

Proposed Activities for Koi Herpesvirus Disease at the SEAFDEC Aquaculture Department

Kazuya NAGASAWA

*Southeast Asian Fisheries Development Center, Aquaculture Department
Tigbauan 5021, Iloilo, PHILIPPINES*

Correspondence, e-mail: nagasawa@aqd.seafdec.org.ph

Abstract

The Southeast Asian Fisheries Development Center (SEAFDEC) is a regional treaty organization with 11 member countries. This was established in 1967 to promote fisheries development in Southeast Asia. As one of four SEAFDEC departments, the Aquaculture Department based in Tigbauan, Iloilo, Philippines, has conducted activities for aquaculture research and development in the region. Since 2000, the Regional Fish Disease Project has been implemented at the SEAFDEC Aquaculture Department through the Government of Japan Trust Fund. Under this project, research studies were conducted on various aspects of viral, bacterial and parasitic diseases of fishes and shrimps. In East Asia, koi herpesvirus (KHV) disease initially occurred in Indonesia and Taiwan in 2002. KHV infection was also found in Japan in 2003. This disease had a serious, devastating impact on common carp and koi (*Cyprinus carpio*) production in Indonesia and common carp production in Japan. Common carp is an important food resource in the rural areas of the region, while koi is internationally traded as ornamental fish among Southeast Asian countries. Under these situations, the Regional Fish Disease Project identified KHV as a serious, transboundary pathogen in the region and decided to work on KHV disease at the SEAFDEC Aquaculture Department in coordination with the SEAFDEC member countries to prevent the spread of KHV in the region. The planned research includes survey of the distribution of KHV in the region, standardization of the PCR (polymerase chain reaction) detection method, characterization of the virus isolated from the region, mode of transmission of KHV, and pathophysiology of KHV-infected fish. To support establishment of the fish disease quarantine and surveillance in Southeast Asia, the Regional Fish Disease Project has, since 2002, annually conducted a hands-on training at the SEAFDEC Aquaculture Department on viral diseases of fishes and shrimps for scientists and technical staff from the SEAFDEC member countries. The trainees are expected to play key roles in the diagnosis, prompt information exchange, and surveillance of fish diseases, including KHV disease, in their respective countries. The Regional Fish Disease Project organized two meetings in March 2004 and will convene another meeting in June 2004: Pre-KHVD Symposium Meeting, International Symposium on Koi Herpesvirus Disease, and Meeting on Current Status of Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training.

Key words: koi herpesvirus disease, KHV, common carp, koi, *Cyprinus carpio*, Southeast Asian Fisheries Development Center, Southeast Asia, Regional Fish Disease Project, proposed activities

What is the SEAFDEC Aquaculture Department ?

The Southeast Asian Fisheries Development Center (SEAFDEC) is a regional treaty organization established in

1967 to promote fisheries development in Southeast Asia. Its member countries are Japan, Malaysia, Philippines, Singapore, Thailand, Brunei Darussalam, Socialist Republic of Vietnam, Myanmar, Indonesia, Cambodia, and Lao PDR. The Chief

administrator of SEAFDEC is the Secretary-General whose office, the Secretariat, is based in Bangkok, Thailand.

Four departments were established to pursue the objectives of SEAFDEC: (1) the Training Department in Samut Prakan, Thailand, for marine capture fisheries training; (2) the Marine Fisheries Research Department in Singapore for fishery post-harvest technology; (3) the Marine Fishery Resources Development and Management Department in Kuala Terengganu, Malaysia, for the development and management of the marine fishery resources in the exclusive economic zones of the SEAFDEC member countries; and (4) the Aquaculture Department in Tigbauan, Iloilo, Philippines, for aquaculture research and development.

