Note

Spontaneous stomach lymphoma and liver metastases in flower horn fish (hybrid cichlid)

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Abstract

A diseased three-year-old flower horn fish (also known as Hua Lou-Han), a hybrid cichlid pet fish, had poor appetite for more than two weeks according to the owner's description. From the clinical examination, enteritis, enlarged abdomen, and rapid respiration rate were found. The gross findings revealed a volume of about 5 ml of ascites. Numerous nodules (approx. 10 × 10 × 10 mm^3, 2 g/nodule) were found in the liver and stomach. The nodules were found to be causing partial obstruction in the stomach and intestines. The histopathological examination of the nodules in the liver and stomach suggested that the diagnosis was lymphoma. Microbiological examinations did not reveal any significant findings. Therefore, the final diagnosis was spontaneous lymphoma with stomach and liver metastases.

Flower horn fish (Hua Luo-Han) first appeared in 1996 through selective crossbreeding (Ou & Gu, 2002; Koo et al., 2001). The origins of this hybrid fish can be traced from the Cichlid genus Cichlasoma. Although the real flower horn fish family tree is still unclear, most fish breeding workers and amateurs believe that the fish is the result of crossbreeding among *Cichlasoma trimaculatus*, *C. festae*, Jingang Blood Parrot and others. This fish is becoming increasingly popular as a pet, especially in South-East Asia where it is known as the discus fish and arowana (Chao & Chou, 2002; Koo et al., 2001; Lee, 2002). This ornamental fish has been reported to have a high incidence of cancer (Chao & Chou, 2002; Koo et al., 2001).

According to the owner's description, the diseased 3-year-old fish, weighing approximately 980 g had a swollen abdomen for 1 month. It was raised in a glass tank (volume: 120 cm × 45 cm × 60 cm) and fed a commercial Hua Lou-Han diet (1% body weight twice a day) throughout its life. The average temperature of the water was 28°C. The owner had observed mucus stools in the bottom of the tank and fecal cast in the anus of the fish. At autopsy, the diseased fish was found to have 5 ml ascites in its swollen abdomen (Figure 1A). There were several white to yellowish nodules scattered on the liver and stomach (Figures 1B & 1D). These masses compressed and even partially obstructed the digestive tract (Figure 1C). No

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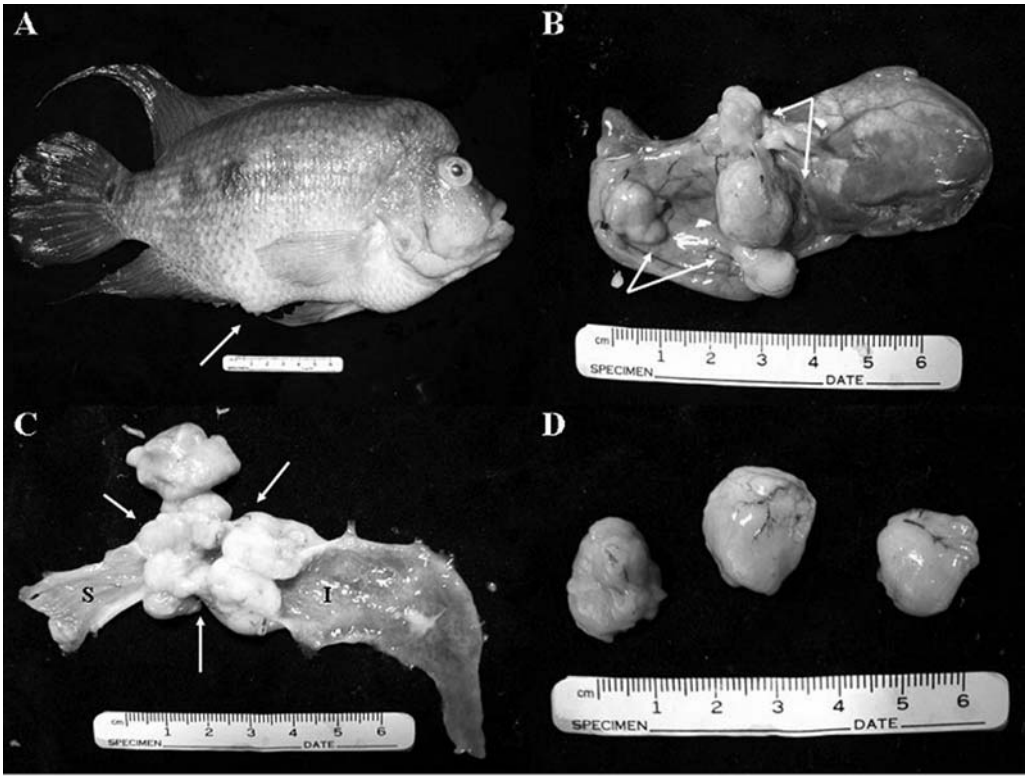

