UNIVERSITI PUTRA MALAYSIA

CONSTRUCTION AND CHARACTERIZATION OF PHAGE-DISPLAY AND BACTERIOPHAGE-MEDIATED VACCINES AGAINST VERY VIRULENT INFECTIOUS BURSAL DISEASE AND GENOTYPE VII NEWCASTLE DISEASE

OMAR BASSIM AHMED AL-TAYYAR

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By

OMAR BASSIM AHMED AL-TAYYAR

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

April 2018

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DEDICATION

I would like to humbly dedicate this work with all appreciations
To My FATHER, To My MOTHER, and To My WONDERFUL WIFE

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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

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April 2018

Chairman : Professor Datin Paduka Dato' Aini binti Ideris, PhD
Faculty : Veterinary Medicine

Newcastle disease (ND) is regarded as one of the most significant viral diseases in the poultry industry and perhaps it presents the larger drainage to the world economy when it is compared to various other animal viruses. The main antigens are the F and HN genes. Infectious bursal disease (IBD) remains a constant intimidation to the world poultry industry. In addition to the high mortalities of very virulent IBD, the most important is the immuno-suppression that it causes. VP2 is the major antigen. Although, attenuated live vaccines and inactivated vaccines have been used in the field since the last century to control these diseases, the need for more safe and more efficacious vaccines is still required.

This study was undertaken to develop phage-display and bacteriophage-mediated vaccines against both Newcastle disease and infectious bursal disease based on F and VP2 protein sequences.

The study hypothesis is that Phage-display vaccines and bacteriophage-mediated vaccines are able to induce protective immune response in vaccinated chickens.

Bacteriophage (phage)-display (PD) and bacteriophage-mediated (BM) immunization are novel vaccines delivery technology. In PD vaccine the inserted gene is displayed as a protein on the surface of the phage. While in BM vaccine the phage act as gene transfer vector. They have many advantages over the conventional vaccines. The first approach was the construction of the vaccines. PD vaccines were constructed using VP2 of vvIBD (UPM0081) as (pha.Dis-VP2) and F for ND genotype VII (IBS002) as (pha.Dis-F) consequently. BM vaccines were constructed using VP2 of vvIBD

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(UPM0081) as (pha.Med-VP2) and F for ND genotype VII (IBS002) as (pha.Med-F) subsequently.

After sequencing and conformation of the vaccines, their functionality was assessed through Western blotting. The results of the Western blot indicate that the antigens in the PD and BM vaccines were successfully expressed in the hd11 cell line.

In the first chicken trial, the pha.Dis-VP2 vaccine was administered via three different delivery routes (orally, subcutaneously and subcutaneously-in oil) into specific pathogen-free (SPF) chickens groups. The vaccination program consisted of two vaccinations with three weeks apart to stimulate the immune response. After each vaccination blood withdrawal from the wing vein was performed to detect the immune respond translated as antibodies titer. The same procedure was accomplished for the pha.Dis-F vaccine were it was administered orally, subcutaneously and subcutaneously-in oil. Three weeks post booster vaccination the challenge was carried out using vvIBDV for the pha.Dis-VP2 vaccinated groups, and vvNDV for the pha.Dis-F groups.

The results of this trial revealed, for the first time, that pha.Dis-VP2 vaccine administered orally provides the best protection (93.34%) for the vaccinated chickens against the challenge with the vvIDBV, while the protection of the subcutaneous and subcutaneous-in oil adjuvant vaccination was (80%) and (66.67%) respectively. The detected antibodies titer was significantly higher ($p< 0.01$) in the oral vaccinated group, using a VP2-based ELISA kit, followed by the subcutaneous and subcutaneous-in oil adjuvant vaccinated groups consequently. The results of the bursal/body weight ratio for the vaccinated chickens indicates that the vaccine in the oral group have no detrimental effects on the bursae and that the vaccine successfully protected the bursae from the challenge impact. The histopathological assessment revealed similar pattern as a bursal lesion scores.

Concerning ND vaccination, unfortunately the pha.Dis-F vaccination did not protect any of the vaccinated chickens groups after the challenge with the highly virulent strain of NDV genotype VII (IBS002), but delayed the mortalities of the chickens.

In the second chicken trial, the pha.Dis-VP2 vaccine was administered via the oral route into SPF chicken group. The vaccination program included two vaccinations with three weeks interval to boost the immune response. Blood withdrawal was carried out from the wing vein to detect the immune respond expressed as antibodies titer. The same procedure was accomplished for the pha.Med-F vaccine. Three weeks after the second vaccination the challenge was carried out using vvIBDV for the pha.Med-VP2 vaccinated group, and vvNDV for the pha.Med-F group.

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The results of this trial revealed, for the first time, that pha.Med-VP2 vaccine administered orally provided a protection of (92.31%) for the vaccinated chickens against the challenge with the vvIDBV. The detected antibodies titer was significantly higher ($p< 0.01$) in the vaccinated group, using a VP2-based ELISA kit. The results of the bursal/body weight ratio for the vaccinated chickens indicates that the vaccine have no detrimental effects on the bursae and that the vaccine successfully protected the bursae from the challenge impact. The histopathological assessment revealed similar pattern as a bursa lesion scores.

Concerning ND vaccination, unfortunately the pha.Med-F vaccination did not protect the vaccinated chickens group after the challenge with the highly virulent strain of NDV genotype VII (IBS002), but delayed the mortalities.

In conclusion, the constructed VP2-recombinant vaccines were immunogenic in SPF chickens. Phage-displayVP2 vaccines (S/C, S/C-in oil and orally) were able to protect the chickens against the vvIBDV challenged virus with the best result via the oral route (93.34%). In addition to that, the phage-mediated VP2 vaccine given orally was able to protect the chickens (92.31%) against the ND genotype VII challenged virus. Phage-display VP2 and phage-mediated VP2 have no detrimental effects on the bursae. The F-based vaccines (pha.Dis.-F and pha.Med.-F) were not able to protect the chickens against the challenged virus. Though F-based vaccines were able to delay chicken mortalities following the challenge.

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

KONSTRUKIS SERTA PENCIRIAN VAKSIN FAJ-PAPARAN DAN BAKTERIOFAG-PERANTARAAN TERHADAP VERY VIRULENT INFECTIOUS BURSAL DISEASE DAN NEW CASTLE DISEASE GENOTIP VII

Oleh

OMAR BASSIM AHMED AL-TAYYAR

April 2018

Pengerusi : Professor Datin Paduka Dato' Aini binti Ideris, PhD
Fakulti : Perubatan Veterinar

Penyakit sampar (ND) dikaitkan sebagai salah satu penyakit virus yang signifikan dalam industri unggas dan ia mungkin menunjukkan ketirisan yang besar ke atas ekonomi apabila dibandingkan dengan pelbagai virus yang lain pada haiwan. Antigen utamanya adalah gen F dan HN. Penyakit bursa berjangkit (IBD) masih menjadi intimidasi malar terhadap industri unggas dunia. Tambahan terhadap kematian yang tinggi daripada IBD yang memudaratkan, perkara terpenting adalah ia merupakan penyebab kepada penindasan imun. Antigen utamanya adalah VP2. Meskipun vaksin hidup yang dilemahkan dan vaksin yang tidak aktif telah digunakan di lapangan sejak beberapa kurun yang lepas untuk mengawal penyakit-penyakit ini, keperluan terhadap vaksin yang lebih selamat dan lebih berkesan masih diperlukan.

Kajian ini dijalankan untuk membangun “phage-display” dan “bacteriophage-mediated vaccines” bagi melaman penyakit Newcastle dan penyakit bursa berinfeksi, berasaskan segnen F dan VP2 protin.

Hipotesis kajian ini ialah vaksin-vaksin “phage-display” dan “bacteriophage-mediated” berupaya memberi imun repons yang datap mengawal penyakit dalm ayam-ayam yang diberi vaksin.

Bakteriofaj paparan-(faj) (PD) dan bakteriofaj-perantaraan (BM) imunisasi adalah teknologi pemberian vaksin yang novel. Bagi gen PD yang dimasukkan akan dipaparkan sebagai protin di permukaan faj. Sementara pada BM faj bertindak sebagai vektor pemindahan gen. Terdapat banyak kelebihan berbanding vaksin konvensional. Tindakan pertama adalah merangka vaksin. Vaksin PD telah dirangka menggunakan VP2 dari vvIBD (UPM0081) sebagai (pha.Dis-VP2) dan F bagi ND genotip VII

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(IBS002) sebagai (pha.Dis-F). Vaksin BM telah dirangka menggunakan VP2 dari vvIBD (UPM0081) sebagai (pha.Med-VP2) dan F bagi ND genotip VII (IBS002) sebagai (pha.Med-F).

Selepas penjujukan dan pengesahan vaksin, fungsi vaksin di nilai menggunakan blot western. Hasil yang diperolehi daripada blot western menunjukkan antigen-antigen pada vaksin PD dan BM telah berjaya di nyatakan pada garisan sel hd11.

Pada kajian pertama dalam ayam, vaksin pha.Dis-VP2 diberikan melalui tiga saluran pemberian yang berbeza (oral, subkutaneus dan subketaneus-dalam minyak) kepada kumpulan-kumpulan ayam bebas pathogen specikic (SPF). Program vaksinasi mengandungi dua vaksinasi dengan tiga minggu jarak bagi merangsang tindakbalas imuniti. Selepas setiap vaksinasi darah diambil dari vena sayap dan digunakan untuk mengesan tindakbalas yang diterjemahkan kepada titer antibodi. Prosedur sama telah digunakan bagi vaksin pha.Dis-F. Tiga minggu selepas vaksinasi penggalak cabaran telah dilakukan menggunakan vvIBDV bagi kumpulan vaksin pha.Dis-VP2 dan vvNDV bagi kumpulan pha.Dis-F.

Hasil yang diperolehi dari kajian ini menunjekkan untuk pertama kalinya vaksin pha.Dis-VP2 yang telah diberikan secara oral memberikan perlindungan terbaik (93.34%) kepada ayam yang telah divaksinasi dicabar dengan vvIBDV, sementara perlindungan bagi vaksinasi subkutaneus dan subkutaneus-adjuvan minyak adalah (80%) dan (66.67%) masing-masing. Titer antibodi yang dikesan menunjukkan signifikan yang lebih tinggi ($p< 0.01$) pada kumpulan vaksinasi menggunakan VP2 berdasarkan kit ELISA, diikuti dengan kumpulan vaksinasi subkutaneus dan subkutaneus-adjuvan minyak. Hasil bagi nisbah bursa/berat badan bagi ayam-ayam yang telah divaksinasi menunjukkan vaksin bagi kumpulan oral tiada kesan-kesan yang merosakkan ke atas bursa dan vaksin berkenaan telah berjaya melindungi bursa daripada impak cabaran. Penilaian histopatologi mendedahkan corak yang serupa seperti skor-skor lesi bursa.

Sebaliknya, vaksinasi pha.Dis-F tidak dapat melindungi sebarang kumpulan ayam-ayam yang telah divaksinasi selepas dicabar dengan strain NDV genotip VII (IBS002) yang sangat virulen tetapi ia memanjangkan tempoh kematian ayam-ayam tersebut.