Since its establishment in 1973, the Aquaculture Department has been mandated to: (1) promote, undertake and coordinate aquaculture research relevant to Southeast Asia; (2) develop human resources for aquaculture development; and (3) disseminate and exchange information in aquaculture. The first mandate is the primary function of the Research Division, which undertakes research on commercially important species of fishes, crustaceans, molluscs, seaweeds, and other aquatic organisms. Research on disease diagnosis and health management of aquatic organisms is one of the main research activities at the department. Under the Research Division is the Fish Health Section with five scientists (4 with PhD and 1 with MS).

The Regional Fish Disease Project Supported by the Government of Japan Trust Fund

The "Regional Fish Disease Project" has been implemented at the SEAFDEC Aquaculture Department through the Government of Japan Trust Fund to address various fish disease problems and food safety

issue in Southeast Asia (Inui, 2002; Nagasawa, 2004). The first phase of the project entitled "Development of Fish Disease Inspection Methodologies for Artificially-Bred Seeds" started in 2000 and will end in 2004. It was initially planned to end in 2003 but it was extended to 2004 because of the urgent need to study KHV infection. After this first 5-year project, the second phase of the Regional Fish Disease Project has been proposed under the title of "Development of Fish Disease Surveillance System" for another 5 years from 2004 to 2008.

The Regional Fish Disease Project aims to: (1) enhance disease diagnosis and health management of aquatic animals in aquaculture in Southeast Asia; (2) promote the healthy and wholesome trading of aquaculture products in the region; and (3) develop a fish disease surveillance network in the region.

To achieve these objectives, the project conducted the following activities from 2000-2004:

1. Research to (1) develop standardized diagnostic methods for major diseases affecting economically important aquaculture species in the region; (2) develop effective prevention and control measures against microbial and parasitic diseases; (3) assess the pathogenesis of newly emerging diseases; and (4) develop monitoring methods for residual chemicals in aquaculture products.

2. Hands-on training to develop capability in aquatic animal health diagnosis and management of technical staff working at research centers and institutions in the region.

3. International meetings to (1) discuss the status of fish disease problems, available diagnostic methods, and prevention and control measures employed in the region; (2) discuss the results of research studies conducted under the project and those generated in other countries in the region; (3) identify and discuss aquatic animal disease issues to be solved for further sustainable aquaculture growth; and (4) discuss

collaboration with other international organizations such as the Office International des Epizooties (OIE).

4. Extension to disseminate research results and technology generated by the Project through (1) training courses on fish diagnosis and health management; (2) production of manuals; (3) publication of primary results in international scientific journals; and (4) presentation of results in international meetings.

Research is the main activity component of the Regional Fish Disease Project. Various aspects of viral, bacterial and parasitic diseases of fishes and shrimps have been studied. When the project started in 2000, research was undertaken only by scientists of the SEAFDEC Aquaculture Department. Subsequently scientists of research institutions under the Department of Fisheries, Thailand, and those of the Marine Fisheries Research Department of SEAFDEC in Singapore joined the project in 2001 and 2002, respectively. A total of 24 research studies were conducted from 2000 to 2003 in terms of (1) establishment and standardization of diagnostic methods, (2) biology and pathogenesis of disease pathogens, (3) disease prevention and control, and (4) establishment of evaluation methods for residual chemicals in aquaculture products. Detailed information on the 24 research studies is given as Appendix.

To coordinate and promote the project, two Japanese senior scientists (designated as "Fish Disease Expert") were dispatched to the SEAFDEC Aquaculture Department (Dr. Yasuo Inui from March 2000 to March 2003; Dr. Kazuya Nagasawa from April 2003 to date).

KHV is a New, Serious Threat to Aquaculture in Southeast Asia

In Southeast Asia, koi herpesvirus (KHV) infection was first detected in common carp and koi (*Cyprinus carpio*) cultured in Indonesia in March 2002 (Sunarto *et al.*,

2002, Sunarto *et al.*, 2005). There is fragmentary information that KHV is also present in Malaysia (see table 1 in Gilad *et al.*, 2003). In neighboring East Asia, KHV disease was found in pond-reared koi from Taiwan in December 2002 (Tu *et al.*, 2004) and in common carp cultured in Japan in November 2003 (Sano *et al.*, 2004, 2005). In Indonesia, there have been numerous cases of KHV-induced mass mortality of common carp and koi since March 2002. Losses were estimated to be more than 15 million US dollars as of December 2003 (Sunarto *et al.*, 2005).