Figure 1. Gross signs of a diseased flower horn fish. **(A)** The fish had enlarged abdomen (arrow) with 5 ml ascites. **(B & C)** Gross lesions showed many nodules in liver and stomach (arrows) and oedematous intestine. Nodules (arrows) caused partial obstruction of stomach (S)-intestine (I). **(D)** The morphology and size of lymphoma mass.

other lesions were found. Nodules and organs were detached, fixed in 10% buffered formalin, and processed to parafin in a vacuum infiltration processor according to the protocol described by Stentiford et al. (2005). The tissue sections were stained with haematoxylin and eosin (H&E) then mounted onto glass slides. The stained sections were observed and analyzed by light microscopy (BX41, Olympus, Tokyo, Japan). The histopathological findings showed diffusely distributed tumor masses, but it was without follicular structures under low magnification (Figure 2E). Moreover, we found that the tumour cells had infiltrated into the submucosa and laminar stratum of the stomach (Figure 2A). Interestingly, the normal hepatopancreatic architecture was obliterated and the parenchyma replaced by an infiltrate of neoplastic lymphocytes of varying sizes (Figure 2E). At high magnification, the tumor was seen to be comprised of medium-sized blast cells with large round or oval pale stained nuclei. Fragments of necrotic nuclei were noted. The amount of cytoplasm in the neoplastic lymphocytes was scant to moderate and a little basophilic (Figures 2B & 2F). Several large non-neoplastic macrophages

