

ACKNOWLEDGEMENTS

VIRUS SCREENING OF CULTURED CARP AND TILAPIA SPECIES AND THE VIRUS SENSITIVITY OF CELL CULTURES FROM INDIGENOUS FISH SPECIES

Throughout the study, I am also grateful to the member of my supervisory committee, Dr. Faleah Ghazwan, for her valuable comments.

I am indebted to Dr. James Richard Arthur, my mentor, for all his specialist help and encouragement. Professor Dr. Mohd. Sheriff Mohd. Din, who gave his unflinching support and assistance to enable me to complete this study.

By

JUAN DEANON ALBALADEJO

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	DNA Deoxyribonucleic Acid	
	EMEM Earle's Balanced Salt Solution	
	EDTA Ethylenediaminetetraacetate	
	EVV Enzootic Hemopoietic Necrosis Virus	
	ER-1 Eel Kidney	
	ELISA Enzyme-linked Immunosorbent assay	
	EO-2 Eel Ovary	
	EPC Epithelioma Papulosum Cyprini	

LIST OF ABBREVIATIONS

AS	Atlantic Salmon Gonad
BGV	Blue Gill Virus
BB	Brown Bullhead Trunk
BF-2	Bluegill Fry
CAR	<i>Carassius auratus</i> Fin
CCO	Channel Catfish Ovary
CCVD	Channel Catfish Virus Disease
CHSE-214	Chinook Salmon Embryo
cm	Centimeter
cm ²	Centimeter Square
CO ₂	Carbon Dioxide
CPE	Cytopathic Effect
CRV	Channel Catfish Reovirus
DNA	Deoxyribonucleic Acid
EBSS	Earle's Balanced Salt Solution
EDTA	Ethylenediaminetetraacetate
EHNV	Enzootic Haemopoietic Necrosis Virus
EK-1	Eel Kidney
ELISA	Enzyme-linked Immunosorbent assay
EO-2	Eel Ovary
EPC	<i>Epithelioma Papulosum Cyprini</i>

EUS	Epizootic Ulcerative Syndrome
EV-2	Eel Virus-2
EVA	Eel Virus American
EVE	Eel Virus European
EVEX	Eel Virus European Rhabdovirus
FAT	Fluorescence Antibody Technique
FBS	Foetal Bovine Serum
FHM	Fathead Minnow Peduncle
FUDR	5-fluoro-2'-deoxyuridine
GCK-84	Grass Carp Kidney
GCRV	Grass Carp Reovirus
GNV	Gill Necrosis Virus
[³ H]	Radio-isotope Uridine
HEPES	(N-[2-hydroxyethyl] piperazine N ¹ - [2-ethanesulfonic acid])
IC	Intracranial
IFAT	Indirect Fluorescence Antibody Technique
IHNV	Infectious Haemopoietic Necrosis Virus
IP	Intraperitoneal
IPNV	Infectious Pancreatic Necrosis Virus
IPT	Immunoperoxidase Antibody Technique
IU/ml	International Units per Milliliter
m	Meter

MEM	Minimum Essential Medium
MEM-4	Minimum Essential Medium with 4 % Serum
MEM-10	Minimum Essential Medium with 10 % Serum
ml	Milliliter
mM	Millimolar
mm	Millimeter
MOI	Multiplicity of Infection
nm	Nanometer
OMV	<i>Oncorhynchus masou</i> Virus
ONB	<i>Oreochromis nilotica</i> Brain
PFRVD	Pike Fry Rhabdovirus Disease
pH	Negative logarithm of Hydrogen Ion
PG	Pike Gonad
PGH	<i>Puntius gonionotus</i> Heart
PGP	<i>Puntius gonionotus</i> Peduncle
PSH	<i>Puntius schwanenfeldii</i> Heart
PSSn	<i>Puntius schwanenfeldii</i> Snout
PTA	Phosphotungstic Acid
RNA	Ribonucleic Acid
rpm	Revolution per minute
RTG-2	Rainbow trout Gonad
SDS-PAGE	Sodium Dodecyl Sulfate- Polyacrylamide Electrophoresis

LIST OF FISH SPECIES

SHF	Striped Snakehead	Fin
SGV	Sand Goby	Virus
STE-137	Steelhead Trout	Embryo
SVCV	Spring Viremia of Carp	Virus or Infectious Dropsy
TCID ₅₀	Tissue Culture Infective Dose at 50%	
TEM	Transmission Electron	Microscopy
TEV	Tadpole Edema	Virus
TK-1	Tilapia	kidney
TmB	<i>Tilapia mossambica</i>	bulbus arteriosus
TO-2	Tilapia	Ovary
UDRV	Ulcerative Disease	Rhabdovirus
µg/ml	Microgram	per milliliter
VEN	Viral Erythrocytic	Necrosis
VHSV	Viral Haemorrhagic Septicaemia	Virus or Egtved virus
VSV	Vesicular Stomatitis	Virus
WC-1	Walleye	Trunk
WO-1	Walleye	Ovary
Crucian Carp	<i>Carassius carassius</i>	
Dab	<i>Limanda limanda</i>	
Discus Fish	<i>Symphysodon discus</i>	
European Eel	<i>Anguilla anguilla</i>	
European Sheatfish	<i>Silurus glanis</i>	

