Detection of Koi Herpesvirus in Koi Cyprinus carpio in Taiwan

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ABSTRACT—Mass mortality occurred in 2-year-old koi (fancy carp) Cyprinus carpio in Taiwan in 2002. Affected fish did not show any external signs except swollen gills. The consistent histological changes were observed in the gills; hyperplasia of epithelial cells, infiltration of eosinophilic granular cells and fusion of the secondary lamellae. Negatively stained nucleocapsids were icosahedral and 112±1 nm in diameter. Koi herpesvirus (KHV) was detected in the diseased fish by PCR using specific primers for KHV, and sequence of the amplicon showed a 99% identity with the published data. This is the first report of KHV infection in Taiwan.

Key words: koi herpesvirus, KHV, histopathology, Cyprinus carpio, koi

Prior to the emergence of koi herpesvirus (KHV), cyprinid herpesvirus (CHV) was the only pathogen known to cause herpesviral diseases in common carp and koi Cyprinus carpio1,2). In 1998, a newly recognized virus, KHV was isolated in Israel and the USA, and shown to be pathogenic for koi and common carp of all ages. It can cause 80-100% mortality, mainly with severe hyperplasia and necrosis of gill epithelia and necrosis of other organs3). Later, outbreaks of KHV disease were reported in the United Kingdom, Germany, Japan and were suspected in Indonesia4)*1,4)*2,4)*3,4)*4) by

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Materials and Methods

In December 2002, koi, approximately 2 years of age, were submitted for examination by a private owner in northern Taiwan. The pond-reared fish were lethargic and lacked appetite. The cumulative mortality during a 14-day period was 90%. The tissues (spleen, kidney and gill) of two fish were homogenized, filtered, and then inoculated onto monolayers of FHM, EPC, and BF-2 cell lines at 25°C, and CHSE-214 and RTG-2 cell lines at 20°C. Bacterial examinations were done with blood agar (5% sheep red blood cell) incubated at 25°C. For light microscopy, tissues were fixed in 10% neutral-buffered formalin for 24 h, embedded in paraffin, sectioned and stained with hematoxylin and eosin. For electron microscopy, tissues from affected koi were processed as previously described5).

For PCR assays and sequencing, a specific fragment was amplified with DNA extracted from tissues using primers and condition as described previously6). The amplicons were cloned, sequenced and analyzed using the BLAST search of GenBank.

Results and Discussion

No CPE appeared in any cell lines inoculated with the organ homogenates as described previously7). No growth of bacteria was found. The most consistent microscopic lesions were in gills, where hyperplastic epithelium and granular eosinophilic cells were observed; there were also fusion of the secondary laminae (Fig. 1). Electron microscopy revealed icosahedral viral nucleocapsids, 112±1 nm in diameter, by negative staining (data not shown). The PCR-amplified 483-bp product (data not shown) showed 99% identity to the American KHV sequence published in GenBank (accession no. AF411803).

In this report, histologically, the lesions we observed in KHV case were consistently found in the gill as first reported by Hedrick et al8). However, in our case, differences of pathological changes were noted that there...
were merely slight nuclear changes in infected cells, of gills, kidney and stomach, and no necrotic lesion in spleen, liver, heart and brain compared to prominent necrosis as previously described\(^6\). This might be due to the small sample size in our case, low copy numbers in some tissues, or the emergence of a new variant of KHV. Since there had been no such mass mortality of adult koi in Taiwan, KHV might have been introduced to Taiwan.

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**References**


**Fig. 1.** Hyperplasia of epithelial cells with infiltration of eosinophilic granular cells (arrow head) and fusion of the secondary lamellae (H&E, bar = 21 µm)