



UNIVERSITI PUTRA MALAYSIA

**VIRAL DISEASES OF CULTURED *PENAEUS SEMISULCATUS*
DE HAAN WITH EMPHASIS ON CONTROL MEASURES OF
PENAEUS MONODON BACULOVIRUS (MBV)
IN THE ISLAMIC REPUBLIC OF IRAN**

MOHAMMAD TOKHMAFSHAN

FPV 2001 2

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By

MOHAMMAD TOKHMAFSHAN

**Thesis Submitted in Fulfilment of the Requirement for the
Degree of Doctor of Philosophy in the Faculty of Veterinary Medicine
Universiti Putra Malaysia**

August 2001



Dedicated to

My parent: Abdolreza Tokhmafshan and Setare Ajafendake

My wife: Kolsoum Golestani

My daughter's: Sedigheh, Maedah, and Fatemah, My son: Masoud

For their Love, Support and Trust



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

**VIRAL DISEASES OF CULTURED *PENAEUS SEMISULCATUS* DE HAAN
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By

MOHAMMAD TOKHMAFSAN

August 2001

Chairman: Professor Dr. Mohamed Shariff Mohamed Din

Faculty: Faculty of Veterinary Medicine

Shrimp aquaculture in Islamic Republic of Iran has a short history. Farmed shrimp production grew slowly from 1992 until 2000 when a number of large farms started to begin production. In 2000, the production was 4,500 MT and estimated that more than 7,000 MT will be produce by 2001.

The present study was carried out to investigate the status of viral shrimp diseases and their impact on the shrimp industry in the I. R. Iran. From August 1997 to March 1998, about two thousand samples of cultured *Penaeus semisulcatus* postlarvae and subadults were collected from five hatcheries and 20 growout farms distributed in three provinces along the coast of Persian Gulf and Oman Sea.

baculovirus was infective at salinity ranging from 5-40 ppt whereas at pH 3 and 12, MBV was completely inactivated. MBV were not affected at pH 5, 7, 8 and 9. Shrimp exposed for 10 hr to MBV at different chlorine concentration (5, 25, 50, 100 and 200 ppm) showed that MBV was completely inactivated after 10 hr of exposure to 200 ppm. Formalin in concentration ranging from 0-100 ppm had no virucidal effect on the infectivity of MBV.

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

**PENYAKIT-PENYAKIT VIRUS UDANG TERNAKAN *PENAEUS*
SEMISULCATUS DE HAAN DI REPUBLIK ISLAM IRAN DENGAN
TUMPUAN TERHADAP CARA-CARA PENGAWALAN BAGI
PENAEUS MONODON BACULOVIRUS (MBV)**

Oleh

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Fakulti: Fakulti Perubatan Veterinar

Ternakan udang di Republik Islam Iran mempunyai sejarah yang singkat. Pengeluaran udang meningkat perlahan daripada tahun 1992, dan mencapai 4,500 MT pada tahun 2000 apabila ladang-ladang besar mula beroperasi. Pengeluaran pada tahun 2001 dijangka mencapai 7,000 MT.

Kajian ini dijalankan untuk menyiasat status penyakit-penyakit virus udang dan kesannya terhadap industri ternakan udang di Republik Islam Iran. Daripada Ogos 1997 hingga Mac 1998, lebih kurang 2,000 sampel pasca larva (PL) dan subdewasa udang *Penaeus semisulcatus* telah dikumpulkan daripada lima pusat penetasan dan 20 ladang ternakan yang terletak di tiga daerah di sepanjang pantai Teluk Persian dan Laut Oman.

