Winter School on

'RECENT ADVANCES IN DIAGNOSIS AND MANAGEMENT OF DISEASES IN MARICULTURE'

7th to 27th November, 2002

Course Manual

Organising Committee

Prof. (Dr) Mohan Joseph Modayil
Director, CMFRI

Course Director

Dr. K. C. George
Principal Scientist,
Division of
Physiology, Nutrition and Pathology

Co-ordinators

Dr. R. Paul Raj, Head, P N P Division

Dr. P. C. Thomas, Principal Scientist

Shri. N.K. Sanil, Scientist (Sr. Scale)

Dr. (Mrs.) K.S. Sobhana, Scientist (Sr. Scale)

Indian Council of Agricultural Research
Central Marine Fisheries Research Institute
P B. No. 1603, Tatapuram P.O., Cochin 682 014

VIRAL DISEASES OF FISH

Dr.K.M.Shankar
Associate Professor, Fish Pathology and Biotechnology Laboratory,
Department of Aquaculture, College of Fisheries, Mangalore-575002

Viruses are submicroscopic particles made up of nucleic acid covered with a protein coat. The nucleic acid is either RNA or DNA and not both. Protein coat is often covered with an envelope and depending on the presence or absence of an envelope, virus can be an enveloped/non enveloped virus. Size of the virus vary from 20-200 nm. Shape of the virus varies from a simple icosahedra to bullet shape to complex brick shape.

Virus are classified based on their nucleic acid (DNA or RNA) and protein profile. However, additional details on type of disease, host range, and geographic distribution of the virus are also used in classification. The classification of the virus is decided by the International committee on taxonomy of virus (ICTV).

Viruses are obligatory intracellular parasites requiring a living cell to replicate. As the virion is lacking its own metabolic machinery, it depends fully on the living host cell for machinery and raw materials for its metabolism and replication. This complex biology of the virus has made it difficult to clearly identify a viral process to design drugs for selective killing without damaging the host cell. Any attempt to kill the intracellular virus affects the host cell.

Replication of Virus in a Cell

There are two kinds of entry into and exit of virus from a cell: a) receptor mediated endocytosis (RME), b) direct entry by fusion. Non-enveloped virus enter the cell by RME (Fig. 1). RME of virus is similar to regular entry of other macromolecules into the cell. With this receptor mediated specified process, virus enter a cell in a vesicle which later fuses with lysosome. Inside the lysosome, viral particles undergo partial changes in protein coat due to digestion by lysosomal enzymes and move to cytoplasm. Finally, this uncoated nucleic acid replicates followed by synthesis of proteins and assembly of viral capsid proteins and genome to full blown virus particles. The site of assembly of the virus may be in the cytoplasm or nucleus of a cell which is referred to as inclusion body. Inclusion bodies which varies in size, shape, location and staining reaction can be used as a diagnostic feature for some viruses. Non-enveloped virus exit from a cell by damaging plasma membrane and hence clearly show cytopathic effect in cell culture.

Enveloped viruses are known to use both RME and direct fusion for entry into and exit from a cell. The virus envelope is a phospholipid bilayer derived from host which is attached with viral derived protein. The protein is used for attachment, while the envelope involves in fusion with the plasma membrane. Enveloped virus exit out of the cell by budding, without damaging the host plasma membrane and hence CPE is usually not clear with an enveloped virus.