Bagi kajian kedua dalam ayam, vaksin pha.Dis-VP2 telah diberikan secara oral kepada kumpulan ayam-ayam SPS. Program vaksinasi merangkumi dua vaksinasi dengan jarak masa tiga minggu bagi menggalakkan tindakbalas imun. Darah telah diambil daripada vena sayap untuk pengesanan tindakbalas imun yang ditunjukkan sebagai titer antibodi. Prosedur sama telah digunapakai bagi vaksin pha.Med-F. Tiga minggu selepas vaksinasi kali kedua cabaran telah diberikan menggunakan vvIBDV bagi kumpulan vaksinasi pha.Med-VP2 dan vvNDV bagi kumpulan pha.Med-F.

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Hasil yang diperolehi dari percubaan ini menunjukkan untuk pertama kalinya vaksin pha.Med-VP2 yang telah diberikan secara oral memberikan perlindungan (92.31%) ke atas ayam-ayam yang telah divaksinasi dan dicabar dengan vvIBDV. Titer antibodi yang telah dikesan adalah signifikan lebih tinggi ($p< 0.01$) bagi kumpulan vaksinasi menggunakan VP2 berdasarkan kit ELISA. Hasil bagi nisbah bursa/berat badan bagi kumpulan ayam-ayam yang telah divaksinasi menunjukkan vaksin tersebut tidak memberi kesan-kesan yang merosakkan ke atas bursa dan vaksin tersebut berjaya melindungi bursa daripada impak cabaran. Penilaian histopatologi mendedahkan corak yang serupa seperti skor-skor lesi bursa.

Sebaliknya, vaksinasi pha.Med-F tidak dapat melindungi kumpulan ayam-ayam yang telah divaksinasi selepas dicabar dengan strain NDV genotip VII (IBS002) yang sangat virulen tetapi ianya melambakkan tempoh kematian.

Sebagai rumusan, keputusam kajian menunjukkan vaksin-vaksin "phage-display' dan "bacteriophage-mediated" adalah efektif melawan vvIBDV, tetap tidak efektif bagi penyakit Newcastle.

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بسم الله الرحمن الرحيم

{ فَلِلَّهِ الْحَمْدُ رَبِّ السَّمَاوَاتِ وَرَبِّ الْأَرْضِ رَبِّ الْعَالَمِينَ (36) وَلَهُ الْكِبْرِيَاء فِي السَّمَاوَاتِ وَالْأَرْضِ وَهُوَ الْعَزِيزُ الْحَكِيمُ (37) }
سورة الجاثية

(In the name of Allah; the gracious, the merciful)

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

Aini binti Ideris, PhD
Professor Datin Paduka Dato'
Faculty Veterinary Medicine
Universiti Putra Malaysia
(Chairman**)**

Abdul Rahman bin Omar, PhD
Professor
Faculty Veterinary Medicine
Universiti Putra Malaysia
(Member)

Tan Wen Siang, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Signature: _____

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TABLE OF CONTENTS

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LIST OF TABLES

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LIST OF FIGURES

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LIST OF ABBREVIATIONS

%	Percentage
BF	Bursa of Fabricius
bp	Base pair
CAM	Chorioallantoic membrane
cDNA	Complementary deoxyribonucleic acid
CFU	colony forming unit
Cm	centimetre
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
DNase I	deoxyribonucleaseI
dNTP	Deoxynucleoside triphosphate
dsDNA	Double-stranded DNA
EDTA	Ethylene diamine tetra acetic acid
EID50	Embryo effective dose fifty
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organisation
FBS	Fetal bovine serum
g	gram
g	gravity
G	G-force
h	hour
HE	Haematoxylin-and-eosin
IBD	Infectious bursal disease
IBDV	Infectious bursal disease virus
IFN	interferon

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IL	interleukin
IPTG	Isopropyl-ß-D-thiogalactosidase
kb	kilobase pair
kDa	kilodalton
LB	Luria Bertani
log	logarithm
M	molar
MgSO4	Magnesium Sulfate
mM	milimolar
ND	Newcastle disease
NDV	Newcastle disease virus
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PEG	polyethylene glycol
PFU	plaque forming unit
pH	Hydrogen ion exponent
pha.Dis-F	Phage Display-F
pha.Dis-VP2	Phage Display-VP2
RNA	Ribonucleic acid
RNase A	ribonuclease A
rpm	Revolution per minute
RT	Room temperature
RT-PCR	Reverse Transcriptase-Polimerase Chain Reaction
S/C	Sub-cutaneous
S/C in oil	Sub-cutaneous in oil
SD	standard deviation
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis

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SPF	Specific pathogen-free chicken
SPF	Specific-pathogen-free
TAE	Tris-acetate-EDTA
TAE	Tris-acetate EDTA
UPM	Universiti Putra Malaysia
V	volt
W	Watt
λ	lambda
µg	Microgram
µl	Microliter
µm	Micrometer

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CHAPTER 1

INTRODUCTION

Poultry diseases caused by viral organisms represent a serious loss to poultry producers globally. The adverse effect of these viral diseases have impacted significantly on both industrialized and subsistence production involving broilers, layers, turkeys as well as other species. Infections of flocks also place a restraint to international trade, as a justifiable mean for preventing the introduction of diseases, as well as protecting domestic poultry industry.

Infectious bursal disease virus (IBDV) is an acute, highly contagious pathogen, which causes intensive severe immunosuppression and high mortality in chickens, mostly at an early age (3-6 weeks) while the B lymphocytes are still developing (Rodríguez-Lecompte et al., 2005). The disease has been of great concern especially in the last decade following the emergence of highly virulent strain, which has caused severe losses to the poultry industry worldwide (Mahgoub et al., 2012). Although, younger chickens are passively protected by maternally derived antibodies against the virus that they acquire through egg yolk while older chickens are capable of mounting immune response against the virus due to fully developed bursa of Fabricious, hence they both rarely develop clinical signs of the disease (Macreadie et al., 1990).

The causative agent is a member of the genus Avibirnavirus of the family Birnaviridae. The most severe form of the disease is caused by the very virulent IBDV (vvIBDV) which is associated with acute clinical disease with 60-100% mortality (Jackwood and Jackwood, 1994; Brown and Skinner, 1996). The virus attacks the immature B-lymphocytes in the bursa of Fabricious leading to the destruction of the tissue which results in the immunodeficiency of its host (Sharma et al., 2000).

In most poultry industries worldwide IBD is controlled by administration of live, inactivated or recombinant IBDV vaccines (Li et al., 2006; Bublot et al., 2007). However, the emergence of IBDV variants and the very virulent strains that are not susceptible to inactivation by MDA has prompted changes in vaccination regimes with broilers getting vaccinated with more virulent vaccines at 2-3 weeks of age when the MDA have declined (Kumar et al., 2000; Rautenschlein et al., 2002).

On the other hand, Newcastle disease (ND) is a highly contagious disease of many avian species that is characterized by considerable losses in infected birds. The disease is caused by avian paramyxovirus 1 commonly referred to as Newcastle disease virus which is an enveloped, single stranded negative-sense RNA virus which belongs to the Avulavirus genus of the family Paramyxoviridae (Alexander, 1997). Infections are manifested by a variety of clinical manifestations from very severe disease with high mortality among birds to mild disease, depending on the virus strains and the host

species infected (Angela et al., 1999). ND is considered one of the two most important avian diseases together with highly pathogenic avian influenza globally (Eterradossi and Saif, 2008). The molecular basis for the pathogenicity of NDV is essentially determined by the amino acid sequence of the protease cleavage site of the F protein as well as the ability of the cellular proteases to cleave the F protein of different pathotypes (Swayne et al., 2003). Cleavage of the precursor glycoprotein F0 to F1 and F2 by host cell proteases is required for the progeny virus to become infective (Xu et al., 1999; Huang et al., 2004). In a endeavour to control the infection in poultry population attenuated and live vaccines developed from lentogenic NDV strains such as the Hitchner B1 and LaSota strains are currently in use globally (Huang et al., 2004). Nonetheless, despite the widespread application of live NDV vaccines and the different regimes of vaccination, vaccinated birds may still develop signs of the disease depending on environmental conditions and the presence of complicating infections (Jinying et al., 2007).

1.1 Research problem

In recent years the number of reported ND viruses has increased significantly (Dimitrov et al., 2016). The number of outbreaks reported, as well as the number of genotypes indicates the broadening of virulent NDV genetic diversity, which might have resulted from the effect of conventional vaccination (Dimitrov et al., 2017). In addition to that, the currently used vaccine strains are old and genetically distant from the currently circulating virulent NDV (Dimitrov et al., 2016). This is seen as one of the reasons why the available vaccines are unable to effectively prevent or reduce the shedding of virulent virus by the vaccinated birds (Miller et al., 2009). Moreover, since the implementation of live ND vaccination, the transfer of antibodies to the offspring that can partially neutralize the live ND vaccines was known to be a complication issue (Miller et al., 2013). Unfortunately, many of the problems encountered during ND control from inception, still persist till present day. The effectiveness of these vaccines is dose dependent and although they provide 100% protection in experimental settings, they have failed to prevent infection and virus replication (Miller et al., 2013). Furthermore, inactivated ND vaccines require a withdrawal period before vaccinated birds can be fit for human consumption (Schijns et al., 2013).

Similarly, the emergence of antigenically variant strains as well as very virulent IBDV is proving to be a stumbling block to a successful control and prevention scheme of the disease among poultry population. The vvIBDV exhibit similar antigenicity to the classical strains and have demonstrated the ability to break through high levels of maternal antibody and cause over 60% mortality in specific pathogen free chickens (Cao et al., 1998). Attenuation of vvIBDV based on site-directed mutagenesis and reverse genetics approach is associated with the risk of reversion of the partially attenuated vvIBDV to wild-type vvIBDV which is viewed as a limiting factor in its application. Another point is the interference of maternally derived antibodies with vaccine uptake is equally proving to be a major problem in early vaccination against IBD with live vaccines (Muller et al., 2012). Based on these problems, it has become imperative to develop new vaccines that combine a straight forward administration

with high efficacy and few side-effects; and in this regard, the development of effective subunit-based vaccines is viewed as a viable alternative due to its excellent properties and high level of biosafety as well as their low environmental risk.

1.2 Study justification

Immunization is considered the most effective, convenient and economical method for prevention of many diseases especially contagious viral diseases. The conventional vaccines which are developed after attenuation or inactivation of the viruses, although they are very effective, their production and administration are considered costly. Their application is also accompanied by side effects such as reversion of virulence due to imperfect attenuation or inactivation or even recombination or mutagenesis that could lead to the generation of pathogens with higher contagious strength during production (Omid, 2013).

However, the identification of protective antigens is thought of a good method. Based on this approach, the safety of a vaccine is combined with the advantages of focusing the immune response on an optimal target. In this vaccination method, only the antigenic part of the pathogen is being extracted and, with or without modification, is applied as vaccine. The emergence of recombinant technologies has provided a very safe and economical way for large-scale production of new generation vaccines (Ribot et al., 2006). This technology facilitates identification, extraction, amplification and modification of DNA/RNA fragments encoding the antigenic part of a pathogen for either recombinant expression or delivery as a DNA vaccine to be expressed in-vivo. Nucleic acid immunization entails inoculation with purified DNA or RNA containing gene(s) under the control of an exogenous eukaryotic promoter, so that protective antigens are expressed within the host (Wolff et al., 1990; Tang et al., 1992). Nucleic acid vaccines have a number of advantages over the conventional vaccines routinely used for immunization of birds against IBDV and Newcastle disease. They are cheaper and easier to produce than recombinant protein vaccines, manifest fewer adverse side effects and can induce both cellular and humoral immune response (Clark and March, 2004). The potentials of DNA based vaccines to stimulate efficient humoral and cell mediated antibody production have been reported in mice, although trials in large animals and primates have not been successful (Dietrich et al., 1999). Nevertheless, a number of modifications have been adopted to improve the responses against DNA vaccines including; gun delivery, microparticles, or the inclusion of cytokines or immunostimulatory CpG motifs in the vaccine vectors (Fynan et al., 1993; Leitner et al., 1999; Singh et al., 2000; Scheerlinck et al. 2001). However, these methods increase both the cost and the easiness of the production.

