Experience on Common Carp Mass Mortality in Japan

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Abstract

The mortality rate among common carp for food reared in net pens in Lake Kasumigaura, the second largest lake in Japan, in Ibaraki Prefecture, increased from early October 2003 and koi herpesvirus (KHV) was detected in the affected fish by the National Research Institute of Aquaculture (NRIA) in late October using PCR methods of Gilad *et al*. (2002) and Gray *et al*. (2002). The Ministry of Agriculture, Forestry and Fisheries of Japan officially announced the first occurrence of KHV disease in Japan. In late October 2003, the water temperature of Lake Kasumigaura was 16-18°C and the fish losses were severe, particularly in market-sized carp. The apparent symptoms of affected fish were presence of mucus-like substance on the body surface, sunken eyes, and pale and necrotic gills, which were similar to those reported by Hedrick *et al*. (2000). Approximately 1,200 metric tons of common carp cultured in the lake were lost by mid-November. Prior to this, however, infected carp cultured in Lake Kasumigaura had already been transferred to farms, wholesalers, restaurants and game fishing facilities. Consequently, the infection spread to other areas in Japan. Independent of the outbreak in Lake Kasumigaura, a massive carp loss of over 10 thousand fish, the cause of which was initially diagnosed as columnaris disease, occurred in some rivers and a lake in Okayama Prefecture from late May to mid-July 2003. In November, the NRIA detected KHV DNA by PCR from samples of the diseased fish stored in a freezer. This demonstrated that KHV was present in Japan before late May 2003. By the end of 2003, KHV was detected in carp from 23 out of 47 prefectures in Japan. No occurrence of the disease was observed during the winter period. However, as the water temperature increased in spring of 2004, KHV reappeared in the area where the disease had been previously recorded, and also in new places. In many of the facilities that experienced KHV outbreak in 2003, the disease was not observed by June 2004 because

all carp had been removed together with other fish species and the facilities were disinfected thoroughly after the outbreaks. From January to the end of May 2004, KHV infections were reported in 24 of 47 prefectures in Japan.

Diagnostic System for Exotic Diseases and Koi Herpesvirus Disease

Some diseases are designated as "Specific Diseases" in the Japanese law. These are principally exotic diseases such as spring viremia of carp (SVC) and viral hemorrhagic septicemia (VHS) of salmonids that have the potential to devastate the aquaculture industry in Japan. For such diseases, protective guidelines have been established in Japan. The guidelines provide etiological information, diagnostic procedures, description of the symptoms and other important characteristics of the diseases. Laboratory diagnosis of the diseases must be conducted in accordance with these guidelines.

A newly isolated herpesvirus, designated as koi herpesvirus (KHV), was first reported as a causative pathogen of mass mortality that occurred among common and ornamental (koi) carp *Cyprinus carpio* cultured in Israel and the USA in 1998 (Hedrick *et al*., 2000). A similar virus was also isolated after massive mortality of carp in Germany (Neukirch and Kunz, 2001) and Israel (Perelberg *et al*., 2003). The virus isolated in Israel was identified as carp nephritis and gill necrosis virus (CNGV) based on the histopathological results (Ronen *et al*., 2003). Subsequently, this viral infection has been observed in western Europe since 2000, Indonesia in the spring of 2002 and Taiwan in the fall of 2002 (Tu *et al*., 2004), revealing that this disease is rapidly spreading worldwide in carp-trading countries. In Japan, there was no such mass mortality of carp before 2003 and KHV was not detected by a survey conducted in the Niigata Prefecture in 2001 (Amita *et al*., 2002). As KHV is highly contagious and virulent in juvenile and adult carp (Hedrick *et al*., 2000; Perelberg *et al*., 2003), KHV infection was designated as a "Specific Disease" by the Japanese law amended on 30 June 2003, and an inspection procedure was established as part of the guidelines (Fig. 1). According to the procedure, Prefectural Fisheries Experimental Stations (PFESs), which

