UNIVERSITI PUTRA MALAYSIA

EXPERIMENTAL INFECTION OF RIVER CATFISH *MYSTUS NEMURUS WITH VIBRIO PARAHAEMOLYTICUS* AND MOLECULAR CHARACTERIZATION OF THE ISOLATES

NAJIAH MUSA

FPV 2002 10

EXPERIMENTAL INFECTION OF RIVER CATFISH *MYSTUS NEMURUS* WITH *VIBRIO PARAHAEMOLYTICUS* AND MOLECULAR CHARACTERIZATION OF THE ISOLATES

By

NAJIAH MUSA

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

October 2002

Dedicated to

Ma & Ayah

& my late Tok

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

EXPERIMENTAL INFECTION OF MALAYSIAN RIVER CATFISH *MYSTUS NEMURUS* WITH *VIBRIO PARAHAEMOLYTICUS* AND MOLECULAR CHARACTERIZATION OF THE ISOLATES

By

NAJIAH MUSA

October 2002

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

Faculty: **Veterinary Medicine**

Declining marine fish resources in Malaysia have led to the innovation of rearing indigenous freshwater river catfish *Mystus nemurus*, locally known as 'baung' in brackishwater. This however will inevitably expose the fish to the pathogen, *Vibrio parahaemolyticus* that is ubiquitous in brackishwater. The present research was undertaken to study the virulence and pathogenicity of clinical and environmental *V. parahaemolyticus* isolates in *M. nemurus*. *Vibrio parahaemolyticus* isolates from various sources and locations in Peninsular Malaysia were identified based on morphological, biochemical and physiological characteristics.

Virulence studies revealed that clinical *V. parahaemolyticus* from clinical cases were more virulent ($p < 0.05$) to *M. nemurus* as compared to environmental isolates. The virulence was categorized as virulent, moderately virulent, weakly virulent and avirulent. The most virulent isolate (F1) was used to infect fish via intraperitoneal (IP), intramuscular (IM) and immersion routes. The LD_{50} results revealed that IP exposure was most pathogenic, following by IM and immersion exposures. Intraperitoneal exposure caused toxemia in fish while IM exposure

caused localized lesions at the injection sites, and immersion exposure caused only mild inflammatory responses on the gills and the scraped skin.

Random amplification polymorphic DNA (RAPD) revealed DNA polymorphism in all isolates tested, indicative of high variability among the *V. parahaemolyticus* isolates. Dendrogram revealed a distant genetic relationship between the virulent (F1) and avirulent (W4) isolates. Antibiogram showed resistance to intermediate to erythromycin, and 90% of the isolates were intermediate to cephalosporins and cefotaxim. The absence of plasmids in all isolates indicated that antimicrobial resistance of the isolates were chromosomally mediated.

Partial sequence analysis of the *tox*R and *tox*S genes of isolates F1 and W4 revealed a very high homology (97%). The genetic variations of *tox*R fragment resulted in 59 to 77% amino acid homology. This might have contributed to the different degrees of virulence of the isolates. The *tox*S fragment showed 100% amino acid homology, indicating that this fragment was more conserved than *tox*R gene fragment.

It appeared that not all *V. parahaemolyticus* isolates could induce infection in *M. nemurus*. However, slight genetic variations in *tox*R gene fragment of *V. parahaemolyticus* isolates could contribute to different degree of virulence. *Mystus nemurus* was least susceptible to the immersion challenge of a virulent *V. parahaemolyticus* isolate.

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

EKSPERIMEN INFEKSI KE ATAS IKAN BAUNG *MYSTUS NEMURUS* DENGAN *VIBRIO PARAHAEMOLYTICUS* DAN PENCIRIAN MOLEKULAR ISOLAT

Oleh

NAJIAH MUSA

Oktober 2002

Pengerusi : Hassan Hj Mohd Daud, Ph.D.

Fakulti : Perubatan Veterinar

Kekurangan sumber ikan marin dari laut di Malaysia telah membawa kepada inovasi menternak ikan sungai tempatan, *Mystus nemurus*, nama tempatannya 'baung' di dalam air payau. Bagaimanapun langkah ini akan menyebabkan ikan ini terdedah patogen, *Vibrio parahaemolyticus* yang sentiasa ada di dalam air payau. Justeru, kajian ini telah dijalankan bagi mengkaji kevirulenan dan patogenisiti isolat-isolat klinikal dan persekitaran *V. parahaemolyticus* ke atas *M. nemurus*. Isolat-isolat *V. parahaemolyticus* ini yang diambil dari pelbagai sumber dan lokasi di Semenanjung Malaysia telah dikenalpasti berdasarkan sifat-sifat morfologi, biokimia dan fisiologi.

