

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



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

DOCTOR OF PHILOSOPHY UNIVERSITI PUTRA MALAYSIA

2001



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

Ву

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 WITH EMPHASIS ON CONTROL MEASURES OF PENAEUS MONODON BACULOVIRUS (MBV) IN THE ISLAMIC REPUBLIC OF IRAN

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

MOHAMMAD TOKHMAFSHAN

Ogos 2001

Pengerusi: Professor Dr. Mohamed Shariff Mohamed Din

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 masingmasing 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 kepakatan 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



ACKNOWLEDGMENTS

Glory and praise be to God, the Omnipotent Omniscient and Omnipresent for providing me with the strength and perseverance to complete this dissertation despite several obstacles encountered throughout the course of this research which at times seemed insurmountable

I would like to express my deep sense of gratitude to my Chief Supervisor Professor Dr Mohamed Shariff Mohamed Din, for his invaluable guidance and constructive suggestions throughout the thesis research. I sincerely appreciate the innumerable hours he spent reading the draft and the suggestions to provided to improve the thesis. Gratitude is also expressed to my co-supervisors. Professor Dr. Fatimah Md. Yusoff, and Dr. Hassan Hj. Mohd. Daud. for their valuable suggestion and kind assistance throughout this study.

I would also like to thank Dr Sohrab Akbari for being the co-supervisor during the period spent in Iran, and for making necessary arrangements for me to spend six months in his laboratory at Shiraz University Iran and for his assistance with electron microscopy

I am grateful to the head and staff of the Persian Gulf Fisheries Research

Centre (PGFRC) for their co-operation during the process of conducting the study



I would like to thank Dr Wang Y G for keen interest in my thesis research. His suggestions throughout the period during my stay in UPM was extremely useful without which the completion of the thesis would have been delayed. I would also like to express my thanks to my colleagues. Devaraja Sanjoy, Najiah, Feenie, Abeer, Agus and those who have helped me in many ways. The camaraderie with these students and friends at UPM is memorable.

I must also thank to Mr Ho Oi Kuan and Ms Azilah Jalil, the staff in Electron Microscope Unit, UPM Their assistance facilitated my electron-microscopic work successfully

Special thanks are extended to my wife, Kolsoum Golestani, for helping and understanding my absence during the complete of thesis. I also appreciate the concern of my daughters Sedigheh, Maedah, and Fatemah, and son Masoud, for their sacrifice of needing attention of their father.

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



TABLE OF CONTENTS

ABST ABST ACKN APPR DECL LIST (LIST (CATION FRACT FRAK NOWLEDGEMENTS ROVAL SHEETS LARATION FORM OF TABLES OF FIGURES OF PLATES OF ABBREVIATION/NOTATIONS	Page III
CHAF	TER	
1	GENERAL INTRODUCTION	1
II	LITERATURE REVIEW Characteristics of the Known Penaeid Shrimp Viruses Baculovirus Mid-gut Gland Necrosis Virus Baculovirus Penaei Hepatopancreatic Parvo-like virus Infection Hypodermal and Hematopoietic Necrosis Virus Shrimp Iridovirus Lymphoidal Parvo-like Virus Lymphoidal Organ Vacuolization Virus Penaeus monodon baculovirus Penaeid Hemocytic Rod-shaped Virus Reo-like Virus Rabdovirus of Penaeid Shrimp Taura Syndrome Virus White Spot Syndrome virus Yellow Head Virus Diagnostic Techniques for Viral Infection Wet mount microscopy Histology and Histochemical Test Transmission Electron Microscopy Molecular Methods In situ Hybridization Polymeraes Chain Reaction	11 12 12 13 14 16 18 19 20 21 25 27 27 28 31 35 36 36 37 38
	Relationship of Environmental Factors with Disease Outreak Salinity	40



	Temperature pH Dissolved Oxygen Biological and physiological Characteristics of <i>Penaeus</i> semisulcatus State of Shrimp aquaculture Industry in Islamic Republic of Iran and other Countries	43 45 48 49 51
II	DETECTION AND IDENTIFICATION OF VIRAL DISEASES OF CULTURED PENAEUS SEMISULCATUS IN I R IRAN Introduction Material and Methods 1 Detection and Identification of Viral Diseases of Cultured Penaeus Semisulcatus in I R Iran Collection of Specimens	55 57 57 57
	Viral Disease Diagnosis Wet Mount Microscopy and Gross Examination of Live Shrimp	58 58
	Histopathological and Histochemical Techniques in Shrimp Disease Diagnosis Transmission Electron Microscopy	62 63
	3 The Prevalence of Viral Infection (ROI) and the Grading of their Severity of Infection (SOI) Estimation of Prevalence Terminology Results	65 65 66 72
	Hepatopancreatic Parvo-like Virus (HPV) Gross Sign and Histopathology Histochemical Studies Ultrastructral Cytopathology and Virus Morphology	72 74 75
	Penaeus Monodon Baculovirus (MBV) Gross Sign and Histopathology Histochemical Studies Ultrastructral Cytopathology and Virus Morphology Prevalence and Severity of MBV and	76 79 79
	HPV in Penaeus Semisulcatus Discussion	81 87
V	SUSCEPTIBILITY OF THE DIFFERENT LARVAL AND POSTLARVAL STAGES OF <i>PENAEUS SEMISULCATUS</i> TO MBV	
	Introduction Materials and Methods Results	114 117 122 135