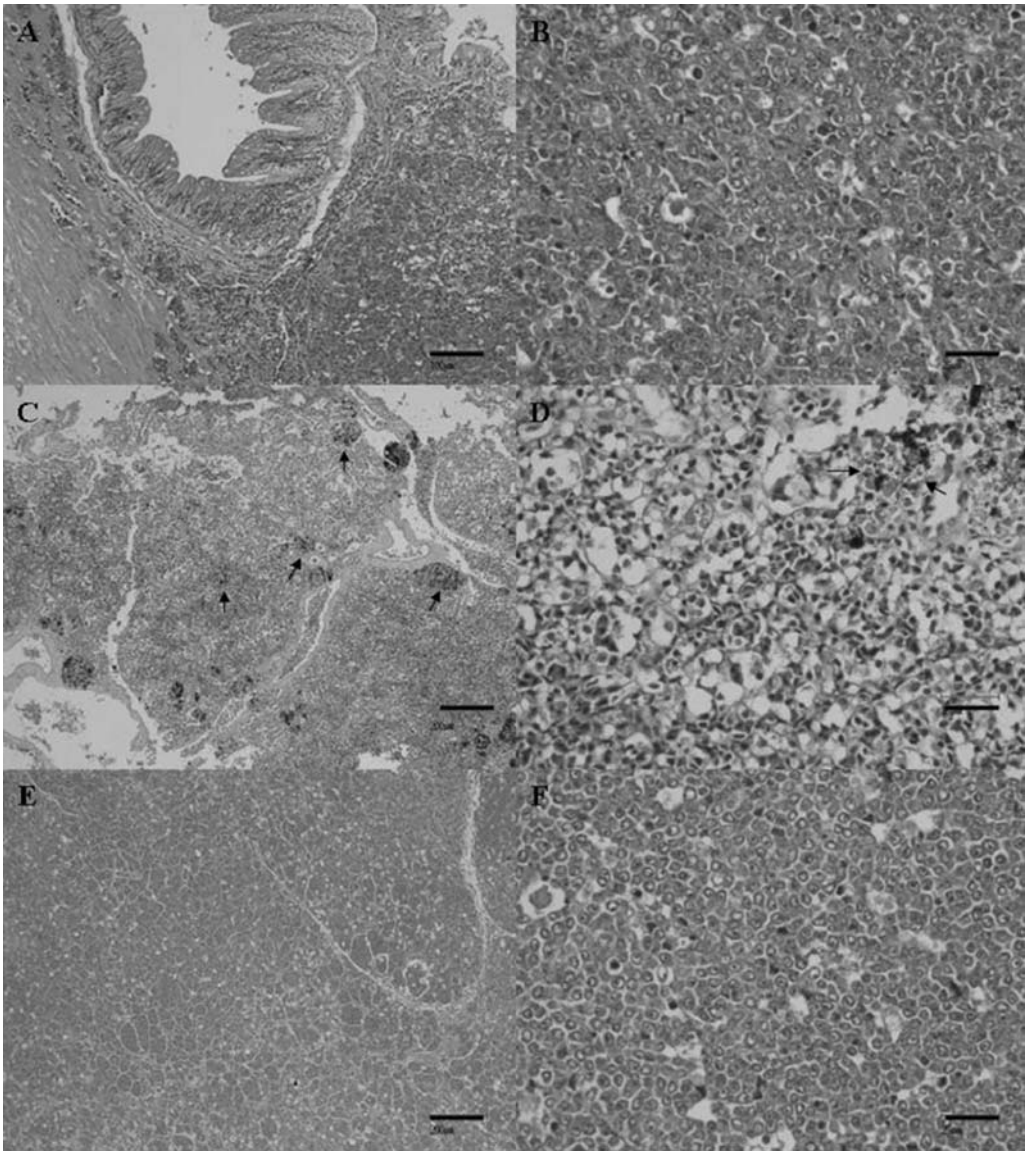

Figure 2. Stomach and liver, H&E **(A)** The tumour cells infiltrated into submucosa and laminar layer of the stomach (scale bar = 100 mm). **(B)** Tumour cells consisted of medium-sized blast cells with large round or oval pale staining nuclei (scale bar = 25 mm). **(C)** At low magnification, most of the melanomacrophage centers (MMC) were paler than normal ones. These reactive macrophages were responsible for 'starry sky' appearance (scale bar = 200 mm). **(D)** At high magnification, several large non-neoplastic macrophages were present and within their abundant cytoplasm there were remnants of ingested cells, including many nuclear fragments (scale bar = 25 mm). **(E)** The tumour appeared to have a diffuse distribution, but without follicular structures at low magnification. The normal hepatopancreatic architecture was obliterated and parenchyma was replaced by an infiltrate of variable sized neoplastic lymphocytes (scale bar = 200 mm). **(F)** At high magnification, tumour consisted of medium-sized blast cells with large round or oval pale staining nuclei. Some pyknotic fragments of necrotic nuclei were noted. The amount of cytoplasm in the neoplastic lymphocytes was scant to moderate and a little basophilic (scale bar = 25 mm).

were present and within their abundant cytoplasm there were remnants of ingested cells, including many nuclear fragments. These reactive macrophages (the cells may be the component of melanomacrophage centers) are responsible for the 'starry sky' appearance (Curran & Crocker, 2000). Most of the melanomacrophage centers (MMC) were paler than normal (Figures 2C & 2D). Based on the cellular morphology and characteristics of the masses from liver and stomach, the cancer was identified as lymphoma. The sections from other organs were without neoplastic cells. The final diagnosis was spontaneous lymphoma with stomach and liver metastases.

Neoplasms of haematopoietic origin are most frequently from lymphoid tissues (Bartfai et al., 2000). Lymphoma in fish is geographically widespread in North America and has been identified from Alaska through Canada to New York State (Banfield et al., 1976). The disease has also been found in abundance in pike in Ireland (Mulcahy, 1963) and in the Baltic Sea along the coasts of Sweden (Ljungberg, 1976). Lymphoma has been reported in several species of fish (Battalora et al., 1990; Earnest-Koons et al., 1997; Bartfai et al., 2000). Epizootics of lymphoma have occurred in northern pike (*Esox lucius*) and muskellunge (*E. masquinongy*) (Banfield et al., 1976; Mulcahy, 1976; Thompson, 1982; Papas et al., 1977). Lymphoma has also been reported in salmonids (Dunbar, 1969; Herman, 1970; Warr et al., 1984, Bernstein, 1984; Bowser et al., 1987), Atlantic cod (*Gadus morhua*) (Wolke & Wyand, 1969), turbot (*Scophthalmus maximus* L.) (Ferguson & Roberts, 1976), annual cyprinodont fish (*Nothobranchius guentheri*) (Cooper et al., 1983), pike-perch (*Stizostedion lucioperca* L.) (Bekesi & Kovacs-Gayer, 1986), bagre pintado (*Pimelodus clarias*) (Urdaneta et al., 1987), and Japanese medaka (*Oryzias latipes*) (Hawkins et al., 1988, Masahito et al., 1989, Okihiro & Hinion, 1989). Other cases of lymphoma have occurred in various fish species that were exposed to chemical carcinogens (Brown et al., 1975; Schwab et al., 1978; Schultz & Schultz, 1982; Chen et al., 1985). Therefore, environmental pollution appears to be a critical factor for lymphoma development in aquatic animals. Furthermore, laboratory experiments have demonstrated that unaffected pike develop cutaneous lymphoma when kept in the same aquarium as lymphoma-bearing pike (Ljungberg, 1976; Sonstegard, 1976). The tumors are easily transplanted from pike to pike by intracutaneous, intramuscular, or intraperitoneal inoculations of viable lymphoma cells (Ljungberg, 1976; Sonstegard, 1976). The fish in the present study was only fed with commercial diet similar to other brands of commercial diets. Hence, we postulate that our case of lymphoma was spontaneous.

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