UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF WHITE SPOT DISEASE IN *PENAEUS MONODON* IN RELATION TO ENVIRONMENTAL CHANGES AND THE OCCURRENCE OF APOPTOSIS

ABEER HASSAN SAHTOUT

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ABEER HASSAN SAHTOUT

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

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SAY, O' MY LORD! ADVANCE ME IN KNOWLEDGE
Holy Quran (Surah Taha-114)

To my beloved parents for all what they do and have done for me
I can never repay them

Thankfulness and Gratefulness

Abstract of thesis presented to the Senate of Universiti Putra Malaysia In fulfillment of the requirement for the degree of Doctor of Philosophy

THE PREVALENCE OF WHITE SPOT DISEASE IN *PENAEUS MONODON* IN RELATION TO ENVIRONMENTAL CHANGES AND THE OCCURRENCE OF APOPTOSIS

By

ABEER HASSAN SAHTOUT

March 2003

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

Faculty: Veterinary Medicine

White spot disease (WSD) is an important viral disease of cultured penaeid shrimp. Despite the amount of research that has been carried out on this disease, much still remains to be done. This study was undertaken to examine the effect of different environmental parameters on the occurrence and severity of outbreaks of WSD. The study also undertook to investigate the occurrence of apoptosis and presence or activation of related genes that might result from virus infection.

Black tiger shrimp *Penaeus monodon* and pond water samples were collected from four shrimp farms located along the coast of Peninsular Malaysia, to investigate the relationship between environmental changes and the occurrence of WSD. In the study, it was found that occurrence of WSD was

more widespread during the wet season, in association with sudden drops in water temperature and salinity.

Laboratory experiments were also conducted to investigate the relationship between salinity, temperature and the occurrence of WSD. All the shrimp exposed to sudden increases in temperature and then returned to normal temperature in association with changed salinity were dead within four days of exposure. However, shrimp maintained at low salinity were less susceptible to disease.

To study the development of white spot lesions during infection, shrimp were exposed to white spot syndrome virus (WSSV) via the water borne route. At one-hour post exposure, electron microscopy revealed the appearance of white spots, which were oval or ovate in shape. White spots were visible by the naked eye three days post infection. The study also showed that there were two types of white spots, one related to viral infection, and the other related to bacterial infection. An epicommensal *Zoothamnium* sp. was able to penetrate the shrimp cuticle at these bacterial white spots.

High numbers of apoptotic cells were identified in moribund *P. monodon* infected with WSSV. Apoptotic cells showed DNA fragmentation by TUNEL fluorescence assay and gel electrophoresis of DNA extracts, while H&E stained sections revealed nuclear enlargement and chromatin condensation and margination in degenerated cells. The numbers of such cells present in

tissues of WSSV infected shrimp increased with increasing severity of infection as determined by gross signs of white spots on the cuticle, number of inclusion bodies in histopathological sections and by single and double-step (nested) PCR assay. The results suggested that apoptosis might be implicated in shrimp death caused by this virus.

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

PREVALEN PENYAKIT BINTIK PUTIH DALAM *PENAEUS MONODON* BERKAIT DENGAN PERUBAHAN PERSEKITARAN DAN KEHADIRAN APOPTOSIS

Oleh

ABEER HASSAN SAHTOUT

Mac 2003

Pengerusi: Hassan Hj. Mohd Daud, Ph.D.

Fakulti: Perubatan Veterinar

Penyakit Bintik Putih (PBP) merupakan satu penyakit virus yang penting dalam udang ternakan. Walaupun telah banyak penyelidikan dijalankan ke atas penyakit ini, masih banyak lagi belum diketahui mengenainya. Kajian ini telah dijalankan untuk memahami kesan parameter persekitaran yang berlainan ke atas kejadian dan keterukan wabak PBP. Kajian ini juga dijalankan untuk menyiasat kejadian 'apoptosis' dan kehadiran atau pengaktifan gen-gen yang berkaitan akibat jangkitan virus.

