus
$TCID_{50}$	50% Tissue Culture Infective Dose
TCR	T cell receptor
Tc	Cytotoxic T cells
TGF-β	Transforming Growth Factor beta
TNF-α	Tumor Necrosis Factor alpha
TP	Terminal Protein

TR	Tandem Repeat
µg	Microgram
µl	Microliter
µm	Micrometer
UV	Ultraviolet
UPM	Universiti Putra Malaysia
V	Volt
V1	Vero cells passage 1
w/v	weight per volume
x-gal	5-bromo-4-chloro-3-indolyl β-D-galactoside
x *g*	gravity

CHAPTER 1

INTRODUCTION

Avian viruses are the major threat in poultry production which may affect the growth of industry either in local or worldwide. Fowl adenoviruses (FAdVs) has been recognized as causative agent of inclusion body hepatitis (IBH) (Morshed *et al.,* 2017; Oliver-Ferrando *et al.,* 2017), hydropericardium syndrome (HPS) (Mettifogo *et al.,* 20147) and gizzard erosion (Domanska-Blicharz, 2011) diseases which affect broilers and layer production in affected farm. The agent was isolated in various geographical regions with different serotype and strains (Steer *et al.,* 2011; Niczyporuk, 2017; Gawel *et al.,* 2016).

FAdV is double stranded DNA virus with non-enveloped structure comprised of three major structural proteins known as hexon, penton and fiber involved in viral replication in host cells. Antigenic determinants for FAdV located in hexon and fiber proteins contain high amino acid variability between serotype and genotype for FAdV classification (Hess *et al.,* 1998; Meulemans *et al.,* 2001a). To date, both proteins are highly associated with virus infectivity and frequently used for analysis to distinguish between pathogenic and non-pathogenic strain previously (Pallister *et al.,* 1996, Grgić *et al.,* 2014a). Epidemiological studies by few researchers revealed that the disease associated with FAdV typically IBH and HPS were kept increased recently with major economic losses due to poor production in infected farms (Zhang *et al.,* 2016; Balamurugan and Kataria, 2004; Hess *et al.,* 1999). In some part of the world, various FAdV serotypes have been isolated from broiler and layer chickens involving serotype 1, 8b and 11(Choi *et al.,* 2012; Kajan *et al.,* 2013).

In year 2005, IBH disease was first reported in Malaysia by Hair-Bejo (2005) in commercial broiler flocks caused high significant impact on economic due to poor performance and productivity. Since then, IBH outbreak continuously reported in several states involving major poultry producing area and molecularly characterised as FAdV serotype 8b (Juliana *et al.,* 2014; Norina *et al.,* 2016). IBH is an acute infectious disease of young broiler chickens of 3 to 7 weeks of age with sudden onset of increased mortality between 2 to 10% and can be great as 30%. The disease transmitted either by vertically through embryonated chicken eggs or horizontally via faecal-oral route, direct contact and fomites (Grgić *et al.,* 2006; Hafez, 2011).

IBH was confirmed as primary disease caused by high pathogenic FAdV as demonstrated in experimental induced in chickens without prior immunosuppression (Gomis *et al.,* 2006; Christensen and Saifuddin, 1989). Recently, in Malaysia, FAdV from serotype 8b has been reported as primary agent of IBH with high mortality in specific pathogen free (SPF) chickens and severe lesions in liver with pale, swollen and yellowish discoloration. Numerous basophilic intranuclear inclusion bodies were detected histologically in liver of dead chickens (Majdi, 2015).

Vaccination against FAdV is the only effective control measures preventing IBH outbreak in various regions (Ali *et al.,* 2015; Kim *et al.,* 2014, Mansoor *et al.,* 2011). It seems that application of live attenuated FAdV vaccine is superior than inactivated vaccine due to high protection rate in vaccinated chickens with long lasting immunity (Mansoor *et al.,* 2011; Kaur *et al.,* 1997).

To develop vaccine candidate, attenuation of FAdV isolate from field strain was attempted in suitable cell cultures or chicken embryos by serial passages to reduced virus virulence with molecular changes in major capsid protein encoded for virus infectivity. As a result, the strain has been attenuated and safe to be used as vaccine candidate in chicken with high antibody response against the challenge FAdV (Ali *et al*., 2015; WO2003039593A1, 2003).

Primary chicken embryo liver (CEL) cells and chicken kidney (CK) cells are the most sensitive medium for FAdV replication and propagation (Soumyalekshmi *et al.,* 2014; McFerran, 1998). Cytopathic effect is indicator of viral replication in host cells and typically in the form of cells rounding and clumping for FAdV (Barua and Rai, 2003). However, some continuous cell lines also provide better medium for FAdV propagation and attenuation such as QT35 quail fibroblast cell line, chicken hepatoma cells and Vero cell which depending much on FAdV strain and virus virulence (WO2003039593A1, 2003; Alexandera *et al.,* 1998; Ali *et al.,* 2015).

Since FAdV is highly pathogenic in Malaysia as demonstrated by previous study, it reflects the importance of vaccine to control the disease outbreak (Majdi, 2015). In addition, the number of clinical IBH and gizzard erosion diseases was increased lately due to unavailable vaccine against the FAdV (Norina *et al.,* 2016). Although several preventive measures have been implemented to prevent immunosuppression, however, the cases of IBH were kept increased mostly in commercial broiler flocks. Thus, it is urgently need a vaccine to prevent and control the disease.

Development of live attenuated isolate has been major focus of attempt since the vaccine is highly protective against the field FAdV strain than inactivated vaccine (Mansoor *et al.,* 2011). Production of safe and efficacious vaccine should be obtained through continuous passages in cell culture or SPF embryonated chicken eggs. However, there is lack of study regarding the influence of attenuation process at high consecutive passages towards molecular changes in major capsid protein. In previous study, the changes at nucleotide and amino acid undetermined specifically in hexon and fiber gene which might influence the virulence of FAdV following adaptation and attenuation in alternative host (Mansoor *et al.,* 2011; Schonewille *et al.,* 2010). Therefore, determination of molecular changes in both proteins is crucial and will support the evidence of virus attenuation after high consecutive passages.

To estimate the influence of molecular changes towards pathogenicity and immunogenicity, experimental trial is definitely important to confirm the attenuation of isolate. Although the isolate remained in same serotype after serial propagation, however, the pathogenicity will be differed as compared to unattenuated strain in both

SPF and commercial broiler chickens. To convince that the isolate is attenuated and immunogenic, in-vivo study in SPF chickens is crucial to ensure that the vaccinated chicken is safe from IBH disease but able to induce antibody response. In addition, to evaluate the effectiveness of the attenuated isolate as vaccine, efficacy study using challenge FAdV strain is necessary.

The hypothesis of the study was Malaysian FAdV isolate, UPM1137, is able to adapt, propagate and attenuate in SPF embryonated chicken eggs and cell cultures. High consecutive passages of the FAdV isolate in SPF embryonated chicken eggs and primary chicken embryo liver (CEL) cells could lead to molecular changes in hexon and fiber genes at both nucleotide and amino acid sequences. The attenuated FAdV isolate from primary CEL cells is non-pathogenic but immunogenic in chickens and able to provide protection against the FAdV infection. Thus, the attenuated FAdV isolate is highly potential to be used as master seed for future production of live attenuated FAdV cell culture based vaccine.

Therefore the objectives of this study were:

1. to isolate, identify and characterize FAdV isolate obtained from field outbreak of the IBH disease in Malaysia.
2. to adapt and determine the pathogenicity and molecular characteristics of the FAdV isolate in primary chicken embryo liver (CEL) cells, Vero cells and SPF embryonated chicken eggs.
3. to determine the pathogenicity, immunogenicity and efficacy of the attenuated FAdV isolate from CEL cells (UPM1137CEL35) in SPF chickens.
4. to determine pathogenicity and immunogenicity of the attenuated FAdV isolate from CEL cells (UPM1137CEL35) and SPF embryonated chicken eggs (UPM1137E20) in commercial broiler chickens.

REFERENCES

Abdul-Aziz, T.A. and Al-Attar, M. (1991). New syndrome in Iraqi chicks. *Veterinary Record*, 129(12): 272-272. doi: 10.1136/vr.129.12.272.

Adair, B.M., Curran, W.L. and McFerran, J.B. (1979). Ultrastructural studies of the replication of fowl adenoviruses in primary cell cultures. *Avian Pathology*, 8: 133-144. doi: 10.1080/03079457908418336.

Adair, B.M. (1978). Studies on the development of avian adenoviruses in cell cultures. *Avian Pathology*, 7: 541-550. doi: http://dx.doi.org/10.1080/03079457808418308.