Common carp is an important food resource in the rural areas of Southeast Asia and abundantly cultured especially in Indonesia. Koi, on the other hand, is internationally traded as ornamental fish among Southeast Asian countries. Considering its high virulence and devastating impact on the aquaculture sector, KHV is regarded as a new, serious threat to common carp and koi aquaculture in the region.

Proposed Activities for KHV Disease under the Regional Fish Disease Project

Under these situations, study leaders involved in the Regional Fish Disease Project assembled at the Annual Progress and Planning Meeting held in Iloilo City, Philippines on 2-3 December 2003 and discussed fish disease issues for 2004 and onward. From the discussion, the following viruses were identified as serious, transboundary pathogens that the Regional Fish Disease Project should tackle as targets for fish disease surveillance in Southeast Asia: for fishes, KHV, spring viremia of carp virus (SVCV) and grass carp (*Ctenopharyngodon idella*) hemorrhagic virus (GCHV), and for shrimps and prawn, white spot syndrome virus (WSSV) and Taura syndrome virus (TSV) of black tiger shrimp (*Penaeus monodon*) and Pacific white shrimp (*Litopenaeus vannamei*) and extra

small virus (XSV) associated with white tail disease of the giant freshwater prawn (*Macrobrachium rosenbergii*). In particular, KHV was recognized as the pathogen that the project must combat urgently.

Based on the output of the meeting, the SEAFDEC Aquaculture Department made a plan to implement various activities for KHV infection through the Regional Fish Disease Project. Some research studies were planned for 2004 under the first phase of the project, while others were for 2004-2008 under the second phase. The SEAFDEC Aquaculture Department believes that the project should proceed efficiently in coordination with the SEAFDEC member countries. Important activities for KHV disease, some of which were initiated in the first half of 2004, are as follows:

1. Research

During the first phase of the project, planned research will involve the survey of the distribution of KHV in the region, standardization of the PCR (polymerase chain reaction) detection method, characterization of the virus isolated from the region, mode of transmission of KHV and pathophysiology of KHV-infected fish. These studies will be undertaken at the SEAFDEC Aquaculture Department.

During the second phase of the project, when new information on KHV infection becomes available in the SEAFDEC member countries, the SEAFDEC Aquaculture Department will dispatch its diagnosis team to the disease site in order to isolate the virus and diagnose the disease together with scientists of the country in question. Also, there will be a study to develop vaccines for KHV, using inactivated virus or recombinant viral envelope protein, at the Fish Health Research Laboratory in Jakarta, Indonesia.

In addition to these activities under the Regional Fish Disease Project, the SEAFDEC Aquaculture Department will join in 2004 a 3-year research project on KHV infection, which will be funded by the

Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan. The National Research Institute of Aquaculture (based in Nansei and Tamaki, Mie, Japan) leads the project. Comparison of characteristics of KHV isolates from Asian countries is a research subject to be tackled by the SEAFDEC Aquaculture Department.

2. Hands-on training

Since 2002, the SEAFDEC Aquaculture Department has annually conducted a hands-on training on viral diseases of fishes and shrimp for scientists and technical staff working at research centers and institutions in Southeast Asia. The training course aims to provide an executive training on the diagnosis of viral diseases to core persons from the SEAFDEC member countries. The trainees are expected to play key roles in the diagnosis, prompt information exchange, and surveillance of fish diseases as well as to serve as national trainers in their respective countries. For 2004, a special training course on KHV and some other important viral pathogens is planned for scientists from some of the SEAFDEC member countries.

3. International meetings

The Regional Fish Disease Project organized two meetings in March 2004 and will convene another meeting in June 2004: (1) Pre-KHVD Symposium Meeting; (2) International Symposium on Koi Herpesvirus Disease; and (3) Meeting on Current Status of Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training.