Sampel udang harimau *Penaeus monodon* dan air kolam telah diambil dari empat buah ladang udang yang terletak di sepanjang persisiran

Semenanjung Malaysia, untuk menyiasat perhubungan di antara perubahan persekitaran dan kejadian PBP. Di dalam kajian ini, di dapati kejadian jangkitan PBP lebih meluas semasa musim hujan, sejajar dengan kejatuhan mengejut suhu dan saliniti.

Eksperimen makmal juga telah dijalankan untuk menyiasat perhubungan di antara saliniti, suhu dan kejadian PBP. Udang yang didedahkan kepada suhu yang meningkat secara mendadak dan kemudiannya diturun semula ke paras normal serentak dengan perubahan saliniti, menunjukkan kematian pada hari ke empat eksperimen dijalankan. Bagaimanapun, udang yang diletakkan di dalam saliniti yang rendah adalah kurang terdedah kepada jangkitan.

Kajian juga telah dijalankan terhadap perkembangan bintik putih semasa jangkitan, di mana udang telah didedahkan kepada virus bintik putih melalui jangkitan air. Pada masa satu jam jangkitan dilakukan, mikroskopi elektron mendedahkan kemunculan bintik-bintik putih yang berbentuk bujur atau bulat. Selepas tiga hari jangkitan, bintik putih dapat dilihat dengan mata kasar. Kajian ini juga menunjukkan terdapat dua jenis bintik, iaitu bintik yang berkait dengan jangkitan virus dan juga yang disebabkan jangkitan bakteria. Satu spesis epikomensal *Zoothamnium* sp, telah menembusi kutikel udang di tempat bintik putih oleh bacteria.

Terdapat bilangan sel apoptotik yang tinggi dalam *Penaeus monodon* nazak yang telah dijangkiti WSSV. Sel apoptotik menunjukkan pecahan DNA dengan asai TUNEL kependarfluoran dan elektroporesis gel ekstrat DNA, manakala pewarnaan H&E mendedahkan hipertropi nukleus, kondensasi dan marginasi kromatin dalam sel yang mengalami degenerasi. Bilangan sel seperti ini yang hadir pada tisu udang yang telah dijangkiti WSSV bertambah dengan meningkatnya darjah kerosakan seperti yang ditunjukkan dengan tanda-tanda kasar bintik putih di atas kutikel, bilangan badan-badan inklusi di dalam bahagian histopatologi, dan melalui asai PCR (tersarang). Keputusan menunjukkan apoptosis mungkin terlibat di dalam kematian udang yang disebabkan oleh virus ini.

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I certify that an Examination Committee met on 14th March 2003 to conduct the final examination of Abeer Hassan Sahtout on her Doctor of Philosophy thesis entitled "Prevalence of White Spot Disease in Penaeus monodon in Related to Environmental Changes and the Occurrence of Apoptosis", 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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This thesis submitted to the senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The member of the Supervisory Committee are as follow:

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DECLARATION

I hereby declare that the thesis 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.

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ABEER HASSAN SAHTOUT

Date:

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LIST OF ABBREVIATIONS

AAHU	Aquatic animal health unit
AcMNPV	*Autographa californica* multiple embedded nucleopolyhedro viruses
ANOVA	Analysis of variance
AO	acridine orange
BmNPV	*Bombyx mori* nuclear polyhedrosis virus
bp	base pair
BSA	Bovine serum albumin
DNA	Deoxyribo nucleic acid
dNTPs	Deoxyribonucleoside triphosphates
dpi	Day post infection
DO	dissolve oxygen
DW	deionized water
EDTA	Ethylene diaminetetraacetic acid
EDAX	Energy Dispersive X-ray
GLS	Gel loading solution
H&E	Hematoxylin and Eosin
hr	Hour
KD	Kilodalton
MBV	Monodon Baculovirus
MSI	Mean severity index
MT	Metric Tone
nPCR	Nested Polymerase Chain Reaction
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PL	Post Larvae
ppt	Parts per thousand
SD	Standard deviation
S. E	Standard error
TBE	Tris-Boric acid. EDTA
TE	Tris-EDTA