UNIVERSITI PUTRA MALAYSIA

IMMUNE RESPONSES TO A LIVE VIRUS VACCINE CANDIDATE AND PROTECTION AGAINST PSEUDORABIES VIRUS INFECTION

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DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

1999

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By

ABDEL-WAHID SAEED ALI BABIKER

Thesis Submitted in Fulfilment of the Requirements for the
Degree of Doctor of Philosophy in the Faculty of Veterinary Medicine
Universiti Putra Malaysia

December 1999

DEDICATED TO MY

PARENTS, *SAEED & SITTANA*
BROTHERS, *ABDEL-GADIR & ALI*
SISTERS, *UMMUHANI, KHADIJA, AISHA, EHSAN, RAHMA, IGBAL, FATIMA & SHAHINDA*

AND TO
MY WIFE, *AWADIA*

FOR THEIR PATIENCE, CONSTANT ENCOURAGEMENT AND SUPPORT

ACKNOWLEDGEMENTS

First and foremost, my heartfelt thanks to Almighty Allah for giving me the strength and willpower to complete this challenging task.

My utmost appreciation and gratitude to Dr. Mohd Azmi Mohd Lila, Chairman of Supervisory Committee, for his invaluable guidance, constructive comments, advice and suggestions which led to the completion of my Ph.D study.

My sincere thanks and appreciation to Professor Dato' Dr. Sheikh-Omar Abdul Rahman, Professor Dr. Aini Ideris and Professor Abdul Rani Bahaman as members of the Supervisory Committee, for their constructive comments and encouragement.

The study was made possible by a scholarship from University of Khartoum (Sudan). I would also like to express my thanks to the Prime Minister's Department (Malaysia) for the research funding and financial assistance to me throughout the course of the study.

Also my special thanks to Associate Professor Dr. Rehana Abdullah Sani, Head of Depatment of Veterinary Pathology and Microbiology, Professor Dr. Tengku Azmi bin Tengku Ibrahim, former Dean and Professor Dato' Dr. Sheikh-Omar Abul Rahman, present Dean of the Faculty of Veterinary Medicine, Universiti Putra Malaysia for allowing me to use the facilities in the Faculty and being helpful whenever I ran into difficulties.

I am indebted to Associate Professor Dr. Henry Too Hing Lee and Dr. Roshidah for their invaluable assistance in obtaining the piglets used in the study and to Associate Professor Dr. Rasheede for his translation the English Abstract of this study to Malay language.

I am also grateful to the staff members of Virology and Vaccine Laboratories, Mr. Mohd Kamarudin Awang, Ms Rodiah Hussein and Mr. Adam for always being so willing to render assistance throughout the course of my study. Special thanks also go to Mr. Fauzi Che Yusof for his assistance and use of his experience to develop the photograph in this study. My thanks also go to all members of the Faculty of Veterinary Medicine who contributed in one way or another toward the completion of my study.

Lastly, my heartfelt appreciation to my parents, brothers, sisters and my wife for their understanding, support and encouragement during the period of my study.

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LIST OF ABBREVIATIONS

Ab(s)	Antibody(ies)
ABTS	2,2-azino-bis (3-ethylbenzthiazoline-6-Sulfonic acid)
AD/ADV	Aujeszky's Disease/Aujeszky's Disease Virus
ATV	Antibiotic Trypsin Versin
BHV	Bovine herpesvirus
bp	Base Pair
BSA	Bovine serum albumin
°C	Degree Celcius
CEF	Chicken Embryo Fibroblast
cm	Centimeter
cm^2	Centimeter square
CMI	Cell-mediated immunity
CNS	Central nervous system
CO_2	Carbon dioxide
CPE	Cytopathic effect
CPS	Cyclophosphamide
D.W	Distilled water
DNA	Deoxyribonucleic Acid
Dr.	Doctor
DTH	Delayed-type Hypersensitivity
DXM	Dexamethasone
e.g.	For example
EBV	Epstein Barr Virus
EDTA	Ethylene Diamine Tetra-acetate
EHV	Equine Herpesvirus
ELISA	Enzyme Linked Immunosorbent Assay
f.p.	Footpad
FC	Final concentration
FCS	Foetal Calf Serum
FLM	Flumethasone
g	Gram
H_2O_2	Hydrogen peroxide
h/hrs	Hour/Hours
HIS	Hyperimmune Serum
HSV	Herpes-simplex Virus
IE	Immediate early
i.e.	That is
i.m.	Intramuscular
i.n.	Intranasal
i.p.	Intraperitoneal
IBRV	Infectious Bovine Rhinotracheitis Virus
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IR	Inverted repeat