Bacteriophages have been used to deliver DNA vaccines (Clark and March, 2004). This is achieved by cloning a DNA vaccine expression cassette consisting of eukaryotic promoter and a vaccine gene into the phage lambda and the whole phage particles are purified and used to immunize the host. This method has been used to generate antibody levels significantly higher than with standard plasmid-based DNA

vaccination in mice and rabbits with HBsAg and other antigens (Clark and March, 2004; March et al., 2006).

Bacteriophages are viral entities that are beginning to attract growing interest as optimal vaccine delivery vehicles. Phages are very suitable for vaccines design as a result of their high stability under harsh environmental conditions, their simple and relatively inexpensive cost for large scale production, in addition to they possess potent adjuvant capacities (Aghebati-Maleki et al., 2016). Phage vaccines have also demonstrated efficient immunostimulatory effects and present a high safety profile due to their long standing relationship with the mammalian body during their evolutionary period (Mateen and Irshad, 2011).

The study hypothesis is that constructed recombinant VP2 and F based Phage-display vaccines and bacteriophage-mediated vaccines are able to induce protective immune response in vaccinated chickens against vvIBDV and NDV genotype VII.

This study was undertaken to develop phage-display and bacteriophage-mediated vaccines against both Newcastle disease and Infectious bursal disease based on F and VP2 protein sequences.

The general objectives of this study are to develop nucleic acid vaccines against ND and IBD to be delivered as phage-display and bacteriophage-mediate particles.

The specific objectives are:

1) To construct phage-display vaccine expressing the VP2 gene of very virulent infectious bursal disease virus (pha.Dis-VP2) and phage-display vaccine expressing the F gene of the genotype VII Newcastle disease virus (pha.Dis-F).
2) To construct bacteriophage-mediated vaccine encoding the VP2 gene of very virulent infectious bursal disease virus (pha.Med-VP2) and bacteriophage-mediated vaccine encoding the F gene of the genotype VII Newcastle disease virus (pha.Med-F).
3) To detect the expressions of the constructed vaccines (pha.Dis-VP2, pha.Dis-F, pha.Med-VP2 and pha.Med-F) based on in vitro studies.
4) To determine the immunogenicity of the constructed vaccines (pha.Dis-VP2, pha.Dis-F, pha.Med-VP2 and pha.Med-F) in SPF chickens.
5) To determine the efficacy of the VP2-based vaccines through the challenge of the vaccinated chickens against vvIBDV and the efficacy of the F-based vaccines through the challenge of the vaccinated chickens against NDV genotype VII.

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REFERENCES

Aamir, S., Tanveer, A., Muhammad, U. and Zahid, H. (2014). Prevention and control of Newcastle disease. Internat. J. Agric. Innov. Res. 3(2), ISSN (Online) 2319-1473.

Abdel-Alim, G. A. and Saif, Y. M. (2001). Immunogenicity and antigenicity of very virulent strains of infectious bursal disease viruses. Avian Dis. 45, 92-101.

Adhya., S., Merril, C.R. and Biswas, B. (2014). Therapeutic and prophylactic applications of bacteriophage components in modern medicine. Cold Spring Harb Perspect Med. 4 (1): a012518.

Adwar, T. and Lukešová, D. (2008). Evaluation of thermostable vaccines against Newcastle disease in village chicken used in tropics and subtropics. Agricultura Trop. Subtrop. 41 (2).

Aghebati-Maleki, L., Babak, B., Motallebnezhad., M., Aghebati-Maleki, A., Nickho., H., Mehdi, Y.. and Majidi, M. (2016). Phage display as a promising approach for vaccine development. J. Biomed. Sci. 23: 66.

Alan, D.T. Barrett. and Galveston, T.X. (2016). Vaccinology in the twenty-first century. npj Vaccines (2016) 16009. doi:10.1038/npjvaccines.2016.9.

Alexander, D. (1997). Newcastle disease and other avian *Paramyxoviridae* infection in: Calnek BW, Barnes HJ, Beard CW, McDougald LR, Saif YM, editors. Diseases of Poultry. 10th ed. Ames: Iowa State University Press; 1997. pp. 541–549.

Alexander, D.J. and Senne, D.A. (2008). Newcastle disease and other avian paramyxoviruses. in: a laboratory manual for the isolation, identification and characterization of avian pathogens. Dufour-Zavala L. (Editor in Chief) Swayne, D.E., Glisson, J.R., Jackwood, M.W., Pearson, J.E., Reed, W.M and Woolcock, P.R., 4th ed., American Association of Avian Pathologists, Athens, GA, 135–141.

Alexander, D.J. (2000). Newcastle disease and other avian paramyxoviruses. Rev. Sci. Sech. Off. Int. Epiz. 19 (2), 443-462.

Al-Garib, S.O., Gielkens, A.L.J., Gruys E. and Koch, G. (2003). Review of Newcastle disease virus with particular references to immunity and vaccination. World's Poult. Sci. J. 59.

Angela, R.O., Egbert, M., Teshome, M., Ursula, J.B. and Thomas, C.M. (1999). Generation of recombinant lentogenic Newcastle disease virus from cDNA. J. Gen. Virol.; 80, 2987–2995

Arora, P., Lakhchaura, B.D., and Garg, S.K. (2010). Evaluation of immunogenic potential of 75kDa and 56kDa proteins of newcastle disease virus (NDV). Indian J. Exp. Biol. 48(9):889-95.

Ayala, A.J., Dimitrov, K.M., Becker, C.R., Goraichuk, I.V., Arns, C.W., Bolotin, V.I., Helena, L. Ferreira., Anton, P. Gerilovych., Gabriela, et al. (2016). Presence of vaccine-derived Newcastle disease viruses in wild birds. PLoS ONE 11(9): e0162484.

Azad, A.A., Jagadish, M.N., Brown, M.A. and Hudson, P.J. (1987). Deletion mapping and expression in E.Coli of the large genomic segment of a birnavirus. Virology. 161:145–152.

Bahey-El-Din, M., Casey, P. G., Griffin, B. T. and Gahan, C. G. (2010). Expression of two Listeria monocytogenes antigens (P60 And LLO) In Lactococcus lactis and examination for use as live vaccine vectors. J. Med. Microbiol. 59: 904–912.

Barrett, A.D.T. and, Stanberry, L.R. (2008). Vaccines for biodefence and emerging and neglected diseases. Academic, Waltham, MA.

Bass, S., Greene, R. and Wells, J.A. (1990). Hormone phage: An enrichment method for variant proteins with altered binding properties. Proteins 8:309-314.

Bastos, R.G., Borsuk, S., Seixas, F.K., and Dellagostin, O.A. (2009). Recombinant mycobacterium bovis BCG. Vaccine. 27: 6495-6503.

Bayliss, C. D., Spies, U., Shaw, K., Peters, R. W., Papageorgiou, A., Müller, H. and Boursnell, M. E. G. (1990). A comparison of the sequences of segment A of four infectious bursal disease virus strains and identification of a variable region in VP2. J. Gen. Virol. 71:1303-1312.

Bazan., J., Całkosiński, I.,. and Gamian, A. (2012). Phage display-A powerful technique for immunotherapy. Hum. Vaccin. Immunother. 1; 8 (12): 1829–1835.

Beal, R. K., Wigley, P., Powers, C., Barrow, P. A. and Smith, A. L. (2006). Cross-reactive cellular and humoral immune responses to Salmonella enterica serovars typhimurium and enteritidis are associated with protection to heterologous re-challenge. Vet. Immunol. Immunopathol. 114: 84–93.

Beard, C.W. and Hanson, R.P (1984). Newcastle disease. In: Hofstad M. S, Barnes H.J, Calnek B.W., Reid W.M., Yoder H. W. (Ed.), Diseases of Poultry, Iowa State University Press, Ames, pp. 452-470.

Becht, H., Muller, H., and Muller, H. K. (1988). Comparative studies on structural and antigenic properties of two serotypes of infectious bursal disease virus. J. Gen. Virol. 69: 631–640.

© COPYRIGHT UPM

Beghetto., E. and Gargano.,N. (2011). Lambda-display: A powerful tool for antigen discovery. Molecules. 16: 3089-3105.

Belakova, J., Horynova, M., Krupka, M., Weigl, E. and, Raska, M. (2007). DNA vaccines: are they still just a powerful tool for the future? Arch. Immunol. Ther. Exp. 55:387-398.

Bell, J.G. (2001). A comparison of the different vaccine available for the control of Newcastle disease in village chickens. In: Alders R.G., Spradbrow P.B. (eds.): SADC Planning Workshop on Newcastle disease control in village chickens. Proceedings of an International Workshop, Maputo, Mozambique, 6-9 March, 2000. ACIAR Proceedings, No. 103: 56–60.

Belshe, R.B., Edwards, K.M., Vesikari, T., Black, S.V., Walker, R.E., Hultquist, M., Kemble, G., and Connor, E.M. (2007). CAIV-T comparative efficacy study group. live attenuated versus inactivated influenza vaccine in infants and young children. N. Engl. J. Med. 356: 685–696.

Benhar, I. (2001). Biotechnological applications of phage and cell display. Biotechnol. Adv. 19: 1–33.

Beukema, E.L., Brown, M.P. and Hayball, J.D. (2006). The potential role of fowl pox virus in rational vaccine design. Exp. Rev Vaccines. 5: 565–577.

Birghan, C., Mundt, E. and Gorbalenya, A.E. (2000). A non-canonical lon proteinase lacking the ATPase domain employs the ser-Lys catalytic dyad to exercise broad control over the life cycle of a double-stranded RNA virus. EMBO J. 19(1):114–123.

Bode, C., Zhao, G., Steinhagen, F., Kinjo, T., and Klinman, D. M. (2011). Cancer and infammation . Exp. Rev.Vaccines; 10(4): 499–511

Borland, L.J. and Allan, W.H. (1980). Laboratory tests for comparing live lentogenic Newcastle disease vaccines. Avian Pathol. 9: 45-59.

Bottcher, B., Kiselev, N.A., Stel-Mashchuk, V.Y., Perevozchikova, N.A., Borisov, A.V. and Crowther, R.A. (1997). Three-dimensiona l structure of infectious bursal disease virus determined by electron cryomicroscopy. J. Virol. 71:325–330.

Boursnell, M.E., Green, P.F., Samson, A.C., Campbell, J.I., Deuter, A., Peters, R.W., Millar, N.S., Emmerson, P.T. and Binns, M.M. (1990). A recombinant fowl pox virus expressing the hemagglutinin-neuraminidase gene of Newcastle disease virus (NDV) protects chickens against challenge by NDV. Virology. 178(1):297-300.

Brandler, S. and Tangy, F. (2008) Recombinant vector derived from live attenuated measles virus: Potential for flavivirus vaccines. Comp. Immunol. Microbiol. Infect. Dis.31: (2–3): 271-291.

Brandt, M., Yao, K., Liu, M., Heckert, R.A. and Vakharia, V.N. (2001). Molecular determinants of virulence, cell tropism, and pathogenic phenotype of infectious bursal disease virus. J. Virol.75:11974–11982.

Brown, M.D. and Skinner, M.A. (1996). Coding sequences of both genome segments of a European 'very virulent' infectious bursal disease virus. Virus Res; 40 (1):1-15.