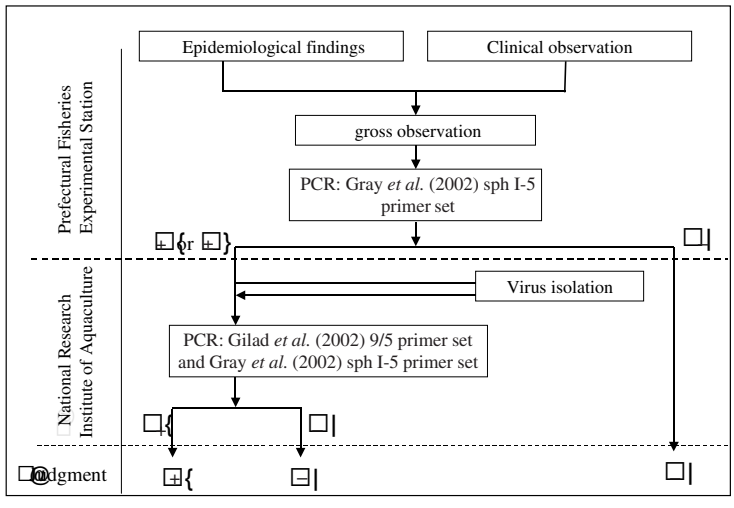

Fig. 1. Inspection procedure for KHV according to the Japanese guidelines

belong to the local government, first conduct an epizootic and routine clinical examination of diseased fish. The most important epizootiological aspect of KHV disease is that it affects only carp *Cyprinus carpio* and occurs apparently only in a limited range of water temperature from 18-28°C (Hedrick *et al*., 2000; Gilad *et al*., 2003). Therefore, the water temperature and susceptible fish species should be determined during a field examination. Few distinguishable external signs are usually visible, but pale and necrotic gills are frequently found. *Flexibacter columnaris* infection and some protozoan parasites, such as *Chilodonella* and *Trichodina*, are sometimes found on necrotic gill lesions, which easily lead to misdiagnosis of KHV disease. In case any doubt remains as to the presence of KHV, a polymerase chain reaction (PCR) test can be used to detect KHV DNA in the tissues of fish. The PCR method described by Gray *et al*. (2002) is adopted in the inspection procedure as the primary examination conducted by PFESs. When the PCR test is positive for KHV, the sample is sent to the National Research Institute of Aquaculture (NRIA) for further examination by PCR methods of both Gilad *et al*. (2002) and Gray *et al.* (2002) for confirmation. Virus isolation on the KF-1 cell line is also attempted using the KF-1 cell line (Hedrick *et al*., 2000). Because of difficult isolation of KHV using the cell line, results of the isolation trial is treated as supplementary data and confirmation of KHV is solely based on the results of the PCR tests.

Occurrence of KHV Disease in Japan and Practical Diagnosis of the Disease

In Lake Kasumigaura, central Japan, the mortality among common carp cultured in net pens increased from early October 2003, when the water temperature of the lake was 16-18°C. The fish were lethargic and swam near the water surface. There were no marked external signs in most of the affected fish, but the appearance of whitish mucous-like substance on the body surface, redness of the fin and body, fin rot, and discoloration of the gill with some necrosis were sometimes observed. Mortality was over 60% in the most severe cases, especially in larger carp over 2 years old. The losses of cultured carp were estimated at 660 metric tons (MT) in early November and this reached approximately 1,200 MT by mid-November. This represents approximately one fourth of the lake's annual production.

External parasites, such as *Chilodonella*, *Trichodina*, and *Gyrodactylus*, were sometimes seen on the necrotic gill of affected fish. Marked histopathological changes were observed in the gill of diseased carp (Fig. 2). The secondary lamellae were often fused with the hyperplastic branchial epithelium where cell necrosis or infiltration of lymphocytes were often observed. Congestion and hemorrhage were sometimes observed. In some cases, the branchial tissues were severely degraded and numerous bacteria were seen in the lesions. These histopathological changes are similar to those previously reported (Hedrick *et al*., 2000; Tu *et al*., 2004). Unlike a previous report (Hedrick *et al*., 2000), however, nuclear changes characterized by hypertrophy and margination of chromatin were rarely observed. No bacteria

were isolated from the kidney of affected fish using trypticase soy agar. The PCR test for KHV revealed specific bands amplified by the methods of Gray *et al*. (2002) and Gilad *et al*. (2002)(Fig. 3). The sequence of the amplicon by the primer set of Gray *et al*. (2002) was identical to the sequence deposited in the GenBank with accession no. AY568951, and that with the primer set of Gilad *et al*. (2002) showed 99% matching to AF411803.

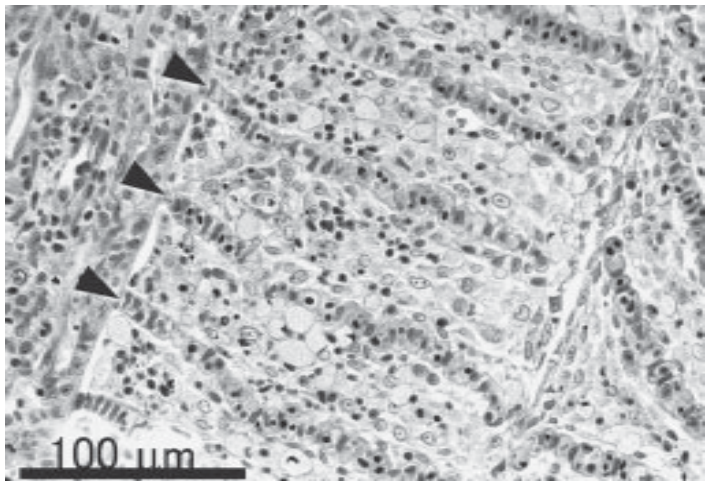

Fig. 2. A tissue section of the gill from affected common carp cultured in Lake Kasumigaura. Arrowheads indicate fused secondary lamellae with hyperplastic branchial epithelium and cell necrosis or infiltration of lymphocytes. H&E stain.

