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The Status of Koi Herpesvirus Disease Outbreaks in Lake Kasumigaura and Kitaura

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Abstract

Lake Kasumigaura and Kitaura in Ibaraki Prefecture is the second largest lake in Japan. In the lake, common fisheries such as trawling and set net as well as net cage culture are practiced. Net cage culture produces about 5,000 tons of edible common carp (*Cyprinus carpio*) per year, which is one half of the total production in Japan. At the beginning of October 2003, deaths of unknown origin of common carp in net cages occurred in Lake Kasumigaura. After inquiry of the cause, PCR testing detected DNA of the Koi herpesvirus (KHV) in the affected fish and natural carp captured in Lake Kitaura, confirming the occurrence of the Koi herpesvirus disease (KHVD). We conducted an interview survey on aquaculturers regarding the date, quantity, and circumstance of death of cultured carp in Lake Kasumigaura and Kitaura, and investigated the circumstances of death in net cage farms. Another interview survey was also conducted on other fishermen regarding the occurrence of abnormality in natural carp, and both cultured and natural carps were sampled for PCR testing. The death of cultured carp in Lake Kasumigaura was generated in two areas dense with net cage farms, and then was likely expanded. The cumulative amount of death according to the interview surveys increased rapidly: 200-300 tons in the middle of October, 660 tons at the end of the same month, and 1,200 tons at the beginning of November. The damage amount reached one quarter that of the annual common carp products. Since the KHVD is one of the designated diseases under the Law to Ensure Sustainable Aquaculture Production, to prevent the spread of this disease according to this law, Ibaraki Prefecture requested aquaculturists of Lake Kasumigaura and Kitaura to exercise restraint on the transfer of cultured carp in net cages on November 2, and issued orders prohibiting transfer on November 12 and implementing incineration and landfill disposal on December 21. The incineration disposal commenced from January 20. This report aims to shed light on the state of mass cultured carp kills in Lake Kasumigaura and Kitaura, caused by this KHV, and the state of outbreaks of abnormal fish among cultured and natural carp.

Key words: koi herpesvirus, KHV, common carp, *Cyprinus carpio*, Lake Kasumigaura and Kitaura

Introduction

Lake Kasumigaura and Lake Kitaura in Ibaraki Prefecture constitute the second largest lake in Japan, with the combined area of 220 km^2, 56 feeder rivers, 2,160km^2 of basin area and 44 municipalities in the basin. With the mean depth and storage volume being four meters and approximately 850 million tons, respectively, the water is used for industrial and agricultural purposes. In the lake（i.e. Kasumigaura, including Lake Kitaura）, general fishery activities, such as trawl and set net fishery, and carp aquaculture are under way. The fish catch in the lake was 17,000 tons in 1978, but has declined and

recently has hovered around 2,000 tons. With carp one of the major fish species subject to capture, the annual catch is between 200 and 300 tons despite a decline in overall catches.

Carp aquaculture in net cages in the lake was launched in the 1960s and thrived due to the use of synthetic fiber, formula feed and automatic feeders, etc. At its peak, approximately 9,000 tons of carp were produced annually, and even now, approximately 5,000 tons per annum, which accounts for half the edible carp in Japan, are shipped to markets nationwide.

In early October 2003, edible carp cultured at net cages in Lake Kasumigaura died from an unknown cause and the deaths spread over the mid October. At that time, various possible causes were discussed, including indigestion caused by excessive feeding at low water temperatures, as well as oxygen deficiency and multiplication of *Skeletonema potamos* which occurred simultaneously. We conducted bacteria tests and bioassays, but stopped short of pinpointing the causes. The authors therefore requested the Training Center of Aquatic Animal Health Division (AAHD), National Research Institute of Aquaculture (NRIA), Fisheries Research Agency (FRA), for the diagnosis to identify the causes of death. Through the PCR test on October 31, KHV DNA was detected in both cultured carp in Lake Kasumigaura and natural carp in Lake Kitaura. As a consequence, it confirmed that the outbreak of the KHVD for the first time in Japan.

This report aims to shed light on the state of mass cultured carp which were killed by the cause of KHV in the lake, and the state of outbreaks of abnormal fish among cultured and natural carps.

