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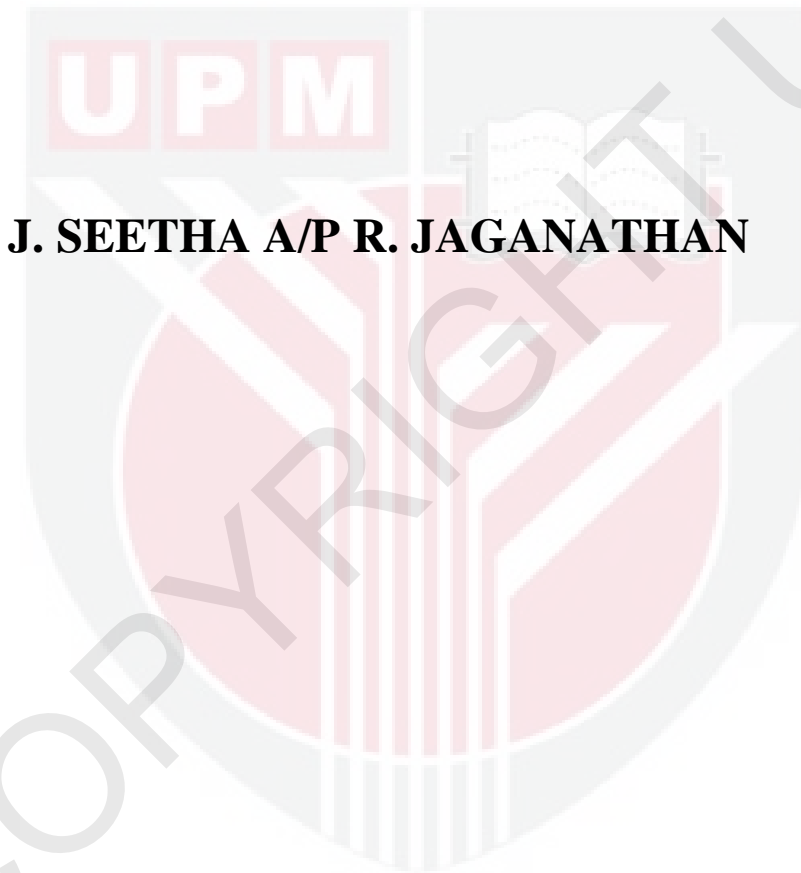
MOLECULAR CHARACTERIZATION OF PORCINE CIRCOVIRUS

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FPV 2012 18

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PORCINE CIRCOVIRUS**

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**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

2012

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

MOLECULAR CHARACTERIZATION OF PORCINE CIRCOVIRUS

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March 2012

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A study was done to determine the occurrence of porcine circoviruses in Malaysia. Porcine Circovirus 2 (PCV2) is the causative agent of Porcine Circovirus associated Diseases (PCVAD), of which Post Weaning Multisystemic Wasting Syndrome (PMWS) is the most important. The virus is of huge economic significance worldwide. Globally, the virus and disease has been reported in Canada, US, several European countries and in Asia. In Malaysia PCV2 was first identified by the Veterinary Research Institute in 2004 by RFLP methods followed by the first case study of PCVAD in 2007 based on clinical features, histopathology findings and PCR screenings. The objectives were to detect PCV2 from selected pig farms and to genetically characterize them. Understanding the presence of the virus may contribute to better vaccination programs against the virus which may control the disease manifestation. At the time the samples were collected, PCV2 vaccines were not available in Malaysia. Therefore, this study will represent the complete phylogenetic characterization of PCV2 reported for the first time in Malaysia from samples collected in 2007. To meet the objective of the study, organ samples were collected from 42 selected pig farms in Malaysia (Penang, Perak, Selangor, Melaka, Johor and Sarawak). The organ samples were collected from animals that