Bruhn, K.W., Craft, N. and Miller, J.F. (2007). Listeria as a vaccine vector. Microb. Infect. 9 (10):1226-1235.

Bruttin, A. and Brussow, H. (2005). Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy. Antimicrob. Agents Chemother. 49:2874–2878.

Bublot, M., Pritchard, N., Le Gros, F.-X. and Goutebroze, S. (2007). Use of a vectored vaccine against infectious bursal disease of chickens in the face of high-titred maternally derived antibody. J. Comp. Path. 137: S81-S84.

Burkhardt, E. and Müller, H. (1987). Susceptibility of chicken blood lymphoblasts and monocytes to IBDV. Arch. Virol. 9: 297-303.

Campbell, A. (2003). The future of bacteriophage biology. Nat Rev. Genet. 4(6):471-7.

Cao., Y.C, Shi., Q., Ma, J., Xie., Q. and Bi, Y. (2005). vaccination against very virulent infectious bursal disease virus using recombinant t4 bacteriophage displaying viral protein VP2. Acta. Biochim. Biophys. Sin. 37:657-664

Centlivre , M. and Combadière, B. (2015). New challenges in modern vaccinology. BMC Immunology. 16:18.

Centre for food security and public health CFSPH (2016). Newcastle disease: Avian paramyxovirus-1 infection, goose paramyxovirus infection, ranikhet disease. http://www.cfsph.iastate.edu/Factsheets/pdfs/newcastle_disease.pdf accessed 28th June, 2017.

Chang, H.C., Lin, T.L., and Wu, C.C. (2001). DNA-mediated vaccination against infectious bursal disease in chickens. Vaccine. 20(3): 328-335.

Chang, H.C., Lin, T.L. and Wu, C.C., (2003). DNA vaccination with plasmids containing various fragments of large segment genome of infectious bursal disease virus. Vaccine. 21:507–513.

Chen, H., Chang., T., Sang, L. L. and Wong C.C. (2002). DNA-mediated vaccination against infectious bursal disease in chickens. Vaccine. 20:328–335.

Cherbonnel, M., Rousset, J. and Jestin, V. (2003) Strategies to improve protection against low-pathogenicity h7 avian influenza virus infection using DNA vaccines. Avian Dis. 47: 1181-1186.

© COPYRIGHT UPM

Chomczynski, P. and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analyt. Biochem.162n (1), 156-159.

Chong, L.K., Omar, A.R., Yusoff, K. Hair-Bejo, M. and Idiris A. (2001). Nucleotide sequence and phylogenetic analysis of A segment of a highly virulent strain of infectious bursal disease virus. Acta Virol. 45:217–226.

Clark, J.R. and March, J.B. (2004a). Bacterial viruses as human vaccines? *Expert Rev Vaccines*, 3(4):463-476.

Clark, J.R. and March, J.B. (2004b). Bacteriophage-mediated nucleic acid immunisation. FEMS Immunol Med Microbiol. 40(1):21–6.

Clark, J.R. and March, J.B. (2006). Bacteriophages and biotechnology: vaccines, gene therapy and antibacterials. Trends Biotechnol. 24(5):212-218.

Clark., J.R., Bartley., K., Jepson., C.D., Craik V. and March J.B. (2011). Comparison of a bacteriophage-delivered DNA vaccine and a commercially available recombinant protein vaccine against hepatitis B. FEMS Immunol Med Microbiol 61 (2011) 197–204.

Cloud, S.S., Lillehoj H.S. and Rosenberger, J.K. (1992). Immune dysfunction following infection with chicken anaemia virus and infectious bursal disease virus. I. Kinetic alterations of avian lymphocytes subpopulations. Vet. Immunol. Immuno-Pathol. 34: 337– 352.

Cosset, F.L., Bouquet, J.F., Drynda, A., Chebloune, Y., Rey-Senelonge, A., Kohen, G., Nigon, V.M., Desmettre, P. and, Verdier, G. (1991). Newcastle disease virus (NDV) vaccine based on immunization with avian cells expressing the NDV hemagglutinin-neuraminidase glycoprotein. Virology. 185(2):862-6.

Coulibaly, F., Chevalier, C., Gutsche, I., Pous, J., Navaza, J., Bressanelli, S., Delmas, B. and Rey, F.A. (2005). The birnavirus crystal structure reveals structural relationships among icosahedral viruses. Cell 120:761-772.

Curslefen, D., Kaufer, I. and Becht, H. (1979). Loss of virulence in a small plaque mutant of the infectious bursal disease virus. Arch. Virol. 59: 39-46.

Daniel, L., Irene, S., Jose´, F. Rodrıguez., N., Verdaguer., D., ´ Garriga., C., San Martín, J., A. Velazquez-Muriel., B., L. Trus., Jose´, L. C. and Caston., R. (2007). Infectious bursal disease virus capsid assembly and maturation by structural rearrangements of a transient molecular switch. J. virol. 81(13): 6869–6878.

Daniel, O.O., Adebowale, I.A., Ibukunoluwa, O., Phyllis, E. and Oluwasanmi, A. (2014). occurrence of newcastle disease and infectious bursal disease virus antibodies in double-spurred francolins in nigeria. J. Vet. Med.; Article ID 106898, 5 pages.

Daral, J., Jackwood., S.E., Sommer-Wagner., T., Stoute., R., Woolcock., M., Crossley., K. and Bruce, R. C. (2009). Characteristics of a very virulent infectious bursal disease virus from California. Avian Dis. 53(4):592-600.

Darrell, R., Kapczynski., C., Afonso, C.L., and Patti, J.M. (2013). Immune responses of poultry to Newcastle disease virus. Devel. Comp. Immunol. 41:447–453.

Davis HL. (2000). CPG motifs for optimization of DNA vaccines. Dev. Biol. (Basel). 104:165-9.

Davis, H.L., Weeratna, R., Waldschmidt, T.J., Tygrett, L., Schorr, J. and Krieg, A.M. (1998). CPG DNA is a potent enhancer of specific immunity in mice immunized with recombinant hepatitis b surface antigen. J. Immunol. 160(2):870-6.

Delmas., B. Mundt., E and Gorbalenya A.E (2013). Birnavirus VP4 Processing Endopeptidase. Handbook of Proteolytic Enzymes. 3:3517–3523.

Dennis, R.B. (1995). Phage display. Immunotechnol. 1(2): 87-94.

Devlin, J.M., Vaz, P.K., Coppo, M.J.C. and, Browning, G.F. (2016). Impacts of poultry vaccination on viruses of wild bird. Curr. Opin. Virol. 9:23–9.

Dimitrov, K.M., Claudio, L.A., Qingzhong, Y. and Patti, J.M. (2017). Newcastle disease vaccines-A solved problem or a continuous challenge? Vet. Microbiol.; 206, 126-136.

Dimitrova., K.M., Afonsoa., C.L., Yub., Q. and Millera P.J. (2016). Newcastle disease vaccines-A solved problem or a continuous challenge? Vet. Microbiol.30:30.

Dietrich, G., Gentschev, I., Hess, J., Ulmer, J.B., Kaufmann, S.H. and Goebel, W. (1999). Delivery of DNA vaccines by attenuated intracellular bacteria. Immunol. Today. 20, 251-253.

Dudek, K., Bednarek, D., Siwicki, A. K., Rokita, E. and Studziński T. (2013). The effect of LPS injections on non-specific immune response in affected pigeons. Polish J. Vet. Sci. 16(4): 723–729.

Dudley, E.G. (2008). In vivo expression technology and signature-tagged mutagenesis screens for identifying mechanisms of survival of zoonotic foodborne pathogens. Foodborne Pathog Dis.5(4):473-85.

El-Mahdy, S., Manal, A. and Helal, A.M. (2013). Evaluation of live gumboro vaccine prepared from local variant strain for control of infectious bursal disease in Egypt. Vet. World. 6.18; EISSN: 2231-0916

Errington, W. and Emmerson, P.T. (1997). Assembly of recombinant Newcastle disease virus nucleocapsid protein into nucleocapsid-like structures is inhibited by the phosphoprotein. J. Gen. Virol. 78:2335-9.

© COPYRIGHT UPM

Eterradossi, N. and, Saif, Y.M. (2008). Infectious Bursal Disease. In: Saif YM, Fadly AM, Glisson JR, Mcdougald LR, Nolan LK, Swayne DE. Diseases of poultry. 12th ed. Ames: Iowa State University Press. 185-208.

Fahey, K.J., McWaters, P., Brown, M.A., Erny, K., Murphy, V.J., and Hewish, D.R. (1991a). Virus-neutralizing and passively protective monoclonal antibodies to infectious bursal disease virus of chickens. Avian Dis. 35:365–373.

Fahey, K.J., Chapman, A.J., Macreadie, I.G., Vaughan, P.R., McKern, N.M., Skicko, J.I., Ward, C.W. and Azad, A.A. (1991b). A recombinant subunit vaccine that protects progeny chickens from infectious bursal disease. Avian Pathol. 20: 447460.

Fahey, K.J., Erny, K. and Crooks, J. (1989). A conformational immunogen on VP2 of infectious bursal disease virus that induces virus-neutralizing antibodies that passively protect chickens. J. Gen. Virol. 70:1473–1481.

Fernandez-Gacio, A., Uguen, M. and Fastrez, J. (2003). Phage display as a tool for the directed evolution of enzymes. Trends Biotechnol. 21, 408–414.

Firouzamandi., M., Moeini., H., Hosseini., D., Hair Bejo., M., Omar., A.R., Mehrbod. P. and Ideris. A. (2016). Improved immunogenicity of Newcastle disease virus inactivated vaccine following DNA vaccination using Newcastle disease virus hemagglutinin-neuraminidase and fusion protein genes. J. Vet. Sci. 17(1): 21–26.

Fynan, E.F., Robinson, H.L. and Webster, R.G. (1993) Use of DNA encoding influenza from escherichia coli. J. Mol. Biol.45: 567-575.

Gao, W., Soloff, A.C., Lu, X., Montecalvo, A., Nguyen, D.C., Matsuoka, Y., Robbins, P. D., Swayne, D.E., Donis, R.O. and Other Authors (2006). Protection of mice and poultry from lethal H5N1 avian influenza virus through adenovirus-based immunization. J. Virol. 80: 1959–1964.

Gao, J., Wang, Y., Liu, Z. and Wang, Z. (2010). Phage display and its application in vaccine design. Annals of Microbiology. 60(1): 13–19.

Garrett, J., Fusselman, R., Hise, J., Chiou, L., Smith-Grillo, D., Schulz, J. and Young, R. (1981). Cell lysis by induction of cloned lambda lysis genes. Mol. Gen. Genet. 182(2):326-31.

Garriga, D.,Querol-Audi, J., Abaitua, F., Saugar, I.,Pous, J., Verdaguer, N., Caston, J. R. and Rodriguez, J. F. (2006). The 2.6-Angstrom structure of infectious bursal disease virus-derived T1 particles reveals new stabilizing elements of the virus capsid. J. Virol. 80:6895-6905.

George, P.C.S. and Fineran, P. C. (2015). A century of the phage: past, present and future. Nat. Rev. Microbiol. 13: 777–786.

Giambrone, J.J., Dormitorio, T. and Brown, T. (2001). Safety and efficacy of in ovo administration of infectious bursal disease viral vaccines. Avian Dis. 45: 144-148.

