

Viral Studies on Carp Disease in China --- with a Special Reference to Herpesvirus on Common Carp---

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Abstract

The present paper mainly deals with the general situation of the viral diseases affected on the Chinese carps in a recent decade in China. The viral disease is highly characterized by the fast transmission, great mortality and being difficult to control which has caused a heavy loss in the aquaculture systems. The paper further and specifically introduces the general status of herpesvirus on carps in China and preventive measures against the outbreak of the disease from the ecological point of view and the environmental issues.

Key words: viral diseases, Chinese carps, herpesvirus on carps, preventive measures

Since 1980s, there have been a rapid development of aquaculture in China, the percentage is getting higher and higher in the position agricultural development and turning one of the four major core production activities (grain, meat fish and eggs). In 1998, the fish production in China was up to 38 million tons. However, the fish diseases, particularly the virus disease becomes more and more prominent, epidemic which lead to a heavy loss in the aquaculture activities in the recent years. Due to a long time-length of incubation period, complex characters and high infection, the fish suffers from a high mortality. Also due to the small size of the pathogenic virus and the copying characters, it has a strong resistance against the antibiotics, while the chemicals application often leads to the death of the host prior to that of the pathogens. Therefore the treatment of the virus diseases is encountered with a great difficulty. The following is the general situation of the virus disease happened in China aquaculture systems.

1. Virus and morphologies

After the isolation of the first strain of the virus affected on fish, there have been reports over 70 virus with 15 families, which are popular with the most animals. In the information collected on virus studies on fish diseases in 1970s-1980s, there was also information on virus diseases with the Chinese grass carps. At the same time there were also new strains of virus discovered and isolated.

Table 1. Some important virus pathogens with fish diseases.

Name of the virus	Host	Classification	Symptoms
Channel catfish virus	Channel catfish	Herpesvirus	Acute haemorrhage
Reoviridae of G/C	Grass carp	Reoviridae	Haemorrhage
Salmo herpesvirus	Salmo & Oncorhynchus	Herpesvirus	Anaemia, bleeding
IHNV	Salmo & Oncorhynchus	Rhabdoviridae	Congestion on fins
IPNV	Salmo & Oncorhynchus	Birnaviridae	Eyes & abdominal enlarged, unbalance in swimming
Japan Iridoviruae	Eels	Iridoviruae	Anus congestion & over mucusing
Oncorhynchus masou virus	Oncorhynchus	Herpesvirus	Unbalance in swimming and abnormal respiration
Bass Iridoviruae	Bass	Iridoviruae	Unbalance in swimming
Spring haemorrhagical virus	C/C,S/C, Cr/C	Rhabdoviridae	Difficult in breathing
Esox lusius (northern pike) Rhabdoviridae	Pikes, G/C	Rhabdoviridae	Sudden death of the fish in mass
VHS virus	Salmo & Oncorhynchus	Rhabdoviridae	Internal bleeding, swollen with liver and spleen
Cod pancreas virus	Cod fish	Adenoviridae	Skin damaged and ulceration
Salmo bladder virus	Salmo	Retroviridae	Rumor causing
Nerve necrosis virus	S/C	Nodaviridae	Nerve necrosis
Siniperca infectious anaemia virus	Siniperca	Paramyxoviridae	Anaemia
Epidermis blood-producer necrosis virus	Oncorhynchus Bass, Channel catfish	Iridoviruae	Blood-producer necrosis

2. *The examine and diagnosis of fish virus diseases*

The earlier examination and diagnosis of the fish diseases will certainly reduce a loss in aquaculture process. The methods of diagnosis are varied but mainly are the following types:

2.1 Biological checks: With the characters of the host that is vulnerable to the disease infection, the host is served as an indicator for testing. One test with crucian carp which is similar to the characters of grass carp sensitive to the disease outbreak showed out immediate results by artificial infection through injection or bathing. The fish got infected in 1-2 weeks with the inoculation. Therefore crucian carp can be used as a biological indicator to induce the outbreak of the disease.

