UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT AND EVALUATION OF BIVALENT DNA VACCINE AGAINST AVIAN INFECTIOUS BRONCHITIS

FARUKU BANDE

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DEVELOPMENT AND EVALUATION OF BIVALENT DNA VACCINE AGAINST AVIAN INFECTIOUS BRONCHITIS

By

FARUKU BANDE

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

June 2015

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DEDICATION

*To my late daughter, **Nana Asmau** and all children died of preventable, but un-diagnosed diseases in Africa and worldwide*!!

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Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

DEVELOPMENT AND EVALUATION OF BIVALENT DNA VACCINE AGAINST AVIAN INFECTIOUS BRONCHITIS

By

FARUKU BANDE

June 2015

Chairperson: Siti Suri Arshad, PhD
Faculty: Veterinary Medicine

Infectious bronchitis (IB) constitutes a major challenge to the poultry industry worldwide. The widely used live attenuated IB vaccines suffer from numerous limitations, including but not limited to, reversion to virulence, recombination and mutations. Whereas killed IB vaccines induce poor immune response and require repeated dosing. DNA vaccines on the other hands, have the potentials to induce both humoral and cell mediated immune responses against infectious pathogens with minimal unwanted effects. In this study, the S1 glycoprotein of M41 and CR88 IBV strains were selected for the development of bivalent DNA vaccine against IB. Bioinformatic analysis revealed structural and phylogenetic differences between the two virus strains. However, several conserved B-cells and T-cells epitopes were predicted to occur among these strains. To develop a broad-based DNA vaccine against the two strains, the full-length S1 glycoprotein genes of M41 and CR88 IBV strains were cloned individually as well as in combination to obtain a pBudCR88-S1, pBudM41-S1 and pBudCR88-S1/M41-S1 plasmids. *In vitro* expressions of the constructed IB-DNA plasmids were confirmed by immunofluorescence and RT-PCR assays 42 hrs post transfection in chicken embryo fibroblast cells. In order to improve the delivery potential of the candidate DNA vaccine plasmid and protect it from endonuclease degradation, a chitosan - saponin (CS-SP) nanoparticle was synthesized, characterized and used for the encapsulation of the bivalent IB DNA plasmids. Characterization of the CS-SP nanoparticle revealed a spherically-shaped nanoparticle with a size below 100 nm and a zeta potential of 38.9 mV. The encapsulated bivalent IB-DNA plasmid was found to be thermostable, less toxic and protected against *in vitro* enzymatic degradation. Subsequently, the efficacy of the candidate DNA vaccine was evaluated by immunizing 3 weeks old SPF chickens. Six groups of chickens comprising of vaccinated and control chicken received 100 µg of plasmid as follows: - PBS-control (A) empty-plasmid control, pBudCE (B); pBudM41-S1 (C); pBudCR88-S1 (D); pBudCR88-S1/M41-S1+nano (E) and pBudCR88-S1/M41-S1-without nano (F). All chickens except those in group E received two booster vaccination at two week intervals. To assess the effect of CS-SP, chickens in group E only received the first booster vaccine. To evaluate the vaccine's protective capacity, the initial six chicken groups (A, B, C, D, E, F) were divided each into 2 parts as M41-challenged (n=6) and CR88-challenge (n=6) subgroups and then challenged accordingly with 10^5 EID50 of M41 and CR88 IBV strains two weeks after the last booster vaccination. All chickens

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were monitored before terminating the experiment two weeks after challenge. Evaluation of immune response was carried out by measuring the IBV specific antibodies (weekly); percentage CD3+, CD4+ and CD3+, CD8+ T-cells (two weeks after the last booster); clinical signs (after the challenge); oropharyngeal and cloacal virus shedding (day 3, day 5, day 10 and day 15) and histopathological lesion scores (two weeks after challenge). Analysis of humoral and cell mediated immune (CMI) response revealed a significant increase in anti-IBV antibody as well as CD3+, CD4+ and CD3+, CD8+ T cells responses in chickens vaccinated with bivalent IB-DNA vaccines as determined by ELISA and flow-cytometry respectively. Vaccinated chickens exhibited milder clinical signs following virus challenge as compared to the control groups. Furthermore, vaccination with a bivalent DNA plasmid especially the nanoencapsulated plasmid (pBudCR88-S1/M41-S1+nanoparticles) protected chickens against heterologous virus challenge as revealed by a significant reduction in the ($p<0.05$) in orophyrengeal and cloacal virus shedding following M41 and CR88-IBV strains. However, monovalent IB-DNA vaccines could only protect against homologous virus challenge ($P<0.05$). Histopathologically, lesion scores tend to be higher in the trachea and kidney of control-unvaccinated chickens (A and B) compared to the vaccinated chicken groups (C-F), although this difference was not statistically significant ($p>0.05$). In conclusion, this study demonstrated that bivalent DNA vaccine expressing S1 glycoproteins from M41 and CR88 IBV strains is able to induce significant humoral and CMI responses that is able to protect vaccinated chickens against challenge with both M41 and CR88 IBV serotypes. These findings highlight the potential applications of bivalent DNA vaccine as an alternative strategy for the control of infectious bronchitis in poultry.

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

PEMBANGUNAN DAN PENILAIAN VAKSIN DNA BIVALEN UNTUK MENENTANG BRONKITIS BERJANGKIT AVIAN

Oleh

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Jun 2015

Pengerusi: Siti Suri Arshad, PhD
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Bronkitis berjangkit (IB) merupakan cabaran besar kepada industri ternakan ayam di seluruh dunia.Vaksin hidup IB yang dilemahkan diguna secara meluas didapati mengalami banyak batasan termasuk, perkembalian kepada virulen, penggabungan semula dan mutasi. Sebaliknya, vaksin mati IB menyebabkan tindak balas imun yang lemah dan memerlukan dos berulang. Vaksin DNA mempunyai potensi untuk mendorong kedua-dua tindak balas imun humoral dan sel pengantara (CMI) terhadap patogen berjangkit. Dalam kajian ini, glikoprotein S1 dari M41 dan CR88 IBV strain telah dipilih untuk pembangunan vaksin DNA bivalen. Analisis bioinformatik mendedahkan perbezaan struktur dan filogenetik antara kedua-dua jenis virus. Walau bagaimanapun, dalam kedua-dua virus, pemuliharaan B-sel dan T-sel epitopes telah diramalkan. Untuk membangunkan calon vaksin DNA, gen S1 glikoprotein berpanjangan penuh telah diklon untuk mendapatkan plasmid bivalen pBudCR88-S1/M41-S1. Ungkapan *in vitro* binaan plasmid IB-DNA telah disahkan oleh esei immunofluorescence dan RT-PCR. Dalam usaha untuk menambah baik penyampaian potensi calon vaksin plasmid, kitosan (CS) dan saponin (SP) telah digunakan untuk menghasilkan nanopartikel CS-SP yang digunakan untuk mengkapsul plasmid DNA. Pencirian nanopartikel CS-SP menunjukkan ianya berbentuk sfera dengan saiz di bawah 100 nm dan potensi zeta 38.9 mV. Kapsul IB-DNA bivalen plasmid didapati tahan panas, kurang toksik dan dilindungi daripada degradasi enzim in vitro. Seterusnya, keberkesanan calon vaksin DNA telah dinilai dengan pelalian ayam SPF berumur 3 minggu. Enam kumpulan ayam terlali dan kawalan yang terdiri daripada 12 ekor setiap kumpulan menerima 100 µg plasmid seperti berikut:- PBS-kawalan (A) plasmid-kosong, pBudCE (B); pBudM41-S1 (C); pBudCR88-S1 (D); pBudCR88-S1 / M41-S1 + nano (E) dan pBudCR88-S1 / M41-S1-tanpa nano (F). Semua ayam kecuali dalam kumpulan E menerima dua booster vaksin selang dua minggu. Cabaran virus dilaksanakan pada minggu kedua selepas booster terakhir. Enam kumpulan ayam terdahulu (A,B,C,D,E, F) di bahagikan kepada dua kumpulan yang dicabar-M41 dan dicabar-CR88, dimana setiap subkumpulan mempuyai 6 ekor ayam. Ayam kemudiannya dicabar dengan 105 EID50 M41 dan CR88 IBV strain dan dipantau selama dua minggu. Penilaian tindak balas imun dan perlindungan dilakukan dengan mengukur antibodi khusus IBV (setiap minggu); peratusan CD3 +, CD4 + dan CD3 +, CD8 + T-sel (dua minggu selepas booster terakhir); petanda klinikal (selepas dicabar);

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rembesan virus orofaringeal dan kloaka (hari ke-3, hari ke-5, hari ke-10 dan hari ke-15) dan skor lesi histopatologi (dua minggu selepas dicabar). Analisis tindak balas humoral dan CMI menunjukkan peningkatan yang ketara dalam anti-IBV antibodi serta CD3 +, CD4 + dan CD3 +, CD8 + T sel pada ayam yang dilalikan dengan vaksin IB-DNA bivalen sebagaimana yang ditentukan oleh ELISA dan aliran sitometri masing-masing. Ayam yang dilalikan memamerkan petanda klinikal yang lebih ringan berikutan cabaran virus berbanding dengan kumpulan kawalan. Tambahan pula, pelalian dengan DNA plasmid bivalen terutama plasmid yang nanoberkapsul (nanopartikel pBudCR88-S1 / M41-S1 +) melindungi ayam daripada cabaran virus heterologus seperti yang ditunjukkan oleh pengurangan yang ketara ($p < 0.05$) dalam rembesan virus M41 dan CR88-IBV strain . Walau bagaimanapun, monovalen vaksin IB-DNA hanya boleh melindungi daripada cabaran homolog virus ($p < 0.05$). Secara histopatologi, skor lesi cenderung untuk menjadi lebih tinggi dalam trakea dan ginjal ayam kawalan tidak dilali (A dan B) berbanding dengan kumpulan ayam yang dilalikan (CF), walaupun perbezaan ini tidak ketara secara statistik ($p > 0.05$). Kesimpulannya, kajian ini menunjukkan bahawa vaksin DNA bivalen yang mempunyai gen S1 dari M41 dan CR88 IBV strain mampu mengaruh tindakbalas humoral khusus untuk IB dan CMI yang melindungi ayam yang dilalikan terhadap cabaran dengan kedua-dua IBV serotip M41 dan CR88. Penemuan ini menyerlahkan potensi aplikasi vaksin IB-DNA bivalen sebagai satu strategi alternatif bagi mengawal bronkitis berjangkit.

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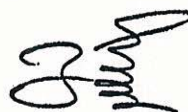

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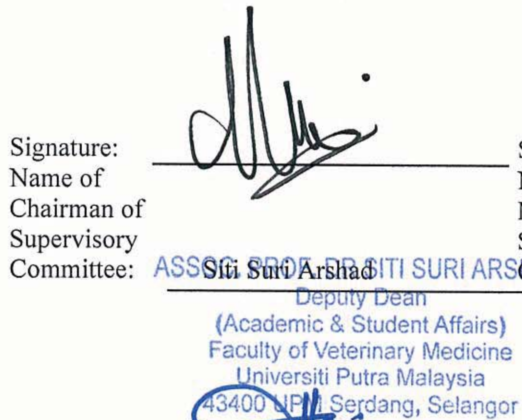

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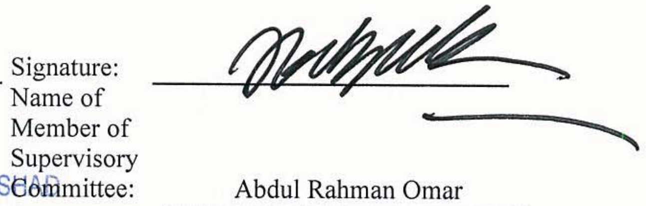

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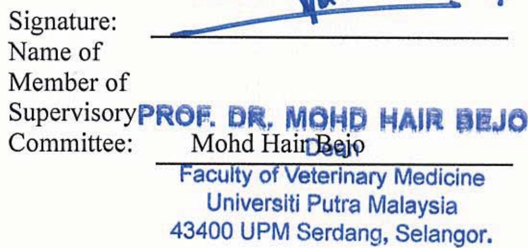

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LIST OF ABBREVIATIONS

+AAs	Amino acids
AF	Allantoic fluid
AI	Aliphatic index
AMP	Adenosine monophosphate
aMPV	Avian metapneumovirus
AMV	Avian Myeloblastosis Virus
APC	Antigen presenting cells
ATCC	American type culture collections
BCoV	Bovine Coronavirus
BCPREDS	B cells epitope prediction server
CD	Cluster of Differentiation (e.g CD3+, CD4+ and CD8+ T-cells)
CDC	Centers for Disease Control
CEACAM1	Carcinoembryonic antigen-cell adhesion molecule
chIL	Chicken interleukin
CMI	Cell mediated immunity
CMV	Cytomegalovirus
CpG-ODNS	Oligodeoxynucleotides
CS	Chitosan
CTD	C terminal domain
CTL	Cytotoxic T-Lymphocytes
DAPI	4', 6-Diamidino-2-Phenylindole
DC	Dendritic cells
DDBJ	DNA databank of Japan
DMEM	Dulbecco Modified Essential Media
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DPP4	Dipeptidyl peptidase 4
DSC	Differential scanning calorimetry
EB	Elution buffer
EF-1α	Human Elongation Factor 1α
EID50	Egg infective dose by 50%
ELISA	Enzyme Linked Immunosorbent Assay
EMBL	European Molecular Biology Laboratory
EMBOOS	European Molecular Biology Open Software Suite
ERGIC	ER/Golgi intermediate compartment
FSEM	Field Emission Scanning Electron Microscopy
GFCoV	Guinea fowl coronavirus
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
GRAVY	Grand average of hydropathicity
HA	Haemagglutinin
HCoV	Human Coronavirus
HI	Haemagglutination inhibition
HIV	Human Immunodeficiency Virus
HS	Heparan sulfate
HVR	hypervariable regions
i.d	Intradermal

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IB	Infectious bronchitis
IBDV	Infectious bursal disease virus
IBV	Infectious bronchitis virus
IC50	Inhibit Cellular Proliferation by 50%
ICTV	International Committee on the Taxonomy of Viruses
IEDB	Immune epitope database
IFNα	Interferon alpha
IFNβ	Interferon beta
IFNγ	Interferon gamma
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHNV	Hematopoietic Necrosis Virus
II	Instability index
IL	Interleukin
IL	Interleukin
ILT	Infectious laryngothrichitis
IM	Intramuscular
IR	Intergenic region
IRF1	Interferon Regulatory Factor 1
ISCOM	Immunostimulatory complex
JAK-STAT	Janus Kinase/Signal Transducer and Activator of Transcription
LB	Luria Bertani
MCP-1	Monocyte Chemoattractant Protein-1
MDA	Maternally derived antibody
MERS	Middle East Respiratory Syndrome
MHC	Major histocompatibility
MHV	Mouse hepatitis virus
MIP	Macrophage inflammatory protein
MIP-1	Macrophage inflammatory protein-1
Mpro	Main protease
mRNA	Messenger Ribonucleic acid
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
MVP	Malaysian Vaccine and Pharmaceutical
MYD88	Myeloid differentiation primary response protein 88
NA	Neuraminidase
NCBI	National Center for Biotechnology Information
NDV	Newcastle Disease Virus
NEB	New England Biolabs
NFKBI	Nuclear Factor of Kappa
NK cells	Natural killer cells
nsp	Nonstructural proteins
NTD	N terminal domain
NMP	N-methl-2-pyrolidone
OD	Optical density
OFR	Open reading frame
OIE	Office International des Épizooties
PAMP	Pathogen-associated molecular pattern
PCR	Polymerase chain reaction
PDB	Protein databank
PLP	Papain-like protease