Gorski, A., Dabrowska, K., Switala-Jelen, K., Nowaczyk, M., Weber-Dabrowska, B., Boratynski, J., Wietrzyk, J. and Opolski, A. (2003) New insights into the possible role of bacteriophages in host defense and disease. Med. Immunol 2: 2–9.

Gray W.L. (2013). Recombinant Varicella-zoster virus vaccines as platforms for expression of foreign antigens. Adv. Virol. 2013, Article ID 219439, 8 Pages.

Grayson., P. and Molineux., I.J. (2007). Is phage DNA "injected" into cells - biologists and physicists can agree. Curr Opin Microbiol. 10 (4): 401–409.

Guittet, M., Le Coq, H., Picault, J.P., Eterradossi, N. and Bennejean, G. (1992). - Safety of infectious bursal disease vaccines: assessment of an acceptability threshold. Dev. biol. Standard.79: 147-152.

Guvenc, T., Hazıroglu, R., Yarım, M. and Tunca, R. (2004). Diagnosis of infectious bursal disease by immunoperoxidase technique. Vet. J. Ankara Univ. 51:237–238.

Hair-Bejo, M. (1992). An outbreak of infectious bursal disease in broilers. J. Vet. Malaysia. 4(2):168.

Hair-Bejo, M. (1994). Disease associated with subclinical infectious bursal disease in broilers. In ASEAN Seminar on Poultry and Their Control. Jan 16-21, 1994, APDRTC, Malaysia. .1-10.

Hamid, H., Campbell, R.S.F. and Lamichhan, C. (1990). The pathology of infection of chickens with the lentogenic V4 of NDV. Avian pathol.19: 687-696.

Hamzeh-Mivehroud., M., Akbar Alizadeh., A., Morris., M.B., and Dastmalchi, S. (2013). Phage display as a technology delivering on the promise of peptide drug discovery. Drug Discov. Tod.18: 23-24.

Haralambieva, I.H. and Poland, G.A (2010). Vaccinomics, predictive vaccinology and the future of vaccine development. Future Microbiol. 5:1757–1760.

Havarstein, L.S., Kalland, K.H., Christie, K.E. and Endresen, C. (1990). Sequence of the large double-stranded RNA segment of the N1 strain of infectious pancreatic necrosis virus: a comparison with other Birnaviridae. J. Gen. Virol. 71: 299– 308.

Hayes, S., Gamage, L.N. and, Hayes, C. (2010). Dual expression system for assembling phage lambda display particle (LDP) vaccine to porcine Circovirus 2 (PCV2). Vaccine. 28(41):6789–99.

© COPYRIGHT UPM

He, C.Q., Ma, L.Y., Wang, D., Li, G.R. and Ding, N.Z. (2009). Homologous recombination is apparent in infectious bursal disease virus. Virology 384, 51–58.

Heckert, R.A., Elankumaran, S., Oshop, G.L. and Vakharia, V.N. (2002). "A Novel transcutaneous plasmid-dimethylsulfoxide delivery technique for avian nucleic acid immunization". Vet. Immuno. Immunopath. 89:67-81.

Müller, H., Rafiqul Islam, B. and Raue, R. (2003). Research on infectious bursal disease—the past, the present and the future. Vet. Microbiol. 97:153–165.

Hoon, S., Takuo, N., Walter, D. and Sim, S. J. (1996). Immune responses in chickens against lipopolysaccharide of Escherichia coli and Salmonella typhimurium. Poult. Sci. 75: 342-5. 10.3382/Ps.0750342.

Hsieh, M. K., Wu. C. C. and Longlin T., (2010). DNA-mediated vaccination conferring protection against infectious bursal disease in broiler chickens in the presence of maternal antibody. Vaccine. 28 (23): 3936-3943. Http://Dx.Doi.Org/10.1155/2013/219439

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

Huang, Z., Elankumaran, S., Panda, A. and. Samal, S. K. (2003). Recombinant Newcastle disease virus as a vaccine vector. Poult. Sci. 82:899–906.

Hudson, P.J., McKern, N.M., Power, B.E. and Azad, A.A (1986). Genomic structure of the large RNA segment of infectious bursal disease virus. Nucleic Acid Res. 14:5001–5012.

Hulse, D.J. and Romero, C.H. (2004). Partial protection against infectious bursal disease virus through DNA-mediated vaccination with the VP2 capsid protein and chicken IL-2 genes. Vaccine 22: 1249-1259.

Ibu, O.J., Aba-Adulugba, A., Adeleke, M.A. and Tijjani, A.Y. (2000). "Activity of Newcastle disease and infectious bursal disease viruses in ducks and guinea fowls in Jos area, Nigeria," .Sokoto J. Vet. Science; 2, pp. 45–46.

Illyés, G., Kovács, K., Kocsis, B. and Baintner, K. (2008). Failure of oral E. coli O83 Lipopolysaccharide to influence intestinal morphology and cell proliferation in rats: Short Communication. Acta. Vet. Hung. 56: 1-3.

Inagawa, H. Kobayashi, Y. Kohchi, C. Zhang, R., Yasuhiro Shibasaki, Y. Soma, G.I. (2016). Primed activation of macrophages by oral administration of lipopolysaccharide derived from Pantoea agglomerans. In Vivo. 30(3):205-11

Inal, J.M. (2003). Phage therapy: a reappraisal of bacteriophages as antibiotics. Arch. Immunol. Ther. Exp.51(4):237-244.

© COPYRIGHT UPM

Inchley, C.J. (1969). The activity of mouse Kupffer cells following intravenous injection of T4 bacteriophage. Clin. Exp. Immunol. 5:173–87.

Islam, M.R., Zierenberg, K., Raue, R. and Muller, H. (2001)., Molecular cloning and sequencing of a Bangladeshi strain of very virulent infectious bursal disease virus and its adaptation in tissue culture by site-directed mutagenesis. In Proceedings of the II. International Symposium on Infectious Bursal Disease and Chicken Anaemia (pp. 3039). Rauischholzhausen, Germany.

Jackwood, D.K., Saif, Y.M. and Hughes, J.H. (1982). Characteristics and serologic studies of two serotypes of infectious bursal disease virus in turkeys. Avian Dis. 26:871-82.

Jackwood, D.J. and Jackwood, R.J. (1994). Infectious bursal disease viruses: molecular differentiation of antigenic subtypes among serotype 1 viruses. Avian Dis.;38(3):531-7.

Jazayeria, S.D., Ideris, A., Zakaria, Z., Yeap, S.K. , Omar, A.R..(2012). Improved immune responses against avian influenza virus following oral vaccination of chickens with HA DNA vaccine using attenuated Salmonella typhimurium as carrier. Comp. Immunol. Microbiol. Infect. Dis. 35: 417– 427.

Jepson, C.D. and March J.B. (2004). Bacteriophage lambda is a highly stable DNA Vaccine Delivery Vehicle. Vaccine. 22: 2413–2419.

Jepson. C.D. and March. J.B. (2004). Bacteriophage lambda is a highly stable DNA vaccine delivery vehicle. Vaccine 22:2413–2419.

Jeurissen, S.H., Janse, E. M., Lehrbach, P.R., Haddad, E.E., Avakian, T A. and Whitfillt, C.E. (1998). The working mechanism of an immune complex vaccine that protects chickens against infectious bursal disease. Immunology. 95: 494-500.

Jiang, Y., Yu, K., Zhang, H., Zhang, P., Li, C., Tian, G., Li, Y., Wang, X., Ge, J., Bu, Z. and Chen, H. (2007) Enhanced protective efficacy of H5 subtype avian influenza DNA vaccine with codon optimized HA gene in a Pcaggs plasmid vector. Antiviral Res. 75: 234-241.

Jinying, G., Guohua, D., Zhiyuan, W., Guobing, T., Yong, W., Jianzhong, S., Xijun, W., Yanbing, L., Sen, H., Yongping, J., Chinglai, Y., Kangzhen, Y., Zhigao, B. and Hualan, C. (2007). Newcastle disease virus-based live attenuated vaccine completely protects chickens and mice from lethal challenge of homologous and heterologous H5N1 Avian Influenza Viruses. J. of virol.; 81,1, p. 150–158.

Johnson, P.V., Blair, B.M., Zeller, S., Kotton, C.N. and Hohmann, E.L. (2011). Attenuated Listeria monocytogenes vaccine vectors expressing influenza A nucleoprotein: Preclinical evaluation and oral inoculation of volunteers. Microbiol. Immunol .55: 304–317.

© COPYRIGHT UPM

Kai, Z., Chen, G., Shi, G., Gao, T., Li, W. Zhao, Y., Zhang, F., Wu, J., Cui, X. and Wang F. (2012). Preparation and efficacy of a live Newcastle disease virus vaccine encapsulated in chitosan nanoparticles. Plos One. 7(12) e53314.

Kapczynski, D. R., Claudio, L.A. and Miller P.J. (2013). Immune responses of poultry to Newcastle disease virus. Devel. Comp. Immunol. 41(3): 447-453.

Kaufer, I. and Weiss, E. (1980). Significance of bursa of Fabricius as target organ in infectious bursal disease. Inject. Immun. 27: 364-367.

Kaur, T., Nafissi, N, Wasfi, O., Sheldon, K., Wettig, S. and Slavcev, R. (2012). Immunocompatibility of bacteriophages as nanomedicines. J. Nanotechnolog. Article ID 247427, 13 pages.

Keller, R. and Engley, F.B. (1958). Fate of bacteriophage particles introduced into mice by various routes. Proc Soc Exp Biol Med. 98:577–80.

Kennedy., R.B. and Poland, G.A. (2011). The top five "game changers" in vaccinology: Toward Rational and Directed Vaccine Development. OMICS. 15(9): 533–537.

Khan., M.S., Gowda, D.V. and Hosakote, G.S. (2011). advancement in vaccinology: New era in formulation strategies. Curr Drug Ther. 6:152-159.

Kibenge, F.S.B., Qian, B., Cleghorn, J.R. and Martin, C.K. (1997). Infectious bursal disease virus polyprotein processing does not involve cellular proteases. Arch. Virol.142: 2401–2419.

Kim, I.J., Karaca, K., Pertile, T.L., Erickson, S.A. and Sharma, J.M. (1998). Enhanced expression of cytokine genes in spleen macro-phages during acute infection with infectious bursal disease virus in chickens. Vet. Immunol. Immunopatho.61:331– 341.

Kim, S. and Samal, S.K. (2016). Newcastle disease virus as a vaccine vector for development of human and veterinary vaccines. Viruses. 8:183.

Klaus, G.G.B. and Humphrey, J.H. (1986) A re-evaluation of the role of C3 in B-cell activation. Imunol Toda.7: 163.

Klaus, G.G.B., Humphrey, J.H., Kunkl, A. and Dongworth, D.W. (1980). The follicular dendritic cell: its role in antigen presentation in the generation of immunological memory. Immunol Rev. 53:1.

Kobayashi, Y., Inagawa, H., Kohchi, C., Okazaki, K., Zhang, R., Kobara, H., Masaki, T. and Soma, G.I. (2017). Lipopolysaccharides derived from Pantoea agglomerans can promote the phagocytic activity of amyloid β in mouse microglial cells. Anticancer Res. 37(7):3917-3920.

© COPYRIGHT UPM

Kodihalli, S., Haynes, J.R., Robinson, H.L. and Webster, R.G. (1997) Cross-protection among lethal H5N2 influenza viruses induced by DNA vaccine to the hemagglutinin. J. Virol. 71: 3391-3396.

Kodihalli, S., Kobasa, D.L. and Webster, R.G. (2000) Strategies for inducing protection against avian influenza A virus subtypes with DNA vaccines. Vaccine. 18: 2592- 2599.