2.2 Microscopic observation: From the experience and practice, the special characterized data and indicator are summarized. The microscopic observation is necessary and useful tool in checking and diagnosing the diseases. Much reliable information are collected in this way.

2.3 Cell culture: Cell culture is basic in studying the virus. It is also a traditional way in diagnosing the diseases affected with virus. The normal practice follows the better understanding of the cell highly vulnerable to the virus.

2.4 Molecular biology: Establishing the reliable methods for checking and diagnosing the fish disease is a positive way for fish disease prevention and control. The molecular biological approaches are as marked antibodies that are used to determine the viral protein, nuclear acid probing or PCR. All these methodologies are also applicable to all the vertebrate animals.

2.5 *A few methods of virus examination:*

There are quite many new and improved methods for examining IHNV on fish virus. However, it demands much previous preparation. Cell culture is a good approach, but it is not as sensitive as desired.

3. *Prevention and control of the virus disease and preparation of the vaccination*

To the virus disease, till present there is no yet any effective drug for this treatment. The expertise have long insisted that there should not be an overused quantity of the drugs, it is considered that it will reduce the fecundity and quality of the fish spawning and reduce the disease resistance of the animal itself with a heavy residue leading to the pollution of the water and environment. Selection, cyto-engineering, genetic engineering will help breed new varieties against the diseases. The herbs application is also an effective way in controlling the disease outbreak. It is quite practical but vague in its mechanisms.

4. *Fish herpesvirus diseases*

Herpesvirus is available in the natural environment like many other pathogens, it has a specific host selection. In the past few years, success has already been achieved in finding out a few virus with herpesvirus. Some of the herpesvirus are rumor-causative, but some can cause the total body infection with a high mortality. There have been a good number of reports describing infectious status with herpesvirus on *Anguilla japonica* and *Anguilla anguilla*.

Herpesvirus on channel catfish is caused by its fingerlings in the southern part of USA, other countries from Central America that introduced the fish successfully isolated the strains of the virus. But there have been not yet any reports in Asian countries.

In Japan, there have been reports on herpesvirus with common carp. It is confirmed that herpesvirus is a rumor

causative for Cyprinidii and a pathogen of smallpox of European carps. So far there have been found 4 kinds of herpesvirus from Salmoni: NeVTA (from *O. nerka*), OMV (from *O. masou*); YIN (from *Yamame* of *O. masou*) and CSIV (from *O. kisutch*). In a rainbow trout farm in USA, the brooders died at 50% after spawning, it was found that herpesvirus is available in the egg yolk.

4.1 SVC is a secondary disease meanwhile confirmed by OIE, NACA and FAO: The fish suffers from an acute hemorrhage mainly attacking one-year old common carp fingerlings. Other fishes not Cyprinidii are also vulnerable to its outbreak. The dead, diseased fishes and pathogen-carriers are all the causes of the diseases. The fish lice can be for its transmission. The outbreak of the diseases can be only at the temperature of lower than 15°C. The mortality reaches 80-90%.

Pathogen: It is the herpesvirus with a 150nm in diameter in size, there is a capsule outside in spherical shape. It causes a change and mutation in the critical areas of the fish body. Under the conditions of 15-18°C, the fish (common carp) is infected by either scratches on injections, the superficial areas of the fish show a smallpox.

The symptoms and pathological changes:

The typical change is the skin hyperplasia. The diseased fish shows the white spots on the skins like wax called smallpox which are closely attached to the fish skins. The smallpox are getting bigger and more in number till covering the whole fish body (Fig. 1). It leads to a zero value in marketing. The epidemics are popular at the temperature of 15°C. The fish suffers and is overloaded till its death. After the dissection, the guts and kidneys are congested and heavy inflammation, the intestinal mucus membranes fell off, the intestinal content is full of the pus-like substances. The intestines are clear with inflammation with a light blue color, some with reddish color.

