

# Current Status of Koi Herpesvirus Disease in Taiwan

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## **Abstract**

The first reported case of koi herpesvirus disease (KHVD) occurred in northern Taiwan in December 2002. Later, there were three more cases in 2003 and one outbreak of KHVD in 2004. Externally, the affected fish did not show any prominent lesions except swollen gills sometimes accompanied by bleeding. Consistent histopathological findings were in the gill tissues, where hyperplastic epithelia and eosinophilic granular cells were observed within fused secondary lamellae. Electron microscopy revealed negatively-stained icosahedral viral nucleocapsids measuring  $112\pm 1$  nm in diameter. Also, the koi herpesvirus was detected in the homogenate of diseased fish by PCR assay using specific primers for koi herpesvirus (KHV). The amplicon was cloned, sequenced and compared with previously published data. The sequenced data showed 99% identity with the American KHV sequence in the GenBank. The above evidence suggests that KHVD have already invaded carp culture systems in Taiwan.

## **The First Case of KHVD in 2002**

The first outbreak of KHVD occurred in a private carp pond in Taipei County in northern Taiwan. On December 6, 2002, two 2-year-old colored carp were submitted for examination by a private hobbyist to our research facility. The owner had about 30 koi, averaging in age between 2 to 3 years, which were reared in two different ponds. He had bought several of the koi from farmers at Taoyuan County in northern Taiwan about one month before the occurrence. The water temperature in both ponds was approximately 20-22°C when the disease outbreak occurred. At first, the affected fish appeared lethargic and lacked appetite for several days before death. The owner observed a light-reddish discoloration of the pond water so that he decided to submit his fish for examination. Upon necropsy, congestion was

observed at the base of the fins and tail, and the gills were swollen. Bleeding from the gill was observed in one fish. There were no other lesions found in the internal organs. For histological examination, tissues were fixed in 10% neutral-buffered formalin for 24 hours, embedded in paraffin, sectioned and stained with hematoxylin and eosin using all routine techniques. Histopathology of the gills showed hyperplasia and fusion of the secondary lamellae in the diseased fish. Necrotic epithelial cells accompanied by some eosinophilic granular cells were noted among the hyperplastic gill epithelia. Samples for bacterial examination of the liver, spleen and kidney were obtained by stabbing the organs with a sterile loop, inoculating samples onto blood agar (5% sheep red blood cell), then incubating them at 25°C for 48 hours. No bacterial growth was obtained from these samples. For viral isolation, the tissues (spleen, kidney and gill) of two diseased fish were homogenized with 10<sup>7</sup> PBS and centrifuged at 1500<sup>7</sup> g for 15 min. The supernatants were filtered through a 0.45 $\mu$  pore-size filter and inoculated onto a monolayer of FHM, EPC, and BF-2 cell lines at 25°C; CHSE-24 and RTG-2 cell lines at 20°C, and observed for 14 days. There was no CPE in the inoculated cell lines after one blind passage. For electron microscopy, a herpes-like viral particle was found in the negatively-stained samples. For PCR assay, specific amplicons were produced using specific primers designed by Gilad *et al.* (2002) and Gray *et al.* (2002). The amplicons were cloned, sequenced and compared with all the data submitted to GenBank. Our sequenced result had 99% identity with that of American KHV in Genbank. KHV was diagnosed as the etiologic agent of this outbreak.

As soon as KHVD was confirmed in the National Animal Research Institute, the Taipei County Livestock Disease Control Center and the Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ) were notified of the results. These facilities are responsible for controlling the spread of newly emerging exotic animal diseases. Upon receiving our notice BAPHIQ sent an official document to all local County Livestock Disease Control Centers requesting the centers to investigate the current status of carp cultures in their respective counties. The public veterinarians began an investigation and discovered no new disease outbreaks among cultured carp in Taiwan. In addition, upon advise of the owner of the diseased fish, we also visited the suspected farm at Taoyuan County as the possible source of infection. Our investigation was unsuccessful because the owner of the fish farm had already closed his farm and fled the premises. Therefore, the source of the KHVD introduction to Taiwan is still unknown.

