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# *Trials for preparation of a combined inactivated oil emulsion vaccine against avian influenza virus H9N2 strain and infectious bronchitis virus in chickens*

**A thesis presented by**
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# *LIST OF ABBREVIATIONS*

| Abbreviation | Complete word |
|---|---|
| **AGPT** | Agar Gel Preceptation Test |
| **AHRI** | Animal Health Research Institute |
| **AI** | Avian Influenza |
| **AIV** | Avian Influenza Virus |
| **BPL** | Beta Probiolactone |
| **CEK** | Chicken Embryo Kidney |
| **CPE** | Cytopathic effect |
| **CT** | Cycle Threshold |
| **CTLs** | Cytotoxic T-Lymphocyte |
| **ECE** | Embryonated Chicken Egg |
| **EDS** | Egg Drop Syndrome |
| **$EID_{50}$** | Embryo Infective Dose 50% |
| **ELISA** | Enzyme Linked Immuno-sorbant Assay |
| **EM** | Electron Microscope |
| **FAO** | Food Agriculture Organization |
| **FC** | Fowl Cholera |
| **HA** | Haemagglutinin |
| **HA Test** | Haemagglutination Test |
| **HAU** | Haemagglutination Unit |
| **HI or HAI** | Haegglutination Inhibition |
| **HPAI** | Highly Pathogenic Avian Influenza |
| **Hrs** | Hours |
| **HVR** | Hyper Variable Region |
| **IB** | Infectious Bronchitis |
| **IBV** | Infectious bronchitis virus |
| **IBD** | Infectious Bursal Disease |
| **IF** | Interferon |
| **IFA** | Imunoflourescence assay |
| **Ig** | Immunoglobulin |
| **IL** | Interleukin |
| **ILT** | Infectious Laryngeotrachitis |
| **ISA** | Incomplete SEPIC Adjuvant |
| **IU** | International Unit |
| **IZSVe** | Istituto Zooprofilattico Sperimentale delle Venezie |
| **LPAI** | Low Pathogenic Avian Influenza |
| **MABs** | Monoclonal antibodies |
| **Mass** | Massachusetts |
| **MBL** | Manos binding lectin |

| | |
|---|---|
| **MDCK** | Madin-Darby Canine Kidney |
| **MHC** | Major Histocomptability Complex |
| **min** | Minutes |
| **µL** | Microliter |
| **MPAI** | Mild Pathogenic Avian Influenza |
| **NA** | Neuraminidase |
| **NAI** | Notifiable Avian Influenza |
| **NAMRU-3** | Naval Medical Research Unit No. 3 |
| **ND** | Newcastle Disease |
| **NI** | Neutralization Index |
| **NK** | Natural Killer Cell |
| **NP** | Nucloprotein |
| **OE** | Oil Emulsion |
| **OIE** | The Office International des Epizooties |
| **ORFs** | Open reading Fram |
| **PAMPs** | Pathogen Associated Molecular Pattern |
| **PCR** | Polymerase Chain Reaction |
| **PI** | Post Inoculation or Post Infection |
| **P.M** | Post Mortum |
| **PRRs** | Pattern Recognition Receptors |
| **PV** | Post Vaccination |
| **RBCs** | Red Blood Cells |
| **RFLP** | Restriction fragment length polymorphism |
| **RNP** | Ribonucloprotein |
| **rpm** | Round Per Minute |
| **RT-PCR** | Real Time Polymerase Chain Reaction |
| **S1** | Spike glyoprotein 1 |
| **SAN** | Specific Antibodies Negative |
| **SNT** | Serum Neutralization Test |
| **SPF** | Specific Pathogen Free |
| **TCR** | T- Cell Receptor |
| **TLR** | Toll Like Receptor |
| **TOCs** | Tracheal organ cultures |
| **UK** | United Kindom |
| **USA** | United States Of America |
| **VN** | Virus Neutralization |
| **VNT** | Virus neutralization test |
| **VSVRI** | Veterinary Serum and Vaccine Research Institute |
| **WPV** | Week Post Vaccination |
| **W/O** | Water in Oil |
| **WHO** | World Health Organization |

# 7. Summary

Avian infectious bronchitis and Avian Influenza subtype H9N2 are from the most important diseases that affect chickens in all ages causing high economic losses. In this study, five different inactivated OE combined and monovallent vaccines were prepared against AIV H9N2 and/or IBV.

