

Preventive Measures against Koi Herpesvirus Disease in Fancy Carp in Niigata Prefecture

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Objectives

Following by the outbreaks of koi herpesvirus (KHV) in worldwide including Israel, many areas of Europe, the United States, and Indonesia, the Fisheries Division of the Niigata Prefectural Government started implementing a polymerase chain reaction (PCR) tests on the prefecture's carp in October of 2001.

The aim of the test was twofold--to prevent the entry of the KHV into the prefecture and to prevent the spread of the virus within and out of the prefecture, both domestic and abroad, if the virus is detected in carp in Niigata. Although there have been no reports of a KHV outbreak in Niigata's fancy carp, the prefecture's fancy carp industry has voluntarily suspended all of its shipments temporarily. This measure has been taken to prevent the possible transfer of the virus following the detection of KHV in common carp in Niigata on November of 2003 (Note: These KHV-affected fish, originally imported from Ibaraki prefecture, were obtained from an entertainment fishing pond and a breeder of common carp in Niigata prefecture.).

Method

A polymerase chain reaction (PCR) tests were conducted in Niigata prefecture during the following three periods: from October to November of 2001 on fancy

carp owned by twenty carp breeders, in December of 2002 on fancy carp owned by thirty carp breeders, and from July to August of 2003 on fancy carp owned by 114 breeders. (Note: Many of the breeders have participated in the PCR tests in multiple years.)

The procedures of PCR-test were as follows (Fig. 1): 1) To avoid a great financial loss, sample fish were selected only for young fancy carps less than one-year old which mean valueless in terms of unable to reproductive activities. 2) These young carps were kept in tank during 3 weeks under maintaining condition of water-temperature from 20°C to 25°C, together with adult ones that would be shipped for commercial purposes. 3) Sample fish for examined were maintained by designated staff members who conduct PCR tests under the supervision of the Niigata Prefectural Inland-water Fisheries Experimental Station. 4) Afterwards tissues from the gill, spleen, kidney, and heart (sometimes just a sample from the gill tissue) were taken from two to five of each sample fish. 5) These tissues were homogenized to extract DNA, which was obtained by using a DNA extraction kit. 6) Each sample was mixed with an agent that contained types of primers such as 9/5 and SphI-5. 7) After this stage, the samples received an agarose gel electrophoresis treatment to examine the DNA fragments. and 8) A KHV DNA sample, which Niigata had received from Dr. R. P. Hedrick, was used to compare the

results of the samples taken in Niigata.