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PRRs	Pattern-recognition receptors
RBD	Receptor binding domain
RdRp	RNA-dependent RNA-polymerase
RFLP	Restriction Fragment Length polymorphism
rN	Recombinant nucleocapsid
RNA	Ribonucleic Acid
RNPC	ribonucleoprotein complex
rS	Recombinant spike
RT	Reverse transcriptase
SARS	Severe Acute Respiratory Syndrome
SC	Subcutaneous
SEM	Scanning Electron Microscopy
sgRNA	Subgenomic RNA
SNP	Single nucleotide polymorphism
SP	Saponin
SPF	Specific Pathogen Free
ssRNA	Single stranded RNA
STAT1	Signal Transducer and Activator of Transcription 1
Ta	Annealing temperature
TCoV	Turkey Coronavirus
TEM	Transmission Electron Microscopy
TGA	Thermal Gravimetric Analysis
TGEV	Transmissible gastroenteritis virus
TLRs	Toll-like receptors
Tm	Melting temperature
TNF	Tumour Necrosis Factor
UK	United Kingdom
USA	United State of America
USDA	United State Department of Agriculture
UTR	Untranslated region
VNT	Virus neutralization test

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

The demand in the consumption of animal proteins has been predicted to increase to about two-third by 2050 and most of the protein is expected to come from the poultry industry (Farrell, 2013). However, infectious pathogens, especially viruses constitute a major challenge to global poultry production (Omar 2014). In particular, infectious bronchitis (IB) is one of the most highly contagious, economically important poultry disease with worldwide distribution (Cavanagh et al., 2005). The disease mainly affects domestic chickens, however, serological and clinical evidences of IB have been reported in non-domestic galliform, exotic and ornamental birds (Liu et al., 2005c, Chen et al., 2013). The causative agent of the disease is infectious bronchitis virus (IBV), which is classified as a single stranded RNA virus belonging to a *gammacoronavirus* subfamily, family *Coronaviridae* (Cavanagh, 2007). Currently, over 50 IBV serotypes which constitute the classical and variant strains are distributed worldwide. Such a large number of different serotypes has contributed to viral diversity as well as difficulty in diagnosis, control and prevention (De Wit et al., 2000, Montassier et al., 2008).

Typically, IBV infects three important body systems and results in various sequelae as follows:- (i) the respiratory system; resulting in tracheal rales, gasping, coughing, watery eyes and nasal discharges; (ii) the renal system; leading to kidney disorders such as nephritis, nephrosis, and urate deposits, (iii) the reproductive system; leading to a decrease in egg production and egg shell quality (Butcher et al., 1990, Ignjatović and Sapats, 2000, Chousalkar and Roberts, 2007a, 2007b, Cook et al., 2001). Some enterotropic IBV strains such as the Moroccan "IBV-G" strains have been reported to cause gastrointestinal tract disorders, thus leading to diarrhoea, fulminating disease and erratic viral shedding (Ambali and Jones, 1990, Ambali, 1992, Liais et al., 2014).

In order to control IBV infection, live attenuated vaccines are currently administered in broilers and for breeder chickens. However, variation exists among countries on the type of IB vaccine strain approved for use. This is usually determined by the epidemiological knowledge of the prevailing local or regional strain. For example, in the USA, the M41, H120, Arkansas, Delaware, Florida and JMK-derived vaccines are used frequently. In Australia, the B and C strains remain used; in Europe vaccine strains, including M41, 4/91 and CR88 are common. In the Netherlands, vaccination is usually carried out using the D274 and D1466 strain vaccine (King and Cavanagh, 1991). For logistics reasons, some commercially available IB vaccines have been combined with other virus vaccines such as the Newcastle disease virus (NDV), Marek's disease infectious bursal disease virus (IBDV) vaccines. However, it is not clear whether this combination affects the immune response to either of the combination antigens (Vagnozzi et al., 2010). Few examples of commercially available live attenuated IB vaccines include, Norbilis IB-Ma5 (MSD Animal Health, UK) from Mass serotype; AviPro IB H120 from Dutch H120 strain (Lohmann Animal Health, Germany); Norbilis 4-91 (MSD Animal Health, UK); Gallivacc-CR88 (Merial, USA from CR88 IBV isolated in European strains. Live attenuated vaccine, POULVAC IB

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QX has been produced against the recently endemic QX-like strains (Pfizer, France). Other forms of vaccines include adenovirus-based vaccines, however, like in the case of live attenuated vaccines, this technology does have limitations that include neutralization or interference by the existing maternal antibodies (Faulkner et al., 2013). Additionally, lack of proper protein folding and glycosylation in the host system; and post-translational modifications that alter the conformation and epitope arrangement, may affect the immunogenicity of the vaccine antigen as well.

1.2 Statement of the problems

Immunization of chickens against IB has so far been dominated by the use of live attenuated and killed vaccines. This is the case in most poultry producing countries, including Malaysia (de Wit et al., 2010, 2011). However, the use of live attenuated IB vaccines is plagued by a number of limitations. These include potential reversion to virulence which may lead to different pathologies and facilitates secondary bacterial infection (Tarpey et al., 2006). Another important limitation is the potential mutation and recombination between vaccine strains and virulent field viruses, which may favour the emergence of the new IBV serotypes (McKinley et al., 2008, Lee et al., 2012, Lee et al., 2010). Moreover, difficulties in adapting some IBV strains in egg or cell cultures have limited the production of live attenuated IBV vaccines. Thus, such viruses may require the use of egg culture which takes longer period and consume huge resources, making the vaccine more expensive for farmers to acquire. The presence of maternal antibodies able to neutralize live attenuated vaccines has also been a daunting challenge to the use of live attenuated vaccines (Klieve and Cumming, 1988). Another means of controlling IB is the use of killed or inactivated vaccines (Finney et al., 1990). This type of vaccine is less effective, especially, when used alone as killed vaccine only induces humoral but not cell mediated immunity (CMI). Despite an increase in the number and diversity of IBV strains, most of the research focus on a single but not multiple serotype based vaccines. There is thus the need for alternative vaccines that are capable of providing adequate immunity against broad IBV strains.

1.3 Justification of the research

The emergence of multiple IBV genotypes invariably hampered control and preventions of IBV disease, thus indirectly affecting poultry production and global protein supply. Genetic variation between field IBV and vaccine variant might contribute to lack of protection and sporadic outbreaks observed in the field. Since the use of conventional IB vaccines such as the live attenuated vaccines has not only proved ineffective, but complicated viral diversity, and increased the burden of controlling the disease. Lack of cross protection and other limitations therefore necessitate the needs to develop alternative, safe and broad based vaccines for the control of IB. DNA vaccines are relatively safe, thermostable, cheaper and faster to produce compared to live attenuated vaccines. Since the use of DNA vaccines requires only immunogenic protein and not whole virus, this may overcome the effect of reversion to virulence associated with live attenuate vaccines. The ability to clone and express more than one immunogenic protein in a single vector is another advantage of using DNA vaccines. Currently, there are limited information on multiserotypes IB-DNA vaccines. Since the M41 (classical strain) and CR88 (variant strain) are two globally important serotypes, these strains may serve as a model for multiple vaccines.

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1.4 Research hypotheses

A bivalent IB-DNA vaccine carrying S1-glycoprotein of M41 and CR88 IBV will significantly induce IB specific immune response and capable of protecting vaccinated chickens from challenge with M41 and CR88 IBV strains.

1.5 Research objectives

1.5.1 General objectives

To develop a bivalent DNA vaccine against infectious bronchitis

1.5.2 Specific objectives

i. to determine the structural characteristics and predict the presence of B cells and T cell epitopes in the S1 glycoprotein of M41 and CR88 IBV strains using bioinformatic tools.

ii. to construct a bivalent IB-DNA plasmid containing S1-glycoprotein genes from classical M41 and variant CR88 IBV strains for use as a vaccine candidate.

iii. to study the transcription and translation of the candidate IB-DNA plasmid in chicken secondary cells (DF1 cells) by RT-PCR and immunoflourescence assays, respectively.

iv. to synthesize and characterize a chitosan-saponin (CS-SP) nanoparticle for application in IB-DNA vaccine delivery.

v. to determine the efficacy of a bivalent IB DNA vaccine in Specific Pathogen Free (SPF) chickens.

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REFERENCES

Abdel-Moneim, A.S., Giesow, K., Keil, G.M., 2014. High-Level Protein expression following single and dual gene cloning of infectious bronchitis virus N and S genes using baculovirus systems. Viral Immunol. 27, 75-81.

Abro, S.H., Renström, L.H.M., Ullman, K., Isaksson, M., Zohari, S., Jansson, D.S., Belák, S., Baule, C., 2012a. Emergence of novel strains of avian infectious bronchitis virus in Sweden. Vet. Microbiol. 155, 237-246.

Abro, S.H., Ullman, K., Belák, S., Baule, C., 2012b. Bioinformatics and evolutionary insight on the spike glycoprotein gene of QX-like and Massachusetts strains of infectious bronchitis virus. Virol J. 9, 211.

Adams, L.G., Babiuk, L., McGavin, D., Nordgren, R., 2009. Chapter 16 - Special Issues around Veterinary Vaccines. In, Alan D.T. Barrett, Lawrence R. Stanberry (Eds.), Vaccines for biodefense and emerging and neglected diseases. Academic Press, London, pp. 225-254.

Adzhar, A., Gough, R., Haydon, D., Shaw, K., Britton, P., Cavanagh, D., 1997. Molecular analysis of the 793/B serotype of infectious bronchitis virus in Great Britain. Avian Pathol. 26, 625-640.

Adzhar, A., Shaw, K., Britton, P., Cavanagh, D., 1996. Universal oligonucleotides for the detection of infectious bronchitis virus by the polymerase chain reaction. Avian Pathol. 25, 817-836.

Ahmed, A.M., Priscila, Z., Jutta, V., Günther, K., Jens, T., 2009. Immunohistochemistry for detection of avian infectious bronchitis virus strain M41 in the proventriculus and nervous system of experimentally infected chicken embryos. Virol J. 6:15.

Akira, S., Takeda, K., Kaisho, T., 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol. 2, 675-680.

Al Sagheer, F., Al-Sughayer, M., Muslim, S., Elsabee, M.Z., 2009. Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf. Carbohydr. Polym. 77, 410-419.

Albert, M.L., Sauter, B., Bhardwaj, N., 1998. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. Nature 392, 86-89.

Alexander, D.J., Gough, R.E., 1977. Isolation of avian infectious bronchitis virus from experimentally infected chickens. Res Vet Sci. 23, 344-347.

Allione, A., Consalvo, M., Nanni, P., Lollini, P.L., Cavallo, F., Giovarelli, M., Forni, M., Gulino, A., Colombo, M.P., Dellabona, P., 1994. Immunizing and curative potential of replicating and nonreplicating murine mammary adenocarcinoma

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cells engineered with interleukin (IL)-2, IL-4, IL-6, IL-7, IL-10, tumor necrosis factor alpha, granulocyte-macrophage colony-stimulating factor, and gamma-interferon gene or admixed with conventional adjuvants. Cancer Res. 54, 6022-6026.

Allison, A.C., Byars, N.E., 1991. Immunological adjuvants: desirable properties and side-effects. Mol Immunol. 28, 279-284.

Alvarado, I., Villegas, P., El-Attrache, J., Jackwood, M., 2006. Detection of Massachusetts and Arkansas serotypes of infectious bronchitis virus in broilers. Avian Dis. 50, 292-297.

Ambali, A., 1992. Recent studies on the enterotropic strain of avian infectious bronchitis virus. Vet. Res. Commun. 16, 153-157.

Ambali, A., Jones, R., 1990. Early pathogenesis in chicks of infection with an enterotropic strain of infectious bronchitis virus. Avian Dis. 34, 809-817.

Ammayappan, A., Upadhyay, C., Gelb Jr, J., Vakharia, V.N., 2009. Identification of sequence changes responsible for the attenuation of avian infectious bronchitis virus strain Arkansas DPI. Arch. Virol. 154, 495-499.

Antonov, P.A., Maximova, V.A., Pancheva, R.P., 1993. Heat shock and osmotically dependent steps by DNA uptake after *Escherichia coli* electroporation. Biochimica et Biophysica Acta.16, 286-288.

Ariaans, M.P., Matthijs, M.G., van Haarlem, D., van de Haar, P., van Eck, J.H., Hensen, E.J., Vervelde, L., 2008. The role of phagocytic cells in enhanced susceptibility of broilers to colibacillosis after infectious bronchitis virus infection. Vet. Immunol. Immunopathol. 123, 240-250.

Armesto, M., Evans, S., Cavanagh, D., Abu-Median, A., Keep, S., Britton, P., 2011. A recombinant avian infectious bronchitis virus expressing a heterologous spike gene belonging to the 4/91 serotype. PloS one 6, e24352.

Arshad S.S., 2006. Infectious Bronchitis. In, Zamri-Saad M, (Eds). Diseases of Poultry in South East Asia. Serdang Malaysia, Universiti Putra Malaysia Press. 199-206.

Aspden, T.J., Mason, J.D., Jones, N.S., Lowe, J.,Skaugrud, Ø, Illum, L., 1997. Chitosan as a nasal delivery system: the effect of chitosan solutions on in vitro and in vivo mucociliary transport rates in human turbinates and volunteers. J. Pharm. Sci. 86, 509-513.

Auvigne, V., Gibaud, S., Léger, L., Malher, X., Currie, R., Riggi, A., 2013. A longitudinal study of the incidence of Avian Infectious Bronchitis in France using strain-specific haemagglutination inhibition tests and cluster analysis. Rev. Med. Vet. 164, 417-424.

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Babcock, G.J., Esshaki, D.J., Thomas, W.D.,Jr, Ambrosino, D.M., 2004. Amino acids 270 to 510 of the severe acute respiratory syndrome coronavirus spike protein are required for interaction with receptor. J. Virol. 78, 4552-4560.

Banat, G.R., Tkalcic, S., Dzielawa, J.A., Jackwood, M.W., Saggese, M.D., Yates, L., Kopulos, R., Briles, W., Collisson, E.W., 2013. Association of the chicken MHC B haplotypes with resistance to avian coronavirus. Dev Comp Immunol. 39, 430-437.

Barlan, A., Zhao, J., Sarkar, M.K., Li, K., McCray, P.B.,Jr, Perlman, S., Gallagher, T., 2014. Receptor variation and susceptibility to Middle East respiratory syndrome coronavirus infection. J. Virol. 88, 4953-4961.

Barry, M.E., Pinto-Gonzalez, D., Orson, F.M., McKenzie, G.J., Petry, G.R., Barry, M.A., 1999. Role of endogenous endonucleases and tissue site in transfection and CpG-mediated immune activation after naked DNA injection. Hum. Gene Ther. 10, 2461-2480.