Virus sama hepatopankreatik parvo (HPV) dan virus baculo monodon (MBV) adalah kecil (diameter 20-24nm) dan merupakan virus DNA, kerana terdapat jasad rangkuman intranukleus di dalam hepatopankreas Virus *Penaeus monodon* baculovirus adalah virus DNA berbentuk rod dengan diameter 300 ± 25 nm Organ-organ sasarannya ialah hepatopankreas dan epitelium usus tengah Di ladang udang, jumlah kes MBV dan HPV di ladang udang masing-masing adalah 50 dan 27% Jumlah kes MBV di pusat-pusat penetasan adalah 60% dan HPV 5%

Rentan larva dan pasca larva pelbagai peringkat kepada MBV telah disiasat dengan kaedah inokulasi melalui air ke atas zoea, mysis dan PL1 hingga 12 Kadar jangkitan (ROI), severiti jangkitan (SOI), mortaliti kumulatif dan kadar tumbesaran telah ditentukan Zoea dan mysis masing-masing tidak menunjukkan jangkitan MBV yang boleh dikesan lima dan tiga hari lepas inokulasi Pada peringkat mysis 2 dan PL1, MBV boleh didiagnosis dua hari lepas inokulasi Nilai ROI dan SOI bagi PL yang lebih matang (PL7 hingga 12) adalah lebih tinggi berbanding dengan PL yang lebih muda

Kesan penyahkuman pH, saliniti, formalin dan kalsium hipoklorid ke atas MBV telah disiasat dengan kaedah inokulasi melalui air dengan mendedahkan *P semisulcatus* selama 10 jam, dan memantau perkembangannya selama 10 hari MBV menunjukkan infektiviti terhadap saliniti 5 hingga 40 ppt dan membunuh semua udang yang didedahkan Virus baculovirus monodon boleh berjangkit pada tahap saliniti 5-40ppt tetapi takaktif pada pH 3 dan 12 Virus

Penaeus monodon baculovirus pada udang yang terdedah kepada berbagai kepekatan hipoklorid (5, 25, 50, 100 dan 200 mg/L) didapati tidak aktif selepas 10 jam terdedah kepada 200 ppm. Formalin pada tahap 0 hingga 100 ppm tiada kesan sebagai penyahvirus atas jangkitan MBV.

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Praise be to the Almighty Allah. Had it not been His will I would not have the patience to withstand the pressure of my research and completion of study would not have been possible.



I certify that an Examination Committee met on 17th August 2001 to conduct the final examination of Mohammad Tokhmashan on his Doctor of Philosophy thesis entitled "Viral Diseases of Cultured *Penaeus semisulcatus* de Haan with Emphasis on Control Measures of *Penaeus monodon* Baculovirus (MBV) in the Islamic Republic of Iran" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 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 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.



MOHAMMAD TOKHMAFSHAN

Date: 14/9/01

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LIST OF ABBREVIATIONS

AM	accumulative mortality
AO	acridine orange
BBV	Bennettiae baculovirus
BKC	benzalkonium chloride
BMNV	baculoviral midgut gland necrosis
BP	<i>Baculovirus penaei</i>
CAI	Cowdery type A inclusion body
CPV	canine parvovirus
DO	dissolved oxygen
DNV	densonucleosis virus
dsRNA	double-stranded ribonucleic acid
ELISA	enzyme-linked immunosorbent assay
EMU	electron microscopy unite
ER	endoplasmic reticulum
FAO	Food and Agriculture Organisation
GR	growth rate
GV	granular virus
H&E	hematoxylin and eosin
HP	hepatopancreas
HPC	hepatopancreatocyte
HPV	hepatopancreatic parvo-like virus

IB	inclusion body
ICTV	International Committee on Taxonomy of Viruses
IHHNV	infection hypodermal & hematopoietic necrosis virus
IHNV	infection hematopoietic necrosis virus
IPNV	infection pancreatic necrosis virus
IRDO	shrimp iridovirus
Kb	kilo base
KDa	kilo Dalton
LM	light microscopy
LO	lymphoid organ
LOVV	lymphoid organ vacuolization virus
LPV	lymphoid parvo-like virus
MBV	monodon baculovirus
ML	membranous labyrinth
MT	metric tons
NBF	nuclear buffered formalin
NPV	nuclear polyhedrosis virus
OB	occlusion body
PBV	plebejus baculovirus
PCR	polymerase chain reaction
PGFRC	Persian Gulf Fisheries Research Centre
PHRV	penaeid haemocytic rod-shaped virus
PI	post inoculation
PIB	polyhedral inclusion body