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Preparation nanoparticles and polymer based mucosal inactivated vaccines against avian influenza (H5N1) and newcastle disease viruses

A Thesis Submitted By

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Abstract.

In this study, two formulations of mucosal bivalent vaccines for Avian Influenza (AIV-H5N1) and Newcastle disease virus (NDV- genotype VIId) were prepared based on the use of polymer (Gel02) and nanoparticles (IMS1313) adjuvants. Mucosal vaccination with the prepared vaccines through intraocular route in one or two doses (10 days' interval) in SPF chickens induced a significant up-regulation of IFNɣ, IL1-β and IL-6 genes in the vaccinated groups compared to the control non-vaccinated group. Also, enhanced lymphocytes proliferation was recorded specially in the chickens vaccinated with two doses of the prepared vaccines. Gel 02 – vaccinated groups demonstrated higher HI titers and protection percent ranged from 50% – 60% with different levels of virus shedding as measured by qRT-PCR assay. However, when these vaccines were used in a prime-boost protocol followed by the use of inactivated oil-based bivalent vaccine (ISA71), Gel 02/ inactivated-vaccinated chicken group induced 100% protection against NDV and AIV challenge with no virus shedding whereas IMS1313/inactivated-vaccinated chicken group induced 100% and 90% for NDV and AIV;

respectively with no shedding of the NDV-challenge virus and only there was shedding of the AIV-challenge virus on the 3rd day post challenge. Indeed, the use of gel 02 as mucosal adjuvant in a prime boost protocol with inactivated vaccine demonstrated the potentiality of polymers compared to nanoparticles adjuvants.

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This thesis is special dedication to…,

My great father,

My mum's soul,

My dear step- mother

My lovely sisters,

Nancy, Nesreen and Nehal

My beloved husband… Kareem

And

My lovey son…Ali

Thank you all for being supportive

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AI	*Avian Influenza*
AIV	*Avian influenza virus*
HPAIV	*Highly pathogenic Avian influenza virus*
ChIFN	*Chicken interferon*
ChIL	*Chicken interleukin*
CTL	*$CD8^+$ cytotoxic T-lymphocyte*
ECEs	*Embryonated chicken eggs*
EID_{50}	*Embryo infective dose 50 %*
ELISA	*Enzyme linked immunosorbent assay*
HA	*Haemagglutinin antigen*
HA test	*Hemagglutination Test*
HI	*Hemagglutination inhibition Test*
I/M	*Intra muscular*
I/O	*Intra ocular*
I/N	*Intranasal*
IBD	*Infectious bursal disease*
IFN-y	*Interferon gamma*
Ig	*Immonoglobulin*
IL-6	*Interleukin 6*
IU	*International unit*
min	*Minute*
Ml	*milliliter*
NA	*Neuraminidase antigen*
ND	*Newcastle Disease*
NDV	*Newcastle disease virus*
NDV	*Newcastle Disease Virus*
NP/HA	*Nucleoprotein/Hemagglutinin*
NS1	*The conserved non-structural protein of influenza A virus*
OIE	*The Office International des Epizooties*
PBMCs	*Peripheral Blood Mononuclear cells*
PBS	*Phosphate Buffered Saline*
PCR	*Polymerase Chain Reaction*
P.C	*Post-Challenge*
PV	*Post-Vaccination*
RBCs	*Red Blood Cells*
RNP	*Ribonucleoprotein*
qRT-PCR	*Quantitative reverse transcriptase PCR*
Sec	*second*
SPF	*Specific pathogen free*
temp	*temperature*
TLRs	*Toll like receptors*
VVND	*Very virulent Newcastle disease*
WPV	*Weeks post Vaccination*

1. Introduction

Avian influenza virus (AIV) is a member or *Orthomyxoviridae* family. Genetic traits and/or severity of the disease in poultry determine if the infection is classified as low pathogenic avian influenza (LPAI) or high pathogenic avian influenza (HPAI) ***(Lee et al., 2013).*** Infections with HPAI virus are identified as a serious threat to the poultry industry and can cause devastating economic losses due to mortality and morbidity rates that reach up to 100% ***(Kapczynski et al., 2015).*** Biosecurity practices, education about prevention, preventive culling, early diagnosis, and surveillance to detect the disease and infection are considered the main procedures to combat and prevent further infections and outbreaks ***(Kayali et al., 2016).*** However, this approach is not applicable in developing countries for several reasons ***(Peeters et al., 2014).*** Implementation of extensive vaccination programs in commercial poultry farms is used to help control AI virus and limit losses in areas where the virus is endemic ***(Lee et al., 2013).*** Inactivated, oil adjuvanted, whole virus vaccines represent the majority of the vaccines available for AIV. Nonetheless, poor quality vaccines and inappropriate application have led to vaccine failures in the field ***(Cattoli et al., 2011).*** The widespread and prolonged use of inactivated AI vaccines promotes the emergence of antigenic variants against which the vaccines are ineffective ***(Kim at al., 2010).*** Attenuated live influenza vaccines in poultry are not recommended due to the potential risk of assortment or mutations ***(Kim & Samal, 2017).*** In Egypt, despite regular vaccination of the suspected poultry species; HPAI H5N1 viruses have been endemic in poultry since 2008, following the first wave of the virus outbreak in 2006 ***(Kandeil el al., 2017).***

Newcastle disease virus (NDV) belongs to avian paramyxovirus type 1, which is a member of the genus Avulavirus of the *Paramyxoviridae* family ***(Miller & Koch, 2013; Dimitrov et al., 2016).***

NDV is as equally important as Avian influenza, owing to its highly contagious nature and worldwide distribution it is an economic burden ***(Ferreira et al., 2014; Kim and Samal, 2017).*** Based on the severity of the disease symptoms, NDVs strains have been classified as lentogenic (avirulent), mesogenic (intermediately virulent) and velogenic (virulent) ***(Miller & Koch, 2013)***. Velogenic strains of the virus are responsible for the sudden deaths of the fully susceptible chickens without major clinical signs. Commonly, NDV infection characterizes by respiratory, enteric and/ or neurological manifestations with high mortality rated that reaches 100% ***(Fentie et al., 2014; Wen et al., 2017).*** Moreover, vaccinated chickens may remain infected while showing no signs ***(Degefa et al., 2004).*** NDV isolates are classified into two classes (I and II) with different genotypes. Class I, comprises 9 genotypes that are avirulent and mainly affect wild birds, while Class II, which is currently divided into 18 genotypes (V, VI, VII, and XII through XVIII) with multiple sub-genotypes, including both virulent and avirulent isolates and can be isolated from wild and domestic birds ***(Dimitrov et al., 2016).*** In Egypt, the sub-genotype VIId is predominant since 2011 causing several ND outbreaks in poultry ***(Hussein et al., 2014).***

Control of Newcastle disease by vaccination is a common strategy in most countries where poultry are raised commercially and where the disease is endemic to keep the disease under control ***(Wen et al., 2017).*** However, several outbreaks were reported worldwide and in Egypt ***(Cattoli et al., 2010; Hussein et al., 2014)***, despite the fact that vaccination is widely applied and this indicates that the current vaccines and vaccination campaigns are not effective in preventing infection and transmission ***(Miller et al., 2010).***

An ideal vaccine against AIV and/or NDV is evaluated after experimental challenge by the prevention of mortality and morbidity and the reduction in the quantity of challenge virus shed from respiratory and digestive tracts, as well as the prevention of transmission of the virus between