Beach, J., Schalm, O., 1936. A filterable virus, distinct from that of laryngotracheitis, the cause of a respiratory disease of chicks. Poult Sci. 15, 199-206.

Beaudette, F., Hudson, C., 1937. Cultivation of the virus of infectious bronchitis. J. Am. Vet. Med. Assoc. 90, 51-60.

Belouzard, S., Millet, J.K., Licitra, B.N., Whittaker, G.R., 2012. Mechanisms of coronavirus cell entry mediated by the viral spike protein. Viruses 4, 1011-1033.

Bentley, K., Keep, S.M., Armesto, M., Britton, P., 2013. Identification of a noncanonically transcribed subgenomic mRNA of infectious bronchitis virus and other gammacoronaviruses. J. Virol. 87, 2128-2136.

Benyeda, Z., Mato, T., Süveges, T., Szabo, E., Kardi, V., Abonyi-Toth, Z., Rusvai, M., Palya, V., 2009. Comparison of the pathogenicity of QX-like, M41 and 793/B infectious bronchitis strains from different pathological conditions. Avian Pathol. 38, 449-456.

Bezuidenhout, A., Mondal, S.P., Buckles, E.L., 2011. Histopathological and immunohistochemical study of air sac lesions induced by two strains of infectious bronchitis virus. J. Comp. Pathol. 145, 319-326.

Bhumkar, D.R., Pokharkar, V.B., 2006. Studies on effect of pH on cross-linking of chitosan with sodium tripolyphosphate: a technical note. Aaps Pharmscitech 7, E138-E143.

Bijlenga, G., Cook, J.K.A., Gelb Jr, J., De Wit, J., 2004. Development and use of the H strain of avian infectious bronchitis virus from the Netherlands as a vaccine: a review. Avian Pathol. 33, 550-557.

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Bonavia, A., Zelus, B.D., Wentworth, D.E., Talbot, P.J., Holmes, K.V., 2003. Identification of a receptor-binding domain of the spike glycoprotein of human coronavirus HCoV-229E. J. Virol. 77, 2530-2538.

Boroomand, Z., Asasi, K., Mohammadi, A., 2012. Pathogenesis and tissue distribution of avian infectious bronchitis virus isolate IRFIBV32 (793/B serotype) in experimentally infected broiler chickens. ScientificWorldJournal. 2012:402537.

Boscarino, J.A., Logan, H.L., Lacny, J.J., Gallagher, T.M., 2008. Envelope protein palmitoylations are crucial for murine coronavirus assembly. J. Virol. 82, 2989-2999.

Bouchie, A., 2003. DNA vaccine deployed for endangered condors. Nat. Biotechnol. 21.

Boyle, J.S., Silva, A., Brady, J.L., Lew, A.M., 1997. DNA immunization: induction of higher avidity antibody and effect of route on T cell cytotoxicity. Proc. Natl. Acad. Sci. U. S. A. 94, 14626-14631.

Breslin, J.J., Smith, L.G., Fuller, F.J., Guy, J.S., 1999. Sequence analysis of the turkey coronavirus nucleocapsid protein gene and 3′ untranslated region identifies the virus as a close relative of infectious bronchitis virus. Virus Res. 65, 187-193.

Britton, P., Armesto, M., Cavanagh, D., Keep, S., 2012. Modification of the avian coronavirus infectious bronchitis virus for vaccine development. Bioeng Bugs. 3, 114-119.

Britton, P., Cavanagh, D., 2008. Nidovirus genome organization and expression mechanisms. Nidoviruses, 29-46.

Bui, H.H., Sidney, J., Li, W., Fusseder, N., Sette, A., 2007. Development of an epitope conservancy analysis tool to facilitate the design of epitope-based diagnostics and vaccines. BMC Bioinformatics 8, 361.

Burkatovskaya, M., Tegos, G.P., Swietlik, E., Demidova, T.N., P Castano, A., Hamblin, M.R., 2006. Use of chitosan bandage to prevent fatal infections developing from highly contaminated wounds in mice. Biomaterials 27, 4157-4164.

Bushnell, L., Brandly CA, 1933. Laryngotracheitis in chicks. Poult Sci. 12, 55-60.

Butcher, G., Winterfield, R., Shapiro, D., 1990. Pathogenesis of H13 nephropathogenic infectious bronchitis virus. Avian Dis. 34, 916-921.

Byars, N., Allison, A., 1990. Immunologic adjuvants: general properties, advantages, and limitations. In: Zola, H. (Ed.), Laboratory Methods in Immunology. Boca Raton, CRC Press, p.39-51.

© COPYRIGHT UPM

Cambridge, C.D., Singh, S.R., Waffo, A.B., Fairley, S.J., Dennis, V.A., 2013. Formulation, characterization, and expression of a recombinant MOMP Chlamydia trachomatis DNA vaccine encapsulated in chitosan nanoparticles. Int J Nanomedicine, 8, 1759.

Cao, H.P., Wang, H.N., Yang, X., Zhang, A.Y., Li, X., Ding, M.D., Liu, S.T., Zhang, Z.K., Yang, F., 2013. Lactococcus lactis anchoring avian infectious bronchitis virus multi-epitope peptide EpiC induced specific immune responses in chickens. Biosci. Biotechnol. Biochem. 77, 1499-1504.

Cao, H.P., Wang, H.N., Zhang, A.Y., Ding, M.D., Liu, S.T., Cheng, H., Zhou, Y.S., Li, X., 2012. Expression of avian infectious bronchitis virus multi-epitope based peptide EpiC in Lactococcus lactis for oral immunization of chickens. Biosci. Biotechnol. Biochem. 76, 1871-1876.

Capua, I., Gough, R., Mancini, M., Casaccia, C., Weiss, C., 1994. A 'novel'infectious bronchitis strain infecting broiler chickens in Italy. Zentralbl Veterinarmed B. 41, 83-89.

Carlos F. Barbas III, Dennis R. Burton, Jamie K. Scott, and Gregg J. Silverman. 2007. Quantitation of DNA and RNA. Cold Spring Harb Protoc; doi:10.1101/pdb.ip47

Caron, L., 2010. Etiology and immunology of infectious bronchitis virus. Revista Brasileira de Ciência Avícola 12, 115-119.

Casais, R., Davies, M., Cavanagh, D., Britton, P., 2005. Gene 5 of the avian coronavirus infectious bronchitis virus is not essential for replication. J. Virol. 79, 8065-8078.

Casais, R., Dove, B., Cavanagh, D., Britton, P., 2003. Recombinant avian infectious bronchitis virus expressing a heterologous spike gene demonstrates that the spike protein is a determinant of cell tropism. J. Virol. 77, 9084-9089.

Caux, C., Dezutter-Dambuyant, C., Schmitt, D., Banchereau, J., 1992. GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells. Nature. 360, 258-258.

Cavanagh, D., 1997. Nidovirales: a new order comprising Coronaviridae and Arteriviridae. Arch. Virol. 142, 629-633.

Cavanagh, D., 2003. Severe acute respiratory syndrome vaccine development: experiences of vaccination against avian infectious bronchitis coronavirus. Avian Pathol. 32, 567-582

Cavanagh, D., 2005. Coronaviridae: a review of coronaviruses and toroviruses. Coronaviruses with Special Emphasis on First Insights Concerning SARS , 1-54.

© COPYRIGHT UPM

Cavanagh, D., 2007. Coronavirus avian infectious bronchitis virus. Vet. Res. 38, 281-297.

Cavanagh, D., Casais, R., Armesto, M., Hodgson, T., Izadkhasti, S., Davies, M., Lin, F., Tarpey, I., Britton, P., 2007. Manipulation of the infectious bronchitis coronavirus genome for vaccine development and analysis of the accessory proteins. Vaccine 25, 5558-5562.

Cavanagh, D., Davis, P., Cook, J., 1992. Infectious bronchitis virus: evidence for recombination within the Massachusetts serotype. Avian Pathol. 21, 401-408.

Cavanagh, D., Gelb, J., 2008. Infectious bronchitis. In, Diseases of poultry 12, 117-135.

Cavanagh, D., Mawditt, K., Welchman, D.d.B., Britton, P., Gough, R., 2002. Coronaviruses from pheasants (Phasianus colchicus) are genetically closely related to coronaviruses of domestic fowl (infectious bronchitis virus) and turkeys. Avian Pathol. 31, 81-93.

Chen, F., Pan, L., Zhang, J., Zhou, X., Li, J., Yu, W., 2014. Allele-dependent association of chicken MHC class I molecules with the invariant chain. Vet. Immunol. Immunopathol. 160,273-280

Chen, G., Zhuang, Q., Wang, K., Liu, S., Shao, J., Jiang, W., Hou, G., Li, J., Yu, J., Li, Y., 2013. Identification and survey of a novel avian coronavirus in ducks. PloS one 8, e72918.

Chen, H., Wurm, T., Britton, P., Brooks, G., Hiscox, J.A., 2002. Interaction of the coronavirus nucleoprotein with nucleolar antigens and the host cell. J. Virol. 76, 5233-5250.

Chen, H., Yang, M., Cui, B., Cui, P., Sheng, M., Chen, G., Wang, S., Geng, J., 2010. Construction and immunogenicity of a recombinant fowlpox vaccine coexpressing S1 glycoprotein of infectious bronchitis virus and chicken IL-18. Vaccine 28, 8112-8119.

Chousalkar, K.K., Roberts, J., 2007. Ultrastructural study of infectious bronchitis virus infection in infundibulum and magnum of commercial laying hens. Vet. Microbiol. 122, 223-236.

Chousalkar, K.K., Roberts, J., Reece, R., 2007b. Comparative histopathology of two serotypes of infectious bronchitis virus (T and N1/88) in laying hens and cockerels. Poult. Sci. 86, 50-58.

Chousalkar, K.K., Roberts, J.R., Reece, R., 2007a. Histopathology of two serotypes of infectious bronchitis virus in laying hens vaccinated in the rearing phase. Poult. Sci. 86, 59-62.

© COPYRIGHT UPM

Chu, V.C., McElroy, L.J., Chu, V., Bauman, B.E., Whittaker, G.R., 2006. The avian coronavirus infectious bronchitis virus undergoes direct low-pH-dependent fusion activation during entry into host cells. J. Virol. 80, 3180-3188.

Circella, E., Camarda, A., Martella, V., Bruni, G., Lavazza, A., Buonavoglia, C., 2007. Coronavirus associated with an enteric syndrome on a quail farm. Avian Pathol. 36, 251-258.

Clementz, M.A., Chen, Z., Banach, B.S., Wang, Y., Sun, L., Ratia, K., Baez-Santos, Y.M., Wang, J., Takayama, J., Ghosh, A.K., 2010. Deubiquitinating and interferon antagonism activities of coronavirus papain-like proteases. J. Virol. 84, 4619-4629.

Cohen, A.D., Boyer, J.D., Weiner, D.B., 1998. Modulating the immune response to genetic immunization. FASEB J. 12, 1611-1626.

Cohen, S.N., 2013. DNA cloning: a personal view after 40 years. Proc. Natl. Acad. Sci. U. S. A. 110, 15521-15529.

Cohen, S.N., Chang, A.C., Boyer, H.W., Helling, R.B., 1973. Construction of biologically functional bacterial plasmids in vitro. Proc. Natl. Acad. Sci. U. S. A. 70, 3240-3244.

Collisson, E.W., Pei, J., Dzielawa, J., Seo, S.H., 2000. Cytotoxic T lymphocytes are critical in the control of infectious bronchitis virus in poultry. Dev Comp Immunol. 24, 187-200.

Cong, F., Liu, X., Han, Z., Shao, Y., Kong, X., Liu, S., 2013. Transcriptome analysis of chicken kidney tissues following coronavirus avian infectious bronchitis virus infection. BMC Genomics 14, 743.

Cook, J., Chesher, J., Baxendale, W., Greenwood, N., Huggins, M., Orbell, S., 2001. Protection of chickens against renal damage caused by a nephropathogenic infectious bronchitis virus. Avian Pathol. 30, 423-426.

Cook, J.K., Orbell, S.J., Woods, M.A., Huggins, M.B., 1999. Breadth of protection of the respiratory tract provided by different live-attenuated infectious bronchitis vaccines against challenge with infectious bronchitis viruses of heterologous serotypes. Avian Pathol. 28, 477-485.

Cook, J.K.A., Darbyshire, J.H., Peters, R.W., 1976. Growth kinetic studies of avian infectious bronchitis virus in tracheal organ cultures. Res. Vet. Sci. 20, 348-349.

Cook, J.K.A., Jackwood, M., Jones, R., 2012. The long view: 40 years of infectious bronchitis research. Avian Pathol. 41, 239-250.

Copaja, S., Blackburn, C., Carmona, R., 2003. Variation of saponin contents in Quillaja saponica Molina. Wood Sci. Technol. 37, 103-108.

© COPYRIGHT UPM

Corse, E., Machamer, C.E., 2003. The cytoplasmic tails of infectious bronchitis virus E and M proteins mediate their interaction. Virology 312, 25-34.

Crinion, R., Hofstad, M., 1972. Pathogenicity of four serotypes of avian infectious bronchitis virus for the oviduct of young chickens of various ages. Avian Dis. , 351-363.

Cumming, R., 1969. The control of avian infectious bronchitis/nephrosis in Australia. Aust. Vet. J. 45, 200-203.

Curtis, K.M., Yount, B., Baric, R.S., 2002. Heterologous gene expression from transmissible gastroenteritis virus replicon particles. J. Virol. 76, 1422-1434.

Dar, A., Potter, A., Tikoo, S., Gerdts, V., Lai, K., Babiuk, L.A., Mutwiri, G., 2009. CpG oligodeoxynucleotides activate innate immune response that suppresses infectious bronchitis virus replication in chicken embryos. Avian Dis. 53, 261-267.

Darbyshire, J.H., Peters, R.W., 1985. Humoral antibody response and assessment of protection following primary vaccination of chicks with maternally derived antibody against avian infectious bronchitis virus. Res. Vet. Sci. 38, 14-21.

Davis, H.L., McCluskie, M.J., 1999. DNA vaccines for viral diseases. Microb. Infect. 1, 7-21.

Davis, H.L., Schleef, M., Moritz, P., Mancini, M., Schorr, J., Whalen, R.G., 1996. Comparison of plasmid DNA preparation methods for direct gene transfer and genetic immunization. BioTechniques 21, 92-4, 96-9.

de Haan, C.A., Vennema, H., Rottier, P.J., 2000. Assembly of the coronavirus envelope: homotypic interactions between the M proteins. J. Virol. 74, 4967-4978.

de Haan, C.A.M., de Wit, M., Kuo, L., Montalto-Morrison, C., Haagmans, B.L., Weiss, S.R., Masters, P.S., Rottier, P.J.M., 2003. The glycosylation status of the murine hepatitis coronavirus M protein affects the interferogenic capacity of the virus in vitro and its ability to replicate in the liver but not the brain. Virology 312, 395-406.

de Haan, C.A.M., Rottier, P.J.M., 2005. Molecular interactions in the assembly of coronaviruses. Adv. Virus Res. 64, 165-230.

de Haan, C.A.M., Volders, H., Koetzner, C.A., Masters, P.S., Rottier, P.J.M., 2002. Coronaviruses maintain viability despite dramatic rearrangements of the strictly conserved genome organization. J. Virol. 76, 12491-12502.

