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Preparation of Mucosal Inactivated Vaccine For ND And H9N2 AI Viruses

A Thesis Submitted By

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(Virology)
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2017

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2017

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Abstract.

The aim of the present study was to develop a mucosal inactivated vaccines for ND and H9N2 viruses to protect against these viruses at sites of infections through mucosal immunity. We prepared two new formulations for mucosal bivalent inactivated vaccine formulations for Newcastle and Avian Influenza (H9N2) based on the use of nanoparticles and polymer adjuvants. The prepared vaccines were delivered via intranasal and spray routes of administration in SPF chickens. Cell mediated and humeral immune responses were measured as well as challenge trial was carried out. In addition ISA71 water in oil was also evaluated.

Results showed that the use of spray route as vaccination delivery method of polymer and nanoparticles Montanide™ adjuvants revealed that it enhanced the cell mediated immune response as indicated by phagocytic activity, gamma interferon and interleukin 6 responses and induce protection against challenge with Newcastle and Avian Influenza (H9N2) viruses. Results after Challenge with velogenic NDV genotype VIId NDV, the protection % for groups received IMS1313 vaccine was 40 %. Groups received the Gel 01 vaccine revealed 50% and 60% for intranasal and spray route; respectively. On the other hand, the chickens vaccinated with ISA71 vaccine demonstrated 100% protection. No shedding was detected in samples from Groups which received Gel 01 and ISA71 vaccines in all interval days after challenge with H9N2.

In conclusion, the results of the present study demonstrate the potentiality of polymer compared to nanoparticles adjuvants when used via spray route. Mass application of such vaccines will be add value to improve the vaccination strategies against NDV and AIV viruses.

Acknowledgment

*I am greatly indebted in all my work and success to our merciful (**ALLAH**)*

*I would like to express my sincere gratitude for the kindness and encouragement to **Prof. DR. Hussein Ali Hussein.** Professor of Virology, Faculty of Veterinary Medicine, Cairo University, under whose supervision, guidance and criticism this work was carried out. I heartily thank him very much for the valuable help. As he gave me the best example what a university professor should be.*

*I express my sincere gratitude to his suggestions and advice given to me and the kind help for offering me much of his precious time and giving me the chance to carry out this work by **Prof. Dr. Mohamed Sayed Madkour.** Chief researcher at New Castle disease department, Serum and vaccine Research Institute, Abbsia , Cairo.*

*Deepest thanks should be applied to **prof Dr Sayed Zeidan** for his help and support.*

*I am highly indebted to all staff members and all my friends of the New Castle disease department, Veterinary serum and Vaccine research institute. Especially **Dr. Ahmed Aly And Dr. Mahmoud Abd El Monem** .*

*I give my sincere acknowledge to **Dr. Ahmed Erfan, Dr. Ahmed Samy** , **Dr. Heba Farouk** and **Dr. Mai Morsy** at NLQP , Animal Health Research Institute, Dokki ,Giza. For giving me hand whenever needed.*

Dedication

Dedicated to my family

…… my Father,

…… Mother

……. Sisters

……Brother

…… my beloved Son Malek

LIST OF CONTENT

LIST OF ABBREVIATION

AI	Avian Influenza
AIV	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	Haemagglutination Test
HBsAg	Hepatitis B surface antigen
HI	Haemagglutination inhibition Test
I/M	Intra muscular
I/N	Intranasal
IBD	Infectious bursal disease
IFN	Interferon
Ig	Immonoglobulin
IL	Interleukin
IU	International unit
min	Minute
Ml	milliliter
N	Number of samples
N or NA	Neuraminidase antigen
NAMRU-3	Naval Medical Research Unit No. 3
ND	Newcastle Disease
NDV	Newcastle disease virus
NDV	Newcastle Disease Virus
NF-kB	Nuclear factor pathway
NP/HA	Nucleoprotein/Hemagglutinin
NS1	The conserved non-structural protein of influenza A virus
OE	Oil emulsion
OIE	The Office International des Epizooties
P/M	Post-Mortem examination
PBMCs	Peripheral Blood Mononuclear cells
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PI	Post-Infection
PV	Post-Vaccination
RBCs	Red Blood Cells
RNP	Ribonucleoprotein
RRT-PCR	Real time RT-PCR
S/P Ratio	Sample/Positive Ratio
Sec	second
SPF	Specific pathogen free
temp	temperature
TLRs	Toll like receptors
VVND	Very virulent Newcastle disease
WPV	Weeks post Vaccination