V	THE EFFECT OF pH AND SALINITY ON THE OCCURRENCE OF MBV IN PENAEUS SEMISULCATUS Introduction Materials and Methods Results Discussion	140 144 148 159
VI	EFFICACY OF CALCIUM HYPOCHLORIDE AND FORMALIN AS DISINFECTANTS FOR MBV IN PENAEUS SEMISULCATUS Introduction Materials and Methods Results Discussion	163 166 169 180
VII	GENERAL DISCUSSION AND RECOMMENDATION	186
	REFERENCES BIOGRAPHICAL SKETCH	197 216



LIST OF TABLES

Table 2 1	Characteristics of the known types of penaeid shrimp virus	Page 33
2 2	The geographical distribution of viral diseases	34
3 1	Health state of specimen (<i>P semisulcatus</i>) collected between March 1997 April 1998 from farms in Three provinces of Iran	60
3 2	Health state of specimen (<i>P semisulcatus</i>) collected between March 1997 to April 1998 from hatchery in Three provinces of Iran	61
3 3	Observed cytopathological stages of development of a MBV infection in P $semisulcatus$ hepatopancreatic and anterior midgut epithelium	67
3 4	A generalised scheme for assigning a numerical qualitative value to severity grade of infection, surface infestation and disease syndrome severity	68
3 5	A generalised scheme for assigning a numerical qualitative value to severity grade of MBV infection	69
3 6	A generalised scheme for assigning a numerical qualitative value to severity grade of HPV infection	70
3 7	ROI and SOI of the viral infection from <i>P semisulcatus</i> postlarvae in Iran (March 1997 to April 1998)	83
3 8	ROI and SOI of the viral infection of P semisulcatus adults in Iran (March 1997 to April 1998)	84
4 1	Severity of infection (expressed as grade of infection) of MBV in <i>P semisulcatus</i> exposed to different stages of zoea	126
4 2	Percentage of infection (ROI) of MBV to P semisulcatus exposed in different stages of zoea	126
4 3	Severity of infection (expressed as grade of infection) of MBV to <i>P semisulcatus</i> exposed in different stages of mysis	127
4 4	Percentage of infection (ROI) of MBV to <i>P semisulcaus</i> exposed in different stages of mysis	127
4 5	Severity of infection (expressed as grade of infection) of MBV to <i>P semisulcatus</i> exposed at different stage of PL	128



46	Percentage of infection (ROI) of MBV to P semisulcatus exposed at different stage of PL	129
4 7	The accumulative mortality rate $(\%)$ in different larval and postlarval stages of P semisulcatus	130
5 1	Rate of infection (ROI) of PL 12 <i>P semisulcaus</i> infected with MBV in different salinity	151
5 2	Severity of infection (SOI) of PL 12 <i>P semisulcatus</i> infecteed with MBV in different salinity	152
5 3	Rate of infection (ROI) of PL 12 <i>P semisulcaus</i> infected with with MBV in different pH concentration	153
5 4	Severity of infection of PL 12 <i>P semisulcatus</i> infected with MBV in different pH concentration	153
6 1	Rate of infection (ROI) of PL 12 <i>P semisulcaus</i> infected MBV in different calcium hypochloride (TC)	172
6 2	Severity of infection (SOI) of PL 12 <i>P semisulcatus</i> infected with MBV in different calcium hypochloride (TC)	173
6 3	Percentage of infection (ROI) of PL12 <i>P semisulcatus</i> infected with MBV in different formalin concentration	174
6 4	Severity of infection (SOI) of PL 12 <i>P semisulcatus</i> infected with MBV in different formalin concentration	175



