Epizootic of Herpes-like Virus Infection in Goldfish, *Carassius auratus* in Taiwan

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In May of 1995, an epizootic occurred in goldfish fry at a breeding colony in northeast Taiwan resulting in 90 percent loss of fry. In the study, we did histopathological and ultrastructural studies in diseased fish and report the association of a herpes-like virus with this epizootic.

Materials and methods

Goldfish (20 to 25 days old, 1.5–2.0 cm body length) from a population with an increased mortality in the breeding colony were subjected to necropsy and for pathogen isolation. Samples were taken from liver, spleen, and kidney of moribund fish and inoculated onto brain heart infusion agar (BHI) (Difco™), which were incubated at 25°C for 48 h. Moribund fish collected from the breeding colony were fixed in 10% neutral formalin, embedded in paraffin, sectioned at 5 µm, and stained with Mayer's hematoxylin and eosin. Samples of liver, spleen, heart, head kidney and trunk kidney of 20 moribund fish were pooled, homogenized in phosphate buffered saline (PBS) to give a 10% (w/v) suspension and centrifuged at 3000 Xg for 20 min at 4°C. The supernatant was centrifuged at 100,000 Xg for 10 min, the pellet was negatively stained with 2% phosphotungstic acid and examined using an electron microscope.

Results

In December 1994, 200 brood goldfish were introduced into a goldfish hatchery in northeast Taiwan. The introduced fish died spontaneously without a cause

being determined, and 120 fish were left by March 1995, the mortality of introduced fish was 40 percent. Selected new brood fish were mixed with established brood fish in the pond for cross breeding at about 22°C water temperature. Eggs were hatched, and fry were transferred to rearing units at 70,000 fry/unit. At twenty days old, fish in one unit began to show anorexia, lethargy, and yellowish discoloration. Fish in the other 2 units showed similar signs a few days later. Most fish began to die at the 4th d in the units and resulted in a mortality of 90% of approximately 2,000,000 fry in four units.

There was yellowish discoloration of skin associated with natural infections of goldfish. No pathogenic bacteria were isolated from moribund fish. The lesions were found in visceral organs and skin of natural infections of fish. The interstitial tissue of trunk kidney revealed multiple focal necrosis with mild infiltration of macrophages. The affected cells in the necrotic area had enlarged nuclei showing prominent chromatin margination (Fig. 1). Similar lesions were found in the myo-

Fig. 1. Natural herpes-like virus infections of goldfish, *Carassius auratus*. The affected trunk kidney showed enlarged nuclei (arrow) in the necrotic cells. H&E. bar = 200 µm.

Fig. 2. Natural herpes-like nucleocapsids (center) and enveloped virion (upper right) in preparation from pooled visceral organs of naturally infected goldfish negatively stained with 2% phosphotungstic acid. bar = 100 µm.

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cardia of heart, lamina propria of small intestine, exocrine tissue of pancreas, head kidney, and epidermis and dermis of skin. There was also cloudy degeneration in some renal tubule epithelia of trunk kidney.

TEM examination on thin sectioned and negatively stained samples showed both nucleocapsid and enveloped virion. The viral particles were hexagonal with intranuclear nucleocapsids of 100 to 110 nm in diameter and intracytoplasmic enveloped virions of 175 to 225 nm (Fig. 2). The morphology and size of the viral particles fell within Herpesviridae^{7,9}.

Discussion

Herpesviruses associated with proliferative conditions of the epidermis^{1,2,4,5,6,8,10} or systemic diseases in fish have been reported^{3,11}. The epidemiology of the case shared some similarity to herpesviral haematopoietic necrosis (HVHN) infection of goldfish in Japan³. Both caused high mortality at about 22°C water temperature. The morphology and the size of the causative virus and host cell range were similar too. In the epizootic, the lesions were characterized by focal necrosis in visceral organs, and epidermis and subcutaneous tissue of skin; the affected cells in the necrotic area had an enlarged nuclei showing prominent chromatin margination. Jung and Miyazaki³ had reported HVHN

virus infected goldfish induced similar focal necrosis in various visceral organs except for the appearance of enlarged nuclei in affected cells.

In this epizootic, the hatchery owner had introduced brood goldfish before spawning. Prior to this introduction there was no known herpesviral infection of goldfish in Taiwan. It is speculated that the virus was introduced with the imported goldfish.

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