Avian influenza H9N2 (A\chicken\Egypt\D4692A\2012) and 3 strains of IBV (M41, H120 and Giza -291-F-2012 variant IBV strain) were propagated and titrated and used for preparation of 3 combined vaccine and 2 monovallent vaccines. Combined vaccine 1 contain AIV H9N2 and 3 strains of IBV (H120, M41 and variant IBV), Combined vaccine 2 contain AIV H9N2 and variant IBV only, combined vaccine 3 contain IBV (H120 and M41) and AIV H9N2, monovallent H9N2 and monovallent variant IBV vaccine by using Montanide ISA 70 oil as adjuvant.

The prepared vaccines were subjected to sterility and safety tests and revealed that they free from any bacterial or fungal growth with no local or systemic finding after double dose vaccination.
The immune response of the prepared vaccines was followed up in SPF chickens for 16 weeks.

HI test was carried out on serum samples that obtained from chickens that vaccinated by combined vaccine 1, 2, 3 (contain AIV H9N2) and monovallent AIV H9N2 vaccine, the results revealed that the antibody titer begin to appear from the 1$^{st}$ WPV and reach to its peak (9.8 $\log_2$) at

$5^{th}$ WPV in group (1) vaccinated by combined vaccine 1, while group (2) vaccinated by combined vaccine 2 showed peak antibody titer at $4^{th}$ WPV (10 $\log_2$). At $5^{th}$ WPV, the antibody titer peak was attained for both groups (3) (vaccinated by combined vaccine 3) and (4) (vaccinated by monovalent H9N2 vaccine) and it was 10.5 $\log_2$ and 8.6 $\log_2$ respectively.

ELISA test was carried out on serum samples obtained from groups vaccinated with combined and monovallent vaccines containing IBV viruses.

Group vaccinated by combined vaccine 1 and combined vaccine 2 showed peak of antibody titer at $12^{th}$ WPV (2314 and 1890 respectively), while group vaccinated by combined vaccine 3 showed increase in antibody titer till reach to its peak (2292) at $16^{th}$ WPV, but group vaccinated by monovallent IBV vaccine reached to peak of antibody titer (1772) at $12^{th}$ WPV.

Results of challenge test demonstrated that the vaccine protect chickens in percentage of 100% in combined vaccine 1 and combined vaccine 3 when challenged against H9N2 and IBV viruses and in percentage of 90% in combined vaccine 2 when challenged against IBV and monovallent H9N2 vaccine when challenged against H9N2, while the vaccine protect the chickens in percentage of 80% in monovallent IBV vaccine.

There were no viral shedding of AIV in groups vaccinated with combined vaccine 1 and 3 in days 2, 4 and 6 post challenge against AIV H9N2, while little viral shedding was observed in group 4 that vaccinated with monovallent H9N2 at days 2 and 4 and group vaccinated with combined vaccine 2 at day 2 post challenge against H9N2 with high viral shedding in control non vaccinated group.

Shedding of IBV was very little in group vaccinated with combined vaccine 2 at day 4 post challenge and in group vaccinated with monovallent IBV vaccine, while no viral shedding of IBV in other group at day 2, 4 and 6 post challenge except control non vaccinated group that showed high viral shedding.

# ***6.Conclusion***

From this study results, it could be concluded that, all of the five prepared vaccines proofed to be sterile, safe and potent. The combined vaccines gave higher immune response than monovalent ones. The combined vaccine 1 (that contained AIV H9N2 and three IBV strains, M41, H120 and variant) and combined vaccine 3 (that contained AIV H9N2 and two IBV strains, H120 and M41) were the most protective ones where it gave 100% protection against both variant IBV and AIV H9N2 challenge and no viral shedding compared to combined vaccine 2 (that contained AIV H9N2 and IBV variant strain).