The Pre-KHVD Symposium Meeting was organized by the project at the Conference Room of the National Research Institute of Fisheries Science (NRIFS), Fisheries Research Agency (FRA) in Yokohama, Japan, on 12 March 2004 as a satellite meeting of the International Symposium on Koi Herpesvirus Disease. Participants to this meeting were nine scientists from the SEAFDEC member countries (one

participant from each country: Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand, Vietnam) and three scientists from the SEAFDEC Aquaculture Department. The scientists from the member countries reported on the current status of KHV disease, fish disease quarantine and surveillance in their respective countries. Participation of 11 scientists from Southeast Asia (excluding Thailand) in the meeting was funded by the project.

The International Symposium on Koi Herpesvirus Disease was co-organized by FRA, SEAFDEC (through the Regional Fish Disease Project), MAFF, and OIE in Yokohama on 13 March 2004. This meeting was participated in by scientists from Japan, the United States, South Korea, China, Israel and the Netherlands as well as scientists from the SEAFDEC member countries and Aquaculture Department. After the keynote lecture by Dr. Ronald P. Hedrick of the University of California, Davis, a total of 15 papers were presented by invited speakers. Four of these invited speakers came from Southeast Asia: Drs. Agus Sunarto (Indonesia), Ling Kai Huat (Singapore), Somkiat Kanchanakhan (Thailand) and Kazuya Nagasawa (SEAFDEC Aquaculture Department). Information presented at the symposium varied from basic knowledge of KHV through epidemiology of KHV infection in Indonesia and Japan, and KHV vaccine development in Israel to fish disease quarantine in Singapore and Thailand. It was useful in understanding of various aspects of KHV infection including control and prevention.

The Meeting on Current Status of Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training in Manila, Philippines, on 23-24 June 2004 is being organized by the project. The meeting aims to exchange the latest information on transboundary fish diseases and fish surveillance, research and training in Southeast Asian countries. The project will

fund 11 scientists from all SEAFDEC member countries, two invited speakers from Taiwan and Canada and 10 scientists from the SEAFDEC Aquaculture Department to participate in the meeting. Two scientists each from OIE in Tokyo and the Network of Aquaculture Centres in Asia-Pacific (NACA) in Bangkok will also attend the meeting. As transboundary fish and shrimp pathogens, KHV, WSSV and TSV will be highlighted. The meeting will consist of five discussion sessions: (1) KHV; (2) WSSV and TSV; (3) quarantine services of aquatic animal diseases; (4) surveillance, monitoring and diagnosis of aquatic animal diseases; (5) research and training on diseases of aquatic animals. For each session, at least one invited lecture will be given, followed by reports from 10 Southeast Asian countries. During the first session, the current status of KHV infection in Indonesia, Taiwan and Japan will be reported by Drs. Agus Sunarto, Chien Tu and Motohiko Sano, respectively. For the second to fifth sessions, invited papers will be presented individually by Drs. Leobert D. de la Peña (SEAFDEC Aquaculture Department), J. Richard Arthur (Canada), Yoshiyuki Oketani (OIE) and Michael J. Phillips (NACA). Through the country reports, detailed information on the current status of KHV, WSSV and TSV as well as fish disease quarantine, surveillance, monitoring, diagnosis, research and training in Southeast Asian countries will be assembled.

4. Extension

Research results will be published in international scientific journals, and standardized PCR diagnostic techniques will be disseminated through manuals and hands-on training. As the output of the Pre-KHVD Symposium Meeting, one report (Nagasawa and Lio-Po, 2004) is available at the SEAFDEC Aquaculture Department. FRA will publish the proceedings of the International Symposium on Koi Herpesvirus Disease in the Bulletin of the Fisheries

Research Agency, Supplement No. 2. By October 2004, the SEAFDEC Aquaculture Department will publish the proceedings of the Meeting on Current Status of Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training (Lavilla-Pitogo and Nagasawa, 2004).

