OCCURRENCE OF IRIDOVIRUSES AND BACTERIAL PATHOGENS IN BIVALVES, SCAVENGING FISH AND TRASH FISH IN MARINE AQUACULTURE FACILITIES IN SABAH, MALAYSIA

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THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

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2015

DECLARATION

I hereby declare that material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

26 August 2015

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ABSTRACT

Study was carried out to determine the occurrence of iridovirus and bacterial pathogen in bivalves, scavenging fish and trash fish collected from marine aquaculture facilities in Sabah. Bivalves and scavenging fish were collected from Kota Marudu, Kuala Penyu and Tuaran over a period of year from May 2012 to April 2013. Meanwhile, trash fish specimens were collected from fisheries landing stations Kudat, Semporna, Lahad Datu, Kuala Penyu and Kota Kinabalu. From the Polymerase Chain Reaction (PCR) analysis, specimens of bivalves and scavenging fish (n=2068) were detected positive for iridovirus (266), *Photobacterium damselae* (53), *Vibrio harveyi* (135), *Vibrio alginolyticus* (44) and *Vibrio parahaemolyticus* (147). Meanwhile, 35 of trash fish (n=221) specimens were also detected positive for iridovirus using PCR. The DNA of positive iridovirus samples was then characterized by DNA sequencing. From the DNA sequencing analysis, it was shown that the iridovirus found on bivalves, scavenging fish and trash fish are from the genus Megalocytivirus (*Iridoviridae*). Further strain divergence analysis suggested that the megaloctivirus from bivalves and scavenging fish are from the same strain that is the Infectious Spleen Kidney and Necrosis Virus (ISKNV) with the average percentage of nucleotide similarity of 98.77%. Similarly,the megalocytivirus from trash fish also showed the highest average percentage nucleotide similarity to ISKNV (99.40%), which suggested that the megalocytivirus in Sabah are from the strain of ISKNV. Finding of this study revealed that some of the bivalves and scavenging fish which are abundance in the aquaculture facilities particularly floating net cage, are carrier of iridovirus, *Vibrio harveyi, Vibrio parahaemolyticus, Vibrio alginolyticus* and *Photobacterium damselae.* These animals can become the hosts of viral and bacterial pathogens and possibly transmit them to cultured fish. Finding of this study also showed that the trash fish can become the carrier of iridovirus and may transmit it to cultured fish during feeding. Therefore, the study suggests that the aquaculture facilities should often be cleaned from biofouling organisms such as bivalves to prevent disease outbreak. Furthermore, the use of trash fish should be minimized for the similar reason.