Afzal, M. and Ahmad, I. (1990). Efficacy of an inactivated vaccine against hydropericardium syndrome in broilers. *Veterinary Record,* 126: 59-60. doi: http://dx.doi.org/10.1136/vr.126.3.59.

Ahmad, M.D., Zaman, S., Mushtaq, M.H., Anjum, A.A. and Akram, M. (2011). Comparative pathogenicity of liver homogenate and cell culture propagated hydropericardium syndrome virus in broiler birds. *Pakistan Veterinary Journal*, 31(4): 321-326.

Ahmad, I., Afzal, M., Malik, M.I., Hussain, Z. and Hanif, W. (1989). Studies on the disease pattern and etiology of hydropericardium syndrome (Angara disease) in broiler chickens in Pakistan. *Pakistan Journal of Agricultural Research,* 10:195-199.

Akopian, T.A., Doronin, K.K., Karpov, V.A. and Naroditsky, B.S. (1996). Sequence of the avian adenovirus FAV 1 (CELO) DNA encoding the hexon-associated protein pVI and hexon. *Archives of Virology,* 141(9): 1759-1765.

Akopian *et al.* (2016). GenBank accession number: Z67970.

Alemnesh, W., Hair-Bejo, M., Aini, I. and Omar A.R. (2012). Pathogenicity of fowl adenovirus in specific pathogen free chicken embryos. *Journal of Comparative Pathology*, 146: 223-229. doi: 10.1016/j.jcpa.2011.05.001.

Alemnesh, W.Y. (2009). Pathogenicity of a Malaysian fowl adenovirus isolate in specific pathogen free embryonated chicken eggs and chicks. Master of Veterinary Science Thesis. Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia.

Alexandera, H.S., Huber, P., Cao, J., Krell, P.J. and Nagy, E. (1998). Growth characteristics of fowl adenovirus type 8 in a chicken hepatoma cell line. *Journal of Virological Methods,* 74: 9-14. doi: https://doi.org/10.1016/S0166-0934(98)00062-7.

Ali, S., Mahmood, M. S., Hussain, I. and Khan, M. N. (2015). Preparation and evaluation of lyophilized live attenuated vaccine of inclusion body hepatitis

hydropericardium syndrome (IBH-HPS) against challenge in broiler chickens. *International Journal of Agriculture and Biology*, 17(3): 658-662.

Alvarado, I.R., Villegas, P., El-Attrache, J., Jensen, E., Rosales, G., Perozo, F. and Purvis, L.B. (2007). Genetic characterization, pathogenicity, and protection studies with an avian adenovirus isolate associated with inclusion body hepatitis. *Avian Diseases*, 51: 27-32. doi: http://www.jstor.org/stable/4493164.

Alvarado *et al*. (2007). GenBank accession number: DQ323984.

Anjum, A., Sabri, M. and Iqbal, Z. (1989). Hydropericarditis syndrome in broiler chickens in Pakistan. *Veterinary Record*, 124: 247-248. doi:10.1136/vr.124.10.247.

Appaiahgari *et al*. (2016). GenBank accession number: KU342001.

Asrani, R.K., Gupta, B.K., Sharma, S.K., Singh, S.P. and Katoch, R.C. (1997). Hydropericardium-hepatopathy syndrome in Asian poultry. *Veterinary Record*, 141: 271-273. doi:10.1136/vr.141.11.271.

Avimex. (1999). Productos Aves. Retrieved from http://www.avimex.co.mx/ productos/aves.

Bai, M., Harfe, B. and Freimuth, P. (1993). Mutations that alter an Arg-Gly-Asp (RGD) sequence in the adenovirus type 2 penton base protein abolish its cell-rounding activity and delay virus reproduction in flat cells. *Journal of Virology*, 67(9): 5198–5205.

Balamurugan, V. and Kataria, J.M. (2004). The hydropericardium syndrome in poultry-a current scenario. *Veterinary Research Communications*, 28: 127-148. doi: 10.1023/b:verc.0000012115.86894.1e.

Barta, V., Springer, W.T. and Millar, D.L. (1984). A comparison of avian and mammalian cell cultures for the propagation of avian reovirus WVU 2937. *Avian Diseases*, 28(1): 216-223. doi: 10.2307/1590144.

Barua, S. and Rai, A. (2004). Phylogenetic analysis of fowl adenovirus 4 hexon gene. *Journal of Immunology and Immunopathology*, 6(2): 64-68.

Barua, S. and Rai, A. (2003). Cultivation of fowl adenovirus 4 in chicken embryo liver cell culture and purification of the virus by ultracentrifugation. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, 24(2): 195-196.

Beall, C., Clark, K. and Johnson, P., Jr. (2009). *U.S. Patent No.US20090275138A1*. Washington, DC: U.S. Patent and Trademark Office.

Benko, M., Harrach, B., Both, G.W., Russell, W.C., Adair, B.M., Adam, E., deJong, J.C., Hess, M., Johnson, M. and other authors (2005). Family adenoviridae. In Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U. and Ball, L.A. (Eds.)

Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses (pp. 213–228). London: Elsevier/Academic Press.

Benko, M., Harrach, B. and Russel, W.C. (2000). Family adenoviridae. In vanRegenmortel, M.H.V. *et al*. (Eds.). *Virus Taxonomy. Classification and nomenclature of viruses. Seventh Report of the International Committee on Taxonomy of Viruses* (pp. 227-238). San Diego, CA: Academic Press.

Benevento, M., Di Palma, S., Snijder, J., Moyer, C.L., Reddy, V.S., Nemerow, G.R. and Heck, A. J. R. (2014). Adenovirus composition, proteolysis, and disassembly studied by in-depth qualitative and quantitative proteomics. *The Journal of Biological Chemistry*, 289(16): 11421–11430. doi: 10.1074/jbc.M113.537498.

Bancroft, J.D. and Stevens, A. (1996). *Theory and practice of histological technique, 4rd edition.* Edinbrugh: Churchill, Livingston Publishers.

Borisov, V.V., Borisov, A.V. and Gusev, A.A. (1997). Hydropericardium syndrome in chickens in Russia. In: *Proceedings of the Tenth International Congress of the WVPA,* Budapest, Hungary. 258: 818–22.

Cao, J.X., Krell, P.J. and Nagy, E´. (1998). Sequence and transcriptional analysis of terminal regions of the fowl adenovirus type 8 genome. *Journal of General Virology*, 79: 2507–2516. doi: 10.1099/0022-1317-79-10-2507.

Chaplin, D.D. (2010). Overview of the immune response. *The Journal of Allergy and Clinical Immunology*, 125(2): 3–23. doi: http://dx.doi.org/10.1016/j.jaci.2009.12.980.

Chomiak, T.W., Luginbuhl, R.E. and Helmboldt, C.F. (1961). Tissue culture propagation and pathology of CELO virus. *Avian Diseases,* 5(3): 313-320. doi:10.2307/1587640.

Chiocca *et al*. (1996). GenBank accession number: U46933.

Chiocca S., Kurzbauer R., Schaffner G., Baker A. and Mautner V. (1996). The complete DNA sequence and genomic organization of the avian adenovirus CELO. *Journal of Virology*, 70: 2939–2949.

Choi, K.S., Kye, S.J., Kim, J.Y., Jeon, W.J., Lee, E.K., Park, K.Y. and Sung, H.W. (2012). Epidemiological investigation of outbreaks of fowl adenovirus infection in commercial chickens in Korea. *Poultry Science,* 91(10): 2502-2506. doi: 10.3382/ps.2012-02296.

Chow, L.T., Lewis, J.B. and Broker, T.R. (1980). RNA transcription and splicing atearly and intermediate times after adenovirus-2 infection. *Cold Spring Harbor Symposia on Quantitative Biology,* 44: 401-414.

Christensen, N.H. and Saifuddin, M. (1989). A primary epidemic of inclusion body hepatitis in broilers. *Avian Diseases,* 33(4): 622–630. doi: 10.2307/1591135.

Cook, J.K.A. (1983). Fowl adenoviruses: studies on aspects of the pathogenicity of six strains for 1-day-old chicks. *Avian Pathology,* 12: 35-43. doi: 10.1080/03079458308436147.

Cook, J.K.A. (1974). Pathogenicity of avian adenoviruses for day old chicks. *Journal of Comparative Pathology,* 84(4): 505-515.

Corredor, J. C., Krell, P. J. and Nagy, E´. (2006). Sequence analysis of the left end of fowl adenovirus genomes. *Virus Genes,* 33: 95–106. doi: 10.1007/s11262-005-0031-y.

Cotten, M. and Weber, J.M. (1995). The adenovirus protease is required for virus entry into host cells. *Virology,* 213: 494-502. doi: 10.1006/viro.1995.0022.

