

**DEVELOPMENT OF A DEOXYRIBONUCLEIC ACID VACCINE AGAINST
ENTEROVIRUS 71**

By

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DEVELOPMENT OF A DEOXYRIBONUCLEIC ACID VACCINE

AGAINST ENTEROVIRUS 71

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Enterovirus 71 (EV71) is a major causative viral agent responsible for large outbreaks of hand, foot and mouth disease (HFMD), a common rash illness in children and infants. There is no effective antiviral treatment for severe EV71 infections and no vaccine is available. The objectives of this study were to design and construct a DNA vaccine against Enterovirus 71 using the viral capsid protein (VP1) gene of EV71 and to verify the functionality of the DNA vaccine *in vitro* and *in vivo*. The VP1 gene of EV71 isolate S2/86/1 and isolate 410/4 obtained from Prof. Mary Jane Cardosa, Universiti Malaysia Sarawak (UNIMAS) were amplified using PCR and then inserted into a eukaryotic expression vector, pVAX1. The 3.9 kb recombinant constructs were transformed into competent *E. coli* cells and the positive clones were screened and selected using PCR analysis, restriction digestion analysis and DNA sequencing. The pVAX1 vector that was successfully cloned with the VP1 gene from each of the isolate (S2/86/1

and 410/4) in the correct orientation and in-frame, were designated as pVAX1/VP1-S and pVAX1/VP1-4, respectively. The DNA vaccine constructs with the VP1 gene were shown to be expressed in a cell-free *in vitro* expression system. The constructs were then tested for protein expression in Vero cells. The VP1 protein was successfully expressed in the mammalian cell line and was detected using RT-PCR, Indirect Immunofluorescence Assay (IFA) and western blotting. Subsequently, in the *in vivo* studies, female Balb/c mice were immunized with the DNA vaccine constructs. Enzyme Linked Immunosorbent Assay (ELISA) was performed to detect the presence of anti-VP1 IgG in mice. The anti-VP1 IgG levels in mice immunized with the DNA vaccine constructs increased after the first booster but declined following the second booster. The anti-VP1 IgG in the mice immunized with the DNA vaccine constructs exhibited neutralising activity against EV71. The promising results obtained in the present study have prompted further testing to improve the expression and immunogenicity of this potential EV71 DNA vaccine.

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

**PEMBANGUNAN SATU VAKSIN ASID DEOKSIRIBONUKLEIK TERHADAP
ENTEROVIRUS 71**

Oleh

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Enterovirus 71 (EV71) merupakan satu agen penyebab yang bertanggungjawab ke atas wabak penyakit tangan, kaki dan mulut, satu penyakit kudis yang biasa dijumpai di kalangan kanak-kanak dan bayi. Setakat ini, tiada rawatan yang berkesan terhadap jangkitan EV71 dan tiada vaksin. Objektif kajian ini adalah untuk mereka bentuk dan membangun satu vaksin DNA terhadap Enterovirus 71 dengan menggunakan gen kapsul protein (VP1) EV71 dan menentusahkan keberkesanan vaksin DNA secara *in vitro* dan *in vivo*. Gen VP1 isolat S2/86/1 dan 410/4 dari EV71 yang didapatkan dari Prof. Mary Jane Cardosa, Universiti Malaysia Sarawak (UNIMAS) digandakan dengan PCR dan kemudian dimasukkan ke dalam satu vektor ekspresi eukariotik, pVAX1. Binaan rekombinasi yang bersaiz 3.9 kb ini ditransformasikan ke dalam sel *E. coli* yang kompeten dan klon positif disaring dan dipilih dengan menggunakan analisis PCR, analisis penghadaman terhad dan penjujukan DNA automasi. Vektor pVAX1 yang berjaya diklonkan dengan gen VP1 dari setiap isolasi (S2/86/1 dan

410/4) dalam orientasi yang betul, telah masing-masing dinamakan sebagai pVAX1/VP1-S dan pVAX1/VP1-4. Binaan vaksin DNA dengan gen VP1 telah ditunjukkan dapat diekspres dalam satu sistem ekspresi *in vitro* bebas sel. Binaan ini kemudiannya diuji untuk ekspresi protein dalam sel Vero. Protein VP1 telah diekspreskan dengan berjaya dalam sel selanjut mamalia dan telah ditentukan dengan menggunakan RT-PCR, asai immunofloresen tidak langsung (IFA) dan blot western. Seterusnya, dalam kajian *in vitro*, mencit Balb/c betina telah diimmunisasikan dengan binaan vaksin DNA. Enzyme Linked Immunosorbent Assay (ELISA) telah dijalankan untuk menentukan kehadiran IgG anti-VP1 dalam mencit. Paras IgG anti-VP1 dalam mencit yang diimmunisasikan dengan vaksin DNA meningkat selepas suntikan penggalak pertama tetapi merosot berikutan suntikan penggalak yang kedua. IgG anti-VP1 dalam mencit yang diimmunisasikan dengan vaksin DNA menunjukkan aktiviti neutralisasi terhadap EV71. Hasil yang menggalakkan yang didapati dalam kajian ini telah mendorong ujian selanjutnya bagi memperbaiki ekspresi dan immunogenisiti vaksin DNA EV71 yang berpotensi ini.

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I certify that an Examination Committee met on 13th December 2005 to conduct the final examination of Wong Siew Tung on his Master of Science thesis entitled "Development of a Deoxyribonucleic Acid Vaccine Against Enterovirus 71" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for equations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

(WONG SIEW TUNG)

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