### **The First Case of KHVD in 2003**

The first occurrence of KHVD in 2003 was at a private koi pond in Taipei. The case was transferred from the Taipei Animal Health Inspection Center to our laboratory. The total number of cultured koi in this incident was 20. Clinically, the affected fish were observed to be very sluggish and after 7-10 days, death occurred. Upon necropsy, only swelling of the gills was observed. Similar histopathological examinations and PCR assays were performed in

this case, and results obtained were the same with findings in the 2002 case. Therefore, this case was also diagnosed as KHVD. All fish were eradicated and buried. As in the previous case, the origin of infection remains unknown.

### **The Second Case of KHVD in 2003**

The second case of KHVD also occurred in Taipei in 2003. The koi were reared in an artificial lake together with tilapia in a public park. The lake had approximately 300 koi with no recent introduction of new fish into the park ponds. The tilapia remained unaffected and had no deaths throughout the koi herpesvirus outbreak. The affected koi (2-3 years old) were lethargic and were floating near the water surface before death. Both dead and moribund fish were sent to our laboratory by the Taipei City Animal Health Inspection Center. The gross lesions were similar to those in the previous two outbreaks. The series of examinations were performed at our laboratory as previously described. Similar histopathological changes and PCR results were also obtained in this case.

Since the park is open to the public for 24 hours, it is very easy to gain access into the artificial lake. Because park officials have not introduced new koi, it was speculated that the outbreak of KHVD might have resulted from unauthorized release of diseased fish into the lake by unknown persons. All the fish in this case were destroyed.

### **The Third Case of KHVD in 2003**

The third outbreak of KHVD in 2003 also occurred in Taipei. Seven hundred 2-3 year old koi that were reared in a lake located at a public memorial hall became infected. Similar pathological changes and PCR results were found during examinations. It was suggested that all the fish be culled to prevent further spread of the disease. Since the public has access to the pond for 24 hours, the origin of this outbreak of KHVD is probably caused by the unintentional (unexpected) introduction of diseased fish by unknown park visitors.

### **The First Case of KHVD in 2004**

The first outbreak of KHVD in 2004 occurred in a suburban area in Taipei County. The owner is a farmer who hatches, raises and sells the koi. The farmer's koi hatchery is located in southern Taiwan. After hatching, the fries are grown to juvenile, moved to the grow-out farm, and later sold in northern Taiwan. Water for the grow-out ponds comes from a spring. The water temperature was 22-23°C when the disease outbreak occurred. The diseased fish did not show any prominent clinical signs or gross lesions during the visual examinations. The histopathological and PCR results were identical with the findings in all previous cases. All the affected fish (about 1000 pieces) were destroyed. After disinfecting the rearing water with chlorine, it was drained and the ponds were left empty for 2 months before

being re-stocked with new fish. This case may have been caused by the owner's acceptance of unhealthy koi returned by his customers.

### **Diagnosis and Control System of Exotic Aquatic Animal Diseases in Taiwan**

The diagnostic system for aquatic animal diseases is a collaboration of the central government and the local county. In the central government, the Animal Health Research Institute (AHRI) of the Council of Agriculture is responsible for the final diagnosis of exotic aquatic animal diseases. In the AHRI, routine monitoring program for animal diseases is in place and it also receives suspected specimens submitted from all over Taiwan. In the local county, the aquatic health section of the county Livestock Disease Control Center (LDCC) is responsible for routine aquatic animal disease diagnosis and control. If a disease is suspected to be caused by a new and emerging disease agent, it will be referred to the AHRI for final confirmation. Furthermore, aside from receiving suspected specimens from local LDCC, AHRI can also accept specimens submitted directly by private individual for diagnosis.

Regarding the control system for exotic aquatic animal diseases, the BAPHIQ in the central government is in charge of the control plan, including confinement, eradication and compensation related to the exotic aquatic animal disease outbreaks. In the local county, the LDCC executes the control plan determined by BAPHIQ.

### **Spread of KHV in Taiwan**

According to our official data, the outbreaks of KHV are found in Taipei and Taoyuan County in northern Taiwan only.

### **Research on KHV in Taiwan**

There are on-going studies in the development of cell lines for viral isolation, development of rapid diagnostic tool, research on viral pathogenesis in the molecular level, and the development of a vaccine against KHVD at AHRI and other universities in Taiwan.

### **References**

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