Cowen, B.S. (1988). Chicken embryo propagation of type I avian adenoviruses. *Avian Diseases,* 32: 347-352. doi:10.2307/1590825.

Cowen, B.S. and Braune, M.O. (1988). The propagation of avian viruses in a continuous cell line (QT35) of Japanese quail origin. *Avian Diseases,* 32(2): 282– 297.

Cowen B. (1992). Inclusion body hepatitis-anemia and hydropericardium syndromes: aetiology and control. *World's Poultry Science Journal,* 48(3): 247-254. doi: https://doi.org/10.1079/WPS19920019.

Crawford-Miksza, L. and Schnurr, D.P. (1996). Analysis of 15 adenovirus hexon proteins reveals the location and structure of seven hypervariable regions containing serotype-specific residues. *Journal of Virology,* 70(3): 1836-1844.

Dahiya, S., Srivastava, R.N., Hess, M. and Gulati, B.R. (2002). Fowl adenovirus serotype 4 associated with outbreaks of infectious hydropericardium in Haryana, India. *Avian Diseases,* 46:230–233. doi: 10.1637/0005-2086(2002)046[0230:FASAWO]2.0.CO;2.

Dar, A., Gomis, S., Shirley, I., Mutwiri, G., Brownlie, R., Potter, A., Gerdts, V. and Tikoo, S. K. (2012a). Pathotypic and molecular characterization of a fowl adenovirus associated with inclusion body hepatitis in Saskatchewan chickens. *Avian Disease,* 56:73-81. doi: 10.1637/9764-041911-Reg.1.

Dar *et al.* (2012b). GenBank accession number: JN112373.

Davison *et al.* (2008). GenBank accession number: AC_000016.

Dey *et al.* (2009). GenBank accession number: FJ949088.

de Jong R.N., van der Vliet P.C. and Brenkman A.B. (2003). Adenovirus DNA replication: Protein priming, jumping back and the role of the DNA binding protein DBP. In: Doerfler, W. and Böhm, P. (Eds.). *Adenoviruses: Model and Vectors in Virus-Host Interactions* (pp.187-221). Springer, Berlin, Heidelberg.

Dhillon, A.S., Winterfield, R.W., Thacker, H.L. and Feldman, D.S. (1982). Lesions induced in the respiratory tract of chickens by serologically different adenoviruses. *Avian Diseases*, 26: 478-486. doi: 10.2307/1589893.

Domanska-Blicharz *et al*. (2012). GenBank accession number: GU952110 and GU952108.

Domanska-Blicharz, K., Tomczyk, G., Smietanka, K., Kozaczynski, W. and Minta, Z. (2011). Molecular characterization of fowl adenoviruses isolated from chickens with gizzard erosions. *Poultry Science*, 90(5): 983–989. doi: https://doi.org/10.3382/ps.2010-01214.

El-Attrache, J. and Villegas, P. (2001). Genomic identification and characterization of avian adenoviruses associated with inclusion body hepatitis. *Avian Diseases*, 45(4): 780-787. doi:10.2307/1592857.

Erny, K.M., Barr, D.A. and Fahey, K.J. (1991). Molecular characterization of highly virulent fowl adenoviruses associated with outbreaks of inclusion body hepatitis. *Avian Pathology*, 20: 597-606. doi: 10.1080/03079459108418799.

Fender, P., Boussaid, A., Mezin, P. and Chroboczek, J. (2005). Synthesis, cellular localization, and quantification of penton-dodecahedron in serotype 3 adenovirus-infected cells. *Virology*, 340: 167-173. doi:10.1016/j.virol.2005.06.030.

Field, A. (2013). *Discovering Statistic using IBM SPSS Statistics*. (4th ed.). London: SAGE Publications Ltd..

Fu *et al*. (2013). GenBank accession number: KF286430.

Ganesh, K., Suryanarayana, V., Raghavan, R. and Gowda, S. (2001). Nucleotide sequence of L1 and part of P1 of hexon gene of fowl adenovirus associated with hydropericardium hepatitis syndrome differs with the corresponding region of other fowl adenoviruses. *Veterinary Microbiology*, 78: 1-11.

Gawel, A., Nowak, M., Ciaputa, R. and Bobrek, K. (2016). Prevalence of inclusion body hepatitis in Poland from 2010-2014. *Polish Journal of Veterinary Sciences*, 19(4): 889-891. doi: 10.1515/pjvs-2016-0111.

Gerlach, H. (1994). Viruses. In: Ritchie, B.W., Harrison, G.J. and Harrison, L.R.(Eds.). *Avian Medicine: Principles and Application* (pp. 862-948). Wingers Publishing, Inc., Lake Worth, FL.

Gelderblom, H. and Maichle-Lauppe, I. (1982). The fibers of fowl adenoviruses. *Archives of Virology*, 72(4): 289-298. doi:10.1007/bf01315225

Ghosh-Choudhury, G., Haj-Ahmad, Y. and Graham, F.L. (1987). Protein IX, a minor component of the human adenovirus capsid, is essential for the packaging of full length genomes. *EMBO J.*, 6: 1733-1739.

Ghatak, S., Muthukumaran, R. B. and Nachimuthu, S. K. (2013). A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. *Journal of Biomolecular Techniques*, 24(4): 224–231. http://doi.org/10.7171/jbt.13-2404-001.

GLOBIVAC IBH. (2010). Retrieved from http://www.globionindia.com/globivacibh.html.

Gomis, S., Goodhope, R., Ojkić, D. and Wilson, P. (2006). Inclusion body hepatitis as a primary disease in broilers in Saskatchewan, Canada. *Avian Diseases*, 50(4): 550-555. doi: 10.1637/7577-040106r.1.

Gomis, S., Tikoo, S. and Ojkić, D. (2013). *U.S. Patent No.US8524249B2*. Washington, DC: U.S. Patent and Trademark Office.

Gowda, R.N.S. and Satyanarayana, M.L. (1994). Hydropericardium syndrome in poultry. *Indian Journal of Veterinary Pathology*, 18:159-161.

Grafl, B., Aigner, F., Liebhart, D., Marek, A., Prokofieva, I., Bachmeier, J. and Hess, M. (2012). Vertical transmission and clinical signs in broiler breeders and broilers experiencing adenoviral gizzard erosion. *Avian Pathology*, 41(6): 599-604. doi: 10.1080/03079457.2012.740614.

Grgić, H., Yang, D-H. and Nagy, E. (2011a). Pathogenicity and complete genome sequence of fowl adenovirus serotype 8 isolate. *Virus Research*, 156: 91-97. doi: 10.1016/j.virusres.2011.01.002.

Grgić, H., Krell, P.J. and Nagy, E. (2014a). Comparison of fiber gene sequences of inclusion body hepatitis (IBH) and non-IBH strains of serotype 8 and 11 fowl adenoviruses. *Virus Genes*, 48: 74-80. doi:10.1007/s11262-013-0995-y.

Grgić, H., Phillippe, C., Ojkić, D. and Nagy, E. (2006). Study of vertical transmission of fowl adenoviruses. *Canadian Journal of Veterinary Research*, 70: 230-233.

Grgić *et al*. (2011b). GenBank accession number: GU734104.

Grgić *et al*. (2014b). GenBank accession number: JQ034217 and JQ034218.

Griffin *et al*. (2011). GenBank accession number:GU188428.

Gunes, A., Marek, A., Grafl, B., Berger, E. and Hess, M. (2012). Real-time PCR assay for universal detection and quantitation of all five species of fowl adenoviruses (FAdV-A to FAdV-E). *Journal of Virological Methods*, 183:147-153. doi: 10.1016/j.jviromet.2012.04.005.

Grimes, T.M., King, D.J., Kleven, S.H. and Fletcher, O.J. (1977). Involvement of a type-8 avian adenovirus in the etiology of inclusion body hepatitis. *Avian Diseases*, 21(1): 26-38.

Hair-Bejo, M. (2005). Inclusion body hepatitis in commercial broiler chickens. *Jurnal Veterinar Malaysia*, 17: 23-26.

Hafez, H.M. (2011). Avian adenovirus infections with special attention to inclusion body hepatitis/hydropericardium syndrome and egg drop syndrome. *Pakistan Veterinary Journal*, 31: 85-92.

Hallenbeck, P.L. and Stevenson, S.C. (2002). Targetable gene delivery vectors. In Habib, N.A. (Ed.). *Cancer Gene Therapy: Past achievement and future challenges* (pp. 37-46). New York: Kluwer Academic.

Harrach, B., Benkö, M., Both, G., Brown, M., Davis, A., Echavarría, M., Hess, M., Jones, M., Kajon, A., Lahmkuhl, H., Mautner, V., Mittal, S. and Wadell, G. (2011). Family adenoviridae. In: King, A., Adams, M., Carstens, E., Lefkowitz, E. (Eds.). *Virus taxonomy: classification and nomenclature of viruses. Ninth report of the International Committee on Taxonomy of Viruses* (pp. 95–111). San Diego, CA: Elsevier.