Kajian kevirulenan menunjukkan bahawa isolat-isolat klinikal *V. parahaemolyticus* adalah lebih virulen ($p<0.05$) kepada *M. nemurus* berbanding dengan isolat-isolat persekitaran. Kevirulenan telah dikategorikan sebagai virulen, virulen sederhana, virulen lemah dan tidak virulen. Isolat yang paling virulen (F1) telah digunakan untuk menjangkiti ikan melalui pendedahan secara intraperitonial (IP), intramuskular (IM) dan rendaman. Keputusan LD_{50} menunjukkan pendedahan

IP adalah paling patogenik, diikuti oleh IM dan rendaman. Pendedahan IP menyebabkan toksemia pada ikan manakala pendedahan IM menyebabkan lesi setempat pada kawasan suntikan dan pendedahan rendaman hanya menyebabkan respon inflamatori ringan pada insang dan kulit ikan yang telah dilukakan.

Amplifikasi secara rawak DNA polimorfik (RAPD) menunjukkan polimorfik DNA dalam semua isolat yang diuji, menandakan variasi yang tinggi pada isolat-isolat *V. parahaemolyticus*. Dendrogram menunjukkan hubungan genetik yang jauh di antara isolat yang virulen (F1) dan tidak virulen (W4). Keputusan kerentanan antibiotik menunjukkan adanya resistan hingga sederhana terhadap eritromycin di kalangan isolat dan didapati 90% daripada isolat-isolat adalah sederhana terhadap cephalosporins dan cefotaxim. Ketiadaan plasmid pada kesemua isolat menunjukkan kerentanan antibiotik adalah bermediasikan kromosom.

Analisa jujukan sebahagian gen *tox*R dan *tox*S pada isolat-isolat F1 dan W4 menunjukkan homologi yang sangat tinggi (97%). Variasi genetik pada fragmen gen *tox*R menyebabkan 59% hingga 77% homologi asid amino. Ini mungkin telah menyumbangkan kepada perbezaan darjah kevirulenan isolat-isolat. Fragmen gen *tox*S menunjukkan 100% homologi asid amino, menandakan fragmen ini adalah lebih terpelihara berbanding fragmen gen *tox*R.

Oleh yang demikian, didapati bukan semua isolat *V. parahaemolyticus* boleh menyebabkan infeksi pada *M. nemurus*. Bagaimanapun, variasi genetik yang sedikit berbeza pada fragmen gen *tox*R boleh menyumbang kepada perbezaan darjah

kevirulenan. *Mystus nemurus* tidak mudah dihinggapi jangkitan isolat *V. parahaemolyticus* yang virulen melalui pendedahan secara rendaman.

ACKNOWLEDGEMENTS

In the name of Allah, The Most Gracious and Most Merciful, I am thankful and grateful to Allah for giving me the strength and capacity to complete this study.

It is my special pleasure to extend my deepest appreciation and gratitude to my main supervisor Dr. Hassan Haji Mohd Daud for his untiring supervision, advices, guidances and kindness, my committee members namely, Prof. Dr. Mohamed Shariff Mohamed Din for his superb supervision, priceless critisms, great opinions and providing emergency fundings, Assoc. Prof. Dr. Mohd Azmi Mohd Lila for his critical thinkings, suggestions, kindness and speedy corrections, and Assoc. Prof. Dr. Mariana Nor Shamsudin for her valuable opinions during the study period.

My sincere thanks to Kolej Universiti Sains dan Teknologi Malaysia (KUSTEM) and Universiti Putra Malaysia (UPM) for providing me the financial support under "Skim Latihan Akademik Bumiputra" (SLAB). Special thanks are also accorded to the Rector of KUSTEM, Prof. Dato' Dr. Mahyuddin Mohamad Dahan, Dean of Faculty of Science and Technology, Assoc. Prof. Dr. Noor Azhar Shazili and previous Dean, Prof. Dr. Haji Mohd Azmi Ambak, Head department of Fisheries and Marine Sciences, Assoc. Prof. Dr. Sakri Ibrahim, previous head of department(s) namely, Prof. Dr. Hajjah Faizah Shaharom, Mr. Aizam Zainal Abidin and Prof. Dr. Lokman Shamsudin for their encouragement and support during my study. I would also like to thank the Dean of Veterinary Medicine Faculty, Prof. Dr. Mohd Zamri Saad and previous Deans, Prof. Dato' Dr. Abdul Salam Abdullah and Prof. Dato' Dr. Sheikh Omar Abd Rahman for their supports and encouragement during my study in this faculty. I would also like to express my gratitudes to Tuan Haji Karim Noh Shah