Acknowledgments

The Regional Fish Disease Project is being funded by the Government of Japan with the SEAFDEC Aquaculture Department as the lead institution. I am most thankful to the Government of Japan for the financial support. I thank Mr. Junichiro Okamoto, Deputy Secretary General of SEAFDEC, for his understanding and encouragement of the project. Special thanks also go to Drs. Shigeo Hayase and Kazuhiro Nakajima of FRA for their support in the participation of SEAFDEC member countries' representatives in the International Symposium on Koi Herpesvirus Disease held in Yokohama, Japan. Dr. Gilda D. Lio-Po of the SEAFDEC Aquaculture Department read an early draft of the manuscript.

References

- Gilad O., Yun S., Adkison M. A., Way K., Willits N. H., Bercovier H. and Hedrick R. P., 2003: Molecular comparison of isolates of an emerging fish pathogen, koi herpesvirus, and the effect of water temperature on mortality of experimentally infected koi. *J. Gen. Virol.*, **84**, 2661-2668.
- Inui Y., 2002: Fish Disease Control Project of SEAFDEC Aquaculture Department in "Disease Control in Fish and Shrimp Aquaculture in Southeast Asia - Diagnosis and Husbandry Techniques" (eds. by Inui Y. and Cruz-Lacierda E. R.), *SEAFDEC Aquaculture Department, Iloilo, Philippines*, pp. 181-185.
- Lavilla-Pitogo C. R. and Nagasawa K., 2004: Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training. *SEAFDEC Aquaculture Department, Iloilo, Philippines*, 254 pp.
- Nagasawa K., 2004: Research and training on fish diseases at the SEAFDEC Aquaculture Department in 2000-2004: a review in "Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training" (eds. by Lavilla-Pitogo C. R. and Nagasawa K.), *SEAFDEC Aquaculture Department, Iloilo, Philippines*, pp. 41-52.
- Nagasawa K. and Lio-Po G. D., 2004: Pre-KHVD Symposium Meeting (A satellite meeting of the International Symposium on Koi Herpesvirus Disease held at the Pacifico Yokohama on March 13, 2004). *SEAFDEC Aquaculture Department, Iloilo, Philippines*, 45 pp.
- Sano M., Ito T., Kurita J., Yuasa K., Miwa S. and Iida T., 2004: Experience on common carp mass mortality in Japan in "Transboundary Fish Diseases in Southeast Asia: Occurrence, Surveillance, Research and Training" (eds. by Lavilla-Pitogo C. R. and Nagasawa K.), *SEAFDEC Aquaculture Department, Iloilo, Philippines*, pp. 13-19.
- Sano M., Ito T., Kurita J., Miwa S. and Iida T., 2005: Diagnosis of koi herpesvirus (KHV) disease in Japan. *Bull. Fish. Res. Agency, Supplement No. 2*, 59-64.
- Sunarto A., Tauhid, Rukyani A., Supriyadi H., Koesharyani I., Huminto H., Agungpriyono D. W., Pasaribu F. H., Widodo, Hardiawan D., Rukmono P., Nilawati, *et al.*, 2002: Field investigations on serious disease outbreak among koi and common carp (*Cyprinus carpio*) in Indonesia. *Abstracts of 5th Symposium on Diseases in Asian Aquaculture. 24-28 November*

2002, Gold Coast, Queensland, Australia.

Sunarto A., Rukyani A. and Itami T., 2005: Indonesian experience on the outbreak of koi herpesvirus in koi and carp (*Cyprinus carpio*). *Bull. Fish. Res. Agency, Supplement No. 2*, 15-21.

Tu C., Weng M.-C., Shiau J.-R. and Lin S.-Y., 2004: Detection of koi herpesvirus in koi *Cyprinus carpio* in Taiwan. *Fish Pathol.*, **39**, 109-110.