Harrach, B. and Kajan, G.L. (2011). Aviadenovirus. In Tidona, C., Darai, G. Eds. *The Springer Index of Viruses, 2nd edition* (pp.13-28). Science + Business Media, New York, USA.

Hasler, P. and Wigand, R. (1978). The susceptibility of vero cell cultures for human adenoviruses. *Medical Microbiology and Immunology*, 164(4): 267-275.

Hess, M., Schachner, A., Marek, A. and Jaskulska, B. (2016). *U.S. Patent No.US20160199484A1*. Washington, DC: U.S. Patent and Trademark Office.

Hess, M., Schachner, A., Marek, A. and Jaskulska, B. (2015). *U.S. Patent No.EP2839841A1*. Washington, DC: U.S. Patent and Trademark Office.

Hess, M. (2013). Avidenovirus infections. In Swayne, D.E., Glisson, J.R., L.R. McDougald, Nolan, L.K., Suarez, D.L. and Nair, V.L. (Eds.). *Diseases of Poultry, 13th edition* (pp. 290–300). Ames, IA: Wiley-Blackwell.

Hess, M. (2000). Detection and differentiation of avian adenoviruses: A review. *Avian Pathology*, 29:195–206. doi: 10.1080/03079450050045440.

Hess, M., Raue, R. and Prusas, C. (1999). Epidemiological studies on fowl adenoviruses isolated from cases of infectious hydropericardium. *Avian Pathology*, 28(5): 433-439. doi: 10.1080/03079459994443.

Hess, M., Prusas, C. and Monreal, G. (1998). Growth analysis of adenoviruses isolated from pigeons in chicken cells and serological characterization of the isolates. *Avian Pathology*, 27: 196-199. doi: 10.1080/03079459808419323.

Hess, M., Cuzange, A., Ruigrok, R.W., Chroboczek, J. and Jacrot, B. (1995). The avian adenovirus penton: two fibers and one base. *Journal of Molecular Biology*, 252(4): 379-385.

Horne, R., Brenner, S., Waterson, A. and Wildy, P. (1959). The icosahedral form of an adenovirus. *Journal of Molecular Biology*, 1:84-86.

Hussain, I., Mahmood, M.S., Arshad, M.I., Akhtar, M., Mahmood, F. and Rafique, A. (2012). Immune system dysfunction in broiler chickens experimentally inoculated with fowl adenovirus serotype-4 associated with inclusion body hepatitis hydropericardium syndrome. *Turkish Journal of Veterinary and Animal Sciences*, 36(3): 223-230. doi: 10.3906/vet-0807-21.

ICTV (International Committee for the Taxonomy of Viruses), 9th report, 2009. http://www.ictvonline.org/virusTaxonomy.asp?version=2009.

Inactivated vaccines. (2016). Retrieved from http://venkys.com/?page_id=1381.

Inactivated IBH. (2017). Retrieved from https://www.hester.in/inactivated-ibh/.

Inclusion Body Hepatitis plus Newcastle Disease Inactivated Vaccine Profile. (2011). Retrieved from http://www.poulvet.com/vetproducts/medicine_detail.php?medii=1301

Itakura, C., Yasuba, M. and Goto, M. (1974). Histopathological studies on inclusion body hepatitis in broiler chickens. *Japanese Journal of Veterinary Science*, 36: 329-340. doi: 10.1292/jvms1939.36.329.

Itakura, C., Matsushita, S. and Goto, M. (1977). Fine structure of inclusion bodies in hepatic cells of chickens naturally affected with inclusion body hepatitis. *Avian Pathology*, 6: 19-32. doi: 10.1080/03079457708418209.

Jaffery, M.S. (1988). A treat is on Angara disease (hydropericardium-pulmonary oedema-hepatonephritis syndrome). *Journal of the Pakistan Veterinary Medical Association*, 34:1-33.

Janeway, C., Travers, J. P., Walport, M. and Schlomchik, M. J. (1999). *Immunobiology: the immune system in health and disease* (5th ed.). London: Harcourt Brace and Company.

Johnson and Pooley (2005). GenBank accession number: AF155911.

Juliana, M.A., Nurulfiza, M.I., Hair-Bejo, M., Omar, A.R. and Aini, I. (2014). Molecular characterization of fowl adenoviruses isolated from inclusion body hepatitis outbreaks in commercial broiler chickens in Malaysia. *Pertanika Journal of Tropical Agriculture Science*, 37(4): 483-497.

Juliana, M.A. (2014). Molecular characterization and genomic organization of fowl adenovirus isolates of Malaysia. Master of Science Thesis. Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia.

Juliana (2016). GenBank accession number: JF917238 and JF917239.

Juliana *et al.* (2016). GenBank accession number: KU517714.

Junnu, S., Lertwatcharasarakul, P., Jala, S., Phattanakulanan, S., Monkong, A.,Kulprasertsri, S., Thivalai, C., Chakritbudsabong, W., Chaichoun, K. and

Songserm, T. (2015). An inactivated vaccine for prevention and control of inclusion body hepatitis in broiler breeders. *Thai Journal of Veterinary Medicine*, 45(1):55-62.

Kajan, G., Davison, A., Palya, V., Harrach, B. and Benkő, M. (2012). Genome sequence of a waterfowl aviadenovirus, goose adenovirus 4. *Journal of General Virol*ogy, 93(11): 2457-2465. doi:10.1099/vir.0.042028-0.

Kajan, G. L., Kecskeméti, S., Harrach, B. and Benkő, M. (2013). Molecular typing of fowl adenoviruses, isolated in Hungary recently, reveals high diversity. *Veterinary Microbiology*, 167: 357-363. doi:10.1016/j.vetmic.2013.09.025.

Kamal, T., Muneer, A., Ali, M., Fatima, Z. and Ullah, A. (2015). Replication and adaptation of hydropericardium virus (adenovirus-4) on QT35-cell line. In *Applied Sciences and Technology (IBCAST), Proceedings of the International Bhurban Conference*, 13-17 Jan, 2015. Islamabad, Pakistan. doi: 10.1109/IBCAST.2015.7058489.

Kaspers, B. and Kaiser, P. (2014). Avian antigen-presenting cells. In Schat, K., Kaspers, B. and Kaiser, P. *Avian Immunology 2nd edition* (pp. 169-188). USA: Elsevier Ltd. doi: https://doi.org/10.1016/B978-0-12-396965-1.00009-1.

Kaur, A., Oberoi, M.S. and Singh, A. (1997). Neutralising antibody and challenge response to live and inactivated avian adenovirus-1 in broilers. *Tropical Animal Health and Production*, 29: 141-146. doi: 10.1007/bf02633009.

Kim, M.S, Lim, T.H., Lee, D.H., Youn, H.N., Yuk, S.S, Kim, B.Y., Choi, S.W., Jung, C.H., Han, J.H. and Song, C. S. (2014). An inactivated oil-emulsion fowl adenovirus serotype 4 vaccine provides broad cross-protection against various serotypes of fowl adenovirus. *Vaccine*, 32(28): 3564-3568. doi:10.1016/j.vaccine.2014.03.015.

Kim, J.N., Byun, S.H., Kim, M.J., Kim, J.J., Sung, H.W. and Mo, I.P. (2008). Outbreaks of hydropericardium syndrome and molecular characterization of Korean fowl adenoviral isolates. *Avian Diseases Digest*, 3(3). doi:10.1637/1933-5334(2008)3[e27:oohsam]2.0.co;2.

Kinloch, R., Mackay, N. and Mautner, V. (1984). Adenovirus hexon: sequence comparison of subgroup C serotypes 2 and 5. *Journal of Biological Chemistry*, 259: 6431-6436.

Kumar, R., Chandra, R., Shukla, S.K., Agrawal, D.K. and Kumar, M. (1997). Hydropericardium syndrome (HPS) in India: A preliminary study on the causative agent and control of the disease by inactivated autogenous vaccine. *Tropical Animal Health and Production*, 29(3): 158-164. doi:10.1007/bf02633014.

Kumar, R., Chandra, R. and Shukla, S.K. (2003). Isolation of etiological agent of hydropericardium syndrome in chicken embryo liver cell culture and its

serological characterization. *Indian Journal of Experimental Biology,* 41:821-826.

Lal B., Maiti N.K., Oberoi, M.S. and Sharma, S.N. (1991). Cell mediated immune responses of chicks following fowl adenovirus type-1 infection. *Comparative Immunology, Microbiology and Infectious Diseases,* 14: 55–58. doi: https://doi.org/10.1016/0147-9571(91)90041-B.

