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**Faculty of Veterinary Medicine**

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# **Preparation of Mucosal Inactivated Vaccine For ND And H9N2 AI Viruses**

**A Thesis Submitted By**

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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 VIIId 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.



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## *Dedication*

*Dedicated to my family*

*..... my Father,*

*..... Mother*

*..... Sisters*

*..... Brother*

*..... my beloved Son Malek*



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## **LIST OF ABBREVIATION**

<b>AI</b>	Avian Influenza
<b>AIV</b>	Avian influenza virus
<b>ChIFN</b>	Chicken interferon
<b>ChIL</b>	Chicken interleukin
<b>CTL</b>	CD8 <sup>+</sup> cytotoxic T-lymphocyte
<b>ECEs</b>	Embryonated chicken eggs
<b>EID<sub>50</sub></b>	Embryo infective dose 50 %
<b>ELISA</b>	Enzyme linked immunosorbent assay
<b>HA</b>	Haemagglutinin antigen
<b>HA test</b>	Haemagglutination Test
<b>HBsAg</b>	Hepatitis B surface antigen
<b>HI</b>	Haemagglutination inhibition Test
<b>I/M</b>	Intra muscular
<b>I/N</b>	Intranasal
<b>IBD</b>	Infectious bursal disease
<b>IFN</b>	Interferon
<b>Ig</b>	Immonoglobulin
<b>IL</b>	Interleukin
<b>IU</b>	International unit
<b>min</b>	Minute
<b>ml</b>	milliliter
<b>N</b>	Number of samples
<b>N or NA</b>	Neuraminidase antigen
<b>NAMRU-3</b>	Naval Medical Research Unit No. 3
<b>ND</b>	Newcastle Disease
<b>NDV</b>	Newcastle disease virus
<b>NDV</b>	Newcastle Disease Virus
<b>NF-<math>\kappa</math>B</b>	Nuclear factor pathway
<b>NP/HA</b>	Nucleoprotein/Hemagglutinin
<b>NS1</b>	The conserved non-structural protein of influenza A virus
<b>OE</b>	Oil emulsion
<b>OIE</b>	The Office International des Epizooties
<b>P/M</b>	Post-Mortem examination
<b>PBMCs</b>	Peripheral Blood Mononuclear cells
<b>PBS</b>	Phosphate Buffered Saline
<b>PCR</b>	Polymerase Chain Reaction
<b>PI</b>	Post-Infection
<b>PV</b>	Post-Vaccination
<b>RBCs</b>	Red Blood Cells
<b>RNP</b>	Ribonucleoprotein
<b>RRT-PCR</b>	Real time RT-PCR
<b>S/P Ratio</b>	Sample/Positive Ratio
<b>Sec</b>	second
<b>SPF</b>	Specific pathogen free
<b>temp</b>	temperature
<b>TLRs</b>	Toll like receptors
<b>VVND</b>	Very virulent Newcastle disease
<b>WPV</b>	Weeks post Vaccination