

Cairo University  
Faculty of Veterinary Medicine  
Department of Virology

رئيس القسم  
د. محمد علي السيد  
6/1905

**“Comparative studies on some adjuvants used in  
production of inactivated antirabies vaccines”**

*A Thesis Presented  
BY*

**NAGLAA IBRAHIM ALY ABDALLA**

B. V. SC. FACULTY OF VETERINARY MEDICINE  
CAIRO UNIVERSITY

**FOR THE DEGREE OF  
MASTER OF VETERINARY SCIENCE  
VIROLOGY**

636.0896  
N. 4

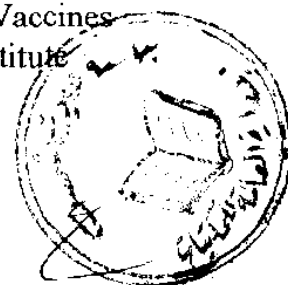
Under The Supervision  
Of

**Prof. Dr. Mohamed S. Sabeh**

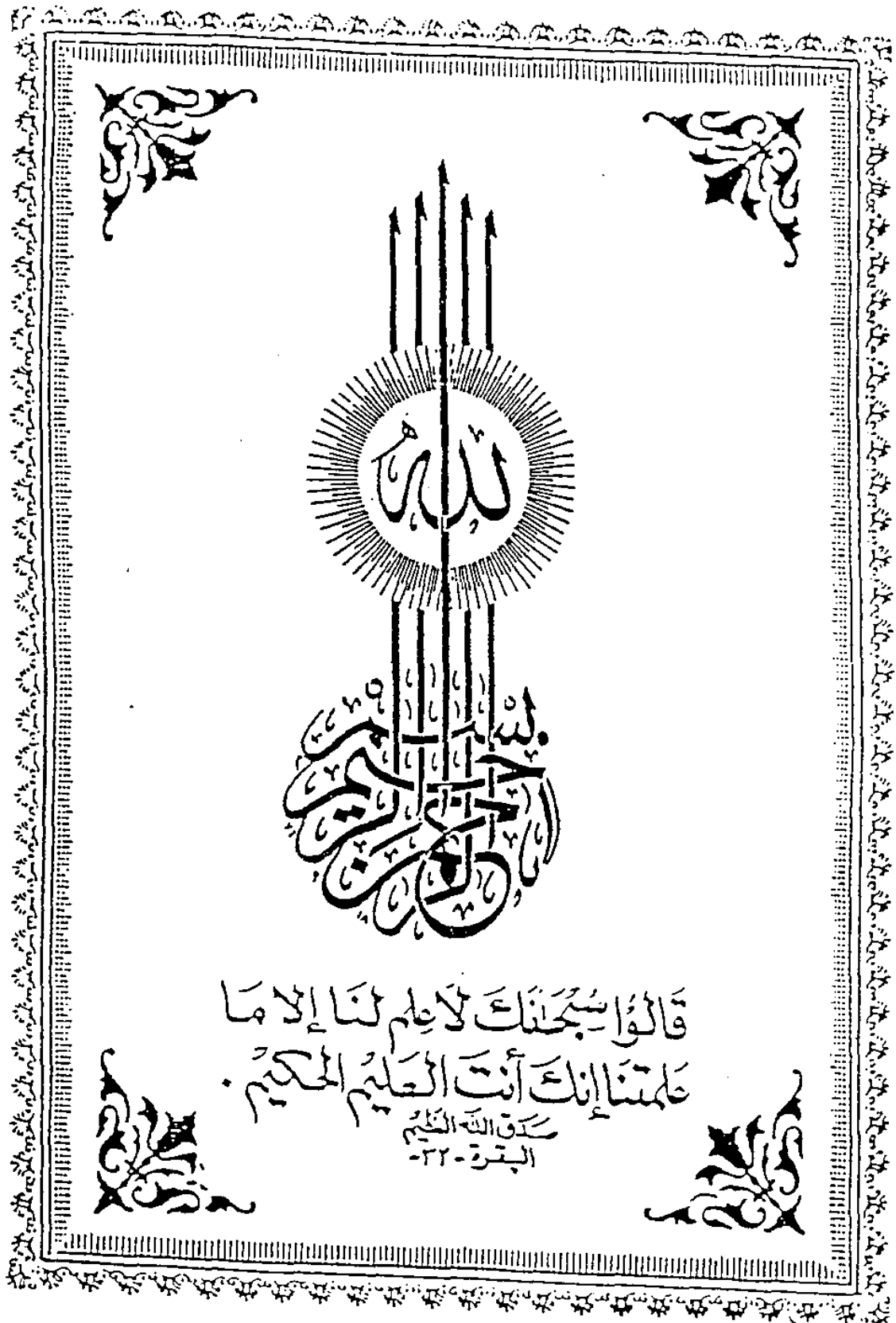
Prof. Of Virology And Chairman  
Of Virology Department  
Faculty Of Veterinary Medicine Cairo University