Diagnosis: The cell tissues are obtained from the liver, spleen, kidney, brain or sperm, eggs inoculated to the FHM or EPC cell for isolating virus (Fig. 2). The cell will meet a good mutation at the temperature of 20°C. The tests will be further conducted with neutralizing test, immune fluorescent test or PCR for final determination (Fig.3).

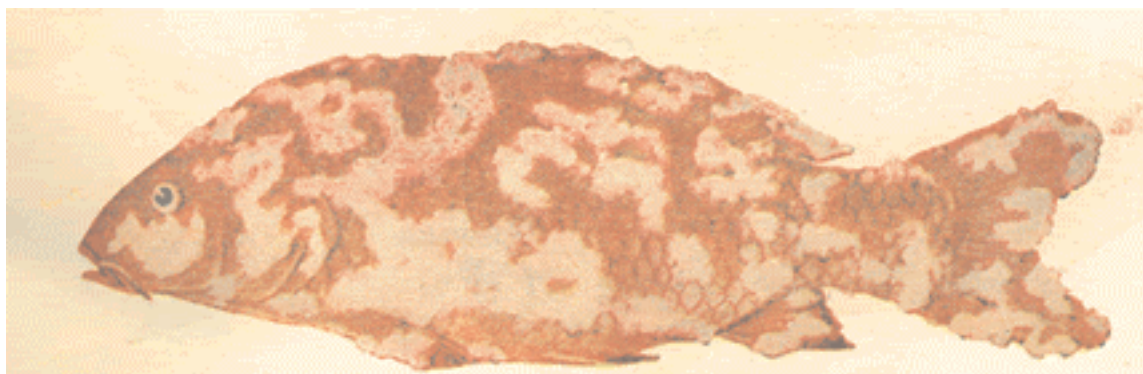


Fig. 1. The fish diseased with smallpox.

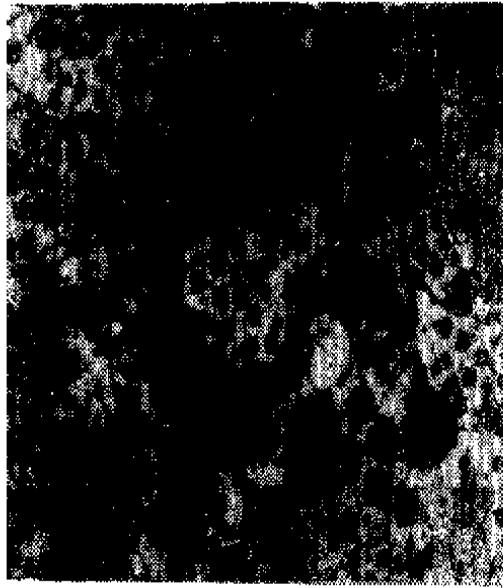


Fig. 2. Large amount of the viral granules are available in the nuclear spasm (x 24600).



Fig. 3. Smallpox: the matured viral grains are released to the superficial areas through follicles (x 13000).

4.2 Eel Virus Disease (Eel mouth disease)

It was firstly reported in China in 1992, it was found out in the HB cell of the eels. The virus is 20-side sphere which is composed of a core and capsule. The average diameter is about 70 nm, while the core diameter is 40 nm in size. The suspended solution with bacteria-free from the heart and spleen of the diseased fish are used to infect the healthy fish. The heart and the liver turned to gray in

color. The fish can not close the mouth after opening.

4.3 Herpesvirus of turbot (*Tarphops oligolepis*)

Characters: The virus is sphere-like with capsules the diameter is 120-220 nm. The available reports on the size of the herpesvirus (turbot) is 200-220 nm in diameter. It is transformed and copied in the host.