Kbp	Kilobase pairs
Kda	Kilodalton
L15	Leibovitz's 15 media
LAT	Latency associated transcripts
M	Molar
Mab	Monoclonal antibody
MLV/ MLVs	Modified live vaccine/ Modified live vaccines
M.W	Molecular Weight
mg	Milligram
MHC	Major histocompatibility complex
min/ mins	Minute/ Minutes
ml	Millilitre
mM	Millimolar
N-Ab(s)	Neutralizing antibody(ies)
NCEM	Negative Contrast Electron Microscope
nm	Nanometer
O.D.	Optical Density
p.f.u.	Plaque Forming Unit
p.i.	Post-Inoculation
PAGE	Polyacrylamide Gel Electrophoresis
PBS	Phosphate Buffer Saline
PBST	Phosphate Buffer Saline Tween 20
PCR	Polymerase chain reaction
pH	Hydrogen-ion Concentration
PrV	Pseudorabies Virus
RE	Restriction Endonuclease
RNA	Ribonucleic Acid
Rpm	Revolution per minute
s.c.	Subcutaneous
SDS	Sodium Dodecyl Sulphate
Sec	Sec
SNT	Serum Neutralization Test
TE	Tris-EDTA
TK	Thymidine kinase
TNE	Tris-NaCl-EDTA
UL	Unique long region of PrV genome
UPM	Universiti Putra Malaysia
US	Unique short region of PrV genome
UV	Ultraviolet
V/V	Volume/Volume
Vero	Cell line derived from kidney tissue of green African monkey
VZV	Varicella Zoster Virus
w/v	Weight/Volume
w/w	Weight/Weight
µg	Microgram
µl	Microlitre
%	Percent

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirements for the degree of Doctor of Philosophy

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By

ABDEL-WAHID SAEED ALI BABIKER

December 1999

Chairman: **Mohd. Azmi Mohd. Lila, Ph.D.**

Faculty: **Veterinary Medicine**

A comparative study of three Malaysian pseudorabies virus (PrV) isolates, one Malaysian PrV strain and one American reference PrV was carried out using restriction endonucleases. Variations were observed among these viruses when their DNAs were digested with only *Kpn* I and *Bam*H I enzymes. The Malaysian strain (PrV-mA1p) showed restriction pattern which was different from its parental virus. An obvious differences were observed between the reference virus and the Malaysian PrVs. When the SDS-PAGE technique was employed, no significant variations in protein profiles of the PrVs but variations in their immunogenic proteins were observed when they were reacted against homologous and heterologous antisera in Western blotting. The viruses were suggested to belong to the same serologic sub-type using SNT and ELISA tests.

Experiments in mice demonstrated that all the viruses were pathogenic to various extents while PrV-mA1p was found to be non-pathogenic even when mice were infected with 10^8 p.f.u. The study showed that 100% protection was obtained following immunization of mice with at least 10^5 p.f.u. However, the

protection level was highly dose and route of immunization dependent. Antibody (Ab) responses to PrV-mA1p were correlated with the protection levels obtained and demonstrated to persist in mouse serum for five consecutive months.

Low levels of neutralizing Ab (N-Ab) were detected in mice following immunization with PrV-mA1p depending on the dose and route of inoculation. They were correlated with the protection levels but not with the ELISA Ab titres. Higher DTH responses were observed in mice immunized with PrV-mA1p especially via intranasal route (i.n.). A second dose of immunization slightly increased the DTH response. A significant ($p<0.05$) increase of PrV-mA1p growth was observed in mice tissues treated with immunosuppressive agents. However, the highest virus titres were detected in cyclophosphamide (CPS)-treated mice followed by dexamethasone (DXM)- and flumethasone (FLM)-treated mice. A significant ($p<0.01$) decline in Ab titres following treatment of mice with all the drugs was noted. However, the highest decline was observed in CPS-treated mice. PrV-mA1p was reactivated in low titres from latently infected nervous tissues of mice using CPS and DXM but not from respiratory tissues.

Increases in the protection levels and Ab responses were observed in mice immunized with the algammulin-adjuvanated PrV-mA1p. PrV-mA1p was also found to be non-pathogenic for swine following inoculation of piglets with up to 10^8 p.f.u. but displayed high levels of immunogenicity in piglets, hence the virus was highly suitable to be proposed as a live virus vaccine against PrV infection.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Doktor Falsafah