**Dr. Youssef Habashi**

Deputy Director Of VSVRI For Virological Products and  
Chief Of Research Dept. Of Pet. Animal Vaccines  
Vet. Serum And Vaccine Research Institute  
Abbasia , Cairo  
Agricultural Research Center



**1996**





CAIRO UNIVERSITY  
FACULTY OF VETERINARY MEDICINE  
DEPARTMENT OF VIROLOGY

## APPROVAL SHEET

This is to approve that the dissertation presented by NAGLAA IBRAHIM ALY ABDALLA to Cairo University for the Master degree has been approved by the examining committee .

**\*Prof. Dr. EMAD KAMEL NAFIE**

Prof. of Microbiology  
Faculty of Medicine  
Asoiut University

*E. Nafie*

**\*Prof. Dr. MOHAMED ABD EL-HAMID SHALABY.**

Prof. of Virology  
Faculty of Vet. Medicine  
Cairo University

*M. Shalaby*

**\*Prof. Dr. Mohamed Sami Saber**

Prof. and Head of Virology Department  
Faculty of Vet. Medicine  
Supervisor of The Thesis

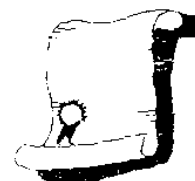
*M. Sami Saber*

**\*Prof. Dr. Youssef Zaki Mohmoud Habashi**

Deputy director of VSVRI for virological vaccine production ,  
chairman of Research Dept. of Pet Animals Vaccines  
Vet. Serum and Vaccine Research Institute  
Abbassia , Cairo Ministry of Agriculture  
Supervisor of The Thesis

*Youssef Habashi*

Date : 29 / 10 / 1996 .





## ACKNOWLEDGMENT

*I am greatly indebted to Prof. Dr. M. Sami Saber prof. and chairman of Virology Dpt. , Faculty of Vet. , Med. , Cairo univesity , for his kind stimulating supervision , planning and interest during the coarse of the present research work .*

*My great pleasure and warm thanks are directed to Prof. Dr. Youssef Z. Mahmoud Habashi , chairman of Res. Dept. of Pet. Animals Vaccines , and deputy director of virology of Vet. Serum and Vaccine Res. Inst. , Abbassia , Cairo , for his valuable guidance supervision , interest , support , kind help , contineous advice and encouragment , as well as his precious time , efforts and supplies which facilitates carrying out this research thesis .*





*Similarly , I don't deny my great thanks to Dr. Ali Fahmy Mohmoud chairman of Rabies production unit , from Gen. Org. of Biological products EL-Agoza , Dokki , Cairo and Dr. S. A. Nakshly , FMD Dept. , V.S.V.R.I. , Abbassia , Cairo , for their kindness help and cooperating in the performance of the specific part concerning Enzyme Linked Immuno-sorbent Assay "ELISAs"*

*Speciefic great appreciations are offered to Prof. Dr. A. M. Dawod , Director of Vet. Serum and Vaccine Res. Inst. , Abbassia , for his valuable help and support .*



# LIST OF CONTENTS

	PAGE
INTRODUCTION.....	1
REVIEW OF LITERATURE.....	6
Historical.....	6
Morphology.....	10
Morphogenesis of rabies virus.....	12
Antigenic composition of rabies virus.....	13
Chemical composition and structure of rabies virus.....	16
Rabies antibody.....	21
Relation of neutralizing antibody to protection.....	22
Types of antirabies vaccines.....	26
Types of commonly used antirabies vaccine.....	35
Types of adjuvants used with the antirabies vaccines.....	43
MATERIAL.....	46
Viruses.....	46
Biological reagents.....	47
Chemical reagents.....	48
Reagents used in ELISA.....	51
Laboratory animals for experimental studies.....	53
METHODS.....	55
Master seed virus propagation.....	55
Titration of the three passages.....	56
Preparing the suitable virus dilution to obtain a high virus titer.....	56
Testing of evaluation of purity and Identity of the seed virus .....	57
Effect of light centrifugation on virus titers.....	58
Vaccines preparation.....	58
Preparation of the inactivator.....	59
Types of vaccines prepared.....	60
Evaluation of the vaccine.....	62
Sterility test.....	62
Safety test.....	62
Potency test.....	63
a- The NIH test.....	63
b- Guinea pig potency test.....	64