(Ex-Deputy Registrar of UPM, currently Director of Development of UPM), Mr. Mohamed Adil Muhammad Tajuddin (Deputy Registrar of UPM) and Mr. Johan Abd Rahim (Registrar Department) for giving me the opportunities of attending an international shrimp pathology course in the University of Arizona, USA, a training workshop on applications of molecular techniques on "White Spot Syndrome Virus" (WSSV) detection in shrimp in the University of Can Tho, Vietnam and presenting my paper in The Third World Fisheries Congress, Beijing, China as well as presenting my papers in the local seminars and conferences during my study.

I am indebted to all my wonderful friends, especially to my best pals Dr. Lee Kok Leong and Ms Nadirah Musa, my colleagues, Ms Abeer Al-Sahtout, Dr. Tan Lee Tung, Dr. Wang Ying Geng and Chen Xia, Dr. Sanjoy Banarjee, Dr. Devaraja, Mrs Fennie Fong, Dr. Samson Soon, Ms Nor Azizah Mohd Taib, Ms Norazila Kassim Shaari, Ms Wan Somarny, Mrs Adnalizawati, Mr Mohd Zulkamal Radzi and Asst. Prof. Dr. Ahmed Jalal Khan Chowdry for their marvelous friendships.

To my parents, Ma and Ayah, whom I owe most of the gratitude, thank you very much for your love, prayers, affections, moral and financial supports throughout these years. My great and best sisters and brothers, Dee, Aid, Cah, Jang and Eee, thank you all for the encouragement, helps, concerns and understandings. Finally, a very special thank you to my BB whom I'm indebted for his patience, understandings, assistance and good heart throughout this project. Most of all, I thank God for making all these possible.

I certify that an Examination Committee met on 11th October 2002 to conduct the final examination of Najiah Musa on her Doctor of Philosophy thesis entitled "Experimental Infection of River Catfish *Mystus nemurus* with *Vibrio parahaemolyticus* and Molecular Characterization of The Isolates " 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:

Abdul Rani Bahaman, Ph.D.
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

Hassan Hj Mohd Daud, Ph.D.
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Mohamed Shariff Mohamed Din, Ph.D.
Professor
Faculty of Veterinary Medicine,
Universiti Putra Malaysia
(Member)

Mohd Azmi Mohd Lila, Ph.D.
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Mariana Nor Shamsudin, Ph.D.
Associate Professor
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia
(Member)

Ansary Ahmed, Ph.D.
Professor
Senior President
Open University Malaysia
(Independent Examiner)

SHAMSHER MOHAMAD RAMADILI, Ph.D.
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 26 NOV 2002

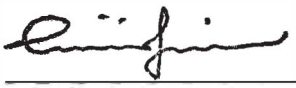
This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Hassan Hj Mohd Daud, Ph.D.
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Mohamed Shariff Mohamed Din, Ph.D.
Professor
Faculty of Veterinary Medicine,
Universiti Putra Malaysia
(Member)

Mohd Azmi Mohd Lila, Ph.D.
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Mariana Nor Shamsudin, Ph.D.
Associate Professor
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia
(Member)



AINI IDERIS, Ph.D.
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 13 FEB 2003

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been dully acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

NAJIAH MUSA

Date: 25.11.2002

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Anova	Analysis of variance
cfu	Colony forming unit
H & E	Hematoxylin and eosin
IP	Intraperitoneal
IM	Intramuscular
pi	Post infection
NaCl	Sodium chloride
RM	Ringgit Malaysia
TSA	Tryptone soya agar
TSB	Tryptone soya broth
TCBS	Thiosulphate citrate bile salt sucrose agar
ppt	Parts per thousand
OD	Optical density
bp	Base pair
°C	Degree celcius
Dh_2O	Distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxynucloside triphosphate
LD_{50}	Lethal dose 50%
kb	Kilobase
M	Molar
µl	Microlitre
min	Minute
PCR	Polymerase chain reaction