Pathology: Herpesvirus caused a great loss over the turbot (at 4-month old) in the farmed conditions. A large number of abnormal giant cells are found in the epidermis of the dorsal part and gill structures. The disease was also found in the farmed turbot in Demark. The fish is not active, move less, seems less oxygen. Dropsy and fallen-off of the intestines are observed. Therefore great pathological changes have taken place over the gills, skins, fish heart, guts and kidneys. Herpesviurs are clearly observed from the gill giant cells. Clinic symptoms are not very clear sometimes. Technical people further observed that turbot fingerlings from France was infected with herpesvirus which are mainly attached to the skin and the gills of the fish. The size of the virus reached over 80um, but it has a less lethal effect in infection and mortality.

5. Countermeasures against virus diseases

The outbreak and epidemics of the fish disease are closely related to the environment and the resistance of the animal itself. In the light of the practical experience gained from both research and the farmers the disease prevention and control should be focused on the improvement of the aquatic environment, removal of the pathogens and increase of the self-immunities.

5.1 Improvement of the aquaculture environment with ecological approaches

A. Water quality improvement: The harmful metabolic substances are washed and diluted with fresh water to improve the water quality. Pond preparation should be done thoroughly to eradicate the wild animals, predators and other food competitors.

B. Water temperature regulations: Most of the parasitic pathogens are only adapted to the temperature of 20°C. The reproduction of the pathogens can be blocked off by water temperature regulations, moreover, it can

accelerate the growth rate of the cultured objects.

C. Minimizing the stress: If water temperature is changed in a big range, pond silt is worsened, the feed quality gets bad, there is a sudden rain like thunderstorm leading to the change of the pH value, disturbance of netting, noise, misuse of the chemicals, there should be a heavy impact over the stress of the cultured animals resulting to the lower resistance of the diseases.

D. Sound ecological prevention and control: This is to maintain the balance between the cultured animals in the controlled conditions. The cultured ponds can be added with the PSB that can turn the harmful substances into good aspects. Predators can be used to remove the disabled, diseased.

5.2 Tight control of the pathogenic transmission.

A. Establishing vaccination system: In the process of the exotic fish introduction, careful steps are taken to prevent the pathogens along with the fish. Generally the virus disease has a long and potential period before its outbreak. The fast diagnosis process should be established to find out immediate roots of the diseases.

B. Sound environmental control: In the cultured conditions, there are large quantities of the disease-caused and non-disease-caused bacteria, while the later is the balance between the cultured environment and the animals. The large quantity of the chemical application in a great extent harms kill the disease-caused bacteria, but meanwhile it also damages and kills the non-disease-caused bacteria which are beneficial to the culturing conditions. Therefore the effective approaches to maintain the sound conditions for the cultured animals are to manipulate, monitor and manage the water quality.

C. Disinfectants: The disinfectants are categorized into two types: oxidized and non-oxidized chemicals, the former like potassium permanganate (PP). The effects are closely related to the water temperature, pH, ammoniac concentration, suspended matters, but it is poor to the virus killing, the later like copper sulphate, formalin is poor in dis-infection, moreover great side-effects, it is no use in virus infection.

D. Biological control: If the polyculture is conducted, some of the cultured animals can help remove the disease pathogens for more important animals.

5.3 *Micro-ecologies to control the outbreak of the diseases*

A. The basic principles: When the cultured animals are under the healthy conditions, there is a dominant population of the micro-organisms with relatively stabilization. This micro-organisms can both be beneficial to the growth of the cultured animals and also restrain the harmful organisms that might cause bad effects. At the normal conditions, the following three components are closely related to the environmental equilibrium: aquatic animals and plants; micro-organisms and environment.

B. Less use of the antibiotics: Over use of the antibiotics often leads to a heavy damage of the environment, high resistance against the new chemicals and greater quantity of the chemical application. The fishery chemicals should be more directive and effectively focusing on specific diseases. Strict avoidance should be made for the general use of the chemicals that might hurt the fish kept in the same areas.

C. Application of the microbiological products: Dominant population of the microbiology is established for the farmed objects internally and externally, for example, PSB can be applied and cultured in the

controlled conditions. It is not only used for purifying water, but also for preventing diseases and promoting fish growth. Another type is the product made of the intestinal anaerobic bacteria (IAB) which helps in reconstruction and regulation of the micro-ecological systems.

