



UNIVERSITI PUTRA MALAYSIA

EPIDEMIOLOGY OF DISEASES OF CULTURED BLACK TIGER SHRIMP (*PENAEUS MONODOM*) IN PENINSULAR MALAYSIA AND EXPERIMENTAL INACTIVATION OF WHITE SPOT SYNDROME VIRUS

WANG YIN GENG

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BY

WANG YIN GENG

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Degree of Doctor of Philosophy in the Faculty of Veterinary Medicine
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Constant dripping wears away the stone!

Where there is a will there is a way!



Abstract of thesis submitted to the Senate of Universiti Putra Malaysia
in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Dr. Hassan Hj. Mohd Daud, Ph.D.

Faculty: Faculty of Veterinary Medicine

A large-scale investigation for detection of shrimp diseases was carried out from 1994 to 1999. Cultured *Penaeus monodon*, were collected from 26 hatcheries and 58 growout farms in 10 states along the coast of Peninsular Malaysia. Six viruses were identified, namely monodon baculovirus (MBV), white spot syndrome virus (WSSV), hepatopancreatic parvovirus (HPV), baculoviral midgut gland necrosis (BMNV), yellow-head virus (YHV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV). *Penaeus monodon* cytoplasmic giant body (PmCGB) was a newly recognized inclusion with unknown aetiology. With the exception of MBV, this is the first confirmation of the presence of these viruses in the Malaysian shrimp farming systems.

With emphasis on viral diseases, the rate of infection (ROI) and severity of infection (SOI) of each disease was evaluated based on the samples pooled for one-year period from 1994 to 1995. The postlarvae



were determined to be infected with MBV with prevalence of 33%, YHV 52% and PmCGB 80%. While in growout, the subadults' prevalence of viral infection was as follows; MBV 76%, YHV 76%, WSSV 23%, BMNV 18%, IHHNV 8%, HPV 3%, and PmCGB 99%. The prevalence of MBV had increased 46% from 1988 to 1994, while the prevalence of WSSV from 23% in 1994 to 80% in the end of 1996.

Other diseases detected were parasitic diseases, including epicommensal ciliates, gregarine and microsporidian; fungal disease was mainly manifested by larval mycosis; bacterial diseases such as filamentous bacterial disease, antennal deformity syndrome, brown spot, red leg, tail rot, luminous disease, bacterial septicaemia, and two newly recognized bacterial diseases named as bacterial white spot syndrome (BWSS) and soft body-white feces syndrome (SBWFS). BWSS was associated with *Bacillus subtilis*, and SBWFS was related to *Vibrio alginolyticus*, *V. parahaemolyticus* and *Aeromonas hydrophila*.

For most viral infections, histopathological and ultrastructural studies showed detail pathogenesis and cytopathic effects. The nucleosome of WSSV was described for the first time in the penaeid viruses, and the mechanism of white spot formation caused by WSSV was preliminarily elucidated. All populations examined were found to have multiple infections with viral and other microbial agents. Based on the cytopathic effect of these infections and associated mortality, viruses were regarded as the major disease agent in growout populations. In consideration to their ROI

and SOI indices, WSSV, MBV, BMNV, YHV, HPV and PmCGB were categorized as important agents. For hatchery-reared larvae, MBV was the significant pathogen in spite of the fact that bacteria, protozoa and fungi were also recorded to cause mortalities in some cases. White spot syndrome and SBWFS were the most risky diseases causing massive losses to shrimp culture.

Infectivity assays on WSSV revealed that the virus could survive in shrimp carcass for 6 days, and the cell-free WSSV survived in seawater for no longer than 2 days. *P. monodon* subadults were proved to resist WSSV infection at salinity 2.5 ppt or lower. While ozone at concentration of 0.03 mg/L, formalin at 30 ppm, and calcium oxide at 75 ppm were found to be effective in inactivating WSSV. Povidone iodine at 0 to 15 ppm showed no effect during the viral penetration period. Based on these findings, the control measures and treatment for white spot syndrome were proposed.

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

**EPIDEMIOLOGI PENYAKIT PADA KULTUR UDANG HARIMAU
(*PENAEUS MONODON*) DI SEMENANJUNG MALAYSIA
DAN UJIKAJI KETIDAKAKTIFAN VIRUS SINDROM BINTIK PUTIH**

Oleh

Wang Yin Geng

Februari 2001

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Penyiasatan penyakit udang melalui kajian berskala besar telah dilakukan dari tahun 1994 hingga 1999. *Penaeus monodon* yang diternak telah dikumpul dari 26 tempat tetasan dan 58 ladang pembesaran yang terdapat dalam 10 negeri di sepanjang pantai Semenanjung Malaysia. Enam jangkitan virus telah dikenalpasti, iaitu monodon baculovirus (MBV), virus sindrom bintik putih (WSSV), parvovirus hepatopankreatik (HPV), baculovirus nekrosis glanda usus tengah (BMNV), virus penyakit kepala kuning (YHV) serta virus infeksius nekrosis hipoderma dan hematopietik (IHHNV). Jasad gergasi sitoplasmik *Penaeus monodon* (PmCGB) ialah satu inklusi yang baru dikenalpasti dengan aetiologi yang belum diketahui. Kesemua virus ini, kecuali MBV, adalah pertama kali dikenalpasti wujud dalam sistem penternakan udang di Malaysia.