ABSTRAK

KEJADIAN IRIDOVIRUS DAN BAKTERIA DI DALAM BIVALVIA, IKAN KECIL DAN MAKANAN IKAN DI DALAM FASILITI AKUAKULTUR MARIN

Kajian telah dijalan untuk memastikan kejadian iridovirus dan bakteria di dalam bivalvia, ikan kecil dan makanan ikan yang dikumpul daripada fasiliti akuakultur. Bivalvia dan ikan kecil diambil daripada Kota marudu, Kuala Penyu dan Tuaran, penggambilan sampel telah dijalankan selama setahun daripada Mei 2012 sehingga April 2013. Manakala sampel makanan ikan diambil daripada Kudat, Semporna, Lahad Datu, Kuala Penyu dan Kota Kinabalu. Daripada analisis Polymerase Chain Reaction (PCR), sampel bivalvia dan ikan kecil (n=2068) telah didapati positif iridovirus (266),Photobacterium damselae (53), Vibrio harveyi (135), Vibrio alginolyticus (44) dan Vibrio parahaemolyticus (147). Di samping itu, 35 daripada sampel makanan ikan (n=221) juga telah didapati positif iridovirus dengan menggunakan PCR. Kemudian, DNA sampel yang positif iridovirus telah dianalisis menggunakan kaedah DNA sequencing untuk pencirian iridovirus yang dijumpai didalam sampel. Keputusan analisis DNA sequencing menunjukkan bahawa iridovirus yang dijumpai di dalam bivalvia, ikan kecil dan makanan ikan adalah daripada genus megalocytivirus (Iridoviridae). Analisis untuk penentuan strain menunjukkan yang megalocytivirus yang dijumpai didalam bivalvia dan ikan kecil adalah daripada strain Infectious Spleen Kidney and Necrosis Virus (ISKNV) dengan purata peratus kesamaan nukleotida sebanyak 98.77%. megalocytivirus yang dijumpai didalam makanan ikan juga adalah daripada ISKNV dengan purata peratus kesamaan nukleotida sebanyak 99.40 yang mana menunjukkan bahawa megalocytivirus di Sabah adalah daripada strain ISKNV. Berdasarkan keputusan kajian; bivalvia dan ikan kecil yang berada di fasiliti akuakultur boleh menjadi pembawa kepada iridovirus, Vibrio harveyi, Vibrio parahaemolyticus,Vibrio alginolyticus dan Photobacterium damselae. Bivalvia dan ikan kecil boleh menjadi perumah kepada virus dan bacteria patogen dan boleh melepaskan patogen tersebut kedalam ikan kultur. Kajian ini juga menunjukkan bahawa makanan ikan juga boleh menjadi pembawa kepada iridovirus. Penggunaan makanan ikan didalam akuakultur boleh menjadi salah satu cara untuk menjangkitkan iridovirus kepada ikan yang dikultur. Oleh itu, kajian ini mencadangkan untuk selalu membersihkan kawasan fasiliti akuakultur untuk mengurangkan kehadiran organisma biofouling seperti bivalvia. Oleh itu, pengunaan makanan ikan harus dikurangkan yang digunakan juga harus dikaji terlebih dahulu untuk memastikan makanan ikan tersebut tidak dijangkiti patogen supaya penyakit dalam akuakultur dapat dikurangkan.

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LIST OF ABBREVIATION

AFCD	Aquaculture Fisheries Division
ATPase	Adenosine Triphosphate
ATV	Ambystoma tifrinum virus
BIV	*Bohle iridovirus*
BLAST	Basic Local Alignment Search Tool
BLASTN	Basic Local Alingment Search Tool Nucleotide
CDS	Coding Sequences
CTAB	Cetyltrimethylammonium Bromide
DNA	Deoxyribonucleotide Acid
DNTPs	Deoxynucleotide Triphosphates
DTAB	Dodecyltrimethylammonium Bromide
dsDNA	Double stranded Deoxyribonucleotide Acid
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic Acid
EtBr	Ethdium Bromide
FAO	Food and Agriculture Organization
FLD	Fish Lymphocystis Disease
FV-3	Frog Virus 3
GIVD	Grouper Iridovirus Disease
GSIVD	Grouper Spawner Iridovirus Disease
IBC	Inclusion Body-bearing Cell
ICTV	International Committe of Taxonomy of Virus
IIV	*Invertebrate iredescent virus*
IIV-1	*Invertebrate iridescent virus 1*
IIV-3	*invertebrate iridescent virus 3*
IIV-6	*Invertebrate iridescent virus 6*
IPTG	Isopropyl ß-D-1-thiogalactopyranoside
ISKNV	Infectious Spleen Kidney Necrosis Virus
LAMP	Loop-mediated isothermal amplification
LB	Luria-Bertani
LCDV	*Lymphocytivirus disease virus*