Lee, J.S. and Hanson, R.P. (1975). Effects of bile and gastrointestinal secretions onthe infectivity of Newcastle disease virus. *Infection and Immunity,* 11(4):692-697.

Lim, T-H., Kim, B-Y., Kim, M-S., Jang, J-H., Lee, D-H., Kwon, Y-K., Lee, J-B., Park, S-Y., Choi, I-S. and Song, C-S. (2012). Outbreak of gizzard erosion associated with fowl adenovirus infection in Korea. *Poultry Science,* 91(5): 1113-1117. doi: 10.3382/ps.2011-02050.

Lim, T. H., Lee, H.J., Lee, D.H., Lee, Y.N., Park, J.K., Youn, H.N, Kim, M.S., Youn, H.S., Lee, J.B., Park, S.Y., Choi, I.S. and Song, C. S. (2011). Identification and virulence characterization of fowl adenoviruses in Korea. *Avian Diseases,* 55(4): 554-560. doi:10.1637/9730-032011-reg.1.

Louis, K.S. and Siegel, A.C. (2011). Cell viability analysis using trypan blue: Manual and automated methods. *Methods in Molecular Biology,* 740: 740-747. doi: 10.1007/978-1-61779-108-6_2.

Lutz, P. and Kedinger, C. (1996). Properties of the adenovirus Iva2 gene product an effector of late-phase-dependent activation of the major late promoter. *Journal of Virology,* 70: 1396- 1405.

Lutz, P., Rosa-Calatrava, M. and Kedinger, C. (1997). The product of the adenovirus intermediate gene IX is a transcriptional activator. *Journal of Virology,* 71: 5102-5109.

Maartens, L.H., Joubert, H.W., Aitchison, H. and Venter, E.H. (2014). Inclusion body hepatitis associated with an outbreak of fowl adenovirus type 2 and type 8b in broiler flocks in South Africa. *Journal of the South African Veterinary Association,* 85: 1-5. doi:10.4102/jsava.v85i1.1146

Mahmood, M.S., Ali, S., Hussain, I., Aslam, A. and Rafique, A. (2014). The development of hydropericardium syndrome vaccines. *World`s Poultry Science Journal,* 70(2): 355-364. doi: 10.1017/s0043933914000361.

Maiti, N.K. and Sarkar, P. (1997). Humoral immune response of chicks to different clinical isolates of avian adenovirus type-1. *Comparative Immunology Microbiology and Infectious Diseases,* 20: 59–62. doi: 10.1016/s0147-9571(96)00027-6.

Majdi, A. (2015). Pathogenicity of fowl adenovirus isolates of Malaysia in specific pathogen free chickens. DVM Final Year Project. Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Manarolla, G., Pisoni, G., Moroni, P., Gallazzi, D, Sironi, G. and Rampin, T. (2009). Adenoviral gizzard erosions in Italian chicken flocks. *Veterinary Record,* 164(24): 754-756. doi: http://dx.doi.org/10.1136/vr.164.24.754

Mansoor, M.K., Hussain, I., Arshad, M. and Muhammad, G. (2011). Preparation and evaluation of chicken embryo-adapted fowl adenovirus serotype 4 vaccine in broiler chickens. *Tropical Animal Health and Production,* 43: 331-338. doi: 10.1007/s11250-010-9694-z.

Mantegazza, A.R., Magalhaes, J.G., Amigorena, S. and Marks, M.S. (2013). Presentation of phagocytosed antigens by MHC class I and II. *Traffic (Copenhagen, Denmark),* 14(2): 135–152. http://doi.org/10.1111/tra.12026.

Marek *et al*. (2016). GenBank accession number: KT862812, FN557186, FN557184 and KT862805.

Marek *et al.* (2012). GenBank accession number: FR872928, FR872911, HE608152, HE608153 and HE608154.

Marek, A., Gunes, A., Schulz, E. and Hess, M. (2010a). Classification of fowl adenoviruses by use of phylogenetic analysis and high-resolution melting-curve analysis of the hexon L1 gene region. *Journal of Virological Methods*, 170: 147-154. doi: 10.1016/j.jviromet.2010.09.019.

Marek, A., Schulz, E., Hess, C. and Hess, M. (2010b). Comparison of the fibers of fowl adenovirus A serotype 1 isolates from chickens with gizzard erosions in Europe and apathogenic reference strains. *Journal of Veterinary Diagnostic Investigation,* 22(6): 937-941. doi:10.1177/104063871002200613.

Mase, M., Nakamura, K. and Minami, F. (2012). Fowl adenoviruses isolated from chickens with inclusion body hepatitis in Japan, 2009–2010. *Journal of Veterinary Medical Science,* 74(8): 1087–1089.

Mase, M., Nakamura, K. and Imada, T. (2010). Characterization of fowl adenovirus serotype 4 isolated from chickens with hydropericardium syndrome based on analysis of the short fiber protein gene. *Journal of Veterinary Diagnostic Investigation,* 22(2): 218-223. doi:10.1177/104063871002200207.

Mathis, J. M., Stoff-Khalili, M. A. and Curiel, D. T. (2005). Oncolytic adenoviruses–selective retargeting to tumor cells. *Oncogene,* 24: 7775-7791. doi:10.1038/sj.onc.1209044.

Mazaheri, A., Prusas, C., Voss, M. and Hess, M. (1998). Some strains of serotype 4 fowl adenoviruses cause inclusion body hepatitis and hydropericardium syndrome in chickens. *Avian Pathology,* 27(3): 269-276. doi:10.1080/03079459808419335.

McFerran J.B. and Adair B.M. (2003). Group I Adenovirus Infections. In: Saif Y.M., Fadly A.M., Glisson J.R., McDougald L.R., Nolan L.K. and Swayne D.E (Eds.). *Diseases of Poultry, 12th edition* (pp. 214-227). Blackwell Publishing, Iowa.

McFerran, J.B. (1997). Group I Adenovirus Infections. In B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif (Eds.). *Diseases of Poultry, 10th Edition* (pp. 608–620). Ames, IA: Iowa State University Press.

McFerran, J.B. (1998). Adenoviruses. In Swayne, D.E., John, R. Jackwood, M.W., Pearson, J.E. and Reed, W.M. (Eds). *A Laboratory Manual for The Isolation and Identification of Avian Pathogens, 2nd edition* (pp. 100-105). Kennett Square, PA: University of Pennsylvania.

Mettifogo, E., Nuñez, Luis F.N., Parra, S.H.S., Astolfi-Ferreira, C.S. and Ferreira, A. J. P. (2014). Fowl adenovirus Group I as a causal agent of inclusion body hepatitis/hydropericardium syndrome (IBH/HPS) outbreak in Brazilian broiler flocks. *Pesquisa Veterinária Brasileira,* 34(8):733-737. https://dx.doi.org/10.1590/S0100-736X2014000800004.

Meulemans *et al*. (2016). GenBank accession number: AF508952, AF508953, AF508951, AF508946, AF508948, AF508949, AF508954, AF508955 and AF508957.

Meulemans, G., Couvreur, B., Decaesstecker, M., Boschmans, M. and Berg, T. P. (2004). Phylogenetic analysis of fowl adenoviruses. *Avian Pathology,* 33(2): 164-170. doi:10.1080/03079450310001652086.

Meulemans, G., Boschmans, M., van den Berg, T.P. and Decaesstecker, M. (2001a). Polymerase chain reaction combined with restriction enzyme analysis for detection and differentiation of fowl adenoviruses. *Avian Pathology,* 30: 655-660. doi: 10.1080/03079450120092143.

Meulemans *et al*. (2001b). GenBank accession number: AF339917, AF339915 and AF339922.

Miller, L.T., Jasty, V. and Yates, V.J. (1972). Cytochemistry and immunofluorescence of CELO virus, an oncogenic avian adenovirus, in chicken embryo kidney and chicken embryo fibroblastic cell cultures. *Avian Diseases,* 16(3): 558–573. doi: 10.2307/1588673.

Mittal, D., Jindal, N. and Khokhar, R.S. (2014). Epidemiological studies on inclusion body hepatitis-hydropericardium syndrome in broiler chicken in Haryana state. *Haryana Vet.*, 53: 34-38.

Muir, W.I., Bryden, W.L. and Husband, A.J. (2000). Immunity, vaccination and the avian intestinal tract. *Development and Comparative Immunology Journal,* 24: 325-342. doi: 10.1016/s0145-305x(99)00081-6.

Monreal, G. (1992). Adenoviruses and adeno-associated viruses of poultry. *Poultry Science Review,* 4: 1– 27.

Morales, G.A., Lucio, D.E. and Puebal, O.N. (1997). Use of immunoglobulins against hydropericardium syndrome in a field outbreak. In: *Proceedings Western Poultry Disease Conference,* 44:34–35.

