CHAPTER I

INTRODUCTION

An annual epizootic in freshwater fish has occurred in Thailand in late 1981, the first,only spread over certain provinces of Southern Thailand. This epizootic occurred in the winter, from September to March. The virulence of disease in December to January was severe than in the other months, especially when the weather was very cool (Taniyavanich 1983, Menasavata 1983, Tonguthai 1985). When the weather became warm, the incidence of diseas decreased. Now, it has spread to every part of Thailand and other countries such as Papau New Guiney, Indonesia, Malaysia, Burma and Laos P.D.R. Fish mortalities were not confined to natural water but spread to the commercial fish ponds. Striped snakehead (Ophicephalus striatus), silver barb (Puntius gonionotus), snakeskin gourami (Trichogaster pectoralis) and freshwater eel (Fluta alba) are very sensitive to this disease. The trunk, head and tail of the sick fish showed ulcers. Some parts of the muscle or tail were lost. Head erosion was also in evidence. The report from National Inland Fisheries Institute, Freshwater Fisheries Division of Department of Fisheries in 1982-1985, showed

that more than twenty species of fish developed clinical signs of this disease such as yellow mystus (Mystus nemurus), walking catfish (Clarias batrachus), climbing perch (Anabas testudineus), red cheek barb (Puntius orphoides), freshwater gar fish (Xenentodon cancila), croaking gourami (Trichopsis vittatus), jullien's mud carp (Cirrhinus jullieni), three spot gourami (Trichogaster trichopterus), etc (Tonguthai 1985). Many investigators found that bacteria and fungi isolated from ulcerative fishes could be the cause of these symptoms (Pittayangkula and Bodhalamik 1983, Sukroongreung et al. 1983, Poonsuk et al. 1983). Virus-like particles were found in many organs (liver, spleen, kidney and muscle) in many kinds of ulcerative fishes eg. snakeskin gourami (Trichogaster pectoralis), freshwater eel (Fluta alba) etc (Ruttanaphani et al. 1983, Wattanavijarn et al. 1982, 1983a,b, 1985). These finding made it necessary prepare freshwater fish cell culture to study the viruses from ulcerated fishes.

Very few reports of fish viruses and fish cell cultures existed in Thailand. The studies that were done differed from those of Europe, the U.S.A. or Canada which had been done more than 30 years ago, since 1951 (Wolf and Quimby 1969). Fish cell lines had been prepared from many speciese of fish. Most of the cell lines were prepared from freshwater or anadromous

species and a few were from strictly marine species. The fish cell lines collected up to 1979 showed about 61 lines of fish cell from 17 families (Wolf and Mann 1980). Fish cell lines could be prepared from many kinds of organs such as gonads (Wolf and Quimby 1962, Robert 1966, Noga and Hartman 1977), ovaries (Ahne 1979a, Bower and Plumb 1980), embryo (Clem et al. 1961, Fryer 1965, Kelly 1973a) and caudal trunk (Gravell and Malsberger 1965).

The important conditions for the growth of fish cells are as follows :

1. Media used in general such as M-199, BME (Eagle's basal medium), NCTC 109, L-15, Eagle's MEM with Earl's BSS and 10 % fetal bovine or calf serum used as supplement.

2. Antibiotics ; 100 IU/ml of penicillin, 100 ug/ml of streptomycin (Wolf and Quimby 1969, 1976b, Wolf 1973a).

3. The physiological saline routine using Hank's BSS, or phosphate buffer saline (PBS) pH 7.2 (Wolf and Quimby 1976ab, Wolf and Ahne 1982).

4. pH 7.2-7.4 is the optimal range for growth of cells, but cells can be grown at a range of pH 7.0-8.0.

5. The optimal temperature for growth of cells from warmwater fish is 25-30 °C but they can be grown

between 15-30 °C.

Many kinds of viruses were isolated from diseased fish by using fish cell lines such as herpes - virus, iridovirus, rhabdovirus,reovirus-like, oncovirus-like. Some kinds of virus could not be isolated but could be detected by electron microscopy as unclassified groups (Mc.Allister 1979).

In general, different kinds of viruses can be grown in different cell cultures (Nim et al. 1970), but some of these cell lines were suitable for many kinds of viruses to replicate eg. RTG-2, BF-2, and some kinds of cell lines did not permit virus to replication such as RTN (Wolf and Mann 1980, Kinkelin 1986). An easy way to know that what kind of cell can be used to culture virus, the virus should be cultivated in the cells and then conducted a blind passage of this virus 2-3 times, noting any of the following : (Schmidt 1973)

1. Cellular degeneration, many viruses manifest their presence in susceptible cell cultures by producing degeneration changes in cells called cytopathic effect (CPE) which can be observed microscopically.

2. Plaque formation, cytopathic viruses, and even some viruses which do not produce CPE in cell cultures maintained on a fluid medium, might be detected macroscopically by their ability to form plaques in the monolayer under a solid medium.

3. Metabolic inhibition, infection of the cell culture with cytopathic virus might be evidence by lack of viral activity a change in color due to metabolic inhibition.

4. Hemadsorption (HAd), a suspension of suitable RBC is added to the cell culture, and after a suitable incubation period, microscopic examination showed that several of the RBC adhered in clumps to the infected host cell monolayer.

Other methods which can be used for detecting the virus are mixed hemadsorption, immunofluorescence or immunoperoxidase staining and interference.

Research Aims:

1. To study primary cell culture preparation from some species of freshwater fish :

   Anabas testudineus (Climbing perch).

   Cyprinus carpio (Common carp).

   Lebeo rohita (Rohu).

   Ophicephalus lucius (Snakehead fish).

2. To study viral multiplication in freshwater fish cell cultures which could be grown and formed good monolayers. The viruses used in this study are as follows :

   Infectious pancreatic necrosis virus (IPNV).

   Viruses from *Ophicephalus striatus* (SHV).

   Viruses from *Hampala sp.* (CV).

Reserch Advantages

1. To discover the kinds of freshwater fish which can be prepared as the primary cell culture.

2. To use these primary cell cultures to study the viruses which may be pathogen in freshwater fish disease.

3. To establish cell lines which can be used for studying viruses from diseased fish.

4. To discover what kinds of freshwater fish cell cultures permit viruses to multiply.

5. To discover method of bilding up primary and continuous cell lines.

6. To discover the results of this study for diagnosis of freshwater fish disease.