LCDV-1	*Lymphocytivirus disease virus 1*
LCDV-2	*Lymphocytivirus disease virus 2*
LCDV-C	*Lymphocytivirus disease virus in China*
LYCIV	Large Yellow Croaker Iridovirus
MCP	Major Capsid Protein
$MgCl_2$	Magnesium Chloride
NaCl	Natrium Chloride
NCBI	National Center for Biotechnology Information
OIE	World Organization for Animal Health
ORFs	Open Reading Frames
OSGIV	Orange-Spotted Grouper Iridovirus
PCR	Polymerase Chain Reaction
RBIV	Rock Bream Iridovirus
RFLP	Restriction Fragment Length Polymorphism
RSIV	Red Sea bream Iridovirus
RSIVD	Red Sea Bream Iridoviral disease
RT-PCR	Reverse Transciptase Polymeras Chain Reaction
SBIV	Seabass Iridovirus
SGD	Sleepy Grouper Disease
SGIV	Singapore Grouper Iridovirus
SGIVD	Singapore Grouper Iridovirus Disease
SOC	Super Optimum Growth
TAE	Tris-acetate-EDTA buffer
TE	Tris-EDTA buffer
TFV	Tiger Frog Virus
TGIV	Taiwan Grouper Iridovirus
TGIVD	Taiwan Grouper Iridovirus Disease
TRBIV	Turbot Reddish Body Iridovirus
UV	Ultraviolet
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
X-GAL	5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside

LIST OF SYMBOLS

%	Percentage
°C	Degree Celsius
µl	Microliter
µM	Micromolar
2X	2 times concentrated
5X	5 times concentrated
10X	10 times concentrated
1X	working concentration
bp	basepair
et al.	And others
g	Gram
M	Molar
mg/ml	milligram per milliliter
mg/L	milligram per liter
ml	mililiter
mM	Milimolar
ng/ µl	nanogram per microliter
nM	Nanomolar
nm	Nanometer
ppt	Part per Thousand
rpm	Revolution per minute
sp.	Species
U	Unit
V	Voltage

LIST OF APPENDICES

CHAPTER 1

GENERAL INTRODUCTION

1.1 Aquaculture in Malaysia

Aquaculture in Malaysia was first introduced in 1920's. The aim of aquaculture introduction was to improve country's economy in term of exports revenue and alternative seafood supply to satisfy the increasing demand for seafood (FAO, 2008). According to Othman (2008), Malaysia has high potential for aquaculture development because of its favorable climate as well as vast natural resources. Malaysia is also known as one of the countries that has high fish consumption. Furthermore, fishes are one of the cheapest sources of animal protein.

Aquaculture is defined as the farming of aquatic organisms including fish, mollusks, crustaceans and aquatic plants with some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding and protection from predators. Aquaculture can be divided into various stages namely; hatchery operation, nursery operation and grow-out operation. And depending on the species being farmed, aquaculture can be carried out in freshwater, brackish water or marine water. Therefore, aquaculture can be used to produce fish, mollusks, crustaceans and aquatic plants for both human consumption and ornamental purposes.

Aquaculture production in Malaysia is increasing every year. Table 1.1 shows the estimated aquaculture production in Malaysia from 2008 to 2012. While Tables 1.2 and 1.3 shows the estimated production by states from 2008 to 2012 of marine/brackish water aquaculture and freshwater aquaculture, respectively. Obviously, Sabah has been the biggest contributor in term of aquaculture production of marine/brackish water in Malaysia.

Table 1.1: Estimated production from aquaculture, Malaysia, 2008 to 2012

	2008	2009	2010	2011	2012
Number of aquaculture culturist	30,634	23,986	26,291	28,599	29,482
Aquaculture production ('000 Tonnes)	354	473	581	526	634
Freshwater					
Quantity (´000 Tonnes)	96	153	155	122	164
Value (RM million)	471.80	704.30	760.34	684.15	992.39
Brackishwater/Marin					
Quantity (´000 Tonnes)	259	320	426	404	471
Value (RM million)	1,268.25	1,617.66	2,038.46	1,821.22	1,765.71

