
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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**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"!**

**My parents, Haji Allaudin and Hajjah Halimah**

**Who are fond of saying "Knowledge is Power", are the ones who started me all those years ago on the journey of knowledge which has brought me to where I am today.**

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| | |
|---|---|
| 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 | cytidosine 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 |
| $H_2O_2$ | hydrogen peroxide |
| hr | hour |
| HSV | herpes simplex virus |
| HVS | saimiriine herpesvirus |
| HVT | herpesvirus of turkey |
| IE | immediate early gene |
| i m | intramuscular |
| i n | 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 | milimetre |
| mM | milimolar |
| m o i | 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 phosphonoformate |
| 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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**May 1999**

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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, *Hin*dIII, *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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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). Perbedaan 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, *Hin*dIII, *Sac*I dan *Bgl*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 perbedaan ketara AEP antara PrV-mAIP dan PrV-CD. Ketiadaan fragmen *Bam*HI 7 pada PrV-BUdR1 danPrV-BUdR7 menunjukkan kemungkinan ketiadaan glikoprotin 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 glikoprotin 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 dijujukkan untuk mengenalpasti sebarang mutasi. Tiada perbedaan didapati pada jujukan bersaiz 399 bp tersebut. Jujukan ini mempamerkan perbedaan 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