Materials and Methods

Interview surveys

Interview surveys, including questions about the period when cultured carp died, the number of such deaths and the conditions at the time of death in the lake were conducted with individuals engaged in the aquaculture business. At the same time, conditions at the time of death at fishing grounds with net cages were also investigated. Interview surveys on the state of abnormal natural fish occurrences were also carried out with general fishery operators and the PCR test was performed on cultured and natural captured carps.

Toxicity test

Water was sampled at five locations in the lake, and concentrated solutions (5 x and 10 x) were prepared using the freeze concentration equipment.

Himedaka (orange-red variety of *Oryzias latipes*) and *Paratya compressa improvisa* were used as test materials and observed for 96 hours.

Results and Discussion

Fig. 1 indicates the location of fishing grounds with net cages in the lake. Net cages crisscross in the lake and the total number of such cages is approximately 2,442: 2,148 cages in Lake Kasumigaura and 294 in Lake Kitaura. 58 aquaculture operators produce carp and other fishes by the net cage culture. Kasumigaura and Tamatsukuri towns have numerous farms and a total of 1,800 net cages are in use in towns. Net cages used for carp culture are 25 m^2 in area and approximately 2 m in water depth. Production in highly efficient since the baseline average production is 1.5 tons per net cage.

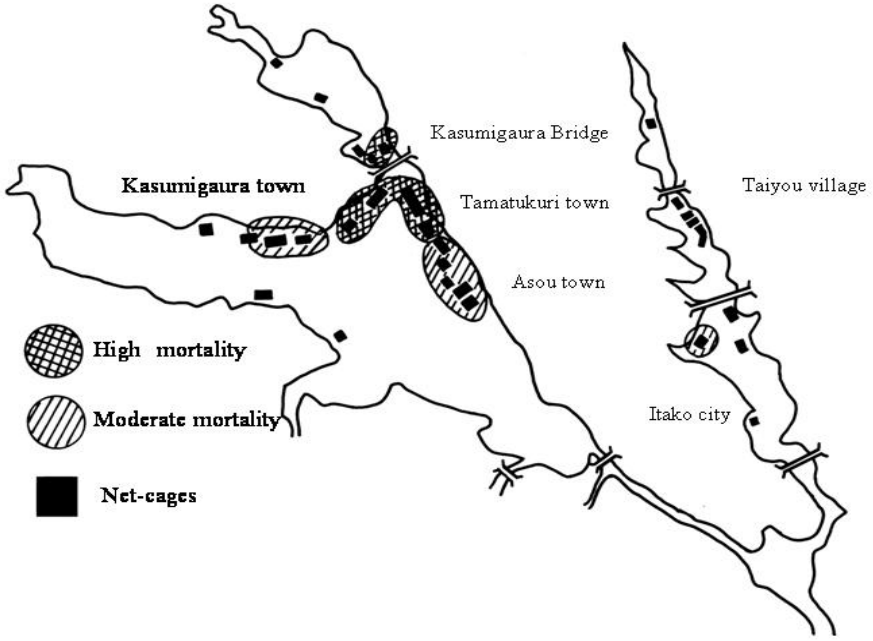

Fig. 1. Distribution of net-cages and KHVD damages in the lake.

Fig. 2 describes carp diagnosed with KHVD. Although diseased carp showed a variety of external symptoms, many of them suffered from albinism or friction on the surface or fins, and some appeared entirely red due to rube faction. Sunken eyes, anemia, hemostasis and discoloration of gills or decomposition of gills were identified.

Fig. 2. Symptoms of diseased fish. Symptoms of affected fish included sunken eyes, whitening of the body surface, and branchial anemia, congestion, and decomposition.

Comparison of disease symptoms between cultured carp and natural carp that tested positive to the PCR test appeared to indicate more advanced symptoms in natural carp. Natural carp had significant hollowness of the eyeballs, they were thin all over, rubefaction was frequently detected on the surface of their body and their body surface felt rough due to deficient mucus.

The interview surveys of aquaculture operators on the behavior and conditions at the time of death, among other conditions, carp in net cages revealed the following trends:

・The number of deaths was high in net cages with high lipid content and large amounts of feed. Deaths also occurred, however, where no feed was given.

・In terms of body size, the number of deaths tended to be higher among large-sized carp compared with yearlings, and many aquaculture operators indicated that the number of yearling deaths was small.