GERAKBALAS IMUN TERHADAP CALON VAKSIN VIRUS HIDUP DAN PERLINDUNGAN TERHADAP JANGKITAN VIRUS PSEUDORABIES

Oleh

ABDEL-WAHID SAEED ALI BABIKER

Desember 1999

Pengerusi: **Mohd. Azmi Mohd. Lila, Ph.D.**

Fakulti: **Perubatan Veterinar**

Suatu kajian bandingan terhadap pencilan virus pseudorabies Malaysia (PrV), satu strain PrV Malaysia dan satu PrV rujukan Amerika telah dijalankan mengguna endonuklease pengehadan. Perbezaan telah dicerap di kalangan virus ini apabila DNAnya dicernakan dengan enzim *Kpn* I dan *Bam*H 1 sahaja. Strain Malaysia (PrV-mA1p) menunjukkan pola pengehadan yang berbeza daripada virus induknya. Suatu perberzaan yang nyata dicerapkan di antara virus rujukan dan PrV Malaysia. Apabila teknik SDS-PAGE digunakan, tiada perbezaan tererti didapati dalam profil protein PrV tetapi perbezaan dalam protein imunogen telah dicerapkan apabila ianya ditindakkan dengan antiserum homologus dan heterologus dalam sap Western. Dengan mengguna ujian SNT dan ELISA, virus-virus ini disarankan adalah daripada sub-tip serologi yang sama.

Ujikaji pada mencit menunjukkan yang kesemua virus ini patogen pada tahap yang berbeza-beza, sambil PrV-mA1p pula didapati bukan patogen walaupun mencit ini telah dijangkitkan dengan 10^8 p.f.u. Kajian ini menunjukkan

yang perlindungan 100% diperolehi berikutan pengimunan mencit dengan sekurang-kurangnya 10^5 p.f.u. Bagaimanapun, aras perlindungan ini paling bersandarkan dos dan cara pengimunan. Gerak balas antibodi (Ab) terhadap PrV-mA1p berkorelasikan dengan aras perlindungan dan ini telah ditunjuk berterusan dalam serum mencit selama 5 bulan.

Aras Ab peneutralan (N-Ab) yang rendah telah dikesan dalam mencit berikutan pengimunan dengan PrV-mA1p, iaitu bergantung kepada dos dan cara penginokulatan. Ini berkorelasi dengan aras perlindungan tetapi bukan dengan titer Ab ELISA. Gerak balas DTH dicerap lebih tinggi dalam mencit terimun dengan PrV-mA1p, khususnya dengan cara intranasum (i.n.). Satu dos pengimunan kedua meningkatkan sedikit gerak balas DTH ini. Peningkatan tererti ($p<0.05$) pertumbuhan PrV-mA1p dicerapkan dalam tisu mencit yang diperlakukan dengan agen imunotindas. Bagaimanapun, titer virus paling tinggi dikesan dalam mencit terperlaku siklofosfamida (CPS) diikuti dengan mencit terperlaku deksametason (DXM) dan flumetason (FLM). Penurunan tererti ($P<0.01$) dalam titer Ab berikutan perlakuan mencit dengan kesemua drug ini telah dilihat. Bagaimanapun, penurunan paling besar dicerapkan dalam mencit terperlaku CPS. PrV-mA1p telah diaktif semula sekadar titer rendah daripada tisu saraf mencit terjangkit pendam mengguna CPS dan DXM, tetapi perkara ini tidak berlaku daripada tisu pernafasan.

Peningkatan dalam aras perlindungan dan gerak balas Ab dicerapkan dalam mencit yang terimun dengan PrV-mA1p teradjuvan algamulin. PrV-mA1p

juga didapati bukan patogen untuk babi berikutan penginokulatan anaknya dengan setinggi 10^8 p.f.u., tetapi telah menunjukkan keimunogenan aras tinggi dalam anak babi tersebut, dan justeru itu virus ini adalah paling sesuai disarankan sebagai vaksin virus hidup terhadap jangkitan PrV.

CHAPTER I

INTRODUCTION

Pseudorabies virus (PrV) is a neurotropic herpesvirus that causes pseudorabies in domestic and wild animals (Gustafson, 1986; Pensaert and Kluge, 1989). The first description of PrV infectivity was made in 1902 by Aujeszky, a Hungarian scientist (Aujeszky, 1902) hence the name Aujeszky's disease. Pigs are relatively resistant to PrV and are the natural host. Other secondary hosts include cattle, sheep, dogs and cats (Pensaert and Kluge, 1989). Laboratory animals such as rabbits and mice can be infected (Nash *et al.,* 1980).

Aujeszky's disease is characterized by inflammation of the respiratory tract and a severe encephalomyelitis (Crandell, 1982; Eich, 1983). The common route of PrV entry is the nasopharyngeal region. The virus is able to replicate in cells of nasal and pharyngeal mucosa (Pol *et al.,* 1989). After the infection of the peripheral neural cells, the virus is transported to the central nervous system, where it causes a severe encephalitis which is fatal in young pigs (Baskerville *et al.,* 1973; Dow and McFerran, 1962). Clinical signs of pseudorabies depend on the virulence of the virus strain, the infective dose and the age of the animal affected. Infection in older pigs results in respiratory and reproductive disorders. Infection with mild strains of the virus results in an inapparent infection (Kluge *et al.*, 1992).