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DETECTION OF VIRUS IN THE SNAKEHEAD FISH DURING A DISEASE EPIZOOTIC BY LIGHT AND ELECTRON MICROSCOPY

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ABSTRACT

Fish *(Ophicephalus striatus)* from a disease epizootic were examined grossly, by light microscopy, and by scanning and transmission electron microscopy. Affected fish were pale and ulcers occurred over the entire body. Histopathological examination revealed intracytoplasmic inclusion bodies within hepatic cells and intranuclear inclusion bodies within seminiferous tubular cells of the testis. Scanning electron microscopy showed bacteria and fungi on the ulcer surface while transmission electron microscopy showed necrosis with virus particles in the liver, spleen, kidney, muscle at the base of ulcers, and capillaries of affected fish. The viruses and inclusion bodies were not found in normal fish.

INTRODUCTION

A serious epizootic of fresh water fish, particularly the "snakehead" *(Ophicephalus striatus),* occurred in Thailand during the late part of 1982 to the beginning of 1983. Disease outbreaks of this type were reported from more than half of the country, and many species of wild and cultured fish were found to be infected. The economic loss to culture operations was estimated at more than 5 million U.S. dollars, while loss to wild fish could not be estimated. The sick fish were pale and ulcers were distributed over the entire body, especially on the jaws and skull. The affected fish were sometimes found to swim obliquely with the head up; finally they swam spirally and died. The bacteria *Aeromonas hydrophila, A. punctata, Flavobacterium* sp., *Pseudomonas fluorescens, P.* sp., *Edwardsiella tarda, Vibrio parahaemolyticus, Streptococcus* sp. (BOONYARATPALIN, 1983), and fungus, *Achlya* sp. (PITTAYANGKULA & BODHALAMIK, 1983) were reported. Virus-like particles in many organs of sick snakehead fish were also reported by WATTANAVIJARN *et al.* (1984).

In 1984, this type of epizootic was again reported from many parts of the country. The lesions observed were exactly the same as reported previously. A sudden drop of water temperature, pollution, poor water quality, bacteria, fungi, parasites, pesticides, etc. are thought to be related to disease outbreaks. This paper reports on light and electron microscopic observations in fish collected from natural and cultured sources.

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MATERIALS AND METHODS

Histopathology. Thirty-five sick snakehead fish from culture ponds in Suphanburi Province and 5 from natural canals and rivers in Suphanburi, Ayutthaya, and Pathum Thani Provinces were collected during an epizootic and submitted for gross and histopathological examination.

All organs were examined grossly and in sections fixed in 10% buffered formalin. Tissues were routinely processed and embedded in paraplast Luna (ed. 1964). Blocks were cut at 5 microns and stained with hematoxylin and eosin.

Thirty-two normal fish from these provinces and from Samyan market, Patumwan, were collected during non-epizootic times and processed in the same manner as the sick fish.

Scanning electron microscopy. Thirty sections of ulcers from the sick fish and 15 sections of muscle from normal fish were fixed in glutaraldehyde-formaldehyde, pH 7.4, for 24 hours. Samples were washed 3 times with 0.1 M cacodylate buffer then post-fixed with 2% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.4 for 2 hours. After dehydration in a graded ethanol series, they were critical point dried. Samples were fixed on stubs, sputter-coated with gold, then examined in a JEOL T-20 electron microscope at 20 KV.

Transmission electron microscopy. One hundred and ten sections of ulcers and internal organs from the sick fish and 54 sections of muscle and internal organs from the normal fish were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2. Tissues were kept in fixative, then cut into 1-mm^3 cubes and fixed for 24 hours at room temperature. Tissues were washed 3 times with 0.1 M cacodylate buffer and post-fixed with 1% osmium tetroxide in 0.05 M cacodylate buffer for 2 hours. Blocks were stained with 1% aqueous uranyl acetate containing 1% dimethyl sulfoxide for 1 hour, dehydrated in a graded ethanol series and embedded in Epon at 60°C for 2 days. Grids were stained with uranyl acetate and lead citrate. All sections were examined with a JEM-200 CX transmission electron microscope at 80 KV.

RESULTS

In culture operation ponds, the moribund snakehead fish floated at the water surface (Fig. 1). Many sick fish swam abnormally with their heads up (Fig. 2).

The gross lesions were confined to the skin which had lesions varying from hemorrhages to widespread necrotizing pale areas. The necrosis extensively penetrated into muscle and bone. Extensive ulcerations were found all over the body. Destruction of head bones and deformity of body fins were also seen. At necropsy, gills were markedly congested or anemic. Congested and petechial hemorrhages of visceral organs and fat were observed in a few cases.