© COPYRIGHT UPM

Dehghan, S., Kheiri, M.T., Tabatabaiean, M., Darzi, S., Tafaghodi, M., 2013. Dry-powder form of chitosan nanospheres containing influenza virus and adjuvants for nasal immunization. Arch. Pharm. Res. 36, 981-992.

Delmas, B., Gelfi, J., L'Haridon, R., Sjöström, H., Laude, H., 1992. Aminopeptidase N is a major receptor for the enteropathogenic coronavirus TGEV. Nature. 1992 4;357:417-20

den Haan, J.M., Bevan, M.J., 2001. Antigen presentation to CD8+ T cells: cross priming in infectious diseases. Curr. Opin. Immunol. 13, 437-441.

De Wit, J., 2000. Detection of infectious bronchitis virus. Avian Pathol. 29, 71-93.

De Wit, J., Cook, J.K., Van der Heijden, Harold MJF, 2011. Infectious bronchitis virus variants: a review of the history, current situation and control measures. Avian Pathol. 40, 223-235.

Di Fabio, J., Rossini, L.I., Orbell, S.J., Paul, G., Huggins, M.B., Malo, A., Silva, B.G., Cook, J.K., 2000. Characterization of infectious bronchitis viruses isolated from outbreaks of disease in commercial flocks in Brazil. Avian Dis. , 582-589.

Dolz, R., Pujols, J., Ordóñez, G., Porta, R., Majó, N., 2006. Antigenic and molecular characterization of isolates of the Italy 02 infectious bronchitis virus genotype. Avian Pathol. 35, 77-85.

Dolz, R., Pujols, J., Ordóñez, G., Porta, R., Majó, N., 2008. Molecular epidemiology and evolution of avian infectious bronchitis virus in Spain over a fourteen-year period. Virology 374, 50-59.

Dolz, R., Vergara-Alert, J., Pe´ rez, M., Pujols, J Majo´, 2011. New insights on infectious bronchitis virus pathogenesis: characterization of Italy 02 serotype in chicks and adult hens. Vet Microbiol. 156, 256-264.

Domańska-Blicharz, K., Śmietanka, K., Minta, Z., 2007. Molecular studies on infectious bronchitis virus isolated in Poland. Bull. Vet. Inst. Pulawy 51, 449-452.

Donnelly, J.J., Wahren, B., Liu, M.A., 2005. DNA vaccines: progress and challenges. J. Immunol. 175, 633-639.

Doytchinova, I.A., Flower, D.R., 2007. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics 8, 4.

Dranoff, G., Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H., Brose, K., Jackson, V., Hamada, H., Pardoll, D., Mulligan, R.C., 1993. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc. Natl. Acad. Sci. U. S. A. 90, 3539-3543.

© COPYRIGHT UPM

Droual, R., Woolcock, P., 1994. Swollen head syndrome associated with E. coli and infectious bronchitis virus in the Central Valley of California. Avian Pathol. 23, 733-742.

Duewell, P., Kisser, U., Heckelsmiller, K., Hoves, S., Stoitzner, P., Koernig, S., Morelli, A.B., Clausen, B.E., Dauer, M., Eigler, A., 2011. ISCOMATRIX adjuvant combines immune activation with antigen delivery to dendritic cells in vivo leading to effective cross-priming of CD8 T cells. J Immunol.187, 55-63.

Eisenbraun, M.D., Fuller, D.H., Haynes, J.R., 1993. Examination of parameters affecting the elicitation of humoral immune responses by particle bombardment-mediated genetic immunization. DNA Cell Biol. 12, 791-797.

EL-Manzalawy, Y., Dobbs, D., Honavar, V., 2008. Predicting linear B-cell epitopes using string kernels. J Mol Recognit. 21, 243-255.

Estevez, C., Villegas, P., El-Attrache, J., 2003. A Recombination Event, Induced In Ovo, between a Low Passage Infectious Bronchitis Virus Field Isolate and a Highly Embryo Adapted Vaccine Strain. Avian Dis. 47, 1282-1290.

Fan, W., Yan, W., Xu, Z., Ni, H., 2012. Formation mechanism of monodisperse, low molecular weight chitosan nanoparticles by ionic gelation technique. Colloids Surf B Biointerfaces. 90, 21-27.

Fang, S., Chen, B., Tay, F.P., Ng, B.S., Liu, D.X., 2007. An arginine-to-proline mutation in a domain with undefined functions within the helicase protein (Nsp13) is lethal to the coronavirus infectious bronchitis virus in cultured cells. Virology 358, 136-147.

Farrell, D., 2013. The role of poultry in human nutrition. the role of poultry in human nutrition , FAO http://www.fao.org/docrep/013/al713e/al713e00.pdf .

Faulkner, O.B., Estevez, C., Yu, Q., Suarez, D.L., 2013. Passive antibody transfer in chickens to model maternal antibody after avian influenza vaccination. Vet. Immunol. Immunopathol. 152, 341-347.

Feltquate, D.M., Heaney, S., Webster, R.G., Robinson, H.L., 1997. Different T helper cell types and antibody isotypes generated by saline and gene gun DNA immunization. J. Immunol. 158, 2278-2284.

Ferraro, B., Morrow, M.P., Hutnick, N.A., Shin, T.H., Lucke, C.E., Weiner, D.B., 2011. Clinical applications of DNA vaccines: current progress. Clin. Infect. Dis. 53, 296-302.

Finney, P., Box, P., Holmes, H., 1990. Studies with a bivalent infectious bronchitis killed virus vaccine. Avian Pathol. 19, 435-450.

© COPYRIGHT UPM

Fynan, E.F., Webster, R.G., Fuller, D.H., Haynes, J.R., Santoro, J.C., Robinson, H.L., 1993. DNA vaccines: protective immunizations by parenteral, mucosal, and gene-gun inoculations. Proc. Natl. Acad. Sci. U. S. A. 90, 11478-11482.

Gallagher, T.M., Buchmeier, M.J., 2001. Coronavirus spike proteins in viral entry and pathogenesis. Virology 279, 371-374.

Gallardo, R.A., van Santen, V.L., Toro, H., 2012. Effects of chicken anaemia virus and infectious bursal disease virus-induced immunodeficiency on infectious bronchitis virus replication and genotypic drift. Avian Pathol. 41, 451-458.

Gan, Q., Wang, T., 2007. Chitosan nanoparticle as protein delivery carrier—systematic examination of fabrication conditions for efficient loading and release. Colloids and Surfaces B: Biointerfaces 59, 24-34.

Garcia-Camacho, L., Schat, K.A., Brooks Jr, R., Bounous, D.I., 2003. Early cell-mediated immune responses to Marek’s disease virus in two chicken lines with defined major histocompatibility complex antigens. Vet. Immunol. Immunopathol. 95, 145-153..

Garnier J, Osguthorpe DJ, Robson B. 1978. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J Mol Biol. 120, :97-120.

Gascón, A.R., del Pozo-Rodríguez, A., Solinís, M.Á, 2013. Non-viral delivery systems in gene therapy. http://www.intechopen.com/books/gene-therapy-tools-and-potential-applications/non-viral-delivery-systems-in-gene-therapy. Retrieved 21/02/2015.

Gasteiger, E., Hoogland, C., Gattiker, A., Wilkins, M.R., Appel, R.D., Bairoch, A., 2005. Protein identification and analysis tools on the ExPASy server. In Anonymous The Proteomics Protocols Handbook. Springer, pp. 571-607.

Gededzha, M.P., Mphahlele, J.M., Selabe, S.G., 2014. Prediction of T-cell epitopes of hepatitis C virus genotype 5a. Virol J. 11, 187.

Gildea, S., Quinlivan, M., Murphy, B.A., Cullinane, A., 2013. Humoral response and antiviral cytokine expression following vaccination of thoroughbred weanlings—A blinded comparison of commercially available vaccines. Vaccine 31, 5216-5222.

Goldenthal, K., Cavagnaro, J., Alving, C., Vogel, F., 1993. Safety evaluation of vaccine adjuvants: National cooperative vaccine development meeting working group. AIDS Res. Hum. Retroviruses 9, S47-S51.

Gough, R., Cox, W., Winkler, C., Sharp, M., Spackman, D., 1996a. Isolation and identification of infectious bronchitis virus from pheasants. Vet. Rec. 138, 208-209.

© COPYRIGHT UPM

Graham, R.L., Sparks, J.S., Eckerle, L.D., Sims, A.C., Denison, M.R., 2008. SARS coronavirus replicase proteins in pathogenesis. Virus Res. 133, 88-100.

Greenland, J.R., Letvin, N.L., 2007. Chemical adjuvants for plasmid DNA vaccines. Vaccine 25, 3731-3741.

Grgiæ, H., Hunter, D.B., Hunton, P., Nagy, É, 2008. Pathogenicity of infectious bronchitis virus isolates from Ontario chickens. Can J Vet Res 72, 403.

Güçlü-Üstündağ, Ö, Mazza, G., 2007. Saponins: properties, applications and processing. Crit. Rev. Food Sci. Nutr. 47, 231-258.

Guo, J., Hui, D.J., Merrick, W.C., Sen, G.C., 2000. A new pathway of translational regulation mediated by eukaryotic initiation factor 3. EMBO J. 19, 6891-6899.

Guo, S., Kenne, L., 2000. Structural studies of triterpenoid saponins with new acyl components from Quillaja saponaria Molina. Phytochemistry 55, 419-428.

Guo, X., Rosa, A.J.M., Chen, D.G., Wang, X., 2008. Molecular mechanisms of primary and secondary mucosal immunity using avian infectious bronchitis virus as a model system. Vet. Immunol. Immunopathol. 121, 332-343.

Guo, Z., Wang, H., Yang, T., Wang, X., Lu, D., Li, Y., Zhang, Y., 2010. Priming with a DNA vaccine and boosting with an inactivated vaccine enhance the immune response against infectious bronchitis virus. J. Virol. Methods 167, 84-89.

Gupta, R., Brunak, S., 2002. Prediction of glycosylation across the human proteome and the correlation to protein function. 7, 310-322.

Gurjar, R.S., Gulley, S.L., van Ginkel, F.W., 2013. Cell-mediated immune responses in the head-associated lymphoid tissues induced to a live attenuated avian coronavirus vaccine. Dev. Comp. Immunol. 41, 715-722.

Gurunathan, S., Klinman, D.M., Seder, R.A., 2000. DNA vaccines: immunology, application, and optimization*. Annu. Rev. Immunol. 18, 927-974.

Guy, J.S., 2000. Turkey coronavirus is more closely related to avian infectious bronchitis virus than to mammalian coronaviruses: a review. Avian Pathol. 29, 207-212.

Guy, J.S., 2008. Isolation and Propagation of Coronaviruses inEmbryonated Eggs, in Anonymous SARS-and Other Coronaviruses. Springer, pp. 109-117.

Haijema, B.J., Volders, H., Rottier, P.J., 2004. Live, attenuated coronavirus vaccines through the directed deletion of group-specific genes provide protection against feline infectious peritonitis. J. Virol. 78, 3863-3871.

© COPYRIGHT UPM

Hanahan, D., Bloom, F.R., 1996. Mechanisms of DNA transformation. Escherichia coli and Salmonella typhimurium.. Cell Mol Biol, 2449-2459.

Hawley-Nelson, P., Ciccarone, V., Gebeyehu, G., Jessee, J., Felgner, P. 1993. Lipofectamine reagent: A new, higher efficiency polycationic liposome transfection reagent. Focus 15, 73-79.

Haygreen, L., Davison, F., Kaiser, P., 2005. DNA vaccines for poultry: the jump from theory to practice. Expert Rev Vaccines. 4, 51-62

Hewson, K.A., Scott, P.C., Devlin, J.M., Ignjatovic, J., Noormohammadi, A.H., 2012. The presence of viral subpopulations in an infectious bronchitis virus vaccine with differing pathogenicity. A preliminary study. Vaccine 30, 4190-4199.

Hiscox, J.A., Wurm, T., Wilson, L., Britton, P., Cavanagh, D., Brooks, G., 2001. The coronavirus infectious bronchitis virus nucleoprotein localizes to the nucleolus. J. Virol. 75, 506-512.

Hodgson, T., Britton, P., Cavanagh, D., 2006. Neither the RNA nor the proteins of open reading frames 3a and 3b of the coronavirus infectious bronchitis virus are essential for replication. J. Virol. 80, 296-305.

Hodgson, T., Casais, R., Dove, B., Britton, P., Cavanagh, D., 2004. Recombinant infectious bronchitis coronavirus Beaudette with the spike protein gene of the pathogenic M41 strain remains attenuated but induces protective immunity. J. Virol. 78, 13804-13811.

Hogue, B., Machamer, C., 2008. Coronavirus Structural Proteins and Virus Assembly. Nidoviruses , 179.

Hopkins, S.R., Yoder Jr., H.W., 1986. Reversion to virulence of chicken-passaged infectious bronchitis vaccine virus. Avian Dis. 30, 221-223.

Howell, D.P., Krieser, R.J., Eastman, A., Barry, M.A., 2003. Deoxyribonuclease II is a lysosomal barrier to transfection. Mol Ther. 8, 957-963.

Hurst, K.R., Ye, R., Goebel, S.J., Jayaraman, P., Masters, P.S., 2010. An interaction between the nucleocapsid protein and a component of the replicase-transcriptase complex is crucial for the infectivity of coronavirus genomic RNA. J. Virol. 84, 10276-10288.

Ignjatovic, J., Ashton, D., Reece, R., Scott, P., Hooper, P., 2002. Pathogenicity of Australian strains of avian infectious bronchitis virus. J. Comp. Pathol. 126, 115-123.

Ignjatovic, J., Galli, L., 1994. The S1 glycoprotein but not the N or M proteins of avian infectious bronchitis virus induces protection in vaccinated chickens. Arch. Virol. 138, 117-134.

© COPYRIGHT UPM

Ignjatovic, J., McWaters, P., 1991. Monoclonal antibodies to three structural proteins of avian infectious bronchitis virus: characterization of epitopes and antigenic differentiation of Australian strains. J. Gen. Virol. 72, 2915.

Ignjatović, J., Sapats, S., 2000. Avian infectious bronchitis virus. Rev. - Off. Int. Epizoot. 19, 493.

Ignjatovic, J., Sapats, S., 2005. Identification of previously unknown antigenic epitopes on the S and N proteins of avian infectious bronchitis virus. Arch. Virol. 150, 1813-1831.

Imbert, I., Snijder, E.J., Dimitrova, M., Guillemot, J.C., Lécine, P., Canard, B., 2008. The SARS-Coronavirus PLnc domain of nsp3 as a replication/transcription scaffolding protein. Virus Res. 133, 136-148.

Ingale, A.G., Goto, S., 2014. Prediction of CTL epitope, in silico modeling and functional analysis of cytolethal distending toxin (CDT) protein of Campylobacter jejuni. BMC research notes 7, 92.

Ismail, M., Tang, Y., Saif, Y., 2003. Pathogenicity of turkey coronavirus in turkeys and chickens. Avian Dis. 47, 515-522.