Kogut, M.H, Swaggerty, C., He H., Pevzner, I. and Kaiser, P. (2006) Toll-like receptor agonists stimulate differential functional activation and cytokine and chemokine gene expression in heterophils isolated from chickens with differential innate responses. Microb. Infect. 8: 1866–1874. Doi:10.1016/J.Micinf.02.026.

Komori, T., Saito, K., Sawa, N., Shibasaki, Y., Kohchi, C., Soma, G.I. and Inagawa, H. (2015). Innate immunity activated by oral administration of LPS is phylogenetically preserved and developed in broiler chickens. Anticancer. 35 (8): 4461-4466.

Kretzschmar., T. and von Rüden., T. (2002). Antibody discovery: phage display. Curr. Opin. Biotechnol.13(6):598-602.

Krieg, A.M., Yi, A.K., Matson, S., Waldschmidt, T.J., Bishop, G.A., Teasdale, R., Koretzky, G.A. and Klinman, D.M. (1995). CpG motifs in bacterial DNA trigger direct B-cell activation. Nature.374(6522):546–9.

Kumar, K., Singh, K.C. and Prasad, C.B. (2000). Immune responses to intermediate strain IBD vaccine at different levels of maternal antibody in broiler chickens. Trop. Animal Health Product.32:357-360.

Kumar., S. Nayak., B., Collins., P.L. and Samal, S.K. (2007). Evaluation of the Newcastle disease virus F and HN proteins in protective immunity by using a recombinant avian paramyxovirus type 3 vector in chickens. J. Virol. 85(13): 6521–6534.

Kurup D., Wirblich C., Feldmann H., Marzi A., Schnell, M.J. (2015). Rhabdoviral-based vaccine platforms against henipaviruses. J. Virol. 89:144–154.

Kutter, E. and Sulakvelidze, A. (2005). Bacteriophages biology and applications. CRC Press (2005).

Lamb, R. A., and Parks, G. D. (2007). *Paramyxoviridae*: the viruses and their replication, p. 1449-1496. *In* D. M. Knipe and P. M. Howley (ed), Fields virology, 5th ed. Wolters Kluwer-Lippincott Williams and Wilkins, Philadelphia, PA.

Lamb, R.A. and Kolakofsky, D. (2001). Paramyxoviridae: the viruses and their replication. In Knipe,D.M. and Howley,P.M. (eds), *Fields Virology*, 4th edn. Lippincott, Williams and Wilkins, Philadelphia.

© COPYRIGHT UPM

Lamb, R.A. and, Jardetzky, T.S. (2007). Structural basis of viral invasion: lessons from paramyxovirus F. Curr Opin Struct Biol.17(4):427-36.

Lasher, H.N. and Shane, S.M. (1994). Infectious bursal disease. World Poultry Sci. J. 50(2): 133-166.

Lauer, K.B., Borrow, R. and Blanchard, T.J. (2016). Multivalent and multipathogen viral vector vaccines. Clin. Vaccine Immunol .24(1): E00298-16.

Leitner, W.W., Ying, H. and Restifo, N.P. (1999). DNA and RNA-based vaccines: principles, progress and prospects. Vaccine 18, 765-777

Levine, M. M., Galen, J., Barry, E., Noriega, F., Tacket, C., Sztein, M., Chatfield, S., Dougan, G., Losonsky, G. and Kotloff, K. (1997). Attenuated Salmonella typhi and shigella as live oral vaccines and as live vectors. Behring. Inst. Mitt. 98: 120–123.

Lewis, P.J. and Babiuk, L.A. (1992). DNA vaccines: a review. Adv. Virus Res. 54:129–88.

Liang, H., Nishioka, Y., Reich, CF., Pisetsky, DS. and Lipsky, PE. (1996) activation of human B cells by phosphorothioate oligodeoxynucleotides. J. Clin. Invest. 98: 1119–1129.

Liu, Y., Wei, Y., Wu, X. and Yu, L. (2005). Preparation of ChIL-2 and VP2 fusion protein by baculovirus expression system. Cell. Mol. Immunol.2: 231-235.

Loke, C.F., Omar, A.R., Raha, A.R. and 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. 106:259-67.

Lombardo, E., Maraver, A., Casten J.R., Rivera, J., Fernandez-Arias, A., Serrano, A., Carrascosa, J.L. and Rodriguez, J.F. (1999). VP1, the putative RNA-dependent RNA polymerase of infectious bursal disease virus, forms complexes with the capsid protein VP3, leading to efficient encapsidation into virus-like particles. J. Virol. 73: 6973–6983.

Lu., A., Diao., Y., Chen., H., Wang., J., Ge., P., Sun., X. and Hao, D. (2014). Evaluation of histopathological changes, viral load and immune function of domestic geese infected with Newcastle disease virus. Avian Pathol. 43, Iss. 4.

Lüschow, D., Werner, O., Mettenleiter, TC. and Fuchs, W. (2001) Protection of chickens from lethal avian influenza A virus infection by live-virus vaccination with infectious laryngotracheitis virus recombinants expressing the hemagglutinin (H5) gene. Vaccine.19(30):4249-59.

© COPYRIGHT UPM

Mackowiak, M., Maki, J, Motes-Kreimeyer, L., Harbin, T. and van Kampen, K. (1999). Vaccination of wildlife against rabies: successful use of a vectored vaccine obtained by recombinant technology. Adv. Vet. Med. 41:571–583.

Macreadie, I.G., Vaughan, P.R., Chapman, A.J., McKern, N.M., Jagadish, M.N., Heine, H.G., Ward, C.W., Fahey, K.J. and Azad, A.A. (1990). Passive protection against infectious bursal disease virus by viral VP2 expressed in yeast. Vaccine. 8:549-52.

Mahgoub, H.A., Bailey, M. and Kaiser, P. (2012). An overview of infectious bursal disease. Arch. Virol. 157(11):2047-57.

Malanche`Re-Bres, E., Payette, P.J., Mancini, M., Tiollais, P., Davis, H.L. and Michel, M.L. (2001) CPG oligonucleotides with hepatitis B surface antigen (HBsAg) for vaccination in HBsAg-transgenic mice. J Virol. 75: 6482–6491.

Martha, R.J., Andrew, D.M., Andrey, V. L. and Shaun, H. (2011). Phages in nature. Bacteriophage. 1(1): 31–45.

Mason, KA., Ariga, H., Neal, R., Valdecanas, D., Hunter, N., Krieg, A.M., Whisnant, J.K. and Milas, L. (2005). Targeting toll-like receptor 9 with CpG oligodeoxynucleotides enhances tumor response to fractionated radiotherapy. Clin Cancer Res. 11(1):361–9.

Mateen, I. and Saba, I. (2011). A Review on DNA Vaccines. J. Health Science.; 1(1): 1-7.

Mayo, M.A. (2002). A summary of taxonomic changes recently approved by ICTV. Arch. Virol. 147:1655–1663.

Mccluskiea, M.J., Weeratnaa, R.D., Krieg A.M. and Davis, H. L. (2000). CPG DNA is an effective oral adjuvant to protein antigens in mice. Vaccine .19: 950-957.

McFerran, J.B. (1993). Infectious bursal disease. In J.B. McFerran and M.S. McNulty (Eds.), virus infections of birds (pp. 213–228). Amsterdam: Elsevier Science Publishers B.V.

McFerran, J.B. (1993). Infectious bursal disease. In Vims infections of birds (J.B. McFerran and M.S. McNulty, eds). Elsevier Science, Amsterdam, 213-228.

McFerran, J.B., McNulty, M.S., McKillop, E.R., Connor, T.J., McCracken, R.M., Collins, D.S. and Allan, G.M. (1980). Isolation and serological studies with infectious bursal disease virus from fowl, turkeys and ducks: demonstration of a second serotype. Avian Pathol.9:395-403.

Meeusen, E., Walker, J., Peters, A., Pastoret, P. and Jungersen, G. (2007). Current status of veterinary vaccines. Clin. Microbiol. Rev.20 (3) :489–510.

Merril, C.R., Geier, M.R. and Petricciani, J.C. (1971). Bacterial virus gene expression in human cells. Nature. 233: 398–400.

© COPYRIGHT UPM

Michailidis, G., Anastasiadou, M., Guibert, E. and Froment, P. (2014). activation of innate immune system in response to lipopolysaccharide in chicken sertoli cells. Reprod.148: 259-270.

Miedzybrodzki, R., Fortuna, W., Weber-Dabrowska, B. and Gorski, A. (2005) Bacterial viruses against viruses pathogenic for man? Virus Res. 110: 1–8.

Miller, P.J. and Koch, G (2013). Newcastle disease. In: Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V. (Eds.), Diseases of Poultry. Wiley-Blackwell, Hoboken, New Jersey, pp. 89–138.

Miller, P.J., King, D.J., Afonso, C.L. and, Suarez, D.L. (2007). Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge. Vaccine. 25:7238–46.

Mohammadamin, O. G. and Qubih, T. S (2011). Histopathology of virulent Newcastle disease virus in immune broiler chickens treated with IMBO®. Iraqi J. Vet. Scie. 25(1):9-13.

Molineux I.J. and and Panja, D. (2013). Popping the cork: mechanisms of phage genome ejection. Nature Rev.Microbiol. 11: 194-204.

Moraes, H.L.S., Salle, C.T.P., Nascimento, V.P., Salle, F.O., Rocha, A.C.G.P., Souza, G.F., Furian, T.Q. and Artencio, J.O. (2005). Infectious bursal disease: evaluation of maternal immunity and protection by vaccination of one-day old chicks against challenge with a very virulent virus isolate. Rev. Bras. Scie. Avic. 7 (1):51-57.

Morgan, R.W., Gelb, J. Jr., Schreurs, C.S., Lütticken, D., Rosenberger, J.K. and Sondermeijer, P.J. (1992). Protection of chickens from Newcastle and Marek's diseases with a recombinant herpesvirus of turkey's vaccine expressing the Newcastle disease virus fusion protein. Avian Dis.36 (4):858-70.

Mosley., Y.C., Wu., C. and Long T. (2015). Avian viral vector vaccines for infectious bursal disease. Taiwan Vet. J. 41: 153.

Müller, H. and Nitschke, R. (1989). The two segments of the infectious bursal disease virus genome are circularized by a 90,000-Da protein. Virology. 159(1):174–177.

Müller, H. (1986). Replication of infectious bursal disease virus in lymphoid cells. Arch. Virol. 87:191–203.

Müller, H., Mundt, E., Eterradossi, N. and Islam, M.R. (2012). Current status of vaccines against infectious bursal disease. Avian Pathol. 41(2):133–9.

Müller, H., Rafiqul, I.M. and Rüdiger, R. (2003). Research on infectious bursal disease-the past, the present and the future. Vet. Microbiol.97:153–165.

Muller, H., Schnitzler, D., Bernstein, F., Becht, H., Cornelissen, D. and Lutticken, D.H. (1992). Infectious bursal disease of poultry: antigenic structure of the virus and control. Vet. Microbiol. 33:175-183.

Mundt, E. and Muller, H. (1995). Complete nucleotide sequences of 5'- and 3'-noncoding regions of both genome segments of different strains of infectious bursal disease virus. Virology. 209: 10-18.

Mundt, E., Beyer, J. and Muller, H. (1995). Identification of a novel viral protein in infectious bursal disease virus-infected cells. J. Gen. Virol.76: 437– 443.

Mundt, E., Kollner, B. and Kretzschmar, D. (1997). VP5 of infectious bursal disease virus is not essential for viral replication in cell culture. J. Virol. 71: 5647–5651.