Histopathological changes include extensive necrosis and myositis of skeletal muscle and brachitis of the gills with telangiectasis of the secondary lamellae and

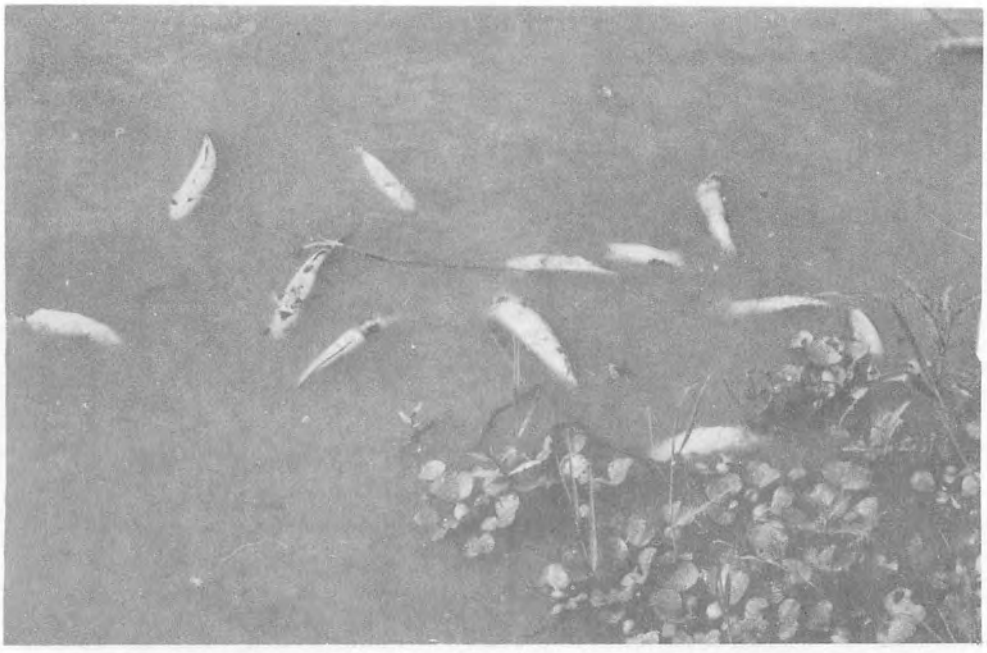

Figure 1. Dead fish at the water surface.

Figure 2. Sick fish swimming with head up.

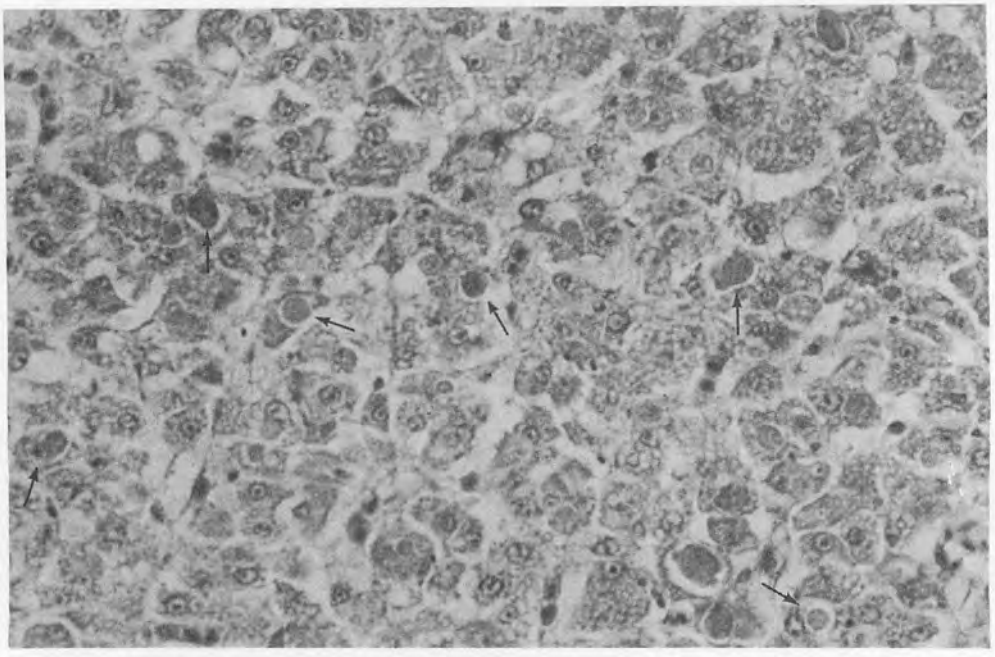

Figure 3. Hepatic cells showing scattered vacuolation and multiple large round cytoplasmic inclusion bodies.