Jackwood, M.W., 1999. Current and future recombinant viral vaccines for poultry. Adv. Vet. Med. 41, 517-522.

Jackwood, M.W., Hall, D., Handel, A., 2012. Molecular evolution and emergence of avian gammacoronaviruses. Infect Genet Evol. 12, 1305-1311.

Jackwood, M.W., Hilt, D.A., Lee, C.W., Kwon, H.M., Callison, S.A., Moore, K.M., Moscoso, H., Sellers, H., Thayer, S., 2005. Data from 11 years of molecular typing infectious bronchitis virus field isolates. Avian Dis. 49, 614-618.

Jacobs, L., Van Der Zeijst, B., Horzinek, M., 1986. Characterization and translation of transmissible gastroenteritis virus mRNAs. J. Virol. 57, 1010-1015.

Jayaram, J., Youn, S., Collisson, E.W., 2005. The virion N protein of infectious bronchitis virus is more phosphorylated than the N protein from infected cell lysates. Virology 339, 127-135.

Jazayeri, S.D., Ideris, A., Zakaria, Z., Shameli, K., Moeini, H., Omar, A.R., 2012. Cytotoxicity and immunological responses following oral vaccination of nanoencapsulated avian influenza virus H5 DNA vaccine with green synthesis silver nanoparticles. J. Controlled Release 161, 116-123.

Jeffers, S.A., Tusell, S.M., Gillim-Ross, L., Hemmila, E.M., Achenbach, J.E., Babcock, G.J., Thomas, W.D.,Jr, Thackray, L.B., Young, M.D., Mason, R.J., Ambrosino, D.M., Wentworth, D.E., Demartini, J.C., Holmes, K.V., 2004. CD209L (L-

© COPYRIGHT UPM

SIGN) is a receptor for severe acute respiratory syndrome coronavirus. Proc. Natl. Acad. Sci. U. S. A. 101, 15748-15753.

Jia, W., Karaca, K., Parrish, C., Naqi, S., 1995. A novel variant of avian infectious bronchitis virus resulting from recombination among three different strains. Arch. Virol. 140, 259-271.

Jia, W., Mondal, S., Naqi, S., 2002. Genetic and antigenic diversity in avian infectious bronchitis virus isolates of the 1940s. Avian Dis. 46, 437-441.

Jiao, H., Pan, Z., Yin, Y., Geng, S., Sun, L., Jiao, X., 2011. Oral and nasal DNA vaccines delivered by attenuated Salmonella enterica serovar Typhimurium induce a protective immune response against infectious bronchitis in chickens. Clin. Vaccine Immunol. 18, 1041-1045.

Johnson, M.A., Pooley, C., Ignjatovic, J., Tyack, S.G., 2003b. A recombinant fowl adenovirus expressing the S1 gene of infectious bronchitis virus protects against challenge with infectious bronchitis virus. Vaccine 21, 2730-2736.

Jonassen, C.M., Kofstad, T., Larsen, I.L., Løvland, A., Handeland, K., Follestad, A., Lillehaug, A., 2005. Molecular identification and characterization of novel coronaviruses infecting graylag geese (Anser anser), feral pigeons (Columbia livia) and mallards (Anas platyrhynchos). J. Gen. Virol. 86, 1597-1607.

Jones, R., 2010. Europe: history, current situation and control measures for infectious bronchitis. Revista Brasileira de Ciência Avícola 12, 125-128.

Kameka, A.M., Haddadi, S., Kim, D.S., Cork, S.C., Abdul-Careem, M.F., 2014. Induction of innate immune response following infectious bronchitis corona virus infection in the respiratory tract of chickens. Virology 450, 114-121.

Kang, S., Min, H., 2012. Ginseng, the'Immunity Boost': The Effects of Panax ginseng on Immune System. J Ginseng Res. 36, 354-368.

Kant, A., Koch, G., Van Roozelaar, D., Kusters, J., Poelwijk, F., van der Zeijst, B., 1992. Location of antigenic sites defined by neutralizing monoclonal antibodies on the S1 avian infectious bronchitis virus glycopolypeptide. J. Gen. Virol. 73, 591.

Kapczynski, D.R., Hilt, D.A., Shapiro, D., Sellers, H.S., Jackwood, M.W., 2003. Protection of chickens from infectious bronchitis by in ovo and intramuscular vaccination with a DNA vaccine expressing the S1 glycoprotein. Avian Dis. 47, 272-285.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: An integrated and extendable desktop

© COPYRIGHT UPM

software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647-1649.

Keeler Jr, C.L., Reed, K.L., Nix, W.A., Gelb Jr, J., 1998. Serotype identification of avian infectious bronchitis virus by RT-PCR of the peplomer (S-1) gene. Avian Dis. 42, 275-284.

Kelley, L.A., Sternberg, M.J., 2009. Protein structure prediction on the Web: a case study using the Phyre server. Nature protocols 4, 363-371.

Kensil, C.R., Patel, U., Lennick, M., Marciani, D., 1991. Separation and characterization of saponins with adjuvant activity from Quillaja saponaria Molina cortex. J. Immunol. 146, 431-437.

Kensil, C.R., Wu, J., Soltysik, S., 1995. Structural and immunological characterization of the vaccine adjuvant QS-21, Pharm Biotechnol. 6, 525-541.

King, A.M., Adams, M.J., Lefkowitz, E.J., Carstens, E.B., 2012. Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses. http://www.trevorwilliams.info/ictv_iridoviridae_2012.pdf.

King, D., Cavanagh, D., 1991. Infectious bronchitis. In, Diseases of Poultry 9, 471-484.

Kirn, J.J., Bagarazzi, M.L., Trivedi, N., Hu, Y., Kazahaya, K., Wilson, D.M., Ciccarelli, R., Chattergoon, M.A., Dang, K., Mahalingam, S., 1997. Engineering of in vivo immune responses to DNA immunization via codelivery of costimulatory molecule genes. Nat. Biotechnol. 15, 641-646.

Kiss, I., Kecskeméti, S., Tanyi, J., Klingeborn, B., Belák, S., 2000. Preliminary studies on feline coronavirus distribution in naturally and experimentally infected cats. Res. Vet. Sci. 68, 237-242.

Klieve, A.V., Cumming, R.B., 1988. Infectious bronchitis: safety and protection in chickens with maternal antibody. Aust. Vet. J. 65, 396-397.

Koch, G., Hartog, L., Kant, A., Van Roozelaar, D., 1990. Antigenic domains on the peplomer protein of avian infectious bronchitis virus: correlation with biological functions. J. Gen. Virol. 71, 1929-1935.

Koch, M., Camp, S., Collen, T., Avila, D., Salomonsen, J., Wallny, H., van Hateren, A., Hunt, L., Jacob, J.P., Johnston, F., 2007. Structures of an MHC class I molecule from B21 chickens illustrate promiscuous peptide binding. Immunity 27, 885-899.

Konietzny, U., Greiner, R., 2003. Phytic acid: Properties and determination. Encyclopedia of Food Sciences and Nutrition.Academic Press, 2nd Edition, EN, 4546-4555.

© COPYRIGHT UPM

Köping-Höggård, M., Tubulekas, I., Guan, H., Edwards, K., Nilsson, M., Vårum, K., Artursson, P., 2001. Chitosan as a nonviral gene delivery system. Structure-property relationships and characteristics compared with polyethylenimine in vitro and after lung administration in vivo. Gene Ther. 8, 1108-1121.

Kozak, M., 1981. Possible role of flanking nucleotides in recognition of the AUG initiator codon by eukaryotic ribosomes. Nucleic Acids Res. 9, 5233-5252.

Kozak, M., 1986. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. Cell 44, 283-292.

Krempl, C., Schultze, B., Laude, H., Herrler, G., 1997. Point mutations in the S protein connect the sialic acid binding activity with the enteropathogenicity of transmissible gastroenteritis coronavirus. J. Virol. 71, 3285-3287.

Krogh, A., Larsson, B., Von Heijne, G., Sonnhammer, E.L., 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J. Mol. Biol. 305, 567-580.

Kubo, H., Yamada, Y.K., Taguchi, F., 1994. Localization of neutralizing epitopes and the receptor-binding site within the amino-terminal 330 amino acids of the murine coronavirus spike protein. J. Virol. 68, 5403-5410.

Kuo, L., Godeke, G.J., Raamsman, M.J., Masters, P.S., Rottier, P.J., 2000. Retargeting of coronavirus by substitution of the spike glycoprotein ectodomain: crossing the host cell species barrier. J. Virol. 74, 1393-1406.

Kuo, S., Wang, C., Hou, M., Huang, Y., Kao, H., Su, H., 2010. Evolution of infectious bronchitis virus in Taiwan: Characterisation of RNA recombination in the nucleocapsid gene. Vet. Microbiol. 144, 293-302.

Kurita, K., 2006. Chitin and chitosan: functional biopolymers from marine crustaceans. Mar Biotechnol. 8, 203-226.

Kusters, J., Jager, E., Niesters, H., Van Der Zeijst, B., 1990. Sequence evidence for RNA recombination in field isolates of avian coronavirus infectious bronchitis virus. Vaccine 8, 605-608.

Kusters, J., Niesters, H., Lenstra, J., Horzinek, M., Van Der Zeijst, B., 1989. Phylogeny of antigenic variants of avian coronavirus IBV. Virology 169, 217-221.

Ladman, B., Pope, C., Ziegler, A., Swieczkowski, T., Callahan, J., Davison, S., Gelb Jr, J., 2002. Protection of chickens after live and inactivated virus vaccination against challenge with nephropathogenic infectious bronchitis virus PA/Wolgemuth/98. Avian Dis. 46, 938-944.

Lai, M., 1992. Genetic recombination in RNA viruses, in Anonymous Genetic Diversity of RNA Viruses. Curr Top Microbiol Immunol. 176, 21-32.

© COPYRIGHT UPM

Lai, M., 1996. Recombination in large RNA viruses: Virology. 7, 381-388.

Lai, M., Cavanagh, D., 1997. The molecular biology of coronaviruses. Adv. Virus Res. 48, 1-100.

Lai, M.M.C., 1990. Corona virus: organization, replication and expression of genome. Annu Rev Microbiol. 44, 303-303.

Lee, C., Hilt, D.A., Jackwood, M.W., 2003. Typing of field isolates of infectious bronchitis virus based on the sequence of the hypervariable region in the S1 gene. Journal of veterinary diagnostic investigation 15, 344-348.

Lee, H.J., Youn, H.N., Kwon, J.S., Lee, Y.J., Kim, J.H., Lee, J.B., Park, S.Y., Choi, I.S., Song, C.S., 2010. Characterization of a novel live attenuated infectious bronchitis virus vaccine candidate derived from a Korean nephropathogenic strain. Vaccine 28, 2887-2894.

Lee, S., Markham, P.F., Coppo, M.J., Legione, A.R., Markham, J.F., Noormohammadi, A.H., Browning, G.F., Ficorilli, N., Hartley, C.A., Devlin, J.M., 2012. Attenuated vaccines can recombine to form virulent field viruses. Science 337, 188-188.

Lehman, I.R., 1974. DNA ligase: structure, mechanism, and function. Science 186, 790-797.

Leitner, W.W., Hwang, L.N., 2002. Alphavirus-based DNA vaccine breaks immunological tolerance by activating innate antiviral pathways. Nat. Med. 9, 33-39.

Lenstra, J.A., Kusters, J.G., Koch, G., van der Zeijst, B.A.M., 1989. Antigenicity of the peplomer protein of infectious bronchitis virus. Mol. Immunol. 26, 7-15.

Li, F., 2012. Evidence for a common evolutionary origin of coronavirus spike protein receptor-binding subunits. J. Virol. 86, 2856-2858.

Li, J., Liang, X., Huang, Y., Meng, S., Xie, R., Deng, R., Yu, L., 2004. Enhancement of the immunogenicity of DNA vaccine against infectious bursal disease virus by co-delivery with plasmid encoding chicken interleukin 2. Virology 329, 89-100.

Li, K., Schuler, T., Chen, Z., Glass, G., Childs, J., Plagemann, P., 2000. Isolation of lactate dehydrogenase-elevating viruses from wild house mice and their biological and molecular characterization. Virus Res. 67, 153-162.

Li, W., Moore, M.J., Vasilieva, N., Sui, J., Wong, S.K., Berne, M.A., Somasundaran, M., Sullivan, J.L., Luzuriaga, K., Greenough, T.C., 2003. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426, 450-454.

© COPYRIGHT UPM

Li, Xiaosong Min, Min Du, Nan Gu, Ying Hode, Tomas Naylor, Mark Chen, Dianjun Nordquist, Robert E. Chen, Wei R., 2013. Chitin, chitosan, and glycated chitosan regulate immune responses: the novel adjuvants for cancer vaccine. Clin Dev Immunol. 2013. 2013:38702

Li, Y.L., Wang, H.N., Zeng, Y.H., Yang, T., Li, J., 2011. Immunogenicity evaluation of IBV DNA vaccine from salmonella typhimurium. Indian Vet. J. 88, 20-22.

Liais, E., Croville, G., Mariette, J., Delverdier, M., Lucas, M.N., Klopp, C., Lluch, J., Donnadieu, C., Guy, J.S., Corrand, L., Ducatez, M.F., Guerin, J.L., 2014. Novel avian coronavirus and fulminating disease in guinea fowl, France. Emerg. Infect. Dis. 20, 105-108.

Lim, K., Ng, L.F.P., Liu, D., 2000. Identification of a novel cleavage activity of the first papain-like proteinase domain encoded by open reading frame 1a of the coronavirus Avian infectious bronchitis virus and characterization of the cleavage products. J. Virol. 74, 1674-1685.

Lim, K.P., Liu, D.X., 2001. The missing link in coronavirus assembly. Retention of the avian coronavirus infectious bronchitis virus envelope protein in the pre-Golgi compartments and physical interaction between the envelope and membrane proteins. J. Biol. Chem. 276, 17515-17523.

Lim, T., Lee, H., Lee, D., Lee, Y., Park, J., Youn, H., Kim, M., Lee, J., Park, S., Choi, I., 2011. An emerging recombinant cluster of nephropathogenic strains of avian infectious bronchitis virus in Korea. Infect Genet Evol. 11, 678-685.

Lindahl, T., Barnes, D.E., 1992. Mammalian DNA ligases. Annu. Rev. Biochem. 61, 251-281.

Lindner, H.A., Fotouhi-Ardakani, N., Lytvyn, V., Lachance, P., Sulea, T., Ménard, R., 2005. The papain-like protease from the severe acute respiratory syndrome coronavirus is a deubiquitinating enzyme. J. Virol. 79, 15199-15208.

Lisziewicz, J., Calarota, S.A., Lori, F., 2007. The potential of topical DNA vaccines adjuvanted by cytokines. Expert Opin Biol Ther. 7, 1563-15674.

Liu, D., Inglis, S., 1992. Identification of two new polypeptides encoded by mRNA5 of the coronavirus infectious bronchitis virus. Virology 186, 342-347.

Liu, J., Gao, G.F., 2011. Major histocompatibility complex: Interaction with peptides. eLS .

Liu, M.A., 2011. DNA vaccines: an historical perspective and view to the future. Immunol. Rev. 239, 62-84.