Morshed, R., Hosseini, H., Langeroudi, A.G., Fard, M.H.B. and Charkhkar, S. (2017). Fowl adenoviruses D and E cause inclusion body hepatitis outbreaks in broiler and broiler breeder pullet flocks. *Avian Diseases*, 61(2): 205-210. doi: https://doi.org/10.1637/11551-120516-Reg.1.

Murphy, F.A., Gibbs, E.P.J., Horzinek, M.C. and Studdert, M.J. (1999). *Veterinary Virology, 3rd Edition.* Academic Press, New York.

Mustaffa-Babjee, A. and Spradbrow, P. (1975). Characterisations of three strains of avian adenoviruses isolated in Queensland. I. Biological properties. *Avian Diseases*, 19: 150-174.

Nakamura, K., Mase, M., Yamamoto, Y., Takizawa, K., Kabeya, M., Wakuda, T., Matsuda, M., Chikuba, T., Yamamoto, Y., Ohyama, T., Takahashi, K., Sato, N., Akiyama, N., Honma, H. and Imai, K. (2011). Inclusion body hepatitis caused by fowl adenovirus in broiler chickens in Japan, 2009–2010. *Avian Diseases,* 55(4): 719-723. doi: https://doi.org/10.1637/9813-052511-Case.1.

Nakamura, T., Sato, K. and Hamada, H. (2003). Reduction of natural adenovirus tropism to the liver by both ablation of fiber-coxsackievirus and adenovirus receptor interaction and use of replaceable short fiber. *Journal of Virology,* 77(4): 2512–2521.

Nakamura, K., Mase, M., Yamaguchi, S., Shibahara, T. and Yuasa, N. (1999). Pathologic Study of Specific-Pathogen-Free Chicks and Hens Inoculated with Adenovirus Isolated from Hydropericardium Syndrome. *Avian Diseases,* 43(3): 414-423. doi:10.2307/1592638.

Niczyporuk, J. S. (2017). Molecular characterisation of fowl adenovirus type 7 isolated from poultry associated with inclusion body hepatitis in Poland. *Archives of Virology,* 162(5): 1325–1333. http://doi.org/10.1007/s00705-017-3240-5.

Nei, M. and Kumar, S. (2000). *Molecular Evolution and Phylogenetics.* New York:Oxford University Press.

Nemerow, G.R., Pache, L., Reddy, V. and Stewart, P.L. (2009). Insights into adenovirus host cell interactions from structural studies. *Virology,* 384(2): 380–388. doi: http://doi.org/10.1016/j.virol.2008.10.016.

Nemerow, G., Wu, E. and Stewart, P. (2007). *U.S. Patent No. US20070003923A1*. Washington, DC: U.S. Patent and Trademark Office.

Norina, L., Norsharina, A., Nurnadiah, A.H., Redzuan, I., Ardy, A. and Nor-Ismaliza, A. (2016). Avian adenovirus isolated from broiler affected with inclusion body hepatitis. *Malaysian Journal of Veterinary Research,* 7(2): 121-126.

Norrby, E., Bartha, A., Boulanger, P., Dreizin, R.S., Ginsberg, H.S., Kalter, S.S., Kawamura, H., Rowe, W. P., Russell, W. C., Schlesinger, W. and Wigand, R. (1976). Adenoviridae. *Intervirology,* 7: 117–125.

Norrby, E. (1969). The structural and functional diversity of adenovirus capsid components. *Journal of General Virology*, 5: 221-236. doi: 10.1099/0022-1317-5-2-221.

Ojkić, D. and Nagy, E´. (2000). The complete nucleotide sequence of fowl adenovirus type 8. *Journal of General Virology*, 81(7): 1833–1837. doi: 10.1099/0022-1317-81-7-1833.

Ojkić, D., Martin, E., Swinton, J., Vaillancourt, J.P., Boulianne, M. and Gomis, S. (2008a). Genotyping of Canadian isolates of fowl adenoviruses. *Avian Pathology*, 37(1): 95-100. doi: 10.1080/03079450701805324.

Ojkić, D., Krell, P.J., Tuboly, T. and Nagy, E. (2008b). Characterization of fowl adenoviruses isolated in Ontario and Quebec, Canada. *Canadian Journal of Veterinary Research*, 72: 236–241.

Ojkić *et al*. (2015). GenBank accession number: NC_000899.

Ojkić, D. and Nagy, É. (2003). Antibody response and virus tissue distribution in chickens inoculated with wild-type and recombinant fowl adenoviruses. *Vaccine*, 22(1): 42-48. doi:10.1016/s0264-410x(03)00544-9.

Ogata, F. and Hamada, C. (1979). Adenovirus type 12 plaque formation in vero cells. *Microbiology and Immunology*, 23(4): 299-301. doi: 10.1111/j.1348-0421.1979.tb00465.x.

Okino, C.H., Alessi, A.C., Montassier, M.D., Rosa, A.J., Wang, X. and Montassier, H.J. (2013). Humoral and cell-mediated immune responses to different doses of attenuated vaccine against avian infectious bronchitis virus. *Viral Immunology*, 26(4): 259-267. doi:10.1089/vim.2013.0015.

Okuda, Y., Ono, M., Shibata, I. and Sato, S. (2004). Pathogenicity of serotype 8 fowl adenovirus isolated from gizzard erosions of slaughtered broiler chickens. *Journal of Veterinary Medical Science*, 66(12): 1561-1566. doi:10.1292/jvms.66.1561.

Okuda, Y., Ono, M., Yazawa, S., Shibata, I. and Sato, S.(2001). Experimental infection of specific-pathogen-free chickens with serotype-1 fowl adenovirus isolated from a broiler chicken with gizzard erosions. *Avian Diseases*, 45:19–25. doi: 10.2307/1593007.

Okuda, Y., Ono, M., Shibata, I., Sato, S. and Akashi, H. (2006). Comparison of the polymerase chain reaction-restriction fragment length polymorphism pattern of the fiber gene and pathogenicity of serotype-1 fowl adenovirus isolates from gizzard erosions and from feces of clinically healthy chickens in Japan. *Journal of Veterinary Diagnostic Investigation*, 18(2): 162-167. doi:10.1177/104063870601800204.

Olson, N.O. (1950). A respiratory disease (bronchitis) of quail caused by a virus. In: *Proceedings of the 54th Annual Meeting of the US Livestock Sanitary Association*.

Ono, M., Okuda Y., Yazawa, S., Shibata I., Sato S. and Okada, K. (2003). Outbreaks of adenoviral gizzard erosion in slaughtered broiler chickens in Japan. *Veterinary Record*, 153:775-779.

Ono, M., Okuda, Y., Shibata, I., Sato, S. and Okada, K. (2004). Pathogenicity by parenteral injection of fowl adenovirus isolated from gizzard erosion and resistance to reinfection in adenoviral gizzard erosion in chickens. *Veterinary Pathology*, 41: 483–489. doi: 10.1354/vp.41-5-483.

Oliver-Ferrando, S., Dolz, R., Calderón, C., Valle, R., Rivas, R., Pérez, M., Biarnés, M., Blanco, A., Bertran, K., Ramis, A., Busquets, N. and Majó, N. (2017). Epidemiological and pathological investigation of fowl aviadenovirus serotypes 8b and 11 isolated from chickens with inclusion body hepatitis in Spain (2011–2013). *Avian Pathology*, 46(2): 157-165. doi: http://dx.doi.org/10.1080/03079457.2016.1232477.

Pallister *et al.* (2000). GenBank accession number: FAU40588.

Pallister, J., Wright, P.J. and Sheppard, M. (1996). A single gene encoding the fiber is responsible for variations in virulence in the fowl adenoviruses. *Journal of Virology*, 70(8): 5115-5122.

Pallister, J.A., Erny, K.M. and Fahey, K.J. (1994). Serological relationships within the group E of fowl adenoviruses. *Intervirology*, 36: 84-90.

Park *et al*. (2017). GenBank accession number: HQ709232 and HQ709231.

Paul, G. and Hess, M. B. (2003). *U.S. Patent No. WO2003039593A1*. Washington, DC: U.S. Patent and Trademark Office.

Parthiban, M., Shalini, K.S., Kumanan, K. and Jayaraman, K. (2013). Identification and differentiation of pathogenic and non-pathogenic serotypes of fowl adenoviruses using PCR-RFLP and sequencing. *Indian Journal of Animal Sciences*, 83(4): 347-350.

Pereira, H. (1958). A protein factor responsible for the early cytopathic effect of adenoviruses. *Virology*, 6: 601-611.

Pettersson, U. and Höglund, S. (1969). Structural proteins and adenovirus type 2 penton antigen. *Virology*, 39: 90-106.