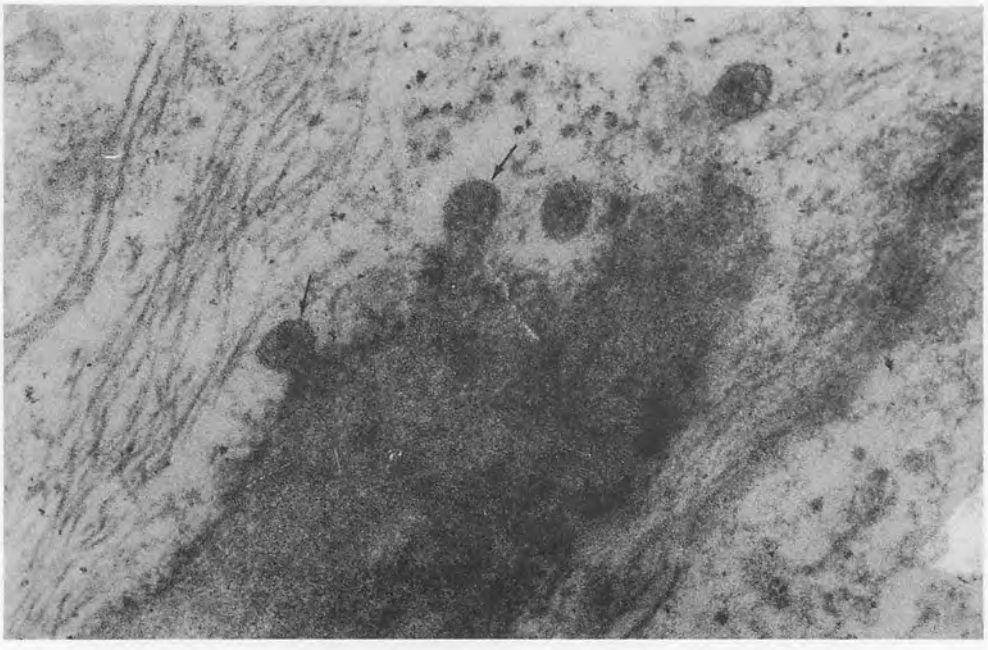

Figure 4. Releasing virus in the cytoplasm of spleen cell (magnification 103,000X).

incidental myxosporidial infection. The liver had multifocal necrosis and large eosinophilic inclusion bodies were present in the cytoplasm of hepatic cells (Fig. 3). Multifocal necrosis of renal tubular cells, hemopoietic tissue, and focal lymphoid necrosis of spleen were also evident. The gastrointestinal tract showed varying degrees of catarrhal inflammation and some nematodes in the small intestine. In male fish there was evidence of tubular degeneration, and numerous intranuclear inclusion bodies were present within the degenerated seminiferous tubule cells of the testis. Thrombosis of the hepatic and splenic blood vessels occurred in two cases.

Scanning electron microscopy revealed necrotic skeletal muscle bundles at the sites of ulceration. Rod-shaped bacteria and fungi were present on the ulcer surface. These bacteria were also found on the outer surface of normal fish in a few cases.

Transmission electron microscopy revealed necrotic muscle myofibrils and sarcoplasm at the sites of ulceration. The liver, spleen, and kidneys had multifocal necrosis and electron dense bodies. Many cell organelles were destroyed. Viruses were found in the endothelium of capillaries, sarcoplasm, cytoplasm of the liver, spleen (Fig. 4), kidney, and in white and red blood cells of the sick fish.

Normal fish had no lesions, inclusion bodies or viruses.

DISCUSSION

The fish disease was very severe and it has proven difficult to control. Although *Aeromonas hydrophila* was isolated from many organs, the presence of inclusion bodies within parenchymatous cells in connection with viruses indicate a more complex etiology.

Surface observation by scanning electron microscopy revealed many bacteria and fungi. These organisms invaded fish muscle from without, not from within; therefore, it is probable that they are opportunistic contaminants in fish ponds.

The viruses observed by transmission electron microscopy revealed particles with budding processes in many damaged organs. Antibody-producing cells have been identified in the spleen of teleosts. Infection by viruses results in reduction of the antibody production, and the fish became easily infected by other kinds of pathogens. Many red blood cells were infected with the viruses, which caused respiratory difficulty and paleness due to lack of oxygen. Infected red blood cells spread the viruses systemically. These viral particles found infecting various tissues and organs appeared to be related to pathogenesis of the disease.

The particles have the mean diameter of about 70 nanometers. Being in the cytoplasm, these agents probably have an RNA genome. Morphologically, they resemble orthomyxovirus, arenavirus, or bunyavirus (Wolf; pers. comm.). With negative staining and accurate size determination, it would be possible to arrive at a better determination. As intranuclear inclusion bodies were also found within the degenerated seminiferous tubule cells of the testis, possibly another DNA virus was contained in the sick fish. However, the viral particles were not clearly seen in the nuclei of the infected fish from these samples.

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