© COPYRIGHT UPM

Liu, S., Chen, J., Chen, J., Kong, X., Shao, Y., Han, Z., Feng, L., Cai, X., Gu, S., Liu, M., 2005a. Isolation of avian infectious bronchitis coronavirus from domestic peafowl (Pavo cristatus) and teal (Anas). J. Gen. Virol. 86, 719-725.

Liu, W., Sun, S., Cao, Z., Zhang, X., Yao, K., Lu, W.W., Luk, K., 2005b. An investigation on the physicochemical properties of chitosan/DNA polyelectrolyte complexes. Biomaterials 26, 2705-2711.

Liu, X., Shao, Y., Ma, H., Sun, C., Zhang, X., Li, C., Han, Z., Yan, B., Kong, X., Liu, S., 2013a. Comparative analysis of four Massachusetts type infectious bronchitis coronavirus genomes reveals a novel Massachusetts type strain and evidence of natural recombination in the genome. Infect Genet Evol 14, 29-38.

Liu, Z., Lv, D., Liu, S., Gong, J., Wang, D., Xiong, M., Chen, X., Xiang, R., Tan, X., 2013b. Alginic acid-coated chitosan nanoparticles loaded with legumain DNA vaccine: effect against breast cancer in mice. PloS one 8, e60190.

Locker, J.K., Griffiths, G., Horzinek, M., Rottier, P., 1992. O-glycosylation of the coronavirus M protein. Differential localization of sialyltransferases in N-and O-linked glycosylation. J. Biol. Chem. 267, 14094-14101.

Loke, C., Omar, A.R., Raha, A., Yusoff, K., 2005. Improved protection from velogenic Newcastle disease virus challenge following multiple immunizations with plasmid DNA encoding for F and HN genes. Vet. Immunol. Immunopathol. 106, 259-267.

Loomis, L.N., Cunningham, C., Gray, M., Thorp Jr, F., 1950. Pathology of the chicken embryo infected with infectious bronchitis virus. Am. J. Vet. Res. 11, 245-251.

Lopez, L.A., Riffle, A.J., Pike, S.L., Gardner, D., Hogue, B.G., 2008. Importance of conserved cysteine residues in the coronavirus envelope protein. J. Virol. 82, 3000-3010.

Lowenthal, J.W., O'neil, T.E., Broadway, M., Strom, A.D.G., Digby, M.R., Andrew, M., York, J.J., 1998. Coadministration of IFN-γ enhances antibody responses in chickens. J Interferon Cytokine Res. 18, 617-622.

Luo, D., Saltzman, W.M., 2000. Synthetic DNA delivery systems. Nat. Biotechnol. 18, 33-37.

Ma, H., Shao, Y., Sun, C., Han, Z., Liu, X., Guo, H., Liu, X., Kong, X., Liu, S., 2012. Genetic diversity of avian infectious bronchitis coronavirus in recent years in China. Avian Dis. 56, 15-28.

Macdonald, J., McMartin, D., 1976. Observations on the effects of the H52 and H120 vaccine strains of infectious bronchitis virus in the domestic fowl. Avian Pathol. 5, 157-173.

© COPYRIGHT UPM

MacGregor, R.R., Boyer, J.D., Ugen, K.E., Lacy, K.E., Gluckman, S.J., Bagarazzi, M.L., Chattergoon, M.A., Baine, Y., Higgins, T.J., Ciccarelli, R.B., Coney, L.R., Ginsberg, R.S., Weiner, D.B., 1998. First human trial of a DNA-based vaccine for treatment of human immunodeficiency virus type 1 infection: safety and host response. J. Infect. Dis. 178, 92-100.

MacLaughlin, F.C., Mumper, R.J., Wang, J., Tagliaferri, J.M., Gill, I., Hinchcliffe, M., Rolland, A.P., 1998. Chitosan and depolymerized chitosan oligomers as condensing carriers for in vivo plasmid delivery. J. Controlled Release 56, 259-272.

Madu, I.G., Chu, V.C., Lee, H., Regan, A.D., Bauman, B.E., Whittaker, G.R., 2007. Heparan sulfate is a selective attachment factor for the avian coronavirus infectious bronchitis virus Beaudette. Avian Dis. 51, 45-51.

Mansouri, S., Lavigne, P., Corsi, K., Benderdour, M., Beaumont, E., Fernandes, J.C., 2004a. Chitosan-DNA nanoparticles as non-viral vectors in gene therapy: strategies to improve transfection efficacy. Eur J Pharm Biopharm 57, 1-8.

Mansouri, S., Lavigne, P., Corsi, K., Benderdour, M., Beaumont, E., Fernandes, J.C., 2004b. Chitosan-DNA nanoparticles as non-viral vectors in gene therapy: strategies to improve transfection efficacy. Eur J Pharm Biopharm 57, 1-8.

Mao, H., Roy, K., Troung-Le, V.L., Janes, K.A., Lin, K.Y., Wang, Y., August, J.T., Leong, K.W., 2001. Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency. J. Controlled Release 70, 399-421.

Martin, J.E., Louder, M.K., Holman, L.S.A., Gordon, I.J., Enama, M.E., Larkin, B.D., Andrews, C.A., Vogel, L., Koup, R.A., Roederer, M., 2008. A SARS DNA vaccine induces neutralizing antibody and cellular immune responses in healthy adults in a Phase I clinical trial. Vaccine 26, 6338-6343.

Matthijs, M., Bouma, A., Velkers, F., van Eck, J., Stegeman, J., 2008. Transmissibility of infectious bronchitis virus H120 vaccine strain among broilers under experimental conditions. Avian Dis. 52, 461-466.

McBride, R., van Zyl, M., Fielding, B.C., 2014. The Coronavirus Nucleocapsid Is a Multifunctional Protein. Viruses 6, 2991-3018.

McKinley, E.T., Hilt, D.A., Jackwood, M.W., 2008. Avian coronavirus infectious bronchitis attenuated live vaccines undergo selection of subpopulations and mutations following vaccination. Vaccine 26, 1274-1284.

McKinley, E.T., Jackwood, M.W., Hilt, D.A., Kissinger, J.C., Robertson, J.S., Lemke, C., Paterson, A.H., 2011. Attenuated live vaccine usage affects accurate measures of virus diversity and mutation rates in avian coronavirus infectious bronchitis virus. Virus Res. 158, 225-234.

© COPYRIGHT UPM

Meeusen, E.N., Walker, J., Peters, A., Pastoret, P., Jungersen, G., 2007. Current status of veterinary vaccines. Clin. Microbiol. Rev. 20, 489-510.

Meir, R., Krispel, S., Simanov, L., Eliahu, D., Maharat, O., Pitcovski, J., 2012. Immune responses to mucosal vaccination by the recombinant S1 and N proteins of infectious bronchitis virus. Viral Immunol. 25, 55-62.

Meulemans, G., Boschmans, M., Decaesstecker, M., Van den Berg, T.P., Denis, P., Cavanagh, D., 2001. Epidemiology of infectious bronchitis virus in Belgian broilers: A retrospective study, 1986 to 1995. Avian Pathol. 30, 411-421.

Miettinen, M., Sareneva, T., Julkunen, I., Matikainen, S., 2001. IFNs activate toll-like receptor gene expression in viral infections. Genes Immun. 2, 349-55..

Miller, L.T., Yates, V.J., 1968. Neutralization of infectious bronchitis virus human sera. Am. J. Epidemiol. 88, 406-409.

Mo, M., Hong, S., Kwon, H., Kim, I., Song, C., Kim, J., 2013. Genetic Diversity of Spike, 3a, 3b and E Genes of Infectious Bronchitis Viruses and Emergence of New Recombinants in Korea. Viruses 5, 550-567.

Moeini, H., Omar, A.R., Rahim, R.A., Yusoff, K., 2011a. Development of a DNA vaccine against chicken anemia virus by using a bicistronic vector expressing VP1 and VP2 proteins of CAV. Comp. Immunol. Microbiol. Infect. Dis. 34, 227-236.

Moeini, H., Rahim, R.A., Omar, A.R., Shafee, N., Yusoff, K., 2011b. Lactobacillus acidophilus as a live vehicle for oral immunization against chicken anemia virus. Appl. Microbiol. Biotechnol. 90, 77-88.

Mondal, S., Chang, Y., Balasuriya, U., 2013. Sequence analysis of infectious bronchitis virus isolates from the 1960s in the United States. Arch. Virol. 158, 497-503.

Mondal, S., Naqi, S., 2001. Maternal antibody to infectious bronchitis virus: its role in protection against infection and development of active immunity to vaccine. Vet. Immunol. Immunopathol. 79, 31-40.

Montassier, H.J., 2010. Molecular epidemiology and evolution of avian infectious bronchitis virus. Rev. Bras. Cienc. Avic. 12, 87-96.

Montassier, M.D.F.S., Brentano, L., Montassier, H.J., Richtzenhain, L.J., 2008. Genetic grouping of avian infectious bronchitis virus isolated in Brazil based on RT-PCR/RFLP analysis of the S1 gene. Pesq. Vet. Bras. 28, 190-194.

Morein, B., Hu, K., Abusugra, I., 2004. Current status and potential application of ISCOMs in veterinary medicine. Adv. Drug Deliv. Rev. 56, 1367-1382.

© COPYRIGHT UPM

Morelli, A.B., Becher, D., Koernig, S., Silva, A., Drane, D., Maraskovsky, E., 2012. ISCOMATRIX: a novel adjuvant for use in prophylactic and therapeutic vaccines against infectious diseases. J. Med. Microbiol. 61, 935-943.

Moreno, S., López-Fuertes, L., Vila-Coro, A., Sack, F., Smith, C., Konig, S., Wittig, B., Schroff, M., Juhls, C., Junghans, C., 2004. DNA immunisation with minimalistic expression constructs. Vaccine 22, 1709-1716.

Moreno, S., Timon, M., 2004. DNA vaccination: an immunological perspective. Inmunologia 23, 41-55.

Mou, H., Raj, V.S., van Kuppeveld, F.J., Rottier, P.J., Haagmans, B.L., Bosch, B.J., 2013. The receptor binding domain of the new Middle East respiratory syndrome coronavirus maps to a 231-residue region in the spike protein that efficiently elicits neutralizing antibodies. J. Virol. 87, 9379-9383.

Mumford, J., Jessett, D., Dunleavy, U., Wood, J., Hannant, D., Sundquist, B., Cook, R., 1994. Antigenicity and immunogenicity of experimental equine influenza ISCOM vaccines. Vaccine 12, 857-863.

Mumper, R.J., Cui, Z., 2003. Genetic immunization by jet injection of targeted pDNA-coated nanoparticles. Methods 31, 255-262.

Narayanan, K., Maeda, A., Maeda, J., Makino, S., 2000. Characterization of the coronavirus M protein and nucleocapsid interaction in infected cells. J. Virol. 74, 8127-8134.

NCBI blast available at http://blast.ncbi.nlm.nih.gov/Blast.cgi. Retrieved 1. 01.2015

Neuman, B.W., Joseph, J.S., Saikatendu, K.S., Serrano, P., Chatterjee, A., Johnson, M.A., Liao, L., Klaus, J.P., Yates, J.R., Wüthrich, K., 2008. Proteomics analysis unravels the functional repertoire of coronavirus nonstructural protein 3. J. Virol. 82, 5279-5294.

Niesters, H.G.M., Bleumink-Pluym, N., Osterhaus, A.D.M.E., Horzinek, M.C., Van Der Zeijst, B.A.M., 1987. Epitopes on the peplomer protein of infectious bronchitis virus strain M41 as defined by monoclonal antibodies. Virology 161, 511-519.

Nix, W., Troeber, D., Kingham, B., Keeler Jr, C., Gelb Jr, J., 2000. Emergence of subtype strains of the Arkansas serotype of infectious bronchitis virus in Delmarva broiler chickens. Avian Dis.44, 568-581.

Oda, K., Matsuda, H., Murakami, T., Katayama, S., Ohgitani, T., Yoshikawa, M., 2000. Adjuvant and haemolytic activities of 47 saponins derived from medicinal and food plants. Biol. Chem. 381, 67-74.

© COPYRIGHT UPM

Ohya, Y., Shiratani, M., Kobayashi, H., Ouchi, T., 1993. Release behavior of 5-fluorouracil from chitosan-gel nanospheres immobilizing 5-fluorouracil coated with polysaccharides and their cell specific cytotoxicity. J Microencapsul.10, 1-9.

OIE:, 2008. Avian infectious bronchitis. Chapter 2, 2.

Okabayashi, T., Kariwa, H., Yokota, S., Iki, S., Indoh, T., Yokosawa, N., Takashima, I., Tsutsumi, H., Fujii, N., 2006. Cytokine regulation in SARS coronavirus infection compared to other respiratory virus infections. J. Med. Virol. 78, 417-424.

Oligo Analyser:- https://sg.idtdna.com/analyzer/Applications/OligoAnalyzer/. Retried 17/01/2015

Omar, A., 2014. Poultry Viruses: From Threat to Therapy. Inaugural lecture: Faculty of Veterinary Medicine, Universiti Putra Malaysia. 23 May 2014.

Omar, A., Schat, K., 1997. Characterization of Marek's disease herpesvirus-specific cytotoxic T lymphocytes in chickens inoculated with a non-oncogenic vaccine strain of MDV. Immunology 90, 579-585.

Onishi, H., Machida, Y., 1999. Biodegradation and distribution of water-soluble chitosan in mice. Biomaterials 20, 175-182.

Otsuki, K., Sakagami, Y., Tsubokura, M., 1987. Serological relationship among ten strains of avian infectious bronchitis virus. Acta Virol. 31, 138-145.

Otsuki, K., Yamamoto, H., Tsubokura, M., 1979. Studies on avian infectious bronchitis virus (IBV). Arch. Virol. 60, 25-32.

Oveissi, S., Omar, A.R., Yusoff, K., Jahanshiri, F., Hassan, S.S., 2010. DNA vaccine encoding avian influenza virus H5 and Esat-6 of Mycobacterium tuberculosis improved antibody responses against AIV in chickens. Comp. Immunol. Microbiol. Infect. Dis. 33, 491-503.

Özcan, İ, Azizoğlu, E., Şenyiğit, T., Özyazıcı, M., Özer, Ö, 2013. Enhanced dermal delivery of diflucortolone valerate using lecithin/chitosan nanoparticles: in-vitro and in-vivo evaluations. Int J Nanomedicine. 8, 461.

Paillot, R., Prowse, L., 2012. ISCOM-matrix-based equine influenza (EIV) vaccine stimulates cell-mediated immunity in the horse. Vet. Immunol. Immunopathol. 145, 516-521.

Pardo, J., Bosque, A., Brehm, R., Wallich, R., Naval, J., Müllbacher, A., Anel, A., Simon, M.M., 2004. Apoptotic pathways are selectively activated by granzyme A and/or granzyme B in CTL-mediated target cell lysis. J. Cell Biol. 167, 457-468.

© COPYRIGHT UPM

Parsons, D., Ellis, M., Cavanagh, D., Cook, J., 1992. Characterisation of an infectious bronchitis virus isolated from vaccinated broiler breeder flocks. Vet Rec. 131, 408-411.