Pettersson, U., Philipson, L. and Höglund, S. (1967). Structural proteins of adenoviruses. I. Purification and characterization of adenovirus type 2 hexon antigen. *Virology*, 33(4): 575-590. doi: https://doi.org/10.1016/0042-6822(67)90057-8.

Pichla-Gollon, S.L., Drinker, M., Zhou, X., Xue, F., Rux, J.J., Gao, G., Wilson, J.M., Ertl, H.C., Burnett, R.M. and Bergelson, J. M. (2006). Structure-based identification of a major neutralizing site in an adenovirus hexon. *Journal of Virology*, 81(4): 1680-1689. doi:10.1128/jvi.02023-06.

Pizzuto, M.S., De Battisti, C., Marciano, S., Capua, I. and Cattoli, G. (2010). Pyrosequencing analysis for a rapid classification of fowl adenovirus species. *Avian Pathology*, 39: 391-398. doi: 10.1080/03079457.2010.510499.

Ramis, A., Latimer, K.S., Niagro, F.D., Campagnoli, R.P., Ritchie, B.W. and Pesti, D. (1994). Diagnosis of psittacine beak and feather disease (Pbfd) viral-infection, avian polyomavirus infection, adenovirus infection and herpesvirus-infection in psittacine tissues using DNA in-situ hybridization. *Avian Pathology*, 23(4): 643-657. doi: 10.1080/03079459408419034.

Raue, R. and Hess, M. (1998). Hexon based PCRs combined with restriction enzyme analysis for rapid detection and differentiation of fowl adenoviruses and egg drop syndrome virus. *Journal of Virological Methods*, 73(2): 211-217. doi: 10.1016/s0166-0934(98)00065-2.

Reed, L.J. and Muench H. (1938). A simple method of estimating fifty per cent endpoints. *American Journal of Hygiene*, 27: 493–497.

Rexroad, J., Evans, R. K. and Middaugh, C. (2006). Effect of pH and ionic strength on the physical stability of adenovirus type 5. *Journal of Pharmaceutical Sciences*, 95(2): 237-247. doi:10.1002/jps.20496.

Robinson, A., Younghusband, H. and Bellett, A. (1973). A circular DNA—Protein complex from adenoviruses. *Virology*, 56: 54-69. doi: 10.1016/0042-6822(73)90287-0.

Roberts, M., White, J., Grutter, M. and Burnett, R. (1986). Three-dimensional structure of the adenovirus major coat protein hexon. *Science*, 232: 1148-1151. doi:10.1126/science.3704642.

Rodriguez, J., Koga, Y., Alvarado, A. and Tinoco, R. (2014). Molecular characterization of Peruvian fowl adenovirus (FAdV) isolates. *Advances in Microbiology*, 4: 595-603. doi:10.4236/aim.2014.410065.

Rosenberger, J.K., Klopp, S., Eckroade, R.J. and Krauss, W.C. (1975). The roles of the infectious bursal agent and several avian adenoviruses in the hemorrhagic-aplastic-anemia syndrome and gangrenous dermatitis. *Avian Diseases*, 19: 717-729. doi:10.2307/1589185.

Roy, P., Koteeswaran, A. and Manickam, R. (1999). Efficacy of an inactivated oil emulsion vaccine against hydropericardium syndrome in broilers. *Veterinary Record*, 145(16): 458-459. doi:10.1136/vr.145.16.458.

Russell, W.C. (2009). Adenoviruses: update on structure and function. *Journal of General Virology*, 90: 1-20. doi:10.1099/vir.0.003087-0.

Russell, W.C., Pereira, H. G. and Valentine, R.C. (1967). The effect of heat on the anatomy of the adenovirus. *Journal of General Virology*, 1:509-522. doi: 10.1099/0022-1317-1-4-509.

Sadeeq-Ur-Rahman, S., Rabbani, M., Shah, M.K., Naseem, S., Khan, S.H. and Muhammad, K. (2008). Development of standard protocols for preparation and evaluate of homogenate liver vaccines against hydropericardium syndrome virus in poultry. *Pakistan Veterinary Journal*, 28(4): 163-166.

Saifuddin, M. and Wilks, C. (1990). Reproduction of inclusion body hepatitis in conventionally raised chickens inoculated with a New Zealand isolate of avian adenovirus. *New Zealand Veterinary Journal*, 38(2): 62-65. doi:10.1080/00480169.1990.35618.

Saifuddin, M. and Wilks, C.R. (1992). Effect of fowl adenovirus infection on the immune system of chickens. *Journal of Comparative Pathology*, 107:285-294.

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. doi: https://doi.org/10.1093/oxfordjournals.molbev.a040454.

Schachner *et al*. (2016). GenBank accession number: LN907576, LN907578, LN907574, LN907584 and LN907582.

Schade, B., Schmitt, F., Bohm, B., Alex, M., Fux, R., Cattoli, G., Terregino, C., Monne, I., Currie, R.J. and Olias, P. (2013). Adenoviral gizzard erosion in broiler chickens in Germany. *Avian Diseases*, 57(1): 159-163. doi:10.1637/10330-082312-Case.1.

Schijns, V. E.J.C., Zande, S. van de., Lupiani, B. and Reddy, S.M. (2013). Practical Aspects of Poultry Vaccination. In Schat, K.A., Kaspers, B. and Kaiser, P. *Avian Immunology*, *2nd edition* (pp. 345-362). London, UK: Elsevier Ltd..

Schmid, S.I. and Hearing, P. (1995). Selective encapsidation of adenovirus DNA. *Current Topic in Microbiology and Immunology*, 199: 67-80.

Schonewille, E., Jaspers, R., Paul, G. and Hess, M. (2010). Specific pathogen free chickens vaccinated with a live FAdV-4 vaccine are fully protected against a severe challenge even in the absence of neutralizing antibodies. *Avian Diseases*, 54(2): 905-910. doi:10.1637/8999-072309-Reg.1.

Schonewille, E., Singh, A., Göbel, T. W., Gerner, W., Saalmüller, A. and Hess, M. (2008). Fowl adenovirus (FAdV) serotype 4 causes depletion of B and T cells in lymphoid organs in specific pathogen-free chickens following experimental infection. *Veterinary Immunology and Immunopathology*, 121(1-2): 130-139. doi:10.1016/j.vetimm.2007.09.017.

Senne, D.A. (1989). Virus propagation in embryonating eggs. In Purchage, H.G., Arp, L.H., Domermuth, C.H. and Pearson, J.E. *A Laboratory Manual for the Isolation and Identification of Avian Pathogens* (pp 176-181). American Association of Avian Pathologist, Pennsylvania, USA.

Shane, S.M. (1996). Hydropericardium-hepatitis syndrome-the current world situation. *Zootecnica International*, 18: 20-27.

Sharpless, G. R., Levine, S., Davies, M. and Englert, M. E. (1961). GAL virus: Its growth cycle in tissue culture and some of its properties. *Virology,* 13:315-322. doi:10.1016/0042-6822(61)90151-9.

Shayakhmetov, D. M., Eberly, A. M., Li, Z. Y. and Lieber, A. (2005). Deletion of penton RGD motifs affects the efficiency of both the internalization and the endosome escape of viral particles containing adenovirus serotype 5 or 35 fiber knobs. *Journal of Virol*ogy, 79: 1053–1061. doi: 10.1128/JVI.79.7.4553.2005.

Sheppard, M., Mccoy, R. J. and Werner, W. (1995). Genomic mapping and sequence analysis of the fowl adenovirus serotype 10 hexon gene. *Journal of General Virology,* 76(10): 2595-2600. doi: 10.1099/0022-1317-76-10-2595

Sheppard, M. and Trist, H. (1992). Characterization of the avian adenovirus penton base. *Virology,* 188: 881-886. doi: 10.1016/0042-6822(92)90546-2.

Singh, A., Grewal, G.S., Maiti, N.K. and Oberoi, M.S. (2006). Effect of fowl adenovirus-1 (IBH isolate) on humoral and cellular immune competency of broiler chicks. *Comparative Immunology, Microbiology and Infectious Dis*eases, 29: 315–321. doi: 10.1016/j.cimid.2006.08.001.

Songserm, T. (2007). Inclusion body hepatitis. *Journal of Kasetsart Veterinarians,* 17: 97-102.

Soumyalekshmi, S., Ajith, M.K. and Chandraprakash, M. (2014). Isolation of fowl adenovirus in chicken embryo liver cell culture and its detection by hexon gene based PCR. *Indian Journal of Scientific Research and Technology,* 2(3): 33-36.

Steer *et al.* (2017). GenBank accession number: KT037703 and KT037711.

Steer *et al.* (2016). GenBank accession number: EU979377, EU979378 and EU979374.

