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

By

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LIST OF ABBREVIATIONS

AS	Atlantic Salmon Gonad
BGV	Blue Gill Virus
BB	Brown Bullhead Trunk
BF-2	Bluegill Fry
CAR	*Carassius auratus* 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	*Epithelioma Papulosum Cyprini*

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 . . . . . *Oncorhynchus masou* Virus

ONB . . . . . *Oreochromis nilotica* Brain

PFRVD . . . . . Pike Fry Rhabdovirus Disease

pH . . . . . Negative logarithm of Hydrogen Ion

PG . . . . . Pike Gonad

PGH . . . . . *Puntius gonionotus* Heart

PGP . . . . . *Puntius gonionotus* Peduncle

PSH . . . . . Puntius schwanenfeldii Heart

PSSn . . . . . *Puntius schwanenfeldii* Snout

PTA . . . . . Phosphotungstic Acid

RNA . . . . . Ribonucleic Acid

rpm . . . . . Revolution per minute

RTG-2 . . . . . Rainbow trout Gonad

SDS-PAGE . . . . Sodium Dodecyl Sulfate- Polyacrylamide Electrophoresis

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 . . . . . *Tilapia mossambica* 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

LIST OF FISH SPECIES

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

Fathead Minnow . . . . . *Pimephales promelas*

Goldfish . . . . . *Carassius auratus*

Gold Dust Tilapia . . . . . *Sarotherodon aureus*

Golden Ide . . . . . *Leuciscus idus*

Grass Carp . . . . . *Ctenophyngodon idella*

Grayling . . . . . *Thymallus thymallus*

Gudgeon . . . . . *Gobio gobio*

Guppy . . . . . *Poecilia reticulata*

Japanese Eel . . . . . *Anguilla japonica*

Japanese Flounder . . . . . *Paralicthys olivaceus*

Javanese Carp . . . . . *Puntius gonionotus*

Lamprey . . . . . *Lampetra fluviatilis*

Minnows . . . . . *Phoxinus phoxinus*

Mossambique Mouthbrooder . . . *Oreochromis mossambica*

Muskellunge . . . . . *Esox masquinongy*

Nase . . . . . *Chonrostoma nasus*

Nile Tilapia . . . . . *Oreochromis nilotica*

Northern Pike . . . . . *Esox lucius*

Orange Chromide Cichlid . . . . . *Etroplus maculatus*

Paradise Fish . . . . . *Macropodus opercularis*

Pacific Cod . . . . . *Gadua macrocephalus*

Rainbow Trout . . . . . *Oncorhynchus mykiss*

Ramirez Dwarf Cichlid . . . . . *Apistogramma ramirezi*

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

Yamamae . . . . . *Oncorhynchus masou*

Yellow Tail . . . . . *Seriola quinqueradiata*

Zebra Fish . . . . . *Brachydano rerio*

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 $TCID_{50}$/ml. Relatively high virus titres were also recorded

from the other two isolants, with a mean virus titres of 7.16 X 10^5 $TCID_{50}$/ml for Herpesvirus-like and 6.46 X 10^5 $TCID_{50}$/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.

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. schwanenfeldii* 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.