5.4 *Eradicating the enemies protecting the farmed objects*

The enemies of the farmed animals are mainly referring to predating fishes, aquatic insects, amphibians, reptiles and birds. The eradicating of these animals should follow different approaches:

A Control the number of the predating fishes: The normal predating fishes are, snakehead (*Channa argus*), *Siniperca chuatsi*, catfish, *Pseudobagrus fulvidraco*, *Monopterus albus*, *Opsariichthys bidens*. The eradication of the wild predating fishes should be done prior to the stocking of the fingerlings. Necessary measures should be also taken to prevent the inflow of the additional predating fishes when the pond is irrigated. An understanding should be made on the spawning ground and conditions of the predating fishes so that manipulations should be continued to destruct the spawning process. Large size fingerlings stocked are also effective in controlling the predating fishes.

B. Eradicating aquatic insects. The normal insects are *Laccotrephes sp.*, *Ranatra chinensis*, *Kirkaladyia deyrouei*, *Hydra vulgaris*, *Cyclestheria*. Some of the insects catch the fish, some eat the fish eggs. Reproduction of these aquatic animals will also consume a great deal of the oxygen and nutrients in the water bodies.

C. Capture of the unwanted animals:

Snakes, water mice, frogs and tadpoles and water birds should be timely removed and driven off. These animals are not only predated but also carriers of the disease pathogens.

5.5 Use of the growth promoters to speed up the growth rate of the fish**A. Application of the growth substances:**

The growth substances are mixed up with the feeds to feed the fish to activate the fish growth, regulate the metabolic rate of the nuclear acid and prevent the expansion and the transmission of the fish diseases. *Undaria pinnatifida* can nicely be used as feed promoter and at the same time it is also used for the treatment of the ulceration of the fish.

B. Rare soil: A certain concentration of the rare soil is applied to inhibit the growth and reproduction of the diseased pathogens. Fish appetite is stimulated, anti-resistance is enhanced. All that is good and effective for the red skin disease, enteritis and gill rot of grass carp.

C. Bio-activated products: The bio-activated products are the substrates of the plants, functioning on the nerve systems and the balance of the internal enzymes for better immunity against the mutation, bacteria and virus.

5.6 An appropriate use of the fish chemicals

A. Right use of the fish chemicals: The fish chemicals should be used not only for the disease prevention and control but also good for the regulation of the metabolisms, improvement of the digestion, promotion of the growth and enhancement of the feed efficiency. The right use of the fish chemicals should be based on the understanding of the needs of the fish. A good understanding

should also be made on the source of the pathogens, obstacles should be created to stop the transmission of any disease. The application of the fish chemicals should be based upon the less pollution, less residues, less toxicants and low cost.

B. An appropriate method in applying the chemicals:

The methods of the chemical application are summarized as the following three types: A. mixture with water. It is diluted in the water for fish to bathe or pond spray. B. Medicated feeds. The medicated feeds should be evenly mixed up with an appropriate concentration. C. Injection.

C. Better quality of the fish chemicals:

The anti-resistance of the fish chemicals has received much attention from the growers. The general recommendation of the fish chemicals should be in an alternative use of different kinds to avoid the resistance of the pathogens. On the other hand, more research needs should be carried out for better quality and more efficiency of the chemicals.

D. Application of the herbs.

The herbs are the traditional methodologies in helping the treatment and prevention of the fish diseases without any residues. It is also served as a health flourisher. In China there have been a good result by using the herbs for the fish treatment.

5.7 Development and application of the fish vaccines

Development of the effective vaccines for the fish is a half way in achieving the success. However, it takes a long time in the research findings. In 1980s, vaccines of SVCV were for marketing in Europe. It is necessary and urgent to develop good quality with a good effect of the vaccines that can be turned for general marketing.