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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 Preception 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
EID₅₀	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 Histocompatibility 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	Nucleoprotein
OE	Oil Emulsion
OIE	The Office International des Epizooties
ORFs	Open reading Frame
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	Ribonucleoprotein
rpm	Round Per Minute
RT-PCR	Real Time Polymerase Chain Reaction
S1	Spike glycoprotein 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 Kingdom
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 monovalent 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 monovalent 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, monovalent H9N2 and monovalent 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 monovalent AIV H9N2 vaccine, the results revealed that the antibody titer begin to appear from the 1st WPV and reach to its peak (9.8 log₂) at

5th WPV in group (1) vaccinated by combined vaccine 1, while group (2) vaccinated by combined vaccine 2 showed peak antibody titer at 4th WPV ($10 \log_2$). At 5th 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 monovalent vaccines containing IBV viruses.

Group vaccinated by combined vaccine 1 and combined vaccine 2 showed peak of antibody titer at 12th WPV (2314 and 1890 respectively), while group vaccinated by combined vaccine 3 showed increase in antibody titer till reach to its peak (2292) at 16th WPV, but group vaccinated by monovalent IBV vaccine reached to peak of antibody titer (1772) at 12th 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 monovalent H9N2 vaccine when challenged against H9N2, while the vaccine protect the chickens in percentage of 80% in monovalent 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 monovalent 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 monovalent 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).