Seroconversion.....	65
ELISA techniques.....	65
RESULTS.....	70
Results of titration of the four passages of the seed virus.....	70
Detection of the most suitable virus dilution to obtain a high virus titer in mice brain.....	72
Evaluation of purity and identity of the seed virus used in preparation of binary inactivated mice brain vaccine.....	73
Effect of light centrifugation on virus titer.....	74
Inactivation of challenge rabies virus strain by binary ethylamin.....	75
Results of adsorbance of rabies virus on rehydragel.....	78
Potency tests	
a- Results of NIH test.....	79
b- Guinea pig potency test.....	81
Estimation of specific rabies antibody in vaccinated guinea pig using ELISA.....	85
DISCUSSION.....	89
SUMMARY.....	101
REFERENCES.....	103
ARABIC SUMMARY.....	



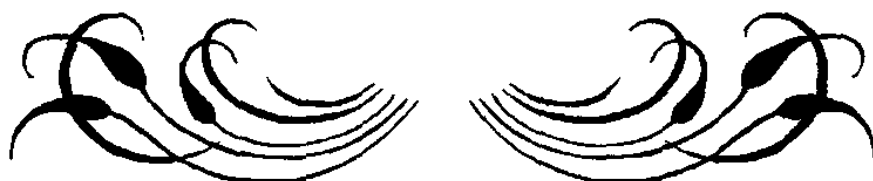
## LIST OF TABLES

	page
1 - Titer of the four serial passage of the seed virus in mice brain.....	70
2 - Detection of the most virus dilution.....	72
3 - Purity and identity of the seed virus.....	73
4 - Effect of light centrifugation on virus titer.....	74
5 - Effect of different dilution of BEI on the challenge virus rabies strain ....	76
6 - Adsorbance of rabies virus on rehydragel.....	78
7 - Result of NIH test applied on the tested vaccines.....	79
8 - Results of guinea pig potency test applied on the tested vaccines .....	82
9 - Guinea pig potency test estimated by ED <sub>50</sub> .....	84
10 - ELISA antibody titers in sera of vaccinated guinea pigs.....	86

## LIST OF ABBREVIATIONS

AEI	==	Acetyl Ethyleneimine
BEI	==	Binary Ethyleamine
BHK	==	Baby Hamster Kidney Cell Line
BPL	==	Beta Propiolactone
CAV	==	Cell Associated Virus
CDC	==	Center Of Disease Control
CEO	==	Chicken Embryo
CFV	==	Cell Free Virus
CVS	==	Challenge Virus Strain
DEAD-D	==	Diethylaminoethyl -Dextran
DEV	==	Duck Embryo Vaccine
DDW	==	Double Dist Water
ELISA	==	Enzyme Linked Immuno Sorbant Assay
FRV	==	Fixed Rabies Virus
HEP	==	High Egg Passage
HDCS	==	Human Diploid Cell Strain
HSA	==	Human Serum Albumin
IL-2	==	Interleukin- 2
LEP	==	Low Egg Passage
MLV	==	Modified Living Virus
NT	==	Nervous Tissue
NIH	==	National Institute Of Health
OPD	==	Orthophenyl Diamine
PBS	==	Phosphate Buffer Saline
RFFIT	==	Rapid Flurescent Focus Inhibition
RP	==	Relative Potency
SAD	==	Street Alabama Dufferine Strain
TC	==	Tissue Culture
VNT	==	Virus Neutralization Test
VNI	==	Virus Neutralization Index

# ***INTRODUCTION***



## **INTRODUCTION**

Rabies is world wide , highly mortality disease affecting mammalian species . Rabies virus can infect all warm blooded animals and in nearly all instances , the infection ends in death .

It is caused by a virus belonging to the family Rabdoviridae and transmitted through abrasions and wounds contaminated with infected saliva of rabid animals.

The clinical features of rabies are similar in most species , but there is great variation between individuals .

The disease characterized by marked nervous excitement and irritability followed by loss of consciousness and ending with paralysis and death.