Pasquini, S., Xiang, Z., Wang, Y., He, Z., Deng, H., Blaszczyk-Thurin, M., Ertl, H., 1997. Cytokines and costimulatory molecules as genetic adjuvants. Immunol. Cell Biol. 75, 397-401.

Patterson, S., Bingham, R., 1976. Electron microscope observations on the entry of avian infectious bronchitis virus into susceptible cells. Arch. Virol. 52, 191-200.

Paul, N., Shum, J., Le, T., 2010. Hot start PCR, In, RT-PCR Protocols. 630, 301-318.

Pei, J., Sekellick, M.J., Marcus, P.I., Choi, I., Collisson, E.W., 2001. Chicken interferon type I inhibits infectious bronchitis virus replication and associated respiratory illness. J Interferon Cytokine Res. 21, 1071-1077.

Peng, G., Sun, D., Rajashankar, K.R., Qian, Z., Holmes, K.V., Li, F., 2011. Crystal structure of mouse coronavirus receptor-binding domain complexed with its murine receptor. Proc. Natl. Acad. Sci. U. S. A. 108, 10696-10701.

Peng, G., Xu, L., Lin, Y., Chen, L., Pasquarella, J.R., Holmes, K.V., Li, F., 2012. Crystal Structure of Bovine Coronavirus Spike Protein Lectin Domain. J Biol Chem. 287, 41931-41938.

Perlman, S., Gallagher, T., Snijder, E.J., 2008. Nidoviruses. Amer Society for Microbiology.

Pertmer, T.M., Roberts, T.R., Haynes, J.R., 1996. Influenza virus nucleoprotein-specific immunoglobulin G subclass and cytokine responses elicited by DNA vaccination are dependent on the route of vector DNA delivery. J. Virol. 70, 6119-6125.

Picault, J., Drouin, P., Lamande, J., Allee, C., Toux, J., Coq, H.L., Guittet, M., Bennejean, G., 1995. L'epizootie recente de bronchite infectieuse aviaire en France: importance, evolution et etiologie. Proceedings of the 1eres Journée de la Recherche Avicole, Angers 28–29 March. 1995.pp.177–179.:http://www.journees-de-la-recherche-avicole.org/JRA/Contenu/Archives/1_JRA/53epizootieJRA1.pdf

Plachy, J., Pink, J.R., Hala, K., 1992. Biology of the chicken MHC (B complex). Crit. Rev. Immunol. 12, 47-79.

Pohjola, L.K., Ek-Kommonen, S.C., Tammiranta, N.E., Kaukonen, E.S., Rossow, L.M., Huovilainen, T.A., 2014. Emergence of avian infectious bronchitis in a non-vaccinating country. Avian Pathol. , 1-18.

© COPYRIGHT UPM

Polakos, N.K., Drane, D., Cox, J., Ng, P., Selby, M.J., Chien, D., O'Hagan, D.T., Houghton, M., Paliard, X., 2001. Characterization of hepatitis C virus core-specific immune responses primed in rhesus macaques by a nonclassical ISCOM vaccine. J. Immunol. 166, 3589-3598.

Poland, G.A., Ovsyannikova, I.G., Kennedy, R.B., Haralambieva, I.H., Jacobson, R.M., 2011. Vaccinomics and a new paradigm for the development of preventive vaccines against viral infections. Omics: 15, 625-636.

Promkuntod, N., van Eijndhoven, R., de Vrieze, G., Gröne, A., Verheije, M., 2014. Mapping of the receptor-binding domain and amino acids critical for attachment in the spike protein of avian coronavirus infectious bronchitis virus. Virology 448, 26-32.

Promkuntod, N., Wickramasinghe, I., de Vrieze, G., Gröne, A., Verheije, M., 2013. Contributions of the S2 spike ectodomain to attachment and host range of infectious bronchitis virus. Virus Res. 177, 127-137.

Purcell, D., McFerran, J., 1972. The histopathology of infectious bronchitis in the domestic fowl. Res. Vet. Sci. 13, 116-122.

Quail, M.A., Otto, T.D., Gu, Y., Harris, S.R., Skelly, T.F., McQuillan, J.A., Swerdlow, H.P., Oyola, S.O., 2012. Optimal enzymes for amplifying sequencing libraries. Nat Methods 9, 10-11.

Quaroni, L., Chumanov, G., 1999. Preparation of polymer-coated functionalized silver nanoparticles. J. Am. Chem. Soc. 121, 10642-10643.

Raafat, D., von Bargen, K., Haas, A., Sahl, H.G., 2008. Insights into the mode of action of chitosan as an antibacterial compound. Appl. Environ. Microbiol. 74, 3764-3773.

Raj, V.S., Mou, H., Smits, S.L., Dekkers, D.H., Müller, M.A., Dijkman, R., Muth, D., Demmers, J.A., Zaki, A., Fouchier, R.A., 2013. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature 495, 251-254.

Rao, S.B., Sharma, C.P., 1997. Use of chitosan as a biomaterial: studies on its safety and hemostatic potential. J. Biomed. Mater. Res. 34, 21-28.

Ravi Kumar, M.N., 2000. A review of chitin and chitosan applications. React Funct Polym 46, 1-27.

Reed, L.J., Muench, H., 1938. A simple method of estimating fifty per cent endpoints. Am. J. Epidemiol. 27, 493-497.

© COPYRIGHT UPM

Reed, M.L., Dove, B.K., Jackson, R.M., Collins, R., Brooks, G., Hiscox, J.A., 2006. Delineation and modelling of a nucleolar retention signal in the coronavirus nucleocapsid protein. Traffic 7, 833-848.

Rejinold, N.S., Muthunarayanan, M., Muthuchelian, K., Chennazhi, K., Nair, S.V., Jayakumar, R., 2011. Saponin-loaded chitosan nanoparticles and their cytotoxicity to cancer cell lines in vitro. Carbohydr. Polym. 84, 407-416.

Restriction mapper http://www.restrictionmapper.org/ retrieved 17.01.205

Richardson, S.W., Kolbe, H.J., Duncan, R., 1999. Potential of low molecular mass chitosan as a DNA delivery system: biocompatibility, body distribution and ability to complex and protect DNA. Int. J. Pharm. 178, 231-243.

Richter, E., Wick, G., 1977. Flora-immuno-cytoadherence (FICA): A new method for the identification and enumeration of antigen-binding cells. Zeitschrift für Immunitätsforschung: Immunobiology 152, 351-362.

Riguera, R., 1997. Isolating bioactive compounds from marine organisms. J. Mar. Biotechnol. 5, 187-193.

Rimmelzwaan, G., Claas, E., Van Amerongen, G., De Jong, J., Osterhaus, A., 1999. ISCOM vaccine induced protection against a lethal challenge with a human H5N1 influenza virus. Vaccine 17, 1355-1358.

Robinson, H., Hunt, L., Webster, R., 1993. Protection against a lethal influenza virus challenge by immunization with a haemagglutinin-expressing plasmid DNA. Vaccine 11, 957-960.

Rönnberg, B., Fekadu, M., Morein, B., 1995. Adjuvant activity of non-toxic< i> Quillaja saponaria Molina components for use in ISCOM matrix. Vaccine 13, 1375-1382.

Rossi, R., Montecucco, A., Ciarrocchi, G., Biamonti, G., 1997. Functional characterization of the T4 DNA ligase: a new insight into the mechanism of action. Nucleic Acids Res. 25, 2106-2113.

Rottier, P., Rose, J.K., 1987. Coronavirus E1 glycoprotein expressed from cloned cDNA localizes in the Golgi region. J. Virol. 61, 2042-2045.

Rovere, P., Vallinoto, C., Bondanza, A., Crosti, M.C., Rescigno, M., Ricciardi-Castagnoli, P., Rugarli, C., Manfredi, A.A., 1998. Bystander apoptosis triggers dendritic cell maturation and antigen-presenting function. J. Immunol. 161, 4467-4471.

Rowe, C., Baker, S., Nathan, M., Sgro, J., Palmenberg, A., Fleming, J., 1998. Quasispecies development by high frequency RNA recombination during MHV persistence. In, Coronaviruses and Arteriviruses. Springer, pp. 759-765.

© COPYRIGHT UPM

Saïed, N., Aider, M., 2014. Zeta Potential and Turbidimetry Analyzes for the Evaluation of Chitosan/Phytic Acid Complex Formation. J Food Res. 3, 71.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406-425.

Sallusto, F., Lanzavecchia, A., 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J. Exp. Med. 179, 1109-1118.

Salonius, K., Simard, N., Harland, R., Ulmer, J.B., 2007. The road to licensure of a DNA vaccine. Curr Opin Investig Drugs. 8, 635-641.

Sambrook, J., and David W.R., 2006. Preparation and transformation of competent E. coli using calcium chloride. CSH Protoc. 1, pii: pdb.prot3932.

Sambrook, J., Russell, D.W., Russell, D.W., 2001. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory.

Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U. S. A. 74, 5463-5467.

Sasipreeyajan, J., Sirikobkul, T.P.N., 2012. Efficacy of Different Vaccination Programs against Thai QX-like Infectious Bronchitis Virus. Thai J Vet Med 42, 73-79.

Scaglione, F., Ferrara, F., Dugnani, S., Falchi, M., Santoro, G., Fraschini, F., 1990. Immunomodulatory effects of two extracts of Panax ginseng C.A. Meyer. Drugs Exp. Clin. Res. 16, 537-542.

Schalk, A., Hawn, M., 1931. An apparently new respiratory disease of baby chicks. J. Am. Vet. Med. Assoc. 78, 19.

Scheepers, K., Becht, H., 1994. Protection of mice against an influenza virus infection by oral vaccination with viral nucleoprotein incorporated into immunostimulating complexes. Med. Microbiol. Immunol. (Berl.) 183, 265-278.

Scheerlinck, J.Y., 2001. Genetic adjuvants for DNA vaccines. Vaccine 19, 2647-2656.

Schultze, B., Herrler, G., 1992. Bovine coronavirus uses N-acetyl-9-O-acetylneuraminic acid as a receptor determinant to initiate the infection of cultured cells. J. Gen. Virol. 73 (Pt 4), 901-906.

Sedegah, M., Charoenvit, Y., Minh, L., Belmonte, M., Majam, V., Abot, S., Ganeshan, H., Kumar, S., Bacon, D., Stowers, A., 2004. Reduced immunogenicity of DNA vaccine plasmids in mixtures. Gene Ther. 11, 448-456.

© COPYRIGHT UPM

Seder, R.A., Hill, A.V., 2000. Vaccines against intracellular infections requiring cellular immunity. Nature 406, 793-798.

Seo, S.H., Collisson, E.W., 1998. Cytotoxic T lymphocyte responses to infectious bronchitis virus infection. Adv Exp Med Biol. 440, 455-460.

Seo, S.H., Pei, J., Briles, W.E., Dzielawa, J., Collisson, E.W., 2000. Adoptive transfer of infectious bronchitis virus primed αβ T cells bearing CD8 antigen protects chicks from acute infection. Virology 269, 183-189.

Seo, S.H., Wang, L., Smith, R., Collisson, E.W., 1997. The carboxyl-terminal 120-residue polypeptide of infectious bronchitis virus nucleocapsid induces cytotoxic T lymphocytes and protects chickens from acute infection. J. Virol. 71, 7889-7894.

Shahwan, K., Hesse, M., Mork, A., Herrler, G., Winter, C., 2013. Sialic acid binding properties of soluble coronavirus spike (S1) proteins: differences between infectious bronchitis virus and transmissible gastroenteritis virus. Viruses 5, 1924-1933.

Shehzadi, A., Ur Rehman, S., Idrees, M., 2011. Promiscuous prediction and conservancy analysis of CTL binding epitopes of HCV 3a viral proteome from Punjab Pakistan: an in silico approach. Virol J 8, 55.

Shen, S., Wen, Z., Liu, D., 2003. Emergence of a coronavirus infectious bronchitis virus mutant with a truncated 3b gene: functional characterization of the 3b protein in pathogenesis and replication. Virology 311, 16-27.

Shi, X.., Zhao, Y., Gao, H.., Jing, Z., Wang, M., Cui, H.., Tong, G.., Wang, Y., 2011. Evaluation of recombinant fowlpox virus expressing infectious bronchitis virus S1 gene and chicken interferon-7 gene for immune protection against heterologous strains. Vaccine 29, 1576-1582.

Shimazaki, Y., Watanabe, Y., Harada, M., Seki, Y., Kuroda, Y., Fukuda, M., Honda, E., Suzuki, S., Nakamura, S., 2009. Genetic analysis of the S1 gene of 4/91 type infectious bronchitis virus isolated in Japan. J Vet Med Sci. 71, 583-588.

Sola, I., Alonso, S., Zuniga, S., Balasch, M., Plana-Duran, J., Enjuanes, L., 2003. Engineering the transmissible gastroenteritis virus genome as an expression vector inducing lactogenic immunity. J. Virol. 77, 4357-4369.

Somvanshi, P., Singh, V., Seth, P., 2008. In silico prediction of epitopes in virulence proteins of Mycobacterium tuberculosis H37Rv for diagnostic and subunit vaccine design. J Proteomics Bioinform 1, 143-153.

Song, C.S., Lee, Y.J., Lee, C.W., Sung, H.W., Kim, J.H., Mo, I.P., Izumiya, Y., Jang, H.K., Mikami, T., 1998. Induction of protective immunity in chickens

© COPYRIGHT UPM

vaccinated with infectious bronchitis virus S1 glycoprotein expressed by a recombinant baculovirus. J. Gen. Virol. 79, 719-723.

Song, J.E., Jeong, W.G., Sung, H.W., Kwon, H.M., 2013. Sequencing, phylogenetic analysis, and potential recombination events of infectious bronchitis viruses isolated in Korea. Virus Genes 46, 371-374.

Takeuchi, O., Akira, S., 2009. Innate immunity to virus infection. Immunol. Rev. 227, 75-86.

Tamura, K., Nei, M., Kumar, S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Natl. Acad. Sci. U. S. A. 101, 11030-11035.

Tan, B., Wang, H., Shang, L., Yang, T., 2009. Coadministration of chicken GM-CSF with a DNA vaccine expressing infectious bronchitis virus (IBV) S1 glycoprotein enhances the specific immune response and protects against IBV infection. Arch. Virol. 154, 1117-1124.

Tang, D., DeVit, M., Johnston, S.A., 1992. Genetic immunization is a simple method for eliciting an immune response. Nature 356, 152-154.

Tang, M., Wang, H., Zhou, S., Tian, G., 2008. Enhancement of the immunogenicity of an infectious bronchitis virus DNA vaccine by a bicistronic plasmid encoding nucleocapsid protein and interleukin-2. J. Virol. Methods 149, 42-48.

Tang, M.J., Wang, H.N., Zhou, S., Huang, Y., Liu, P., 2007. [Potent immune responses elicited by a bicistronic IBV DNA vaccine expressing S1 and IL-2 gene]. Wei Sheng Wu Hsueh Pao 47, 1055-1059.

Tarpey, I., Orbell, S., Britton, P., Casais, R., Hodgson, T., Lin, F., Hogan, E., Cavanagh, D., 2006. Safety and efficacy of an infectious bronchitis virus used for chicken embryo vaccination. Vaccine 24, 6830-6838.