Murakami, M., Tsubata, T., Shinkura, R., Nisitani, S., Okamoto, M., Yoshioka, H., Usui, T., Miyawaki, S. and Honjo, T. (1994) Oral administration of lipopolysaccharides activates B-1 cells in the peritoneal cavity and lamina propria of the gut and induces autoimmune symptoms in an autoantibody transgenic mouse. J. Exp. Med. 180: 111-121.

Nagai, Y., Hamaguchi, M. and Toyoda, T. (1989). Molecular biology of Newcastle disease virus. Prog Vet. Microbiol. Immunol. 5 (1):16-64.

Nagy, E., Krell, P.J., Dulac, G.C. and Derbyshire, J.B. (1991). Vaccination against Newcastle disease with a recombinant baculovirus hemagglutinin-neuraminidase subunit vaccine. Avian Dis. 35 (3):585-90.

Narjes, J. and Saeid, A. (2015). Phage particles as vaccine delivery vehicles: concepts, applications and prospects. Asian Pac. J. Cancer Prev.16 (18):8019-8029.

Nascimento, I.P. and Leite, L.C.C. (2012). Recombinant vaccines and the development of new vaccine strategies. Braz. J. Med. Biol. Res. 45(12): 1102–1111.

Noor, M. (2009). Development of infectious bursal disease virus vaccine candidates by reverse genetics. PhD Thesis, Bangladesh Agricultural University, Mymensingh (Bangladesh).

Nunoya, T., Otaki, Y., Tajima, M., Hiraga, M. and Saito, T. (1992). Occurrence of acute infectious bursal disease with high mortality in Japan and pathogenicity of field isolates in SPF chickens. Avian Dis. 36: 597– 609.

Ogawa, M., Wakuda, T., Yamaguchi, T., Murata, K., Detiyono, A., Fukushi, H. and Hirai H. (1998). Seroprevalence of infectious bursal disease virus in free-living wild birds in Japan. J. Vet. Med. Sci. 60:1277–1279.

OIE Terrestrial Manual (Alexander 2012). Newcastle disease (infection with Newcastle disease virus). Chapter 2.3.14. :555-574.

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Oketani, K., Inoue, T. and Murakami, M. (2001). Effect of E3040, an inhibitor of 5-lipoxygenase and thromboxane synthase, on rat bowel damage induced by lipopolysaccharide. Eur. J. Pharmacol. 427: 159-166.

Omid, T. (2013). Expression and characterization of infectious bursal disease virus protein for poultry vaccine development and application in nanotechnology. PhD dissertation.

Oppling, V., Muller, H. and Becht, H. (1991). Heterogeneity of the antigenic site responsible for the induction of neutralizing antibodies in infectious bursal disease virus. Arch.Virol. 119:211– 223.

Oshop, G.L., Elankumaran, S., Vakharia, V.N. and Heckert, R.A. (2003). In ovo delivery of DNA to the avian embryo. Vaccine. 21:1275-1281.

Palya V., Kiss, T., Tatár-Kis, T., Mató, B. Felföldi and Y. Gardin (2012). Advancement in vaccination against Newcastle disease: Recombinant HVT NDV provides high clinical protection and reduces challenge virus shedding with the absence of vaccine reactions. Avian Dis. 56(2):282-287.

Park, J.H., Sung, H.W., Yoon, B.I. and Kwon, H.M. (2009). Protection of chicken against very virulent IBDV provided by in ovo priming with DNA vaccine and boosting with killed vaccine and the adjuvant effects of plasmid-encoded chicken interleukin-2 and interferon gamma. J. Vet. Sci.10:131-139.

Perozo, F., Villegas, P., Estevez, C., Alvarado, I. and Purvis, L. (2007). A recombinant avian adeno-associated virus as a vector for infectious bursal disease vaccination. Rev. Cient. (Maracaibo) 17:423–434.

Phong, S.F., Hair-Bejo, M., Omar, A.R. and Idiris A. (2003). Sequence analysis of Malaysian infectious bursal disease virus isolate and the use of reverse transcriptase nested polymerase chain reaction enzyme-linked immunosorbent assay for the detection of VP2 hypervariable region. Avian Dis 47:154–162.

Pitcovski, J., Gutter, B., Gallili, G., Goldway, M., Perelman, B., Gross, G., Krispel, S., Barbakov, M. and Michael, A. (2003). Development and largescale use of recombinant VP2 vaccine for the prevention of infectious bursal disease of chickens. Vaccine 21:4736–4743.

Qin., Y. and Zheng, S.J. (2017). Infectious bursal disease virus-host interactions: Multifunctional viral proteins that perform multiple and differing jobs. Int. J. Mol. Sci. 18(1): 161.

Rajawat, Y.S., Sundaresan, N.R., Ravindra, P.V., Kantaraja, C., Ratta, B., Sudhagar, M., Rai, A., Saxena, V.K., Palia, S.K. and Tiwari, A.K. (2008). Immune responses induced by DNA vaccines encoding Newcastle virus haemagglutinin and/or fusion proteins in maternal antibody-positive commercial broiler chicken. Br. Poult. Sci. 49(2):111-7.

Rautenschlein, S., Kraemer, C., Vanmarcke, J. and Montiel, E. (2005). Protective efficacy of intermediate and intermediate plus infectious bursal disease virus (IBDV) vaccines against very virulent IBDV in commercial broilers. Avian Dis.49:231-237.

Rautenschlein, S., Yeh, H.Y. and Sharma, J.M. (2002). The role of T cells in protection by an inactivated infectious bursal disease virus vaccine. Vet. Immunol. Immunopathol. 89: 159-167.

Rautenschlein, S.and Alkie, T.N. (1982). Infectious bursal disease virus in poultry: current status and future prospects. Veterinary Medicine: Research and Reports, Dove press, volume 2016 pages 9-18.

Ren, Z.J. and, Black, L.W. (1998). Phage T4 SOC and HOC display of biologically active, full-length proteins on the viral capsid. Gene. 215: 439-444.

Ribot, W.J., Powell, B.S., Ivins, B.E., Little, S.F., Johnson, W.M., Hoover, T.A., Norris, S.L., Adamovicz, J.J., Friedlander, A.M. and Andrews, G.P. (2006). Comparative vaccine efficacy of different isoforms of recombinant protective antigen against Bacillus anthracis spore challenge in rabbits. Vaccine, 24; 24(17):3469-76.

Riedel, S. (2005). Edward Jenner and the history of smallpox and vaccination. Proc. Bayl Univ. Med. Cent. 18: 21–25.

Rimmelzwaan, G.F. and Sutter, G. (2009). Candidate influenza vaccines based on recombinant modified vaccinia virus Ankara. Exp. Rev. Vaccines. 8: 447–454.

Rinaudo, C., John, L., Rappuoli, R. and Seib, K.T. (2009). Vaccinology in the genome era. J. Clin. Invest. 119 (9): 2515–2525.

Robert Brasseur., L.L., Wemers., C., Meulemans.G. and Burny., A. (1988). Fusion (F) protein gene of Newcastle disease virus: Sequence and hydrophobicity comparative analysis between virulent and avirulent strains. Virus genes. 1:333-350.

Rodenberg, J., Sharma, J., Belzer, S.W., Nordgren, R.M. and Nagi, S. (1994). Flow cytometric analysis of B cell and T cell subpopulations in virus. Avian Dis. 38:16-21.

Rong, J., Jiang, T., Cheng, T., Shen, M., Du, Y., Li, S., Wang, S., Xu, B. and Fan, G. (2007). Large-scale manufacture and use of recombinant VP2 vaccine against infectious bursal disease in chickens. Vaccine. 25:7900-7908.

Rout, S.N. and Samal, S.K. (2008). The large polymerase protein is associated with the virulence of Newcastle disease virus. J. Virol. 82(16):7828-36.

Russell, P. (1988). Monoclonal antibodies in research, diagnosis and epizootiology of Newcastle disease. Kluwer Acedemic Press, Boston, MA.

Russell, P.H. and Ezeifeka, G.O. (1995). The Hitchner B1 strain of Newcastle disease virus induces high levels of IgA, IgG and IgM in newly hatched chicks. Vaccine. 113:61-66.

Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R and Swayne, D.E. (2005). Diseases of poultry, 11th ed., Ames: Iowa State University Press. pp 66–78.

Sakaguchi, M., Nakamura, H., Sonoda, K., Hamada, F. and Hirai, K. (1996). Protection of chickens from Newcastle disease by vaccination with a linear plasmid DNA expressing the F protein of Newcastle disease virus. Vaccine; 14(8):747-52.

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

Schijns, V.E.J.C., Sharma, J. and Tarpy, I. (2008). Practical aspects of poultry vaccination. In F. Davison, B. Kaspers and K.A. Schat (Eds.). Avian Immunology 1st edn (pp. 373393). London: Academic Press.

Schijns, V.E.J.C., van De Zande, S., Lupiani, B. and Reddy, S.M (2013). Practical aspects of poultry vaccination. In: Schat, K.A., Kaspers, B., Kaiser, P. (Eds.), Avian Immunology. Elsevier Science, pp. 345–362.

Schnitzler, D., Bernstein, F., Müller, H. and Becht, H. (1993). The genetic basis for the antigenicity of the VP2 protein of the infectious bursal disease virus. J. Gen. Virol. 74(8):1563–1571.

Schryvers, A.B., Schollaardt, T., Woods, D.E. Williams, K. and Bryan, LE. (1987). Efficacy of oral immunization with Pseudomonas aeruginosa lipopolysaccharide. Serodiag. Immunother. Infect. Dis.1: 379-392.

Sellaoui, S., Alloui, N., Mehenaoui, S. and Djaaba, S (2012). Evaluation of size and lesion scores of bursa cloacae in broiler flocks in Algeria. J. World's Poult. Res. 2:37–39.

Sharma.,J.M., Kim.,I.J., Rautenschlein.,S. and Yeh. H.Y. (2000). Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. Develop. Comp. Immunol. 24; 223-235.

Shata, M.T., Stevceva, L., Agwale, S., Lewis, G.K. and Hone, D.M. (2000). Recent advances with recombinant bacterial vaccine vectors. Mol. Med. Tod. 6(2):66-71.

Shaw, I. and Davison, T.F. (2000). Protection from IBDV-induced bursal damage by a recombinant fowl pox vaccine, fpIBD1, is dependent on the titre of challenge virus and chicken genotype. Vaccine. 18:3230–3241.

Sherwood, R.C. and Roger, W.H. (2015). Bacteriophage lambda: early pioneer and still relevant. Virology. 10: 310–330.

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Siegrist, C.A. (2008). Vaccine immunology. In: Plotkin S, Orenstein W, Offit P, editors. Vaccines. 5th ed. Philadelphia, PA: Elsevier Inc; 2008. p. 17–36.

Singh, J., Banga, H.S., Brar, R.S., Singh, N.D., Sodhi, S. and Leishangthem, G.D. (2015). Histopathological and immunohistochemical diagnosis of infectious bursal disease in poultry birds. Vet. World. 8(11): 1331–1339.

Skeeles, J.K., Slavik, M., Beasley, J.N., Brown, A.H., Meinecke, C.F., Maruca, S. and Welch, S. (1980). - An age-related coagulation disorder associated with experimental infection with infectious bursal disease virus. Am. J. vet. Res. 41 (9): 1458-1461.

Smith, G.P. and Scott, J.K. (1993). Libraries of peptides and proteins displayed on filamentous phage. Meth. Enzymol. 217:228-257.