Appendix

Under the Regional Fish Disease Project, the following research studies were conducted during first phase of the project from 2000 to 2003 in four categories:

A. Establishment and Standardization of Diagnostic Methods

1. Standardization of diagnostic methods for viral diseases of shrimp (SEAFDEC Aquaculture Department [AQD], 2000)
2. Standardization of PCR technique as the detection method for WSSV infection in *Penaeus monodon* (SEAFDEC/AQD, 2001)
3. Development of shrimp cell culture *in vitro* (Marine Shrimp Research and Development Center [MSRDC], Thailand, 2001-2002)
4. Standardization of diagnostic methods for monodon baculovirus (MBV) and hepatopancreatic parvovirus (HPV): Establishment of monoclonal antibodies (MAbs) against MBV and HPV (SEAFDEC/AQD, 2001-2003)
5. Epizootiology of economically important viral diseases of wild *Penaeus monodon* (SEAFDEC/AQD, 2001-2003)
6. Viral diseases of cultured marine fishes in Southeast Asia
 - 6-1. Detection and identification of viral pathogens in cultured marine finfish in the Philippines (SEAFDEC/AQD, 2000-2003)

6-2. A viral survey in diseased grouper in Thailand using virus isolation and polymerase chain reaction (PCR) technique (Aquatic Animal Health Research Institute [AAHRI], Thailand, 2001-2002)

6-3. Survey of iridoviral disease in freshwater fish in Thailand (AAHRI, Thailand, 2003)

7. Establishment of preventive measures against viral nervous necrosis (VNN) in fish broodstocks: (1) grouper, (2) milkfish, (3) red snapper, and (4) sea bass (SEAFDEC/AQD, 2001-2003)

B. Biology and Pathogenesis of Disease Pathogens

1. Parasitosis in marine and freshwater fish: diagnosis, pathology, prevention and control of infection
 - 1-1. Screening of important parasites in economically important aquaculture fish (SEAFDEC/AQD, 2000-2003)
 - 1-2. Biology and pathology of the gill monogenean parasitic to grouper (SEAFDEC/AQD, 2000-2003)
 - 1-3. Leech infestation and its associated blood parasitic protozoans (SEAFDEC/AQD, 2000-2003)
 - 1-4. Establishment/application of prevention and control methods against parasites (SEAFDEC/AQD, 2000-2003)
2. Study on parasites of grouper in Thailand (AAHRI, Thailand, 2001-2002)
3. Screening of important parasites of freshwater fish in Thailand and neighboring countries (AAHRI, Thailand, 2003)

C. Disease Prevention and Control

1. Use of bacteria as biological control agent against microbial diseases in shrimp (*Penaeus monodon*) and crab (*Scylla serrata*) hatcheries (SEAFDEC/AQD, 2000-2003)
2. Screening of probiotics as biocontrol/bioremediation in the rearing

- of *Penaeus monodon* I. Tank experiment (SEAFDEC/AQD, 2001-2003)
3. Antibacterial metabolites in the microbial and phytoplankton flora of the “green water” cultured *Penaeus monodon* (SEAFDEC/AQD, 2000-2003)
 4. Investigation on the mechanism of the effect of tilapia culture water on luminous bacteria (SEAFDEC/AQD, 2001-2003)
 5. Screening of *Vibrio harveyi* bacteriophage for controlling luminous disease in marine shrimp hatchery (Samutsakhon Coastal Aquaculture Development Center [SCADC], Thailand, 2001-2003)
 6. Development of immuological indices for monitoring health status in *Penaeus monodon* (SEAFDEC/AQD, 2001-2003)
- D. Establishment of Evaluation Methods for Residual Chemicals in Aquaculture Products**
1. Establishment and monitoring on antimicrobial usage in shrimp aquaculture (SCADC, Thailand, 2001-2003)
 2. Detection of pesticide residues in aquaculture products (SEAFDEC/AQD, 2000-2003)
 3. Detection of antibiotic residues in aquaculture products (SEAFDEC/Marine Fisheries Research Department, 2002-2003)