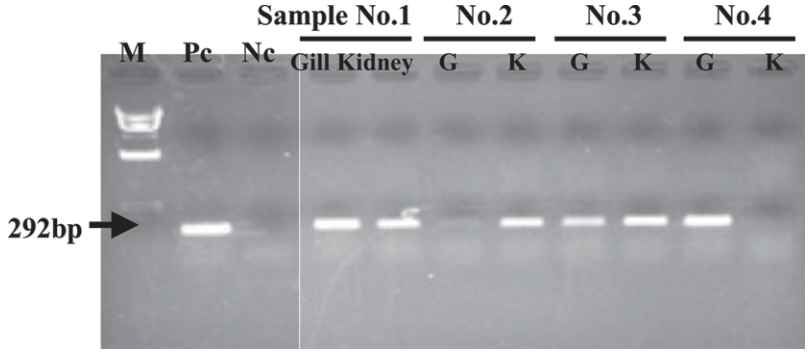

Fig. 3. Gel electrophoresis of the products (292bp) amplified with the primer set sph I-5 of Gray *et al.* (2002) from the extracted sample of gill and kidney of affected fish cultured in Lake Kasumigaura. Pc: positive control, Nc: negative control. 1% agarose gel stained with ethidium bromide.

The Ministry of Agriculture, Forestry and Fisheries of Japan officially announced the first occurrence of KHV disease in Japan on 2 November 2003. It was also reported to the Office International des Epizooties (OIE). According to the law, the Ibaraki Prefectural Governor prohibited any shipment or removal of cultured carp from the lake and ultimately ordered that all carp cultured in the lake would be destroyed by the end of March 2004.

Evidence of the Presence of KHV before the Outbreak in Lake Kasumigaura

Independent of the outbreak in Lake Kasumigaura, a massive loss exceeding 10,000 pieces of carp occurred in some rivers and a lake in Okayama Prefecture in late May to mid-July 2003. In November 2003, the NRIA detected KHV DNA by PCR in samples of diseased fish stored in a freezer. This demonstrates that KHV had been introduced into Japan before May 2003, much earlier than the Lake Kasumigaura outbreak.

The Spread of KHV in Japan

KHV-infected common carp cultured in Lake Kasumigaura were transferred to other areas in Japan before the first detection of KHV resulting in the spread of the virus. Mortalities of carp with KHV were reported in some facilities, but there were many facilities where KHV was detected in carp without mortality. This could be attributed to the fact that the water temperature was gradually decreasing at the time of investigation. By the end of 2003, the NRIA examined 529 carp in 87 cases, and KHV was found in 23 out of 47 prefectures in Japan. Half of the KHV positive cases had no obvious relations with the Lake Kasumigaura.

There was no occurrence of KHV disease during the winter period. However, as the water temperature increased in the spring of 2004, KHV reappeared in those areas where the disease was recorded in 2003, and also in new places. However, in many of the facilities that experienced KHV outbreak in 2003, the disease was not observed by June 2004. This is because in these places, all carp were removed together with other fish species, and the facilities were thoroughly disinfected after the outbreaks. There has been no occurrence of KHV disease in ornamental (koi) carp farms to date. From January to May 2004, KHV infection was reported in 24 of 47 prefectures in Japan.

Research Activity for KHV Infection at the NRIA

The NRIA and other research groups, including some universities and the Southeast Asian Fisheries Development Center (SEAFDEC), began to conduct a research project funded by the Ministry of Agriculture, Forestry and Fisheries of Japan to control KHV infection. This project will last for 3 years and consists of three major research aspects: 1) molecular virology and histopathology of KHV infection, including viral behavior in infected fish at different temperatures and at a carrier state, 2) development and evaluation of diagnostic tools such as the loop-mediated isothermal amplification (LAMP, Eiken Chemical Co.) method or immunofluorescence technique, and 3) control measures of the infection, including efficacy of disinfectants, vaccination and elevation of the rearing water temperature. The results could contribute to the control of KHV infection in both wild and cultured carp populations.

Acknowledgements

We are grateful to Prof. Dr. Ronald P. Hedrick of the University of California, Davis, USA, for his invaluable suggestions and for providing KF-1 cell line and a KHV isolate. We thank the staff of the Ibaraki Prefectural Experimental Station and Okayama Prefectural Experimental Station for providing the sample fish, and also the staff of the Aquatic Animal Health Division, National Research Institute of Aquaculture (NRIA), for running the diagnosis of KHV. This study was partially supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan.

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