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Production of Anti-rabies Immunoglobulin in Chicken Egg Yolk (anti-rabies IgY)

A thesis submitted by

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Abstract

Rabies is the most serious and most feared infectious disease affecting human and animals. The currently available types of anti-rabies antibodies have many disadvantages. The present work provides an attempt to produce anti-rabies immunoglobulin in chicken egg yolk. In this study, a total number of 40 egg laying hens were involved where 30 birds were immunized with rabies vaccine and the remaining 10 birds were kept as a control group. Birds have received five doses of the vaccine; the first booster dose was given at one week post 1st immunization then birds were repeatedly boosted three times at two weeks intervals. Eggs of immunized hens were collected and used for extraction of anti-rabies IgY. Three methods of IgY extraction were used; chloroform, poly ethylene glycol (PEG) and chloroform-PEG methods. The three methods were compared in terms of purity and protein content, where chloroform method was found to give the highest protein yield followed by chloroform-PEG and then the PEG method. In SDS-PAGE analysis, PEG and chloroform-PEG methods were found to give better purity than chloroform method. By western blotting, the anti-chicken antibodies specifically detected the heavy and light chains of the IgY only confirming that the majority of the extracted proteins are IgY. By using ELISA, it was confirmed that the prepared IgY antibodies are specific to rabies virus, the anti-rabies antibodies were detected at two weeks post immunization and increased gradually by boosting. In vivo testing of the prepared antibodies revealed that the antibodies could neutralize the rabies virus infectivity in mice experimentally infected with challenge virus standard (CVS). At 9th week post immunization the antibodies could neutralized 100 LD₅₀ of CVS and protected 100% of inoculated mice. ELISA could be used for calculating antibodies titer instead of the currently used neutralization technique after calculating a correction factor between ELISA and neutralization.

Dedication

I dedicate this to my lovely family (mother, father, my sisters Jihan and Asmaa and my brother Mo'taz); Thanks for always being there for me, for your motivation and endless support, for your prayer for my success, for your patience throughout the long period of the research and your confidence and belief in my abilities throughout my life. Thank you, father and mother for teaching me self-confidence and to be a responsible person. God bless you all and give you good health and happiness.

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LIST OF ABBREVIATIONS

ARS	Anti-Rabies Serum
BSA	Bovine serum albumin
CCEEVs	Cell Culture Vaccines and Embryonated egg-based vaccines
CDC	Centers for Disease Control and Prevention
CNS	Central nervous system
CVS	Challenge virus standard
DAB	3,3'-diaminobenzidine
DRG	Dorsal root ganglion
ED₅₀	Median effective dose
ELISA	Enzyme linked immune sorbent assay
ERIG	Equine rabies immune globulin
FAT	Fluorescent antibody test
FBS	Fetal bovine serum
FCS	Fetal calf serum
FRV/K	Fixed rabies virus/Karamany
G	Glycoprotein
HDCV	Human diploid cell vaccine

List of abbreviations

HRIG	Human rabies immune globulin
HRP	Horse radish peroxidase
IgY	Immunoglobulin of yolk
ID	Intra dermal
IM	Intra muscular
L	Large protein (RNA Polymerase)
LD₅₀	Median lethal dose
M	Matrix protein
MEEREB	Middle East and Eastern Europe rabies Expert Bureau
mRNA	Messenger Ribonucleic Acid
N	Nucleoprotein
NFDM	Non-fat dry milk
NIBSC	National Institute for Biological Standards and Control
NIH	National Institutes of Health
OD	Optical density
P	Phosphoprotein
PBS	Phosphate buffered saline

List of abbreviations

PEG	Polyethylene glycol
PEP	Post-exposure prophylaxis
PD	Proportional distance
RIG	Rabies Immunoglobulin
RNA	Ribonucleic Acid
RNP	Ribonucleoprotein
RT	Room temperature
RV	Rabies virus
RVA	Rabies vaccine adsorbed
SC	Sub coetaneous
SD	Standard deviation,
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEA	Southeast Asia
TEMED	Tetramethylethylenediamine
TPC	Total protein content
TPI	Time post immunization
VACSERA	The Holding Company for Production of Vaccines, Sera and Drugs.
WHO	World health organization

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