Dengan menekankan kepada penyakit virus, kadar jangkitan (ROI) dan keparahan jangkitan (SOI) bagi setiap penyakit telah dikaji

berdasarkan sampel yang dikumpul dari tahun 1994 hingga 1995. Pascalarva dikenalpasti dijangkit oleh MBV dengan kekerapan 33%, 52% oleh YHV dan 80% oleh PmCGB. Manakala kekerapan jangkitan virus pada peringkat tumbesaran subdewasa adalah seperti berikut; MBV 76%, YHV 76%, WSSV 23%, BMNV 18%, IHHNV 8%, HPV 3% dan PmCGB 99%. Kekerapan jangkitan MBV telah meningkat sebanyak 46% dari tahun 1988 hingga 1994, manakala kekerapan jangkitan WSSV pula meningkat daripada 23% dalam tahun 1994 kepada 80% pada hujung tahun 1996.

Penyakit lain yang turut dikenalpasti termasuklah penyakit parasit yang melibatkan siliat epicommercial, gregarine dan microsporidian; penyakit kulat yang disebabkan oleh larva mikosis; penyakit bakteria seperti penyakit bakteria berfilamen, sindrom kecacatan antena, bintik perang, kaki merah, kereputan ekor, penyakit berpendarfluor, septisemia bakteria dan dua jenis penyakit bakteria yang baru dikenalpasti iaitu bakteria sindrom bintik putik (BWSS) dan sindrom badan lembut-tinja putih (SBWFS). BWSS adalah berkaitan dengan *Bacillus subtilis*, dan SBWFS pula dikaitkan dengan *Vibrio alginolyticus*, *V. parahaemolyticus* dan *Aeromonas hydrophila*.

Bagi kebanyakan jangkitan virus, kajian histopatologi dan struktur-ultra menunjukkan patogenesis yang terperinci dan kesan sitopatik ketara. Nukleosom pada WSSV telah ditunjukkan buat pertama kalinya dalam virus penaeid dan mekanisme dalam pembentukan bintik putih

yang disebabkan oleh WSSV juga telah dijelaskan. Kesemua populasi yang dikaji didapati mempunyai jangkitan majmuk berbilang dengan virus dan agen mikrob yang lain. Berdasarkan kesan sitopatik dan morbiditi berkaitan, virus telah dikenali sebagai agen penyakit yang utama dalam populasi yang sedang membesar. Dengan mengambilkira indeks ROI dan SOI, WSSV, MBV, BMNV, YHV, HPV dan PmCGB telah dikategorikan sebagai agen yang penting. Bagi tempat tetasan larva, MBV merupakan patogen penting selain daripada bakteria, protozoa dan kulat yang juga direkodkan sebagai penyebab kematian dalam sesetengah kes. Sindrom bintik putih dan SBWFS adalah penyakit yang paling berisiko menyebabkan kerugian besar-besaran dalam penternakan udang.

Asai jangkitan WSSV menunjukkan bahawa virus boleh hidup dalam bangkai udang selama enam hari dan virus bebas sel dapat hidup dalam air laut tidak lebih daripada dua hari. *Penaeus monodon* subdewasa telah dibuktikan rentang kepada jangkitan WSSV pada kadar saliniti 2.5 ppt atau ke bawah. Ozon pada kadar 0.03 mg/L, formalin pada kadar 30 ppm dan kalsium oksida pada kadar 75 ppm telah didapati berkesan dalam mengurangkan keaktifan WSSV. Povidone iodine pada kadar 0 sehingga 15 ppm tidak menunjukkan sebarang kesan ke atas tempoh penyerapan virus. Berdasarkan penemuan ini, langkah pengawalan dan perawatan untuk sindrom bintik putih telah dicadangkan.

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I certify that an Examination Committee met on 12th February 2001 to conduct the final examination of Wang Yin Geng on his Doctor of Philosophy thesis entitled "Epidemiology of Diseases of Cultured Black Tiger Shrimp (*Penaeus monodon*) in Peninsular Malaysia and Experimental Inactivation of White Spot Syndrome Virus" 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 dissertation is based on my original work except for quotations 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.



WANG YIN GENG

Date: *20 Feb. 2001*

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