Terregino, C., Toffan, A., Serena Beato, M., De Nardi, R., Vascellari, M., Meini, A., Ortali, G., Mancin, M., Capua, I., 2008a. Pathogenicity of a QX strain of infectious bronchitis virus in specific pathogen free and commercial broiler chickens, and evaluation of protection induced by a vaccination programme based on the Ma5 and 4/91 serotypes. Avian Pathol. 37, 487-493.

Terregino, C., Toffan, A., Serena Beato, M., De Nardi, R., Vascellari, M., Meini, A., Ortali, G., Mancin, M., Capua, I., 2008b. Pathogenicity of a QX strain of infectious bronchitis virus in specific pathogen free and commercial broiler chickens, and evaluation of protection induced by a vaccination programme based on the Ma5 and 4/91 serotypes. Avian Pathol. 37, 487-493.

Thompson, J.D., Gibson, T., Higgins, D.G., 2002. Multiple sequence alignment using ClustalW and ClustalX. Curr Protoc Bioinformatics, 2.3. 1-2.3. 22.

© COPYRIGHT UPM

Thor, S.W., Hilt, D.A., Kissinger, J.C., Paterson, A.H., Jackwood, M.W., 2011. Recombination in avian gamma-coronavirus infectious bronchitis virus. Viruses 3, 1777-1799.

Tian, L., Wang, H., Lu, D., Zhang, Y., Wang, T., Kang, R., 2008. The immunoreactivity of a chimeric multi-epitope DNA vaccine against IBV in chickens. Biochem. Biophys. Res. Commun. 377, 221-225.

Tighe, H., Corr, M., Roman, M., Raz, E., 1998. Gene vaccination: plasmid DNA is more than just a blueprint. Immunol. Today 19, 89-97.

Timms, L.M., Bracewell, C.D., 1981. Cell mediated and humoral immune response of chickens to live infectious bronchitis vaccines. Res. Vet. Sci. 31, 182-189.

Titomirov, A.V., Sukharev, S., Kistanova, E., 1991. In vivo electroporation and stable transformation of skin cells of newborn mice by plasmid DNA. Biochim Biophys Acta. 1088, 131-134.

Tomkinson, A.E., Vijayakumar, S., Pascal, J.M., Ellenberger, T., 2006. DNA ligases: structure, reaction mechanism, and function. Chem. Rev. 106, 687-699.

Toro, H., Godoy, V., Larenas, J., Reyes, E., Kaleta, E., 1996. Avian infectious bronchitis: viral persistence in the Harderian gland and histological changes after eyedrop vaccination. Avian Dis. , 114-120.

Toro, H., Pennington, D., Gallardo, R.A., Van Santen, V.L., Van Ginkel, F.W., Zhang, J., Joiner, K.S., 2012. Infectious bronchitis virus subpopulations in vaccinated chickens after challenge. Avian Dis. 56, 501-508.

Ulmer, J.B., Donnelly, J.J., Parker, S.E., Rhodes, G.H., Felgner, P.L., Dwarki, V.J., Gromkowski, S.H., Deck, R.R., DeWitt, C.M., Friedman, A., 1993. Heterologous protection against influenza by injection of DNA encoding a viral protein. Science 259, 1745-1749.

Vagnozzi, A., García, M., Riblet, S.M., Zavala, G., 2010. Protection induced by infectious laryngotracheitis virus vaccines alone and combined with Newcastle disease virus and/or infectious bronchitis virus vaccines. Avian Dis. 54, 1210-1219.

Van der Meide, P., Schellekens, H., 1996. Cytokines and the immune response: In cytokine Yearbook Volume 1. Springer, pp. 243-249.

Van Hemert, M.J., van den Worm, S.H.E., Knoops, K., Mommaas, A.M., Gorbalenya, A.E., Snijder, E.J., 2008. SARS-coronavirus replication/transcription complexes are membrane-protected and need a host factor for activity in vitro. PLoS pathogens 4, e1000054.

© COPYRIGHT UPM

Van Roeckel, H., Bullis, K.L., Flint O S., Clarke, M.K., 1942. Poultry disease control service. Massachusetts Agricultural Experiment Station, MA. Annual Report. Bulletin 388, 99-103.

Vigerust, D.J., Shepherd, V.L., 2007. Virus glycosylation: role in virulence and immune interactions. Trends Microbiol. 15, 211-218.

Vimal, S., Abdul Majeed, S., Taju, G., Nambi, K., Sundar Raj, N., Madan, N., Farook, M., Rajkumar, T., Gopinath, D., Sahul Hameed, A., 2013. Chitosan tripolyphosphate (CS/TPP) nanoparticles: Preparation, characterization and application for gene delivery in shrimp. Acta Trop. 128, 486-493.

Vlasak, R., Luytjes, W., Spaan, W., Palese, P., 1988. Human and bovine coronaviruses recognize sialic acid-containing receptors similar to those of influenza C viruses. Proc. Natl. Acad. Sci. U. S. A. 85, 4526-4529.

Waeckerle-Men, Y., Allmen, E.U., Gander, B., Scandella, E., Schlosser, E., Schmidtke, G., Merkle, H.P., Groettrup, M., 2006. Encapsulation of proteins and peptides into biodegradable poly (d, l-lactide co-glycolide) microspheres prolongs and enhances antigen presentation by human dendritic cells. Vaccine 24, 1847-1857.

Wang, L., Parr, R., King, D., Collisson, E., 1995. A highly conserved epitope on the spike protein of infectious bronchitis virus. Arch. Virol. 140, 2201-2213.

Wang, W., Li, W., Ma, N., Steinhoff, G., 2013. Non-viral gene delivery methods. Curr. Pharm. Biotechnol. 14, 46-60.

Wang, X., Jiang, P., Deen, S., Wu, J., Liu, X., Xu, J., 2003. Efficacy of DNA vaccines against infectious bursal disease virus in chickens enhanced by coadministration with CpG oligodeoxynucleotide. Avian Dis. 47, 1305-1312.

Wang, X., Schnitzlein, W.M., Tripathy, D.N., Girshick, T., Khan, M.I., 2002. Construction and immunogenicity studies of recombinant fowl poxvirus containing the S1 gene of Massachusetts 41 strain of infectious bronchitis virus. Avian Dis. 46, 831-838.

Wang, Z., Gu, M., Li, G., 2005. Surface properties of gleditsia saponin and synergisms of its binary system. J. Dispersion Sci. Technol. 26, 341-347.

Wentworth, D.E., Holmes, K.V., 2007. Coronavirus binding and entry. Coronaviruses: molecular and cellular biology.Caister Academic Press, Norfolk, United Kingdom , 3-32.

Wickramasinghe, I.A., van Beurden, S., Weerts, E., Verheije, M., 2014. The avian coronavirus spike protein. Virus Res. 194:37-48.

© COPYRIGHT UPM

Wickramasinghe, I.N.A., de Vries, R.P., Gröne, A., de Haan, C.A.M., Verheije, M.H., 2011. Binding of avian coronavirus spike proteins to host factors reflects virus tropism and pathogenicity. J. Virol. 85, 8903-8912.

Williams, A., Wang, L., Sneed, L., Collisson, E., 1993. Analysis of a hypervariable region in the 3'non-coding end of the infectious bronchitis virus genome. Virus Res. 28, 19-27.

Williams, A.K., Li, W., Sneed, L.W., Collisson, E.W., 1992. Comparative analyses of the nucleocapsid genes of several strains of infectious bronchitis virus and other coronaviruses. Virus Res. 25, 213-222.

Williams, R.K., Jiang, G.S., Holmes, K.V., 1991. Receptor for mouse hepatitis virus is a member of the carcinoembryonic antigen family of glycoproteins. Proc. Natl. Acad. Sci. U. S. A. 88, 5533-5536.

Wilson, L., Gage, P., Ewart, G., 2006. Hexamethylene amiloride blocks E protein ion channels and inhibits coronavirus replication. Virology 353, 294-306.

Wilson, N.S., Yang, B., Morelli, A.B., Koernig, S., Yang, A., Loeser, S., Airey, D., Provan, L., Hass, P., Braley, H., 2011. ISCOMATRIX vaccines mediate CD8+ T-cell cross-priming by a MyD88-dependent signaling pathway. Immunol. Cell Biol. 90, 540–552.

Winter, C., Herrler, G., Neumann, U., 2008. Infection of the tracheal epithelium by infectious bronchitis virus is sialic acid dependent. Microb. Infect. 10, 367-373.

Winter, C., Schwegmann-Wessels, C., Cavanagh, D., Neumann, U., Herrler, G., 2006. Sialic acid is a receptor determinant for infection of cells by avian Infectious bronchitis virus. J. Gen. Virol. 87, 1209-1216.

Winterfield, R.W., Thacker, H.L., Badylak, S.F., 1984. Effects of subtype variations in the Holland strain of infectious bronchitis virus when applied as a vaccine. Poult. Sci. 63, 246-250.

Wolff, J.A., Malone, R.W., Williams, P., Chong, W., Acsadi, G., Jani, A., Felgner, P.L., 1990. Direct gene transfer into mouse muscle in vivo. Science 247, 1465-1468.

Worthington, K.J., Currie, R., Jones, R.C., 2008. A reverse transcriptase-polymerase chain reaction survey of infectious bronchitis virus genotypes in Western Europe from 2002 to 2006. Avian Pathol. 37, 247-257.

Wurm, T., Chen, H., Hodgson, T., Britton, P., Brooks, G., Hiscox, J.A., 2001. Localization to the nucleolus is a common feature of coronavirus nucleoproteins, and the protein may disrupt host cell division. J. Virol. 75, 9345-9356.

© COPYRIGHT UPM

Xiao, X., Chakraborti, S., Dimitrov, A.S., Gramatikoff, K., Dimitrov, D.S., 2003. The SARS-CoV S glycoprotein: expression and functional characterization. Biochem. Biophys. Res. Commun. 312, 1159-1164.

Xie, H., Liu, T., Chen, H., Huang, X., Ye, Z., 2007. Evaluating the vaccine potential of an influenza A viral hemagglutinin and matrix double insertion DNA plasmid. Vaccine 25, 7649-7655.

Yager, E.J., Dean, H.J., Fuller, D.H., 2009. Prospects for developing an effective particle-mediated DNA vaccine against influenza.

Yan, F., Zhao, Y., Hu, Y., Qiu, J., Lei, W., Ji, W., Li, X., Wu, Q., Shi, X., Li, Z., 2013. Protection of chickens against infectious bronchitis virus with a multivalent DNA vaccine and boosting with an inactivated vaccine. J Vet Sci. 14, 53-60.

Yang, T., Wang, H., Wang, X., Tang, J., Lu, D., Zhang, Y., Guo, Z., Li, Y., Gao, R., Kang, R., 2009a. The protective immune response against infectious bronchitis virus induced by multi-epitope based peptide vaccines. Biosci. Biotechnol. Biochem. 73, 1500-1504.

Yang, T., Wang, H.-., Wang, X., Tang, J.-., Gao, R., Li, J., Guo, Z.-., Li, Y.-., 2009b. Multivalent DNA vaccine enhanced protection efficacy against infectious bronchitis virus in chickens. J Vet Sci. 71, 1585-1590.

Yoshikawa, M., Murakami, T., Kishi, A., Kageura, T., Matsuda, H., 2001. Medicinal flowers. III. Marigold.(1): hypoglycemic, gastric emptying inhibitory, and gastroprotective principles and new oleanane-type triterpene oligoglycosides, calendasaponins A, B, C, and D, from Egyptian Calendula officinalis. Chem Pharm Bull. 49, 863-870.

Youn, S., Collisson, E.W., Machamer, C.E., 2005. Contribution of trafficking signals in the cytoplasmic tail of the infectious bronchitis virus spike protein to virus infection. J. Virol. 79, 13209-13217.

Yu, D., Han, Z., Xu, J., Shao, Y., Li, H., Kong, X., Liu, S., 2010. A novel B-cell epitope of avian infectious bronchitis virus N protein. Viral Immunol. 23, 189-199.

Yudong, W., YongLin, W., Zichun, Z., GenChe, F., Yihai, J., Xiange, L., Jiang, D., Shushuang, W., 1998. Isolation and identification of glandular stomach type IBV (QX IBV) in chickens. Chinese Journal of Animal Quarantine 15, 1-3.

Zanella, A., Lavazza, A., Marchi, R., Martin, A.M., Paganelli, F., 2003. Avian infectious bronchitis: characterization of new isolates from Italy. Avian Dis. 47, 180-185.

© COPYRIGHT UPM

Zhai, Y., Sun, F., Li, X., Pang, H., Xu, X., Bartlam, M., Rao, Z., 2005. Insights into SARS-CoV transcription and replication from the structure of the nsp7–nsp8 hexadecamer. Nat Struct Mol Biol. 12, 980-986.

Zhang, D., Zhou, J., Chen, W., Chen, J., 2009. Co-expression of IBV structural proteins and chicken interleukin-2 for DNA immunization. Vet. Med. 54, 169-174.

Zhang, W., Yin, Z., Liu, N., Yang, T., Wang, J., Bu, Z., Wu, D., 2010. DNA-chitosan nanoparticles improve DNA vaccine-elicited immunity against Newcastle disease virus through shuttling chicken interleukin-2 gene. J. Microencapsul. 27, 693-702.

Zhao, K., Chen, G., Shi, X., Gao, T., Li, W., Zhao, Y., Zhang, F., Wu, J., Cui, X., Wang, Y., 2012. Preparation and efficacy of a live newcastle disease virus vaccine encapsulated in chitosan nanoparticles. PloS one 7, e53314.

Zhou, F., Song, S., Chen, W.R., Xing, D., 2011. Immunostimulatory properties of glycated chitosan. J Xray Sci Technol. 19, 285-292.

Zhou, J.Y., Wu, J.X., Cheng, L.Q., Zheng, X.J., Gong, H., Shang, S.B., Zhou, E.M., 2003. Expression of immunogenic S1 glycoprotein of infectious bronchitis virus in transgenic potatoes. J. Virol. 77, 9090-9093.

Zhou, Y.S., Zhang, Y., Wang, H.N., Fan, W.Q., Yang, X., Zhang, A.Y., Zeng, F.Y., Zhang, Z.K., Cao, H.P., Zeng, C., 2013. Establishment of reverse genetics system for infectious bronchitis virus attenuated vaccine strain H120. Vet. Microbiol. 162, 53-61.

Zhu, J., Qian, H., Zhang, Y., Hua, X., Wu, Z., 2007. Analysis of similarity of the S1 gene in infectious bronchitis virus (IBV) isolates in Shanghai, China. Arch. Med. Vet. 39, 223.

Ziebuhr, J., 2008. Coronavirus replicative proteins. Nidoviruses.ASM Press, Washington, DC , 65-81.

Zulperi, Z.M., Omar, A.R., Arshad, S.S., 2009. Sequence and phylogenetic analysis of S1, S2, M, and N genes of infectious bronchitis virus isolates from Malaysia. Virus Genes 38, 383-391.

Zur, H., Tuller, T., 2013. New universal rules of eukaryotic translation initiation fidelity. PLoS Comput Biol 9, e1003136.

Zwaagstra, K.A., van der Zeijst, B.A., Kusters, J.G., 1992. Rapid detection and identification of avian infectious bronchitis virus. J. Clin. Microbiol. 30, 79-84.

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