LIST OF FISH SPECIES

Atlantic Cod	<i>Gadus morhua</i>
Atlantic Menhaden	<i>Brevoortia tyrannus</i>
Atlantic Salmon	<i>Salmo salar</i>
Ayu	<i>Plecoglossus altivelis</i>
Bait Minnow	<i>Notemigonus crysoleucas</i>
Barbel	<i>Barbus barbus</i>
Bighead Carp	<i>Aristichthys nobilis</i>
Bluegill	<i>Lepomis macrochirus</i>
Blue-eyed Cichlid	<i>Cichlasoma spirillum</i>
Bream	<i>Abramis abramis</i>
Brown Bullhead	<i>Ictalurus nebulosus</i>
Channel Catfish	<i>Ictalurus punctatus</i>
Chinook Salmon	<i>Oncorhynchus tshawytscha</i>
Chum Salmon	<i>Oncorhynchus keta</i>
Cobitid Loach	<i>Misgurnus anguillicaudatus</i>
Common Carp	<i>Cyprinus carpio</i>
Convict Cichlid	<i>Cichlosoma nigrofasciatum</i>
Crucian Carp	<i>Carassius carassius</i>
Dab	<i>Limanda limanda</i>
Discus Fish	<i>Symphysodon discus</i>
European Eel	<i>Anguilla anguilla</i>
European Sheatfish	<i>Silurus glanis</i>

Fathead Minnow	<i>Pimephales promelas</i>
Goldfish	<i>Carassius auratus</i>
Gold Dust Tilapia	<i>Sarotherodon aureus</i>
Golden Ide	<i>Leuciscus idus</i>
Grass Carp	<i>Ctenophyngodon idella</i>
Grayling	<i>Thymallus thymallus</i>
Gudgeon	<i>Gobio gobio</i>
Guppy	<i>Poecilia reticulata</i>
Japanese Eel	<i>Anguilla japonica</i>
Japanese Flounder	<i>Paralichthys olivaceus</i>
Javanese Carp	<i>Puntius gonionotus</i>
Lamprey	<i>Lampetra fluviatilis</i>
Minnows	<i>Phoxinus phoxinus</i>
Mossambique Mouthbrooder	<i>Oreochromis mossambica</i>
Muskellunge	<i>Esox masquinongy</i>
Nase	<i>Chonrostoma nasus</i>
Nile Tilapia	<i>Oreochromis nilotica</i>
Northern Pike	<i>Esox lucius</i>
Orange Chromide Cichlid	<i>Etroplus maculatus</i>
Paradise Fish	<i>Macropodus opercularis</i>
Pacific Cod	<i>Gadua macrocephalus</i>
Rainbow Trout	<i>Oncorhynchus mykiss</i>
Ramirez Dwarf Cichlid	<i>Apistogramma ramirezi</i>

Redbelly Tilapia	<i>Tilapia zilli</i>
Red Fin Perch	<i>Perca fluviatilis</i>
Red-Fin Cigar Shark	<i>Leptobarbus hoevenii</i>
Red Tilapia Hybrid	<i>Oreochromis nilotica</i> X <i>Oreochromis mossambica</i>
Rio Grande Cichlid	<i>Cichlasoma cyanoguttatum</i>
Roach	<i>Rutilus rutilus</i>
Rudd	<i>Scardinius erythrophthalmus</i>
Sand Goby	<i>Oxyeleotris marmoratus</i>
Seabass	<i>Dicentrarchus labrax</i>
Silver Bream	<i>Blicca bjoernka</i>
Sockeye Salmon	<i>Oncorhynchus nerka</i>
Smelt	<i>Osmerus eperlanus</i>
Smooth Dogfish	<i>Mustelus canis</i>
Steelhead Trout	<i>Salmo gairdneri</i>
Striped Snakehead	<i>Ophiocephalus striatus</i>
Sucker	<i>Catostomus commersoni</i>
Tench	<i>Tinca vulgaris</i>
Tilapia Hybrid	<i>Oreochromis nilotica</i> X <i>Oreochromis aureus</i>
Tin-Foil Barb	<i>Puntius schwanenfeldii</i>
Turbot	<i>Scophthalmus maximus</i>
Walking Catfish	<i>Clarias batrachus</i>
Walleye	<i>Stizostedion vitreum vitreum</i>