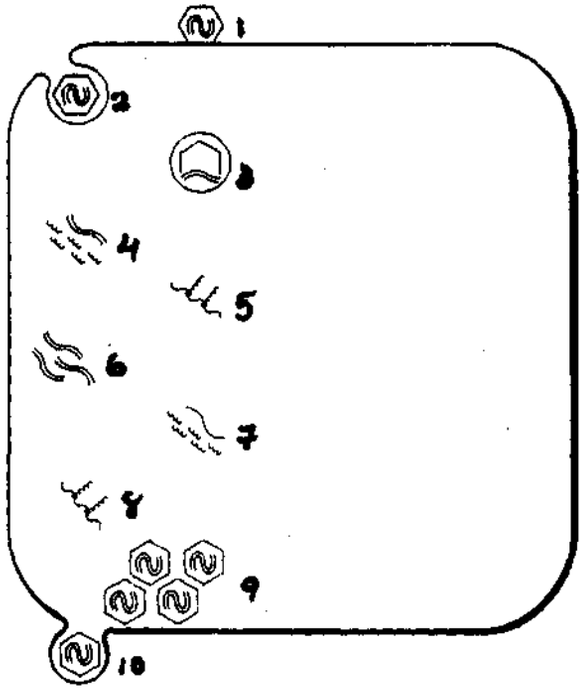

Fig. 1: Entry, replication and exit of a virus from a cell.

1. Attachment, 2. Penetration, 3. Uncoating, 4. Transcription of mRNA, 5. Translation of early proteins, 6. Viral DNA replication, 7. Transcription of mRNA, 8. Translation of late proteins, 9. Assembly of virions, 10. Exit by budding

Aquatic viruses are transmitted mainly by two routes; horizontal and vertical. In the vertical route virus is transmitted from parents to offspring through sex products. Depending on whether virus is present inside or on sex products, it could be true or false vertical transmission. More commonly, virus is present on sex products and in plasma fluid. In horizontal transmission, virus is transmitted from one individual to another through water, feed, contact with asymptomatic host and other carriers. The mode of transmission has a key role in determining severity and rapidity of virus infection and disease manifestation, spreading and persistence of the virus in an area. Overall, the amount of virus (infectious dose) in brood, seed, water or other infected material determines severity of virus infection in a population. Normally, virus enters fish through skin, lateral line, gills and gut and reach target organs through blood.

Virus Pathogenecity

Virus is a highly obligatory intracellular parasite. Virus replication is invariably at the cost of the host cell, either the cell is affected partly or fully. At host level, virus affect target organ partially damaging and impairing its function or fully destroying it leading to morbidity and death of the host.

Outcome of disease in a fish population due to virus infection is complex and depends on several factors. Among the various factors, immune status of individuals and infectious dose of virus (quantity of virus in water, feed, infected material) are very

important in the outcome of disease. Depending on these variables, outcome of virus infection are of several types; virus infects host but get aborted in some individuals; virus infects and cause disease but host overcomes; virus infects and cause disease and death; virus infects and host survives infection and remain as carrier. Hence, clinical signs and mortality pattern of viral infection in a population differs between individuals.

In a population viral infection need not always cause 100% mortality due to differences in the individual host immune status and infectious dose. In some cases, virus remain at a low level of infection establishing a delicate balance with the host. In addition, there are carriers which are survivors of a mass scale infection and mortality. Carriers will be spreading the virus life long to the environment and are potential risk and concern in viral disease management. Other types of carriers are non-target hosts which are not affected at all but become asymptomatic carriers. Latent infection of virus at very low level, which express late in the life of an host is also risky. Usually it is difficult to detect virus in carrier or latent infection stage.

Virus Variants

Replication of virus is very rapid. In general, virus requires 6-10 h for one complete cycle which is faster in RNA virus compared to DNA virus. Because virus replicate rapidly, they undergo mutation very often. Mutation in RNA virus is higher than in DNA virus. Intensive aquaculture measures such as high stocking density, extensive culture area, stress to animals and high traffic provide ideal conditions for outbreak of viral disease and resulting mutation leading to several strains. The new strains of virus may be virulent or avirulent which differ in pathogenicity and mortality pattern in hosts. Due to rapid evolution of virus, designing vaccines or standardising diagnosis becomes difficult.

Viral Disease Diagnosis

Diagnostic methods include both traditional and modern molecular biological tools. Traditional methods such as histopathology, electron microscopy, cell culture assay, virus neutralisation tests have their own importance. Electron microscopy provides vital useful information on size, shape and site of replication of the virus inside host cell. However, the method is cumbersome, time consuming and hence cannot be recommended for routine diagnosis. Histopathology gives idea about target organs and cells involved. The replication sites are identified by the presence of inclusion bodies. Greater specific details on viral pathology can be obtained by immunohistochemistry or DNA based *in situ* hybridisation.