Smith, L.L., Rebecca, B. and Patricia, L. (2014). Diagnostic Immunization with Bacteriophage ΦX174 in Patients with Common Variable Immunodeficiency /Hypogammaglobulinemia. Front. Immunol. 5: 410.

Snyder, D. B., Lana, D. P., Savage, P. K., Yancey, F. S., Mengel, S.A and Marquardt, W.W (1988). Differentiation of infectious bursal disease viruses directly from infected tissues with neutralizing monoclonal antibodies: evidence of a major antigenic shift in recent field isolates. Avian Dis. 32:535-539.

Stoyanov, C. T., Boscardin, S. B., Deroubaix, S., Barba-Spaeth, G., Franco, D., Nussenzweig, R. S., Nussenzweig, M. and Rice, C. M. (2010). immunogenicity and protective efficacy of a recombinant yellow fever vaccine against the murine malarial parasite Plasmodium yoelii. Vaccine. 28: 4644–4652.

Summers, W.C. (2001). Bacteriophage therapy. *Annu*. *Rev*. *Microbiol*. 55: 437-451.

Sun, H.L., Wang, Y.F., Tong, G.Z., Zhang, P.J., Miao, D.Y., Zhi, H.D., Wang, M. and Wang, M. (2008). Protection of chickens from Newcastle disease and infectious laryngotracheitis with a recombinant fowl pox virus co-expressing the F, HN genes of Newcastle disease virus and gB gene of infectious laryngotracheitis virus. Avian Dis. 52(1):111-7.

Swanson., K., Wen., X., George, P. L., Reay, G., Paterson R., Lamb, R.A. and Jardetzky, T.S. (2010). Structure of the Newcastle disease virus F protein in the post-fusion conformation. Virol.5: 402(2): 372–379.

Swayne, D.E. and King, D.J. (2003). Avian influenza and Newcastle disease. J. Am. Vet. Med. Assoc. 222 (11):1534–1540.

Synder, D.B., Vakharia, V.N., Mengel-Whereat, S.A., Edwards, G.H., Savage, P.K., Luttichen, D. and Goodwin, M.A. (1994). Active cross-protection induced by a recombinant baculovirus expressing chimeric infectious bursal disease virus structural protein. Avian Dis. 38:701-7.

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Szardenings, M. (2003). Phage display of random peptide libraries: Applications, limits, and potential. J. Recept. Signal Transduct. Res. 23:307-349.

Tacken, M.G., Rottier, P.J., Gielkens, A.L. and Peters, B.P (2000). Interactions in vivo between the proteins of infectious bursal disease virus: capsid protein VP3 interacts with the RNA-dependent RNA polymerase, VP1. J. Gen. Virol. 81: 209–218.

Takehiro, U., Okuda., K. and Shimada., M. (2014). developments in viral vector-based vaccines. Vaccines (Basel). 2(3): 624–641.

Tang, D., De Vit, M. and Johnston, S.A. (1992). Genetic immunization is a simple method for eliciting an immune response. Nature. 356:152–4.

Taniguchi, Y., Yoshioka, N., Nishizawa, T., Inagawa, H., Kohchi, C. and Soma, G. (2009). Utility and safety of LPS-based fermented flour extract as a macrophage activator. Anticancer Res 29: 859-864.

Tanimura, N., Tsukamoto, K., Nakamura, K., Narita, M. and Maeda, M. (1995). Association between pathogenicity of infectious bursal disease virus and viral antigen distribution detected by immunohistochemistry. *Avian Dis.*, 39:9-20.

Tanimura, N. and Sharma, J.M. (1997). Appearance of T cells in the bursa of Fabricius and cecal tonsils during the acute phase of infectious bursal disease vims infection in chickens. Avian Dis.41(3): 638-645.

Taylor, J., Edbauer, C., Rey-Senelonge, A., Bouquet, J.F., Norton, E., Goebel, S., Desmettre, P. and Paoletti, E. (1990). Newcastle disease virus fusion protein expressed in a fowl pox virus recombinant confers protection in chickens. J. Virol. 64(4):1441-50.

Tesfaheywet, Z., Hair-Bejo, M. and Rasedee, A. (2012). Hemorrhagic and Clotting Abnormalities in Infectious Bursal Disease in Specific-Pathogen-Free Chicks. World Appl. Sci. J. 16(8): 1123-1130.

-Tew, J.G., Phipps, R.P. and Mandel, T.E. (1980). The maintenance and regulation of the humoral immune response: persisting antigen and the role of follicular antigen-binding dendritic cells as accessory cells. Immunol. Reiv. 53:1-75.

Tobias, J., Holmgren, J., Hellman, M., Nygren, E., Lebens, M. and Svennerholm, A.M. (2010). over-expression of major colonization factors of enterotoxigenic Escherichia coli, alone or together, on nontoxigenic E. coli bacteria. Vaccine. 28: 6977–6984.

Tobias, J., Lebens, M., Bo Lin, I., Wiklund, G. and Svennerholm, A.M. (2008). Construction of non-toxic Escherichia coli and Vibrio cholerae strains expressing high and immunogenic levels of enterotoxigenic E. coli colonization factor I fimbriae. Vaccine. 26: 743–752.

Tsukamoto, K., Saito, S., Saeki, S., Sato, T., Tanimura, N., Isobe, T., Mase, M., Imada, T., Yuasa, N. and Yamaguchi, S. (2002). Complete, long-lasting protection against lethal infectious bursal disease virus challenge by a single vaccination with an avian herpesvirus vector expressing VP2 antigens. J. Virol. 76(11): 5637–5645.

Ulmer, J.B., Donnelly, J.J., Parker, S.E., Rhodes, G.H., Felgner, P.L., Dwarki, V.J., Stanislaw, H. Gromkowski., R., Randall, D., Corrille, M., Friedman., A., Linda, A., Karen, R. L., Martinez., D., Helen, C., John, W. S., Donna, L. Montgomery. and Margaret, A. (1993). Heterologous protection against influenza by injection of DNA encoding a viral protein. Science. 259:1745-1749.

Vakharia, P.J. and, Tao, Y.J. (2007). The structure of a birnavirus polymerase reveals a distinct active site topology. Proc. Natl. Acad. Sci. USA. 104(18):7385–7390.

Vakharia, V.N., Snyder, D.B., He, J., Edwards, G.H., Savage, P.K. and Mengel-Whereat, S.A. (1993). Infectious bursal disease virus structural proteins expressed in a baculovirus recombinant confer protection in chickens. J. Gen. Virol. 74: 1201-1206.

Vakharia, V.N., Synder, D.B., Luttichen, D., Mengel, S.A., Savage, P.K., Edwards, G.H. and Goodwin, M.A. (1994). Active and passive protection against variant and classic infectious bursal disease virus strains induced by baculovirus expressed structural protein. Vaccine. 12:452-6.

van den Berg, T.P. and Meulemans, G. (1991). Acute infectious bursal disease in poultry: Protection afforded by maternally derived antibodies and interference with live vaccination. Avian Pathol. 20 (3): 409-421.

van den Berg, T.P., Eterradossi, N., Toquin, D. and Meulemans, G. (2000). Infectious bursal disease (Gumboro disease) Rev. Sci. Tech. Off. Int. Epiz.19 (2): 527-543.

van den Berg, T.P., Gonze, M. and Meulemans, G. (1991). Acute infectious bursal disease in poultry: Isolation and characterisation of a highly virulent strain. Avian Pathol. 20:133–143.

van den Berg, T.P., Gonze, M., Morales, D. and Meulemans, G. (1996). Acute infectious bursal disease in poultry: Immunological and molecular basis of antigenicity of a highly virulent strain. Avian Pathol. 25:751–768.

Van Kampen K.R. (2001). recombinant vaccine technology in veterinary medicine. Vet. Clin. North Am. Small Anim. Pract. 31(3):535-8.

van Loon, A.A., de Haas, N., Zeyda, I. and Mundt, E. (2002). Alteration of amino acids in VP2 of very virulent infectious bursal disease virus results in tissue culture adaptation and attenuation in chickens. J. Gen. Virol. 83:121-129.

Vleugels, B., Ververken, C. and Goddeeris, B. M. (2002). Stimulatory effect of CPG sequences on humoral response in chickens. Poult. Sci. 81:1317–1321.

Wang, L.F. and Yu, M (2004). Epitope identification and discovery using phage display libraries: applications in vaccine development and diagnostics. Curr. Drug Targets. 5:1-15.

Wang, M.Y., Kuo, Y.Y., Lee, M.S., Doong, S.R., Ho, J.Y., Lee, L.H., (2000). Self-assembly of the infectious bursal disease virus capsid protein, rVP2, expressed in insect cells and purification of immunogenic chimeric rVP2H particles by immobilized metal-ion affinity chromatography. Biotechnol. Bioeng. 67:104–111.

Wang, X., Jiang, P., Deen, S., Wu, J., Liu, X., and Xu, J. (2003); Efficacy of DNA vaccines against infectious bursal disease virus in chickens enhanced by coadministration with CPG oligodeoxynucleotide, Avian Dis.47(4):1305-1312.

Wei, Y., Li, J., Zheng, J., Xu, H., Li, L. and Yu, L. (2006). Genetic reassortment of infectious bursal disease virus in nature. Biochem. Biophys. Res. Commun. 350: 277–287.

Westbury, H.A., Parsons, G. and Allan, W.H (1984). Comparison of the residual virulence of Newcastle disease vaccine strains V4, Hitchner B1 and La Sota. Austra. Vet. J. 61(2): 47–9.

White, J.M., Delos, S.E., Brecher, M. and Schornberg, K. (2010). Structures and mechanisms of viral membrane fusion proteins: multiple variations on a common theme. Crit. Rev. Biochem. Mol. Biol. 43(3):189-219.

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

Whitfill, C.E., Haddad, E.E., Ricks, C.A., Skeeles, J.K., Newberry, L.A., Beasley, J.N., Andrews, P.D., Thoma, J.A. and Wakenell, P.S. (1995). Determination of optimum formulation of a novel infectious bursal disease virus (IBDV) vaccine constructed by mixing bursal disease antibody with IBDV. Avian Dis. 39: 687–699.

Xu, X., Subbarao, K., Cox, N. and Guo, Y. (1999). Genetic characterization of the pathogenic influenza A/Goose/Guangdong/1/96 (H5N1) virus: similarity of its hemagglutinin gene to those of H5N1 viruses from the 1997 outbreaks in Hong Kong. Virology 261:15–19.

Yao, K., Goodwin, M.A. and Vakharia, V.N. (1998). Generation of a mutant infectious bursal disease virus that does not cause bursal lesions. J. Virol. 72: 2647–2654.

Zachary, S. C., Michael, K. G. and Susan, L. J. (2015). Chicken immune-related gene expression after stimulation with bacterial component. Animal Industry Report: AS 661, ASL R2999.

Zhang, J. and Gauger, P.C. (2014). Isolation of swine influenza virus in cell cultures and embryonated chicken eggs. Meth. Mol. Biol. 1161:265-76.

Zhang, Q.X., Chen, Y., Gao, L., Wu, G., Qin, L., Wang, Y., Ren, X., Gao, Y. and Gao, H. (2003). Mutations of residues 249 and 256 in VP2 are involved in the replication and virulence of infectious Bursal disease virus. PLoS ONE. 8, e70982.

Zorman-Rojs O., Barlic-Maganja D., Mitevski D., Lübke W. and Mundt E. (2003). Very virulent infectious bursal disease virus in Southeastern Europe. Avian Dis. 47(1):186-92.

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