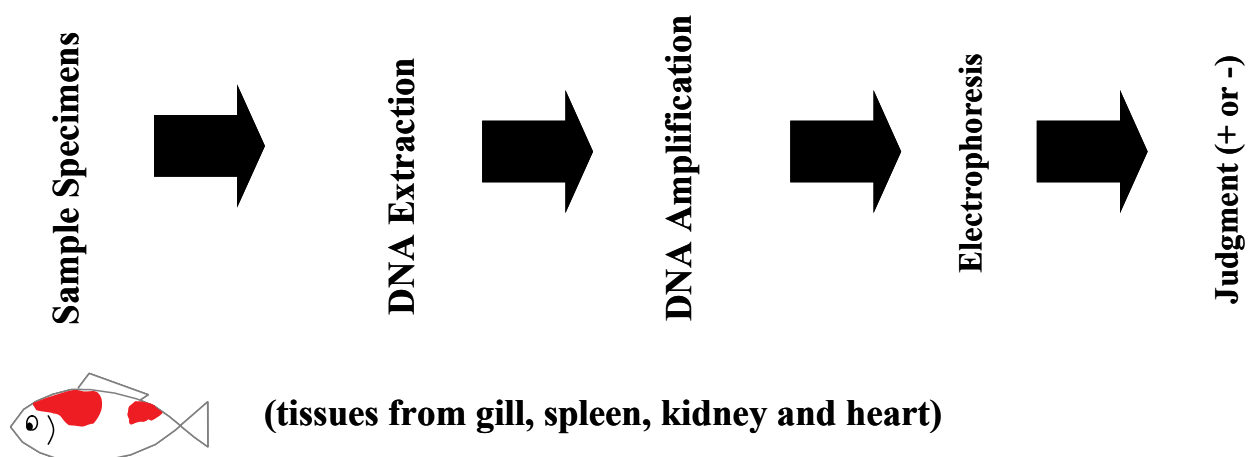
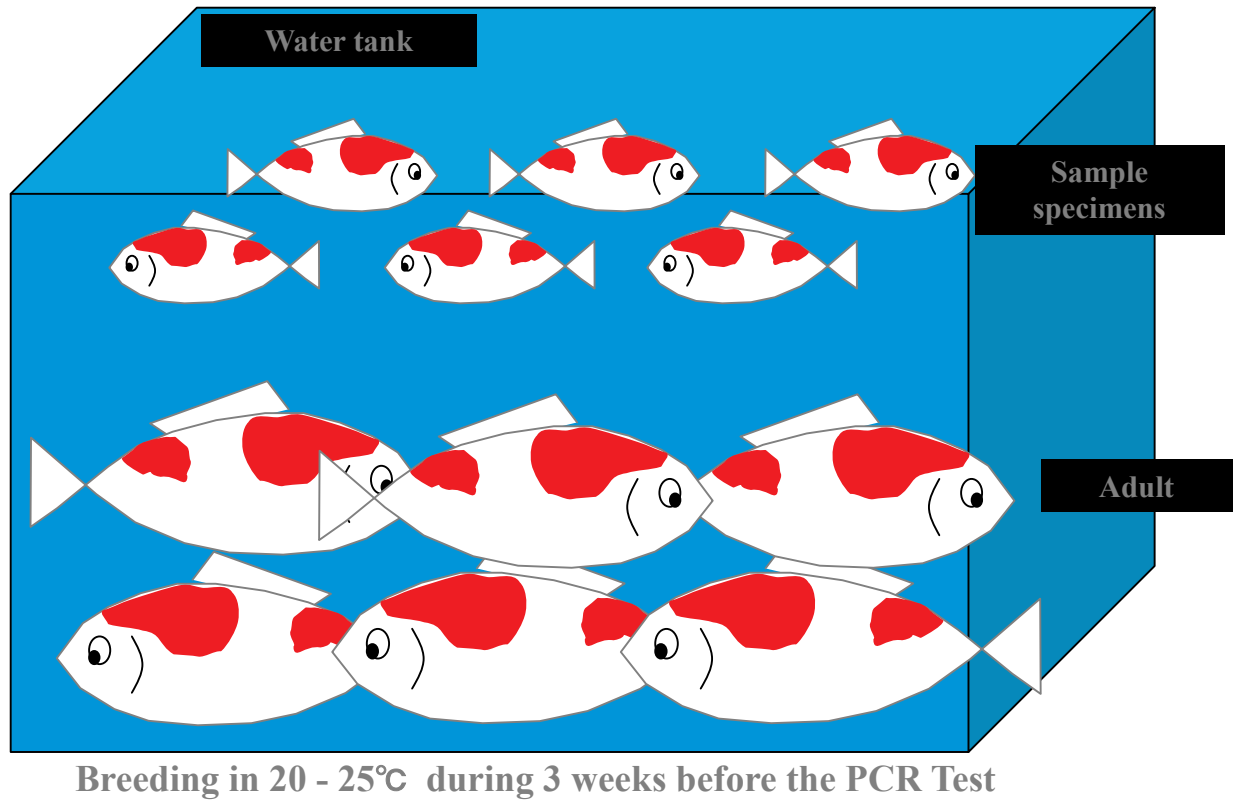


Fig. 1. PCR-Test Method.

Results

All the fish that were examined in the PCR tests during the previously specified periods tested negative. During these test periods also, Niigata provided carp business owners in the region with meetings and lectures about KHV to enable the breeders to be able to detect KHV-affected carp at an early stage of the disease and thus minimize the risk of spreading the virus.

Currently PCR tests are being conducted on the largest scale ever in

Niigata with the participation of 150 carp breeders, each of which presented tissue samples from thirty-one fish from their farm. Originally, the voluntary policy to temporarily suspend ornamental carp shipments prohibited any shipments of fancy carp until the test results of the fish from all the breeders concerned were confirmed; however, the voluntary policy to temporarily suspend fancy carp shipments has been revised to allow overseas shipments of fish upon confirmation of favorable test results of the fish from each individual breeder (Table 1).

Table 1. Results of PCR-testing in Niigata Prefecture.

Test-Date	Oct.-Nov., 2001	Nov.-Dec., 2002
Method	PCR (9/5 Primer)	PCR (9/5 Primer)
DNA Extraction Kit (Agents)	ISOGEN	ISOGEN
A tissues (section) from	Gill, Brain, Spleen, Kidney	Gill, Brain, Spleen, Kidney
No. of samples examined	205 individuals (42 specimens)	420 inds (84 specimens)
No. of enterprise examined	20 enterprise	30 enterprise
Results of PCR-test	All negative (-)	All negative (-)

Upon Niigata Prefectural Government's request, the carp industry in Niigata is creating a KHV prevention policy that instructs carp breeders how to avoid exposing fish to the virus. Under this policy, any fish that is shipped from another breeder will have to be isolated from other fish in a low temperature water tank for three weeks during which time the breeder will check to see whether or not

the fish is infected with the virus. It also advises the breeder to avoid using river water when maintaining the fish and to sanitize all equipment that comes in contact with fish water or the fish themselves, including staff's boots and gloves. Staff members are also required to wash their hands at their facility and to keep a detailed journal of the conditions of their fish.