Diagnosis by cell culture is the first step in identification and study of viruses. Virus replicates only in living cell and hence cell lines are essential for study, diagnosis, purification and vaccine development. Cell lines of many cultivable species of fish are available now. Since list of cultivable species is growing, there is emergence of new viral disease, and hence there is a need for development of more cell lines. Further, cell lines from various organs of fish such as gonad, gill, spleen, liver, skin and heart are required for efficient culture and detection of virus. Virus enters cells, replicates and damage the cell monolayer in a culture. The damage is termed as cytopathic effect (CPE). In some cases virus besides destroying the cells change the morphology of cells. Nature of CPE and inclusion body give preliminary idea about the involvement of a virus and to some

extent type of the virus. Quantity of the virus in the infected fish can be estimated by cell culture by two methods; tissue culture infectious dose 50% ($TCID_{50}$) assay and plaque assay. The $TCID_{50}$ is a quantal assay which indirectly gives virus concentration in terms of the dose causing 50% cell death. The plaque assay gives actual number of infectious particles present in the fish. The $TCID_{50}$ assay is ideal for mass scale quantitation of virus, while plaque assay is ideal for small scale studies. Once virus is identified by histopathology, cell culture CPE, further specific tests are required to confirm the type of the virus. This involves specific serological tests such as neutralisation test (NT) employing rabbit antisera or monoclonal antibodies. A suspected virus should be treated with available antisera against established viruses and the neutralisation of the virus is detected by inoculating the reactants to cell culture. Depending on the occurrence of CPE in the neutralisation test, virus can be identified. A new virus cannot be neutralised by the antisera and hence cause CPE in cell monolayer. Neutralisation test is the most specific and invariably required in virological investigations. In addition to NT, other serological tests available for fish viruses are haemagglutination, hemeadsorption and complement fixation.

Antibody based tests such as ELISA, immunodot, Western blot are commonly used in diagnosis of viral disease. Other antibody based *in situ* tests such as immunoperoxidase (IPO), immunofluorescence (IFT) give more specific information about the target organs and cells involved in pathogenesis, besides diagnosis. DNA based methods such as DNA hybridisation and PCR are also becoming popular in diagnosis. Several simplified versions of these tests are available as kits for field diagnosis in developed countries.

Common Viral Diseases of Fish in the World

In some countries in Europe and North America, scientific fish culture particularly salmonid culture started half a century ago. In salmonid culture, where intensive hatchery practice is common, several viral disease of very serious nature have been recorded. Economically important fish virual diseases are given in Table 1.

Among the various fish viruses, infectious pancreatic necrosis (IPN) virus which was reported back in 1940 in salmonid hatcheries is very well studied. Information on this virus will be useful in fish and shellfish viral disease management in developing countries. IPN virus is an RNA virus, affecting young ones of salmonids in hatcheries causing mortality ranging from 60-100%. The virus cause disease by damaging pancreas and intestine of young fish and hence the name infectious pancreatic necrosis. Juveniles and adults are refractory to infection. Young ones which survive the infection will become life long carriers spreading the virus. In addition to salmonids, the virus has been isolated from non-salmonids such as tilapia, carp with little or no mortality. The virus has also been recorded in molluscs and rotifers. The virus which originated in North America and Europe gradually spread to some of the Asian countries through transport of salmonid eggs and juveniles. Feral population of salmonids in remote areas of North America acting as cariers of virus is also on record. e.g. presence of IPN virus in feral lake trouts of Sub Arctic region of Canada. VR299, SP and Ab are the major serotypes of IPNV. Also several variants of the IPN virus differentiated using gene and monoclonal antibody probes have been reported. Hence, vaccine development is a difficult task. As such, no effective drug is available. Chemicals such as vescodyne is available for washing trout egg which is not 100% effective. Since no effective drug or vaccine is available,

preventing the entry of virus to the culture system has been the best option. Proper screening and certification of seed, brood and water has been the major strategy for control of the virus. If disease is rampant, crop holiday is the suggested measure to reduce the viral load in the culture system.