LIST OF FIGURES

Figure 11	Farmed shrimp production in Iran, 1992 – 2000	Page 8
1 2	Map of I R Iran showing the source of samples Farmed	9
1 3	Farmed shrimp production by species, 1992 - 2000	10
3 1	Farm history record for shrimp viral diseases	59
3 2	General external adult anatomy of shrimp	71
3 3	General internal adult anatomy of shrimp	71
3 4	Prevalence of the viral infection (MBV and HPV) of cultured <i>P semisulcatus</i> based on the sample collected from March 1997 to April 1998	85
3 5	Severity of infection grading of the viral infection (MBV and HPV) of PLs and subadults <i>P semisulcatus</i> based on total number of cases from March 1997 to April 1998	86
4 1	Accumulative mortality in the different larval and postlarval stage of MBV-infected and control groups of <i>P semisulcatus</i>	131
4 2	General growth rate exhibited by the MBV-infected and control groups of different larval and postlarval stages of <i>P semisulcatus</i>	132
5 1	Procedure used to study the effect of different pH level on the viability of MBV	147
5 2	Effect of salinity on MBV infectivity in PL 12 of P semisulcatus. The shrimp were analysed after 6 days post exposure	155
5 3	Effect of pH on MBV infectivity in PL 12 <i>P semisulcatus</i> The shrimp were analysed 6 days post exposure	156
5 4	Accumulative mortality in PL12 P semisulcatus infected with MBV and the control groups maintained at different salinity	157



5 5	Accumulative mortality in PL 12 <i>P semisulcatus</i> infected with MBV and the control groups maintained at different pH	158
6 1	Procedure used to study the efficacy of chlorine as disinfectant for MBV	167
6 2	Severity of infection (express as grade of infection) on MBV infectivity in PL 12 <i>P semisulcatus</i> at different concentration of TC. The shrimp were analysed 6 days post-exposure	176
6 3	Severity of infection (express as grade of infection) on MBV infectivity in PL 12 <i>P semisulcatus</i> at different concentration of formalin. The shrimp were analysed 6 days postexposure	177
6 4	Accumulative mortality in PL12 <i>P semisulcatus</i> of MBV infected and control groups at different Concentration TC	178
6 5	Accumulative mortality in PL 12 P semisulcatus of MBV infected and control groups at different concentration formalin	179



LIST OF PLATES

Plate		Page
3 1	HPV infection in juvenile P semisulcatus	101
3 2	The enlarge inclusion bodies in Juvenile P semisulcatus showing the hypertropied nucleus	101
3 3	The advance stage of HPV infection showing hypertrophied nucleus, large well development intranuclear inclusion bodies, basophilic inclusion bodies	102
3 4	Feulgen staining of inclusion body of HPV infection in P semisulcatus	102
3 5	A higher magnification of the hepatopancraetic tissue showing granulomas	103
3 6	Fluorescent micrograph of AO staining of HPV-infected hepatopancreatic tissue	103
3 7	Methylene blue stained sections showing hepatopancreas tubules heavily infected with HPV	104
3 8	Stage 1 HPV infection	104
3 9	Stage 2 HPV infection	105
3 10	Stage 3 HPV infection	105
3 11	A higher magnification of the HPV inclusion body	106
3 12	High magnification of HPV particles	106
3 13	Wet mount view of tissue squash preparation of the hepatopancreas (HP) from P semisulcatus post larvae with MBV infections	107
3 14	Hepatopancrease of <i>P semisulcatus</i> with MBV infected cells in the developmental stages (stage 1) and (stage 2)	107
3 15	Hepatopancreas of P semisulcatus infected with MBV	108



3 16	Hepatopancreas cells of <i>P semisulcatus</i> in advanced (stage 3) MBV infection	108
3 17	Hepatopancreas of P semisulcatus with MBV infected cells	109
3 18	Methylene blue stain showing typical histopathology of MBV infection in Hepatopancreas	109
3 19	Acridine orange stained section observed by fluorescent microscope showing karyoplasms and OB are in yellow-green	110
3 20	Stage 1 MBV infected hepatopancreatocyte	110
3 21	Hepatopancreatocyte with stage 1 MBV infection	111
3 22	A higher magnification of the nucleus of stage 2 MBV-infected hepatopancratocyte	111
3 23	The basal portion of a stage 2 MBV	112
3 24	Stage 3 MBV infected hepatopancreatocyte	112
3 25	A high magnification of a stage 3 MBV infection	113
3 26	A high magnification of an aggregation of nonoccluded MBV	113
4 1	Hepatopancreas of mysis 3 P semisulcatus showing the normal cell (Grade 0) on the first day	133
4 2	Hepatopancreas of mysis 3 P semisulcatus stage 1 of MBV infection	133
4 3	Hepatopancreas of PL 6 of <i>P semisulcatus</i> showing the stage 2 of MBV	134
4 4	Hepatopancreas of PL 6 of <i>P semisulcatus</i> showing the stage 3 of MBV	134



LIST OF ABBREVATIONS

AM accumulative mortality

AO acridine orange

BBV Bennettae baculovirus

BKC benzalkonium chloride

BMNV baculoviral midgut gland necrosis

BP Baculovirus penaei

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 kılo 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 plebjeus baculovirus

PCR polymerase chain reaction

PGFRC Persian Gulf Fisheries Research Centre

PHRV penaeid haemocytic rod-shaped virus

PI post inoculation

PIB polyhedral inclusion body