Rabies disease is a serious health problem in many parts of the world , in developing countries specially in Egypt , the authorities adopt a wide organized program for the control of canine rabies , such program is very important for a country such Egypt , which has an open desert without any natural boundaries separating it from other neighboring countries .

Since rabies is known to be an incurable disease , so vaccination is the powerful tool in combating the disease , the veterinary rabies vaccines are generally used for pre - exposure immunization of pet and farm animals .

Post - exposure treatment of animals is rarely applied as it is regarded as potentially too dangerous for man .

In human beings most of the vaccines are used in post - exposure therapy , pre-exposure immunization being reserved for the relatively little number of people who run some risk of infection because of their occupation .

**Fermi , [1908]** produced a vaccine consists of 5% aqueous goats or sheep brain suspension containing 0.5% phenol .

**Koprowski and Cox , [1948]** manufactured the chicken embryo vaccine by inoculating of day old chicken embryos intra-yolk sac with LEP virus strain and that type of vaccine is used successfully for pre exposure immunization of dogs only .

**In 1955 , Peck** modified the chicken embryo vaccine , by inoculating seven day old duck embryos free from salmonella , intra-yolk with a variant strain of PM at 12 - 13<sup>th</sup> passage level , then the virus was inactivated with BPL to form the duck embryo vaccine .

**Svet - Moldavsky , [1960]** produced the suckling rat brain vaccine by i/c inoculation suckling rats (4 - 8 day old) with the original fixed virus of Pasteur strain , and inactivated by 1% phenol .

**Ott and Heyke , [1962]** suggested a vaccine in which the virus used is a tissue culture adapted strain by **Kissling [1958]** . The vaccine was used via i/m or s/c to dogs , cats , cattle , horses and sheep, "Hamster Kidney-CVS phenol inactivated vaccine" .

**Fuenzalida , et. al [1964]** succeeded to obtain on a vaccine from suckling mice inoculated with special mice fixed rabies strain and inactivation is carried out by u/v .

**Wiktor , et. al. [1964]** prepared a vaccine for human by the inoculation of HDC-WI38 cell line with a seed virus lot prepared from PM strain and inactivated by BPL 1: 4000 for 18 hours at 4°C .

**Abelseth , [1964]** obtained on a vaccine from virus adapted on swine kidney cells after growing on the hamster kidney cells and chicken embryos.

**Cabasso , et. al [1965]** prepared a vaccine from the LEP virus strain adapted to chicken embryo fibroblast cells . The vaccine was used as prophylactic for dogs only.

**Brown , et. al. [ 1967]** produced a vaccine by growing the HEP virus on a permanent dog kidney cell line .

**Sikes and Larghi , [1967]** produced a vaccine from suckling mice 3 - 4 days old were inoculated with 0.01% ml of fixed rabies virus, the virus was inactivated by BPL .

**Kucera , et. al. , [1969]** used the adapted tissue culture virus strain by **Kissling [1958]** and inactivated with formalin and used oil adjuvants , the vaccine was licensed for s/c use in dogs (2 ml) and cats (1 ml) .

**In 1973 , Seligman** produced a vaccine from rabbit , goat and sheep brains , it is consisted of suspension of 5% inactivated fixed rabies virus , containing 0.25% - 0.4% phenol and 1.0 % thiomersal .

**Slimov and Morogova , [1973]** produced a freeze - dried vaccine containing 5% sheep brain suspension of fixed rabies virus , 3.75% sucrose and not more than 0.25% phenol .

**Lepine , et. al. [1973]** prepared a freeze dried vaccine using the original Pasteur strain or PV<sub>11</sub> strain , containing 75% sucrose and inactivated by BPL in 1: 4000 concentration .

WHO and Californial conference in 1979 recommended the prevention of using all alive vaccine to administrated for vaccination either in human beings or in veterinary use , due to reverse of the virulence of the vaccinated strains which may be happened , or due to incidence of focal infection in a clean area . So the using of inactivated vaccine is most safer , but in order to increase their potency , it must be combined with an adjuvant .

Adjuvant is a substance that , when used in combination within specific antigen , enhanced the levels of immunity beyond those developed with the vaccines alone ( **Roman , 1926** ) .

It may act on a hapten or antigen enhancing its antigenic properties , or on the cells involved in the immune response , ( **Jolles and Paraf , 1973** ) .

The practical application of these substances has also widened from their classical use with vaccines , including tumour vaccines ( **Bartlett and Kreider , 1981** ) .