Viral Disease Management

Specific drugs for viral disease treatment are not available or difficult to develop since virus is host cell dependent for all its metabolic machinery. However, virucidal chemicals capable of killing virus outside the host are available. Chlorine, iodine, ozone and UV rays are some of the commonly employed virucidal agents in aquaculture. In recent years, though several nucleic acid analogue based drugs have been developed, they are found to be cytotoxic and as such they are not successful. Vaccines are the most effective preventive strategies in mammals. However, vaccines in general are not found to be effective in fish viral disease management. Nevertheless, killed, attenuated and recombinant DNA vaccines have been developed. Protection from vaccines against viral disease in fish is found to be for short period with variable results. Poor immune system of the fish and young age at infection are some of the responsible factors for susceptibility to disease. Further, there are several variants of the virus. All these factors contribute to the poor performance of viral vaccines. In the absence of successful drug or vaccine, avoidance of the virus in culture system is the best strategy. This can be achieved by proper sensitive screening of brood, seed and certification programmes. Crop holiday is one of the best strategies to prevent viral disease in aquaculture and is still practised in salmonid culture to prevent IPN disease. During crop holiday, infectious dose in water and carriers gradually comes down due to reduction in host and carrier availability in the system.

Fish Virus in Asian Countries

Compared to viral disease in North America and Europe, fish viruses recorded are few in Asian countries. This could be due to several reasons. Intensive fish culture started in these countries only recently. Furthermore, in some of these countries virological studies are not conducted systematically due to lack of trained persons, facilities and cell lines. Nevertheless, new diseases, suspected to be of viral etiology in carps and aquarium fish have been recorded. Incidences of viral disease in grass carp, grouper have been reported. In EUS primary viral involvement is still suspected.

Investigations on Emerging New Fish Diseases Suspected to be of Viral Etiology

In recent years, several new diseases suspected to be of viral etiology have emerged in tropical aquaculture. Such new diseases may be due to bacteria or viruses. It is always customary to look for primary etiology step by step. Filtering the infected tissue homogenate through 0.2 μ and performing Koch's postulate using the bacteria-free filterate would be the most appropriate preliminary step. Bacteria-free filterate producing clinical disease and mortality will indicate viral involvement. Simultaneously, the filterate is inoculated to cell culture in the presence of antibiotics. Bacteria get separated in 0.2 μ filter and cannot cause CPE in the presence of antibiotics, while the virus can, which will give further indication of involvement of the virus. Further tests such as electron microscopy and virus neutralisation will positively confirm the viral etiology.

Table 1 Viral pathogens of economic importance in aquaculture

Virus	Host	Target tissue	Major pathology	Major clinical signs	Vaccine under development
a. Infectious Pancreatic Necrosis virus (IPNV)	Salmon, Trout	Pancreas and liver	Necrosis of acinar pancreatic tissue, catarrhal exudate	Distended abdomen, blackening of body, swirling movement, exophthalmia	Killed, live attenuated, subunit
b. Infectious Haematopoietic Necrosis virus (IHNV)	Salmon, Trout	Kidney, spleen and liver	Necrosis of haematopoietic tissue, anaemia, internal haemorrhages	(Non-specific)	Subunit, live attenuated
c. Viral Haemorrhagic septicaemia (VHS)	Salmon, Trout	Vascular system, kidney	Haemorrhagic anemia, Necrosis and exudate formation	Dropsy (Non-specific)	Subunit, live attenuated
d. Rhabdovirus carpio	Carp, Pike	Swim bladder	Haemorrhage in the swim bladder	Non-specific	Killed, live attenuated, subunit
e. Channel catfish virus (CCV)	Channel Catfish	Pancreas	Necrosis, spongiosis	(Non-specific)	Live attenuated
f. Lymphocystis virus	Walley	Lymphocytes, Epidermal cells	Hypertrophy of lymphocytes and epidermal cells	(Non-specific)	None