



UNIVERSITI PUTRA MALAYSIA

**CHARACTERISATION OF PSEUDORABIES VIRUS
AND ITS MUTANTS**

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**CHARACTERISATION OF PSEUDORABIES VIRUS
AND ITS MUTANTS**

By

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**Thesis Submitted in Fulfilment of the Requirement for
the Degree of Master of Science in
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Dedicated with love and gratitude to:

My husband, Mohamed Abu Baker Jailani

Who had supported me and had been very understanding throughout this long and demanding project, and was my constant source of encouragement and motivation whenever I got into a “brain-rut”!

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LIST OF ABBREVIATIONS

ABTS	2,2'-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid)
ACV	acyclovir
ADV	Aujeszky's disease virus
Ara-A	adenine arabinoside
Ara-T	1-D-arabinofuranosylthymine
ATP	adenosine triphosphate
ATV	antibiotic trypsin versin
BHK	baby hamster kidney
BHV	bovine herpesvirus
BMC	blood mononuclear cells
bp	base pair
BSA	bovine serum albumin
BU-BHK	BUdR resistant BHK
BUdR	bromodeoxyuridine
BVdU	E-5-(2-bromovinyl)-2'-deoxyuridine
CAV	cell-associated virus
CEF	chicken embryo fibroblast
cm	centimetre
cm ²	centimetre square
CMI	cell mediated immunity
CNS	central nervous system
CP	capsid protein
CPE	cytopathic effect
CTP	cytidine triphosphate
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
e.g	for example
ECV	extracellular virus
EDTA	ethylene diamine tetra-acetate
EHV	equine herpesvirus
ELISA	enzyme linked immunosorbent assay
FBS	foetal bovine serum
FC	final concentration
FCS	foetal calf serum
g	gramme
H ₂ O ₂	hydrogen peroxide
hr	hour
HSV	herpes simplex virus
HVS	saimevine herpesvirus
HVT	herpesvirus of turkey
IE	immediate early gene
im	intramuscular
in	intranasal
IPTG	isopropylthiogalactosidase
IR	inverted repeat



IR _L	internal repeat
IR _R	inverted terminal
IUdR	iododeoxyuridine
kb	kilobase
kDa	kilodalton
L15	Leibovitz's media
LAT	latency-associated transcripts
LB	Lennox medium
LD ₅₀	50% lethal dose
LLT	large latency transcripts
LM(TK-ve)	mouse fibroblast strain deficient in TK activity
M	molar
Mab	monoclonal antibody
MarHV	marmoset herpesvirus
MDV	Marek's disease virus
MEM	Minimum Essential Media
MHC	major histocompatibility complex
min	minute
ml	millilitre
mm	millimetre
mM	millimolar
MOI	multitude of infection
mRNA	messenger ribonucleic acid
MTD	mean time to death
NCEM	negative contrast electron microscope
NIA3	pseudorabies strain NIA-3
nm	nanometre
OD	optical density
ORF	open reading frame
PAA	phosphonoacetic acid
PBS	phosphate buffer saline
PBST	phosphate buffer saline tween 20
pc	post challenge
PCR	polymerase chain reaction
PFA	trisodium phosphonofomate
pfu	plaque forming unit
pH	hydrogen-ion concentration
PI	post infection/post inoculation
PK	pig kidney
pk	protein kinase
PrV	pseudorabies virus
RE	restriction endonuclease
RFP	restriction fragment pattern
RK	rabbit kidney
RNA	ribonucleic acid
rpm	revolution per minute
SDS	sodium dodecyl sulphate
sec	second

SPF	specific pathogen free
T _c	cytotoxic T lymphocytes
TdR	thymidine
TE	Tris-EDTA
TEM	Transmission Electron Microscope
TFT	trifluorothymidine
TK	thymidine kinase
TNE	Tris-NaCl-EDTA
U _L	unique long region
UPM	Universiti Putra Malaysia
U _S	unique short region
UV	ultraviolet
V	volt
v/v	volume/volume
Vero	African green monkey kidney cell
vs	verses
VZV	varicella-zoster virus
w/v	weight/volume
Xgal	5-bromo-4-chloro-3-indolyl-β-D-galactoside
μg	microgramme
μl	microlitre
μm	micrometre

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CHARACTERISATION OF PSEUDORABIES VIRUS AND ITS MUTANTS

By

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May 1999

Chairman: Mohd Azmi Mohd Lila, Ph.D.