・On the other hand, comparison between edible carp and red koi carp revealed that red koi carp began dying earlier.

・With regard to conditions at the time of death, there were cases in which healthy carp fed next to net cages where deaths occurred, cases where breeding groups of healthy carp that were fed until the day before deaths

occurred began to die from the following day and cases where some populations were observed swimming lethargically in the surface layer prior to death.

Since carp kills coincided with the formation of "red water" due to the multiplication of *Skeletonema potamos*, this was suspected as the cause of the deaths. Some individuals believed the deaths were caused by poisoning due to water quality deterioration or indigestion because of excessive feeding at low water temperatures. Since anoxia, pH, low-quality formula feed and various other possibilities were cited as probable causes, a bioassay was performed. No deaths occurred after 96 hours, which led to the conclusion that the carp kills were not caused by acute toxicity.

Taking into account the possibility of bacterial diseases, bacteria tests were conducted, however, no bacteria were separated. The possibilities of indigestion and inferior feed from specific manufacturers were precluded because unfeeding carp also died and deaths of cultured carp were not reported in the Kitaura district during this period.

Since a myriad of test results failed to identify the causes, we requested the NRIA on October 28 to identify the causes of the deaths using specimens submitted by us. As a result of the PCR test, KHV DNA was detected in sick cultured carp in Lake Kasumigaura and natural carp in Lake Kitaura on October 31, and an outbreak of KHVD was suspected.

On November l, the Ministry of Agriculture, Forestry and Fisheries (MAFF) and the NRIA conducted field surveys and concluded that the possibility of KHVD was high. The Ibaraki Prefectural Government held a "Crisis-Response Meeting for Cultured Carp Deaths" on November 2 and called on aquaculture operators to cooperate in voluntary restraint of shipments.

In 2003, water temperatures of Lake Kasumigaura were lower than average during summer, then significantly fluctuated and dropped suddenly in October. The temperature in early October, when carp kills began, was 18℃ and decreased to 14-15℃ from late October to early November. It further declined to 12-14℃ from mid to late November, when deaths had mostly ceased. Deaths ended earlier in aquaculture farms where they began earlier while deaths tended to come to an end later in aquaculture farms where the outbreak occurred later.

An overview of damages in the lake is described in Fig. 1. It appears from the interview survey results that deaths of cultured carp in Lake Kasumigaura began early in Kasumigaura and Tamatsukuri towns, two districts that have numerous fishing grounds with net cages. These two districts also suffered significant damage. Deaths began in fishing grounds with net cages located far away from these two districts in late October. Although the accumulated number of deaths was estimated to be 200 to 300 tons in mid-October, the said number rapidly increased over a short period of time and the affected area also spread in Kasumigaura district: 660 tons in late October and 1,200 (1,124) tons from November 5 to 7, when on-site inspections were conducted. The extent of the damage reached a quarter of the annual production. On-site inspections also confirmed that carp had died in 77% of net cages in the lake. Due partly to the fact that production volume was large in Lake Kasumigaura; deaths were confirmed in 1,828 net cages among 2,148 in Lake Kasumigaura. The quantity of deaths in Lake Kitaura at that time was still low at approximately two tons.

Fig. 3 is a pattern diagram in which the period of death occurrences in two districts in Lake Kasumigaura, where deaths began early, was classified every five days from October 1.

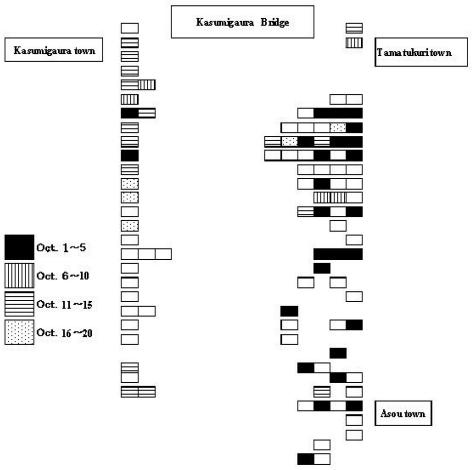

Fig. 3. A pattern diagram in which the period of death occurrences in two districts in Lake Kasumigaura.