Steer *et al.* (2014). GenBank accession number: GU120268 and GU120266.

Steer, P.A., O'Rourke, D. Ghorashi, S.A. and Noormohammadi, A.H. (2011). Application of high-resolution melting curve analysis for typing of fowl adenoviruses in field cases of inclusion body hepatitis. *Australian Veterinary Journal,* 89: 184–192. doi: 10.1111/j.1751-0813.2011.00695.x.

Steer, P.A., Kirkpatrick, N.C., O´Rourke, D. and Noormohammadi, A.H. (2009). Classification of fowl adenovirus serotypes by use of high-resolution meting-curve analysis of the hexon gene region. *Journal of Clinical Microbiology,* 47: 311-321. doi: 10.1128/jcm.01567-08.

Strauss, J.H. and Strauss, E.G. (2008). *Viruses and human disease* (2nd ed.). Canada: Elsevier /Academic Press.

Taharaguchi, S., Fukazawa, R., Kitazume, M., Harima, H., Taira, K., Oonaka, K. and Hara, M. (2012). Biology of fowl adenovirus type 1 infection of heterologous cells. *Archives of Virology,* 157(11): 2223-2226. doi:10.1007/s00705-012-1413-9.

Tamanoi, F. (1986). On the mechanism of adenovirus DNA replication. In Doefler, W. (Ed.) *Adenovirus DNA: The Viral Genome and Its Expression* (pp. 97-128). Boston: Martinus Nijhoff.

Tan, P. K., Cotten, M., Bergelson, J. M. and Michou, A. (2001). Defining CAR as a cellular receptor for the avian adenovirus CELO using a genetic analysis of the two viral fibre proteins. *Journal of General Virology,* 82(6): 1465-1472. doi: 10.1099/0022-1317-82-6-1465.

Tanimura, N., Nakamura, K., Imai, K., Maeda, M., Gobo, T., Nitta, S., Ishihara, T. and Amano, H. (1993). Necrotizing pancreatitis and gizzard erosion associated with adenovirus infection in chickens. *Avian Diseases.* 37(2): 606. doi: 10.2307/1591697.

Tollefson, A.E., Ryerse, J.S., Scaria, A., Hermiston, T.W. and Wold, W.S. (1996). The E3-11.6-kDa adenovirus death protein (ADP) is required for efficient cell death: characterization of cells infected with adp mutants. *Virology,* 220: 152-162. doi: 10.1006/viro.1996.0295.

Toogood, C.I. and Hay, R.T. (1988). DNA sequence of the adenovirus type 41 hexon gene and predicted structure of the protein. *Journal of General Virology,* 69: 2291-2301. doi: 10.1099/0022-1317-69-9-2291.

Toogood, C.I.A., Crompton, J. and Hay, R.T. (1992). Antipeptide antisera define neutalizing epitopes on the adenovirus hexon. *Journal of General Virology,* 73: 1429-1435. doi: 10.1099/0022-1317-73-6-1429.

Toro, H., Gonzalez, C., Cerda, L., Morales, M.A., Dooner, P. and Salamero, M. (2002). Prevention of inclusion body hepatitis/hydropericardium syndrome in progeny chickens by vaccination of breeders with fowl adenovirus and chicken anemia virus. *Avian Diseases,* 46: 547-554. doi: 10.1637/0005-2086(2002)046[0547:POIBHH]2.0.CO;2.

Toro, H., Gonzalez, C., Cerda, L., Hess, M., Reyes, E. and Geisse, C. (2000). Chicken anemia virus and fowl adenovirus: association to induce the inclusion body hepatitis/hydropericardium syndrome. *Avian Diseases,* 44: 51-58. doi: 10.2307/1592507.

Toro, H., Prusas, R., Raue, R., Cerda, L., Geisse, C., Gonzalez, C. and Hess, M. (1999). Characterization of fowl adenoviruses from outbreaks of inclusion body hepatitis/hydropericardium syndrome in Chile. *Avian Diseases,* 43: 262-270. doi: 10.2307/1592616.

Van den Ende, M., Don, P.A. and Kipps, A. (1949). The isolation in eggs of a new filterable agent which may be the cause of bovine lumpy skin disease. *Journal of General Microbiology,* 3: 174-182.

Vellinga, J., Van der Heijdt, S. and Hoeben, R.C. (2005). The adenovirus capsid: major progress in minor proteins. *Journal of General Virology,* 86: 1581–1588. doi: 10.1099/vir.0.80877-0

Voss, M., Vielitz, E., Hess, M., Prusas, C.H. and Mazaheri, A. (1996). Aetiological aspects of hepatitis and HPS caused by pathogenic adenoviruses in different countries. In: *International Symposium on Adenovirus and Reovirus Infection in Poultry*. Rauischholz-hausen.

Wang, C., Yu, S., Ao-ri-ge-le, Jia, D., Yao, H., Zhao, H., Lillehoj, H.S., Si-mu-ji-de, Postnikoff, A.C.L. and Xu, S. (2012). Regulation of T lymphocyte subpopulations in specific pathogen-free chickens following experimental fowl adenovirus-VIII infection. *Brazilian Journal of Microbiology*, 43(4): 1281–1290. doi: http://doi.org/10.1590/S1517-83822012000400007.

Wang, Z., Wang, B., Lou, J., Yan, J., Gao, L., Geng, R. and Yu, B. (2014). Mutation in fiber of adenovirus serotype 5 gene therapy vector decreases liver tropism. *International Journal of Clinical and Experimental Medicine*, 7(12): 4942–4950.

Wiethoff, C.M., Wodrich, H., Gerace, L. and Nemerow, G. R. (2005). Adenovirus protein VI mediates membrane disruption following capsid disassembly. *Journal of Virology*, 79(4): 1992–2000. doi: 10.1128/JVI.79.4.1992-2000.2005.

Wigand, R., Bartha, A., Dreizin, R.S., Esche, H., Ginsberg, H.S., Green, M., Hierholzer, J.C., Katler, S.S., McFerran, J.B., Pettersson, U., Russell, W.C. and Wadell, G. (1982). Adenoviridae: second report. *Intervirology*, 18: 159-175.

Wilcox, G.E., Robertson, M.D. and Lines, A.D. (1985). Adaptation and characteristics of replication of a strain of avian reovirus in vero cells. *Avian Pathology*, 14(3): 321-328. doi: 10.1080/03079458508436234.

Wilcox, W.C. and Ginsberg, H.S. (1963). Structure of type 5 adenovirus : I. Antigenic relationship of virus-structural proteins to virus-specific soluble antigens from infected cells. *The Journal of Experimental Medicine*, 118(2): 295–306.

Xie, Z., Tang, Y., Fan, Q., Liu, J., Pang, Y., Deng, X., Peng, Y., Xie, L. and Khan, M.I. (2011). Rapid detection of group I avian adenoviruses by a loop-mediated isothermal amplification. *Avian Diseases*, 55: 575-579. doi: 10.1637/9897-971911-digest.110.1637/9897-971911-digest.1.

Yates, V.J. and Fry, D.E. (1957). Observations on a chick embryo lethal orphan (CELO) virus. *American Journal of Veterinary Research*, 18: 657-660.

Zadravec, M., Slavec, B., Krape, U., Kaján, G.L., Ranik, J., Juntes, P., Cizerl, R.J., Benko, M. and Rojs, O.Z. (2011). Inclusion body hepatitis associated with fowl adenovirus type 8b in broiler flock in Slovenia – a case report. *Slovenian Veterinary Research*, 48: 107–113.

Zhang, Y. and Bergelson, J.M. (2005). Adenovirus receptors. *Journal of Virol*ogy, 79(19):12125–12131. doi: 10.1128/JVI.79.19.12125-12131.2005.

Zhang, T., Jin, Q., Ding, P., Wang, Y., Chai, Y., Li, Y., Liu, X., Luo, J. and Zhang, G. (2016). Molecular epidemiology of hydropericardium syndrome outbreak-

associated serotype 4 fowl adenovirus isolates in central China. *Virology Journal,* 13: 188. doi: 10.1186/s12985-016-0644-x.

Zhang, W. and Imperiale, M.J. (2003). Requirement of the adenovirus IVa2 protein for virus assembly. *Journal of Virology,* 77: 3586-3594. doi: 10.1128/JVI.77.6.3586-3594.2003.

Zhao, J., Zhong, Q., Zhao, Y., Hu, Y-x. and Zhang, G-z. (2015). Pathogenicity and complete genome characterization of fowl adenoviruses isolated from chickens associated with inclusion body hepatitis and hydropericardium syndrome in China. *PLOS ONE,* 10(7): e0133073. doi: https://doi.org/10.1371/journal.pone.0133073.