Faculty: Veterinary Medicine

A plaque-purified pseudorabies virus (PrV-mAIP) was established from a field isolate. Following exposure to bromodeoxyuridine (BUdR) and iododeoxyuridine (IUdR), six drug resistant mutants namely PrV-BUdR1, PrV-BUdR7, PrV-BUdR10, PrV-IUdR5, PrV-IUdR9 and PrV-IUdR10 were derived from PrV-mAIP. These viruses were passaged for 47 times in chicken embryo fibroblast (CEF) monolayer. These viruses and also including PrV-mAIP and a reference PrV (PrV-CD) were characterised. Differences in their eclipse phase and virus titres were determined based on the virus growth. Prolonged eclipse period (increment of 14 hrs) and reduction of the peak titres were observed in PrV-BUdR7, PrV-IUdR9 and PrV-IUdR10. DNA fingerprinting of the virus conducted with five restriction enzymes (RE) namely *Bam*HI, *Kpn*I, *Hind*III, *Sac*I and *Bgl*II revealed variations in the number, size and migration rate of the restriction fragments only for BUdR resistant mutants. Generally, the RE pattern of all the IUdR resistant mutants appeared similar to PrV-mAIP, perhaps PrV is more stable to IUdR exposure. PrV-mAIP and PrV-CD were different, probably



due to strain diversity of different geographical area. The absence of *Bam*HI fragment 7 in both PrV-BUdR1 and PrV-BUdR7 indicated highly to the loss of glycoprotein gE which is related to virulence. *Bam*HI and *Kpn*I enzymes appeared to be useful in discriminating the viruses. Alteration in the rate and type of cytopathic effect (CPE) were noticed in the viruses. The pronounced syncytium forming CPE in PrV-BUdR1, PrV-BUdR7 and PrV-IUdR10 indicated the loss of glycoprotein gC which usually plays a role in virus adsorption and cell to cell fusion. Similar to PrV-mAIP, the mutants were not pathogenic to mice. However, immunisation with these viruses conferred 100% protection (except PrV-BUdR10) in mice upon challenge infection with the virulent PrV (PrV-CD). Mice immunised with PrV-BUdR10 produced similar antibody levels to those of PrV-mAIP but the degree of protection was reduced by 10%. The non pathogenic nature of the PrVs is known to be related to the deletion or mutation of the thymidine kinase (TK) gene. Thus, the 5' end of the gene of the PrVs was sequenced to identify any mutation. No variation was identified in the 399 bp nucleotide sequence data. However, the sequence showed various percentage of homology when pair-wise homology search were conducted against ten herpesviruses. Homology percentage ranged from 44.4% with herpesvirus of turkey (HVT) to the highly homologous (93.5%) of an established PrV strain NIA-3. These study demonstrate the variation among the mutants. All the mutants (except for PrV-BUdR10) could be exploited for future research work for vaccine development. Genetic manipulation on these viruses such as insertion, deletion or recombination of foreign genes would be a valuable pathway to venture into.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan ijazah Master Sains

PENCIRIAN VIRUS PSEUDORABIES DAN MUTANNYA

Oleh

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Mei 1999

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Virus pseudorabies (PrV) PrV-mAIP telah dihasilkan melalui kaedah penulenan-plak ke atas satu pencilan PrV. Selepas pendedahan kepada bromodeoxyuridine (BUdR) dan iododeoxyuridine (IUdR), enam jenis mutan yang resistan iaitu PrV-BUdR1, PrV-BUdR7, PrV-BUdR10, PrV-IUdR5, PrV-IUdR9 dan PrV-IUdR10 telah dihasilkan daripada PrV-mAIP. Kesemua mutan tersebut telah dipasaj secara berterusan sebanyak 47 kali di atas sel ekalapisan fibroblas embrio avian. Pencirian telah dilaksanakan ke atas mutan, PrV-mAIP dan virus rujukan (PrV-CD). Perbezaan dari segi tempoh senyap dan titer virus telah diperhatikan dari replikasi virus. Peningkatan tempoh senyap (14 jam) dan penurunan titer kemuncak virus telah dipamerkan oleh PrV-BUdR7, PrV-IUdR9 dan PrV-IUdR10. Analisis endonukleas penyekat (AEP) dengan 5 enzim penyekat (*Bam*HI, *Kpn*I, *Hind*III, *Sac*I dan *Bg*II) telah memberikan variasi dari segi bilangan, saiz dan kadar migrasi fragmen terbatas pada mutan yang resistan terhadap BUdR. Corak fragmen terbatas oleh mutan yang resistan terhadap IUdR menyerupai PrV-mAIP, kemungkinan virus ini lebih stabil terhadap