This diagram implies that deaths began at various fishing grounds almost simultaneously, rather than as result of the outbreak of disease spreading in concentric circles from a single net cage. Since it was also conceivable for cultured carp to become infected from natural carp, we investigated the relationship between the period when carp deaths broke out in an aquaculture farm and when natural carp caught elsewhere were placed in the net cage of the farm. Nevertheless, the relationship between the breeding of natural carp and mortality outbreaks was not clear. Fig. 4 shows the distribution of cultured carp and natural carp which tested positive to the PCR test.

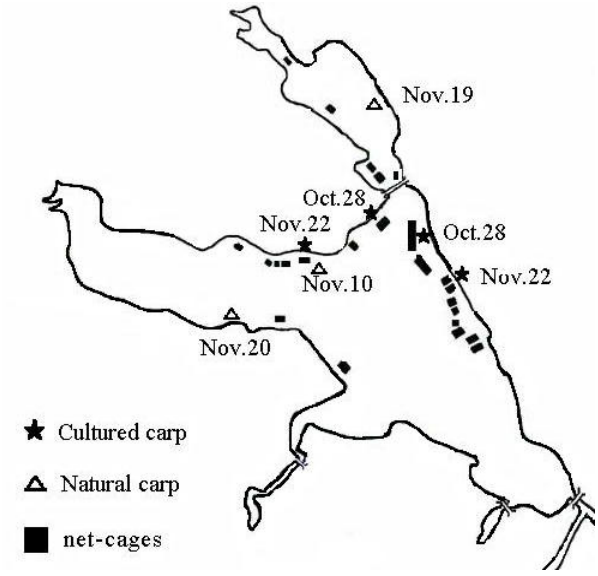

Fig. 4. Detection of PCR positive carp in Lake Kasumigaura.

Interview surveys on the abnormality of natural carp in various places were conducted with general fishery operators from October 23. There was no information on abnormal carp in Lake Kasumigaura until October 31. As a result of the PCR test, KHV DNA was detected in carp caught on November 10, 19 and 20.

Fig. 5 indicates the state of abnormal carp appearances in Lake Kitaura.

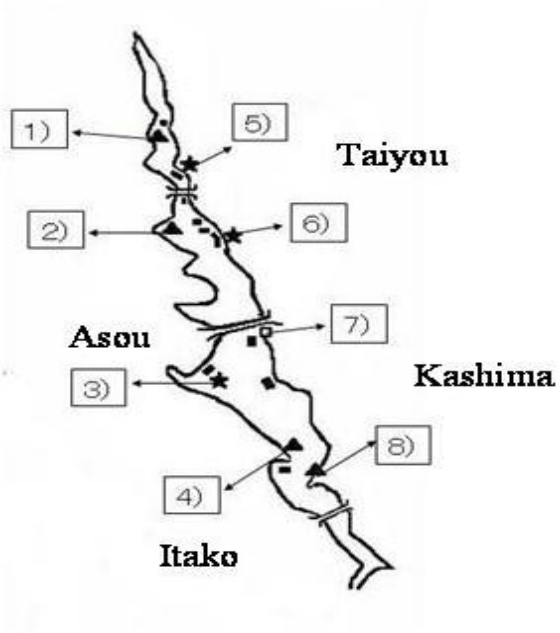

Fig. 5. Detection of PCR positive carp and abnormal natural carp in Lake Kitaura.

1) Abnormal natural carp since Oct. 29
2) Abnormal natural carp since Oct. 20
3) Mortality occurred since Oct. 12
4) Abnormal natural carp since last Sept. PCR positive natural carp on Oct. 28
5) Low mortality.PCR positive cultured carp on Nov. 22
6) Mortality occurred since Nov. 9
   PCR positive cultured carp on Nov. 15
7) Normal natural carp until Oct. 27
8) Abnormal natural carp since Oct. 20

In the Kitaura district, interview surveys were conducted after cultured carp kills occurred. As a result, it was found that abnormal natural carp, which were extremely lean and suffered rubefaction on the surface, had been caught in Itako city in the downstream basin from around late September, i.e. prior to the outbreak of cultured carp deaths. Natural carp caught at identical places on October 28 tested positive to the PCR test.

