QUANTITATIVE HISTOPATHOLOGY AND EPIDEMIOLOGY OF PRAWN VIRAL DISEASES

Thesis submitted by

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Abstract

The interaction of prawns, viruses and the environment in disease development at individual and population levels was studied using histological and statistical analyses based on quantitative models and simple quantitative methods.

At the individual level, quantitative histopathology was utilized to differentiate monodon baculovirus (MBV) in the digestive tissues (the midgut or MG and hepatopancreas or HP) of Australian *Penaeus monodon* and *Penaeus merguiensis*. It seems MBV in *P. monodon* was less damaging than in *P. merguiensis.* Indeed, *P. monodon* sustained significantly lower severity of MBV infection (mean±SE of severity index (SI) = 1.6 ± 0.1) and proportion of abnormal cells (R_{ac} = 34 ± 3%) with a significantly higher proportion of infected nuclei (R_{in} = 64 ± 3%); while *P. merguiensis* had significantly higher SI (2.1 ± 0.1) and R_{ac} (76 ± 5%) with significantly lower R_{in} (46 ± 3%). Besides, the diameter of MBV occlusion bodies (OBs) in *P. monodon* (5.4 ± 0.3 µm*)* was significantly larger than that in *P. merguiensis* (3.5 ± 0.4 µm). Also, the number of MBV OBs per nuclei in *P. monodon* was moderately correlated to the OB diameter (R^2 = 0.34, p << 0.05), while in *P. merguiensis* it was weakly correlated (R^2 = 0.12, p << 0.05). Perhaps, *P. monodon* and MBV mutually determined the size of the OBs, whereas *P. merguiensis* mostly influenced the size of the OBs. Tissue tropism of Australia-type MBV in the AMG of *P. merguiensis* was reported for the first time based on MBV prevalence (91± 6%), SI (2.4± 0.1), R_{cc} (91±3%) and significant dominance of the final stage of nuclear change. Overall, the HP of both penaeid species was more tolerant to MBV than the AMG since the HP contained significantly larger OBs and higher proportion of nuclei with the early stage of MBV infection compared to that in the AMG.

For the first time a transect method was applied to analyse lymphoidal changes in *P. monodon*. This method was simple and cost effective since it detected the changes of the length of lymphoidal tubules and spheroids using a micrometer and a low-power light microscope. Two novel quantitative models were developed and tested here, i.e. the spheroid-total length ratio (STLR) and morphotype (MT) score. The results show that the ratio of spheroid length and morphotypes of the spheroids continually changed in a successive fashion from an early infection type (morphotype A) to a terminal type (morphotype C) in chronic or acute experimental infection by an insect *Autographa californica* nuclear polyhydrosis virus (AcNPV). Overall, this simple technique was sensitive and reliable to detect lymphoidal changes in the prawns.

At the population level, risk factor analysis was applied relative to a declining prawn production in a farm with no written records of prawn diseases. Statistics were employed to investigate any possible correlation between the food budget (proxy measure of prawn health) and 10 parameters of daily water quality (265 working days from 18 crops); the biomass (proxy measure of prawn health) to 5 parameters of prawn biology, 6 parameters of farming management and 1 parameter of production (29 crops within 9 month data span); and 2 parameters of histology (35 sub-adult *P. monodon*). Coherence, analogy and histology show that high salinity (33 ppt), low temperature (26 ^{o}C), young age (< 107 days), high stocking density (> 20 individuals/m^2), systemic bacterial infection (46% prevalence) and muscle necrosis (91% prevalence) were the potential risk factors of declining prawn production in the farm. Non-systemic and minor cellular changes associated with suspected viruses suggest that viral disease was very likely not a risk to the prawn production.

Since molecular techniques can be used to confirm the diagnosis by histology, the histological diagnosis of MBV was compared to non-isotope *in situ* hybridization (NISH) and polymerase chain reaction (PCR) assays. Molecular diagnosis of MBV appears to encounter technical difficulties, which were clarified in terms of possible causes and procedural modifications.

Quantitative histopathology, statistical epidemiology and simple, low-costs, time-efficient and robust quantitative methods in this research may be suitable for places like Indonesia lacking sophisticated diagnostic facilities.

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LIST OF ABBREVIATIONS

ABW	Average body weight
AcNPV	*Autographa californica* nuclear polyhedrosis virus
AMG	Anterior mid-gut
AR	Attributable risk
BBV	Bennettae baculovirus
BMNV	Baculoviral midgut gland necrosis virus
BP	Baculovirus penaei
BWF	Body weight fed
DNA	Deoxyribonucleic acid
d.p.i	Day post injection
DIG	Dixogenin-11-dUTP
EDTA	Ethylenediaminetetracetic acid
ELISA	Enzyme-linked immunosorbant assay
FA	Fluorescent antibody
FCR	Food conversion ratio
FLW	Feed last week (the amount of feed in the last week before sampling)
GAV	Gill-associated virus
HHNBV	Hypodermal and hematopoietic necrosis baculovirus
HP	Hepatopancreas
h.p.i	Hour post injection
HPV	Hepatopancreatic parvo-like virus
ICTV	International Committee on Taxonomy of Viruses
IGR	Individual growth rate
IHHNV	Infectious hypodermal and haematopoietic necrosis virus
LO	Lymphoid organ
LOS	Lymphoid organ spheroid
LOV	Lymphoid organ virus
LOVV	Lymphoidal organ vacuolization virus
LPV	Lympoidal parvo-like virus

MbSNPV	*Metapenaeus bennettae* single nucleocapsid polyhedrosis virus
MBV	Monodon baculovirus
MG	Mid gut
MNPV	Many nucleocapsid polyhedrosis virus
MT score	Morphotype score
NISH	Non-isotope *in situ* hybridization
OB	Occlusion body
OR	Odds ratio
PBV	Plebejus baculovirus
PCR	Polymerase chain reaction
PjNOB I	*Penaeus japonicus* non occluded baculovirus I
PL	Postlarvae
PmSNPV	*Penaeus monodon* single nucleocapsid polyhedrosis virus
PvSNPV	*Penaeus vannamei* single nucleocapsid polyhedrosis virus
PWG	Pond weight gain
R_{cc}	Ratio of cytolytic cells
R_{in}	Ratio of infected nuclei
RNA	Ribonucleic acid
RV-PJ	Rod-shaped nuclear virus of *Penaeus japonicus*
SC	Standing crop
SEM	Scanning electron microscopy
SEMBV	Systemic ectodermal and mesodermal baculovirus
SI	Severity index
SMV	Spawner-isolated mortality virus
SN	Spheroid number
SNPV	Single nucleocapsid polyhedrosis virus
SPF	Specific-pathogen free
SPR	Specific-pathogen resistance
SR	Survival rate
STL	Spheroid-total length
STLR	Spheroid-total length ratio

TEM	Transmission electron microscopy
TRF	Total running feed
TSV	Taura syndrome virus
WSBV	White spot syndrome baculoviruses
WSSV	White spot syndrome virus
YHV	Yellow-head virus