Source: Fisheries Department, Malaysia, 2012

Table 1.2: Production of brackishwater/marine aquaculture by State, 2008 to 2012

State	2008		2009		2010		2011		2012	
	Quantity (tonnes)	Value (RM ´000)	Quantity (tonnes)	Value (RM '000)	Quantity (tonnes)	Value (RM '000)	Quantity (tonnes)	Value (RM '000)	Quantity (tonnes)	Value (RM '000)
Perlis	268	5,799	363	4,851	354	4,330	118	1,462	89	1,241
Kedah	1,468	17,150	4,379	45,133	4,896	43,633	3,281	30,787	2,659	33,467
Pulau Pinang	22,676	174,344	21,497	230,054	31,859	347,837	34,168	433,414	34,498	444,726
Perak	48,884	213,756	46,766	252,917	48,191	270,132	36,279	241,633	31,025	165,348
Selangor	26,629	182,730	43,517	211,424	53,179	222,649	37,792	207,189	20,900	173,759
Negeri Sembilan	447.49	5,381	1,164	15,035	1,405	17,165	1,418	17,394	1,016	12,440
Melaka	183.74	2,612	212	2,391	216	2,063	141	1,993	124	2,987
Johor	27,028	303,266	30,347	289,096	32,278	302,880	18,194	228,919	16,290	217,902
Pahang	5,917	123,243	10,670	224,354	12,260	260,632	4,216	80,632	3,807	67,678
Terengganu	1,953	24,582	2,072	26,778	2,076	26,118	2,165	29,606	4,150	59,453
Kelantan	229	3,278	1,079	14,880	598	7,676	410	5,372	609	8,496
Sarawak	4,143	66,741	5,459	66,305	8,604	129,902	9,592	145,394	9,434	142,529
Sabah	118,754	146,365	152,153	234,445	229,734	403,384	256,514	397,424	364,013	435,594
Total	258,581	1,268,248	319,676	1,617,663	425,650	2,038,402	404,287	1,821,220	470,620	1,765,714

Source: Fisheries Department, Malaysia, 2012

Table 1.3: Production of freshwater aquaculture by State, 2008 to 2012

State	2008		2009		2010		2011		2012	
	Quantity (tonnes)	Value (RM million)	Quantity (tonnes)	Value (RM million)	Quantity (tonnes)	Value (RM million)	Quantity (tonnes)	Value (RM million)	Quantity (tonnes)	Value (RM million)
Perlis	254	1,072	531	1,765	151	511	67	246	153	548
Kedah	869	8,003	6,221	20,453	3,801	20,133	3,154	10,396	3,973	13,419
Pulau Pinang	6,849	6,654	4,817	12,854	6,264	17,661	5,337	17,278	4,994	17,000
Perak	13,889	121,578	51,701	224,381	70,319	329,205	53,618	288,129	92,264	558,629
Selangor	8,629	65,568	15,251	70,150	14,867	65,385	14,365	63,522	14,857	69,946
Negeri Sembilan	431	29,853	12,082	50.787	8,882	41,777	7,187	41,308	6,983	38,627
Melaka	96	21,922	28,605	90,119	13,810	45,588	7,248	23,622	7,966	27,637
Johor	10,816	55,382	12,783	42,772	14,196	47,558	10,628	38,934	10,511	42,131
Pahang	5,842	52,737	7,413	74,814	9,361	78,048	9,341	102,965	8,779	104,080
Terengganu	133	22,568	3,644	20,932	3,561	22,920	3,101	22,006	4,219	30,979
Kelantan	80	3,124	2,162	9,295	2,365	11,061	1,695	8,449	2,033	10,326
Sarawak	3,380	26,238	2,560	27,837	2,929	22,060	2,396	18,374	3,054	31,003
Sabah	5,852	57,099	4,859	58,144	4,871	58,429	4,081	48,921	3.971	47,614
Total	57,118	471,798	152,631	704,303	155,399	760,335	122,219	684,149	163,757	992,386

Source: Fisheries Department, Malaysia, 2012