demonstrated typical clinical signs of PCVAD such as wasting, weight loss, stunted in growth, dyspnea, lymph nodes enlargement, diarrhea, pallor and jaundice. A total of five pigs were sampled from each farm. For the end analysis, the organs were pooled and screened by PCR to represent the data in each farm. Among the 42 farms, 39 were positive for PCV, while three farms indicate negative result. Subsequently, a phylogenetic tree was constructed from 13 representative positive PCV2 samples to represent the six states of which samples were collected from. All 13 selected samples fell into the same clade as other PCV2b isolates from other countries. Amino acid sequence analysis of the putative capsid protein (ORF2) of the PCV2 revealed that there are three clusters found in Malaysia, namely cluster 1C and 1A/1B. The hyper variable regions of the ORF2 of PCV2 was analyzed by utilizing a differentiation of wild-type vs. vaccine-type strain (DIVA) assay by real-time PCR which was developed in this study in order to distinguish and differentiate the strains isolated in this study. Analysis by DIVA assay which targeted the hyper variable regions confirmed that all isolates were wild-type PCV2 with no association with vaccine strains. Overall, our study suggests that there may be a link between movements of animals by import of breeders into the country being the route of entry of the virus. However, more investigations are needed to confirm this.

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

PENCIRIAN MOLEKUL BAGI CIRCOVIRUS BABI

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Satu kajian telah dilakukan untuk menentukan penyebab berlakunya penyakit circovirus babi di Malaysia. Circovirus 2 (PCV2) adalah agen penyebab Penyakit Circovirus babi (PCVAD), di mana sindrom pelbagai sistem pasca cerai susu (PMWS) adalah yang paling penting. Virus ini berkepentingan ekonomi yang besar di seluruh dunia. Di peringkat global, virus dan penyakit ini telah dilaporkan di Kanada, Amerika Syarikat, beberapa negara Eropah dan di Asia. Di Malaysia PCV2 telah mula dikenal pasti oleh Institut Penyelidikan Veterinar pada tahun 2004 oleh kaedah RFLP, diikuti pula dengan kajian kes pertama PCVAD pada tahun 2007 di UPM berasaskan kepada ciri-ciri klinikal, pemeriksaan histopatologi dan PCR. Objektif kajian ini adalah untuk mengesan PCV2 dari ladang-ladang babi yang terpilih dan mengkaji ciri-ciri genetik virus tersebut. Memahami kehadiran virus tersebut boleh menyumbang kepada program pemvaksinan yang lebih baik terhadap virus ini dan kaedah pengawalan ini boleh mengurangkan manifestasi penyakit ini di negara ini. Pada masa sampel untuk kajian ini dikumpulkan, vaksin PCV2 tidak terdapat di Malaysia. Oleh itu, kajian ini akan mewakili pencirian filogenetik lengkap PCV2 yang dilaporkan buat kali pertama di Malaysia dari sampel yang dikumpul pada tahun 2007. Untuk memenuhi objektif kajian, sampel telah diperolehi

daripada 42 ladang terpilih di Malaysia (Pulau Pinang, Perak, Selangor, Melaka, Johor dan Sarawak). Sampel organ telah dikumpul daripada babi yang menunjukkan petanda klinikal lazim PCVAD seperti kehilangan berat badan, tumbesaran terencat, dispnea, nodus limfa yang besar, cirit-birit, kepuccatan, dan jaundis. Sebanyak lima babi telah disampel dari setiap ladang. Untuk analisis akhir, organ-organ telah dikumpulkan sebagai satu sampel dan dianalisa dengan kaedah PCR untuk mewakili data dalam setiap ladang. Sampel organ telah diuji dengan kaedah PCR untuk menyaring organ tersebut. Daripada 42 sampel ladang yang diuji, 39 ladang memberi keputusan positif bagi PCV, manakala tiga ladang menunjukkan keputusan negatif. Seterusnya, suatu analisa filogenetik telah dibina daripada 13 wakil sampel PCV2 positif untuk mewakili 6 negeri dimana sampel-sampel kajian ini telah diperolehi. Kesemua 13 sampel PCV2 yang dianalisa jatuh ke bidang yang sama yakni PCV2b. Analisis jujukan asid amino protein kapsid (ORF2) PCV2 menunjukkan bahawa terdapat tiga kelompok yang ditemui di Malaysia, iaitu kelompok 1C dan 1A/1B. Analisis yang lebih mendalam juga telah dilakukan untuk mengkaji jujukan pembolehubah hiper protein kapsid ORF2 PCV2 dengan menggunakan kaedah pembezaan virus jenis liar dan virus jenis vaksin – (esei DIVA) yang telah dibangunkan dalam kajian ini untuk membezakan virus yang dipencil dalam kajian ini. Analisis melalui esei DIVA mengesahkan bahawa semua sampel dari kajian ini adalah jenis liar. Secara keseluruhannya, kajian ini mencadangkan bahawa mungkin terdapat pautan di antara pergerakan haiwan import penternak yang di import ke dalam negara ini sebagai laluan masuk virus tersebut. Walau bagaimanapun, penyiasatan yang lebih mendalam diperlukan untuk mengesahkan perkara ini.