Abstract of the Thesis submitted to the Senate of the
 University of Pertanian Malaysia in partial fulfillment of the requirements for the degree of
 Doctor of Philosophy
 YAMAMAE *Oncorhynchus masou*
 Yellow Tail *Seriola quinqueradiata*
 Zebra Fish *Brachydanio rerio*

By
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March 1994

Chairman : Dr. Hassan Uj. Mohd. Daud
 Faculty : Fisheries and Marine Sciences

Three virus particles were isolated in vitro and tentatively classified into three groupings, namely Herpesviridae, Birnaviridae and Iridoviridae. Virus groupings were based on the ultrastructural morphology and cytopathology observed. Distinct virus-like cytopathic effects were noted on four established fish cell lines viz. CHSE-214, GCK-84, EPC and TmB, utilized in the study. The virus-like particles, from pelleted infected cell cultures and clarified cell culture supernatant, were visualized under transmission electron microscopy.

Virus titration utilizing EB cells for the Birnavirus-like isolate achieved a mean titre of 7.48×10^6 TCID₅₀/ml. Relatively high virus titres were also recorded

Abstract of the thesis submitted to the Senate of the Universiti Pertanian Malaysia in partial fulfilment of the requirements for the degree of Master of Science

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Three virus particles were isolated *in vitro* and tentatively classified into three groupings, namely *Herpesviridae*, *Birnaviridae* and *Iridoviridae*. Virus groupings were based on the ultrastructural morphology and cytopathology observed. Distinct virus-like cytopathic effects were noted on four established fish cell lines viz. CHSE-214, GCK-84, EPC and TmB, utilized in the study. The virus-like particles, from pelleted infected cell cultures and clarified cell culture supernatant, were visualized under transmission electron microscopy.

Virus titration utilizing BB cells for the Birnavirus-like isolate achieved a mean titre of 7.40×10^5 TCID₅₀/ml. Relatively high virus titres were also recorded

from the other two isolants, with a mean virus titres of 7.16×10^5 TCID₅₀/ml for Herpesvirus-like and 6.46×10^5 TCID₅₀/ml for Iridovirus-like. Cohabitation infection of fish infected with Herpesvirus-like on naive coloured-strain common carp (*Cyprinus carpio*) resulted in the production of epidermal hyperplastic reaction. Correspondingly, infection was amplified in smaller fish (5-6 cm) with mortality recorded at 40% over a 21-day observation period. Epidermal hyperplasia in larger size fish (15-20 cm) was however somewhat restricted. In interspecies comparative study of Herpesvirus-like infection, atypical lesions which were seen as localized petechial haemorrhages and raised scales were noted in Javanese carp (*Puntius gonionotus*). In tin-foiled barb (*P. schwanenfeldii*) infection, the fish exhibited raised sanguinous masses along the dorso-lateral surface of the body. Results from the parenteral inoculation of virus infected cell culture filtrate was inconclusive.

A total of 18 monolayers were started from five commonly cultured species of carp and tilapia. These were grouped according to the cells type observed, i.e.

Amtrak tesis yang kemukakan kepada Pusat Universiti
Pertanian Malaysia sebagai pemenuhi sebahagian
fibroblast, epithelial and mixed-type. Susceptibility
to the isolated viruses and to an established fish virus,
the Infectious Pancreatic Necrosis Virus (IPNV) of Sp
strain, revealed that the cell monolayers from *Oreochromis
nilotica* Brain (ONB) and *P. schwanefeldii* heart (PSH)
successfully supported the virus replication and produced
relatively high titres. Growth requirements of the
established cells, serum concentrations and incubation
temperature were also examined.

Tiga partikel virus telah dipencilkan secara *in vitro*
dan secara tentatif diklasifikasikan kepada tiga kumpulan,
iaitu Herpesviridae, Birnaviridae dan Iridoviridae.
Golongan virus adalah berdasarkan kepada morfologi
struktur ultra dan pemerhatian patologi sel. Kesemua
sitopatik nyata dilihat dalam empat kultur sel ikan iaitu
GSS-214, GCK-84, KPC dan IHB, yang digunakan dalam kajian
ini. Partikel seakan virus dari pelat kultur sel yang
dijangkiti dan juga supernatan kultur sel yang ditapis
telah dilihat di bawah mikroskop elektron pancaran.

Titraasi terhadap isolat seakan Birnavirus menggunakan
sel BB mencapai nilai purata $(7.40 \times 10^6 \text{ DIT}_{50}/\text{ml})$. Titer
yang tinggi juga diperolehi dari dua isolat lain iaitu