Subsequently, deaths of cultured carp began in Aso town around October 12, followed by the catch of carp with rubefaction on the surface at two locations in the upstream and downstream basins where the populations that tested positive to the PCR test were initially caught on October 20. Information came to hand on October 29 that abnormal natural carp had been caught a long way upstream. Cultured carp began dying in the midstream basin on November 9, and most cultured carp that were sampled on November 15 tested positive to the PCR test. Although there were few deaths among cultured carp in the uppermost stream of Lake Kitaura, samples collected on November 22 showed positive reactions to the PCR test.

In the Kitaura district, information that abnormal natural carp had been caught in the downstream areas was first made available.

KHVD was confirmed in natural carp first, and carp that ordinary fishery operators found to be abnormal in appearance were subsequently confirmed in the upstream areas. They were followed by the onset of cultured carp kills and the appearance of populations the tested positive to the PCR test. These were characteristics of the mass carp kills.

In addition to carp, channel catfish, *Ictalurus punctatus*, silver carp, *Hypophthalmichthys molitrix*, crucian carp, C*arassius auratus*, etc. are cultured in the net cage aquaculture. Nevertheless, deaths of the aforementioned fish species were not observed while large quantities of carp died. Aiming to prevent damage from harmful rumors, the PCR test was carried out on lake smelts, (*Hypomesus nipponensis*), ice fish (*Salangichthys microdon*), gobies (*Tridentiger kuroiwae brrevispinis*) and freshwater prawns (*Macrobrachium nipponense*), then all results were negative.

As countermeasures for the spread of the disease, the Ibaraki Prefectural Government called on aquaculture operators for voluntary restraint on shipments based on the Law on Ensuring Sustainable Aquaculture Production on November 2. From November 5 to 7, the prefectural government conducted on-site inspections of all net cages where carp deaths were confirmed and issued an injunction on November 11 against transferring carp in net cages where deaths were confirmed. On

November 30, another injunction was issued against transferring carp from all net cages. On December 20, disposal through incineration and burial was ordered and incineration was initiated from January 20, 2004.

Nevertheless, precritical cultured carp with no symptoms had already been shipped to carp-consuming areas nationwide before the detection of the KHV DNA by PCR test. Because live fish were shipped according to the general pattern of carp distribution; The lake produced half the cultured edible carp in the nation; it coincided with the shipping period; and the latent period of KHVD was long, i.e. for two to three weeks. The first outbreak of KHVD was confirmed at the lake, and outbreaks of KHVD were then confirmed throughout the nation. KHVD outbreaks included a case in which the phenomenon of mass carp kills was recognized in natural waters in Okayama Prefecture prior to the outbreak of KHVD at the lake. The aforementioned mass carp kills were handled as deaths from other causes and KHVD was confirmed in frozen specimens at a later date. And the infection routes were unknown among half cases of 84 KHVD outbreaks in Japan until December (announcement of the MAFF of Japan). These cases highlighted the importance of preventing non-native diseases from entering Japan, the necessity of developing and disseminating diagnostic technology for new diseases and the significance of initial prevention of spread of such diseases. This survey on the state of KHVD outbreaks revealed that the mass deaths in Lake Kasumigaura began with simultaneous multiple deaths at all aquaculture farms in Kasumigaura and Tamatsukuri towns, rather than occurring at specific fishing grounds with net cages and spreading from there. Since abnormality of natural carp broke out in Lake Kitaura before deaths of cultured carp occurred, the possibility of natural carp getting involved in the expanded infection among cultured carp was also taken into consideration. On the other hand, the outbreak of cultured carp deaths in Lake Kasumigaura and the confirmation of abnormal natural carp in Lake Kitaura were almost concurrent, suggesting the possibility that there might have been two routes of infection.

The outbreak of KHVD had never been reported in natural waters and it was confirmed in the lake for the first time. At small-sized aquaculture farms in closed waters, it is possible to eradicated pathogens by killing diseased fish and disinfecting aquaculture farms and to resume aquaculture easily. In open waters with an expansive catchments area, whose water is widely used for industrial and agricultural purposes, for example Lake Kasumigaura, pathogen eradication measures are not practical. Resumption of aquaculture business in the lake, where the production quantity of cultured carp accounts for 50% of the national total, however, urgently requires the development of vaccines, the breeding of disease-resistant fishes, among other measures, in addition to countermeasures for the spread of disease and virus eradication measures.

Acknowledgements

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