TABLE OF CONTENTS

ABSTRACT	Page
ABSTRAK	iii
ACKNOWLEDGEMENT	v
APPROVAL	vii
DECLARATION	viii
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF ABBREVIATIONS	xii
CHAPTER	xiv
1 INTRODUCTION	1
2 LITERATURE REVIEW	4
2.1 <i>Circoviridae</i>	4
2.2 Taxonomy	5
2.3 History of Porcine <i>Circovirus</i>	6
2.4 Genetic homology among PV2 isolates	8
2.5 Morphology and molecular organization	11
2.6 Genetic differences between PCV1 and PCV2	12
2.7 Pathogenicity differences between PCV1 and PCV2	13
2.8 Genetic differences among PCV2 isolates and their emergence	14
2.9 PCV2 infection and PCVAD	16
2.10 Epidemiological and clinical features	16
2.11 PCV2-associated pathology	17
2.12 Pathogenesis	18
2.13 Diagnosis	22
2.14 Control measures	24
2.15 Commercially available vaccines	25
3 THE DETECTION OF PORCINE CIRCOVIRUS FROM THE MALAYSIAN PIG FARMS	
3.1 Introduction	28
3.2 Objectives	31
3.3 Methodology	31
3.3.1 Sampling	31
3.3.2 PCV2 reference controls	33
3.3.3 Nucleic Acid Extraction	33
3.3.4 The detection of PCV1 and PCV2 by conventional multiplex PCR	35
3.3.5 Agarose Gel electrophoresis	37
3.3.6 The detection of PCV2 by real time PCR	37
3.4 Results	38

3.4.1	Detection of PCV1 and PCV2 by conventional Polymerase Chain Reaction (PCR)	38
3.4.2	Detection of PCV2 by real-time PCR	42
3.5	Discussion	45
3.6	Conclusion	47

4 GENETIC CHARACTERIZATION OF PORCINE CIRCOVIRUS 2 FOUND IN MALAYSIA AND ANALYSIS OF THE HYPER VARIABLE REGIONS OF PCV2 BY DIVA ASSAY

4.1	Introduction	48
4.2	Objectives	53
4.3	Methodology	53
4.3.1	Samples for genetic characterization	53
4.3.2	PCR amplification of the complete genome of PCV2	53
4.3.3	Extraction and purification of DNA fragments	55
4.3.4	Sequencing	56
4.3.5	Bioinformatics analysis of the sequences	56
4.3.5.1	Sequence editing and assembly	56
4.3.5.2	Nucleotide sequencing, sequencing analysis and construction of phylogenetic tree	57
4.4	Analysis of the hyper variable regions of PCV2 by the development of a DIVA assay	58
4.5	Validation	58
4.5.1	Sensitivity	59
4.5.2	Specificity	59
4.5.3	Repeatability	60
4.5.4	Sequencing analysis	60
4.6	Results	60
4.6.1	Genetic characterization, amino acid sequence analysis and phylogenetic study of the PCV2 isolates in Malaysia.	60
4.6.2	Nucleotide sequence accession numbers	67
4.6.3	Analysis if the hyper variable regions of PCV2 by the development of a DIVA assay	67
4.6.4	Validation	70
4.6.4.1	Sensitivity	70
4.6.4.2	Specificity	71
4.6.4.3	Repeatability	72
4.6.4.4	Sequencing analysis	73
4.7	Discussion	74
4.8	Conclusion	81

5 SUMMARY, CONCLUSIONS & RECOMMENDATIONS FOR FUTURE RESEARCH **82**

REFERENCES/BIBLIOGRAPHY	85
APPENDICES	104
BIODATA OF STUDENT	122
LIST OF PUBLICATION	123