



UNIVERSITI PUTRA MALAYSIA

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

JUAN DEANON ALBALADEJO

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SPECIES AND THE VIRUS SENSITIVITY OF CELL
CULTURES FROM INDIGENOUS FISH SPECIES**

By

JUAN DEANON ALBALADEJO

Thesis Submitted in Partial Fulfilment of the Requirements for the
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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 |



| | | | |
|--------------------|-----------|--------------------------------------|-------------------------------|
| 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 |



LIST OF FISH SPECIES

| | |
|------------------------------|-------------------------------------|
| Atlantic Cod | <i>Gadus morhua</i> |
| Atlantic Menhaden | <i>Brevoortia tyrannus</i> |
| Atlantic Salmon | <i>Salmo salar</i> |
| Ayu | <i>Plecoglossue 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 abrama</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 anguillacoubulatus</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</i> <i>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> |



Yamamae *Oncorhynchus masou*
Yellow Tail *Seriola quinqueradiata*
Zebra Fish *Brachydano rerio*



Abstract of the thesis submitted to the Senate of the
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March 1994

Chairman : Dr. Hassan Hj. 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 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.



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.



Abstrak tesis yang kemukakan kepada Senat Universiti
Pertanian Malaysia sebagai memenuhi sebahagian
syarat bagi mendapatkan ijazah Master Sains

**SARINGAN VIRUS DALAM SPESIS IKAN KAP DAN TILAPIA DAN
SENSITIVITINYA TERHADAP KULTUR SEL DARI
BEBERAPA SPESIS IKAN TEMPATAN**

oleh

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Mac 1994

Pengerusi : Dr. Hassan Hj. Mohd. Daud
Fakulti : Perikanan dan Sains Samudera

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. Kesan sitopatik nyata dilihat dalam empat kultur sel ikan iaitu CHSE-214, GCK-84, EPC dan TmB, yang digunakan dalam kajian ini. Partikel seakan virus dari pelet kultur sel yang dijangkiti dan juga supernatan kultur sel yang ditapis telah dilihat di bawah mikroskop elektron pancaran.

Titration terhadap isolat seakan Birnavirus menggunakan sel BB mencapai nilai purata (7.40×10^5 DIKT₅₀/ml). Titer yang tinggi juga diperolehi dari dua isolat lain iaitu