pendedahan IUdR. Variasi strain disebabkan kelainan kawasan geografi berkemungkinan menjadi penyebab perbezaan ketara AEP antara PrV-mAIP dan PrV-CD. Ketiadaan fragmen *Bam*HI 7 pada PrV-BUdR1 dan PrV-BUdR7 menunjukkan kemungkinan ketiadaan glikoprotein gE yang berkaitan dengan kevirulenan. Enzim *Bam*HI dan *Kpn*I didapati berguna untuk diskriminasi analisis restriksi. Pertukaran dari segi kadar dan jenis kesan sitopatik juga diperhatikan. Pembentukan kesan sitopatik sinsitium oleh PrV-BUdR1, PrV-BUdR7 dan PrV-IUdR10 menunjukkan kemungkinan ketiadaan glikoprotein gC yang berperanan dalam penyerapan-masuk virus ke dalam sel dan fusi sel. Virus mutan tidak patogenik terhadap mencit seperti PrV-mAIP. Immunisasi mencit dengan virus-virus mutan ini (kecuali PrV-BUdR10) memberikan perlindungan 100%. PrV-BUdR10 menghasilkan tahap antibodi yang menyerupai PrV-mAIP, tetapi tahap perlindungan mencit mengalami penurunan 10%. Sifat ketidakpatogenikan PrV ini berkaitan dengan delesi atau mutasi terhadap gen thymidine kinase (TK). Oleh kerana itu, terminal 5' gen pada kesemua PrV telah diujukkan untuk mengenalpasti sebarang mutasi. Tiada perbezaan didapati pada jujukan bersaiz 399 bp tersebut. Jujukan ini mempamerkan perbezaan peratusan homologi apabila dibandingkan dengan jujukan 10 herpesvirus lain. Peratusan homologi serendah 44.4% dengan HVT kepada yang tertinggi (93.5%) diperolehi dengan PrV NIA-3. Kajian ini mempamerkan variasi diantara mutan. Kesemua mutan kecuali PrV-BUdR10 berkeupayaan untuk dieksploitasikan untuk kajian vaksin masa depan. Manipulasi genetik terhadap virus ini seperti insersi, delesi dan rekombinasi gen asing merupakan suatu era kajian yang produktif.

CHAPTER I

INTRODUCTION

Pseudorabies virus (PrV) (synonym: porcine herpesvirus type 1 or Aujeszky's disease virus (ADV)) is a member of the family *Herpesviridae*, subfamily *Alphaherpesvirinae*. The virus infects domestic and wild animals (Gustafson, 1986) but not humans (Prieto, 1991). The infection in swine is indigenous and wide spread, causing severe economic losses world-wide (Christensen, 1988). Although swine is the primary reservoir of the virus, other animal species including sheep, goat, cattle, dogs and cats may be affected (Gustafson, 1986).

The first report of pseudorabies in scientific literature was done by Aujeszky (1902). He recovered a virus from an ox, a dog and a cat, which was serially transmissible to rabbits and guineapigs. The syndrome was described and differences from rabies were pointed out. Hanson (1954) suggested that the disease was present in the United States as early as 1813. The serologic identity of "mad itch" as it came to be called in the United States, with Aujeszky's disease was established by Shope (1931).



Although some countries, among them Australia, Canada and Norway appear to be free of PrV, the disease is distributed world-wide and has a great economic impact on the pig industry (Van Oirschot *et al.*, 1990). PrV causes multimillion dollar losses annually in the United States and other countries (Gustafson, 1986). For example, the estimated economic loss in The Netherlands was between \$25-\$40 million each year. In the United Kingdom, an acute outbreak in an unvaccinated herd cost about \$250 per sow per year (Muirhead, 1984).

Infections by PrV may run an acute (Baskerville *et al.*, 1973) or subclinical course depending on the virulence of the virus and the age of the pig. The infection in swine is manifested by various degrees of respiratory distress, nervous disorders and mortality (Baskerville *et al.*, 1973). Abortions and stillbirth in sows, neurological disorders in piglets and respiratory signs in fattening pigs are the most prominent symptoms of the disease. Mortality in young piglets approaches 100%, whereas mortality in fattening pigs is usually less than 5% (Van Oirschot *et al.*, 1990a). Infected pigs shed PrV for 2-3 weeks (Van Oirschot *et al.*, 1990a).

Like other herpesviruses, PrV has the propensity to establish a latent infection (Sabo and Rajcani, 1976; Beran *et al.*, 1980). Latently infected swine should be regarded as a putative permanent source for transmission of PrV to other susceptible animals (Rziha *et al.*, 1986). Reactivation of latent PrV may result in excretion of the infectious virus and transmission to other

