

Epitheliocystis in Carp (*Cyprinus carpio*) in South Korea

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ABSTRACT. Epitheliocystis in the carp of a pet fish market were investigated by our diagnostic work and collecting information from department of laboratory animal medicine and fish & shellfish laboratory. The epitheliocystis was identified by using histopathological examination. Epitheliocystis was confirmed as inflammation, epithelial hyperplasia, and lamellar fusion of the gill tissue. Electron microscopic observation showed that the inclusions were filled with Chlamydia-like organism.

KEY WORDS: *Cyprinus carpio*, epitheliocystis, gill infection.

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Epitheliocystis has been described in over 30 species of fish, including both wild and cultured marine and freshwater species [1, 5]. Mortality rates ranging from 4 to 100% in larvae and juveniles of cultured fish with epitheliocystis are common, although the condition is usually benign [1, 5]. Gills and (rarely) skin are the primary target organs [5]. Lesions present as white miliary nodules up to 1 mm in diameter on the skin or gills. Epitheliocystis is characterized by the presence of hypertrophied cells containing fine basophilic granular inclusions in hematoxylin and eosin (HE) staining [5, 7]. The causative agent is an intracellular bacteria, most probably Chlamydia-like organisms or Rickettsia-like organisms [1, 3, 5, 7]. This study is the first report for occurrence of epitheliocystis in carp in Korea.

Seven carp (body length: 7–23 cm, body weight: 20–230 g) with morbidity were collected from a pet fish market (Seosan, Korea) for diagnostic work. Organ samples were immediately fixed in Davidson's fixative over 24 hr with slight shaking. Fixed tissues were dehydrated in an alcohol-xylene series, and embedded in paraffin wax. From each block, 2 μ m sections were prepared and stained with HE for histopathological examination. The HE stained sections containing inclusions were selected for electron microscopic observation. The selected sections were postfixed in 1% OsO₄, and rinsed with 0.1 M sodium cacodylate buffer (pH 7.2) for 30 min. After dehydration in a series of alcohol-propylene oxide, the sections were in situ embedded in an Epoxy resin (Quetol 812, DDSA, MNA, DMP 30) at 60°C. Ultrathin sections were made by diamond knife and double-stained with 1% uranyl acetate and 1% lead citrate, and examined with a transmission electron microscope (JEM 100CX II, JEOL, Japan) at an acceleration voltage of 80 kV.

On histopathological examination, all of the fish samples were diagnosed with epitheliocystis. Round cysts from 10

to 40 μ m diameter were observed at the proliferative lesion between the second gill lamellar (Fig. 1). The inclusion bodies contained a homogenous basophilic granular material. Moderate epithelial hyperplasia of the gill tissue, occasionally leading to lamellar fusions, was observed. Extensive inflammation, including granulocytes and monocytes was seen in all infected gill tissue. Ultrastructural analysis revealed that cysts were filled with polymorphic cells 0.2–0.4 μ m in diameter (Fig. 2). The cells had electron-dense protoplasm and a compact, centrally located round nucleoid. Most of these small cells contained several translucent vacuoles of various size. Epitheliocystis has been reported in numerous fish species such as atlantic salmon [5], sea bass [1], steelhead trout [6], sea bream [2], and white sturgeon [3]. We are the first to report epitheliocystis in carp (*Cyprinus carpio*) in Korea. Cysts usually were present in the middle, base, and tip of the gill filament but mostly concentrated in the middle of the gill filament [1–7]. Although causative agents in the cyst included three distinctive developmental stages; primary elongated cell stage, intermediate elongated cell stage, and small cell stage, we could observe only small cell stage [1–7].

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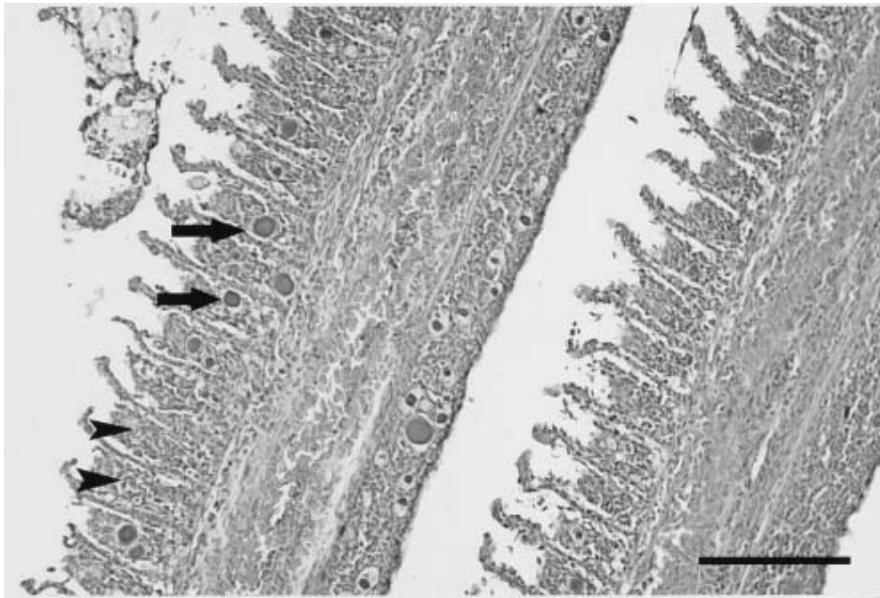


Fig. 1. Epitheliocystis inclusions (arrows) in the between of second gill lamella of carp. Moderate hyperplasia of the gill tissue, leading to lamellar fusion (arrowheads) was observed. Hematoxylin and eosin stain. Scale bar = 200 μm .

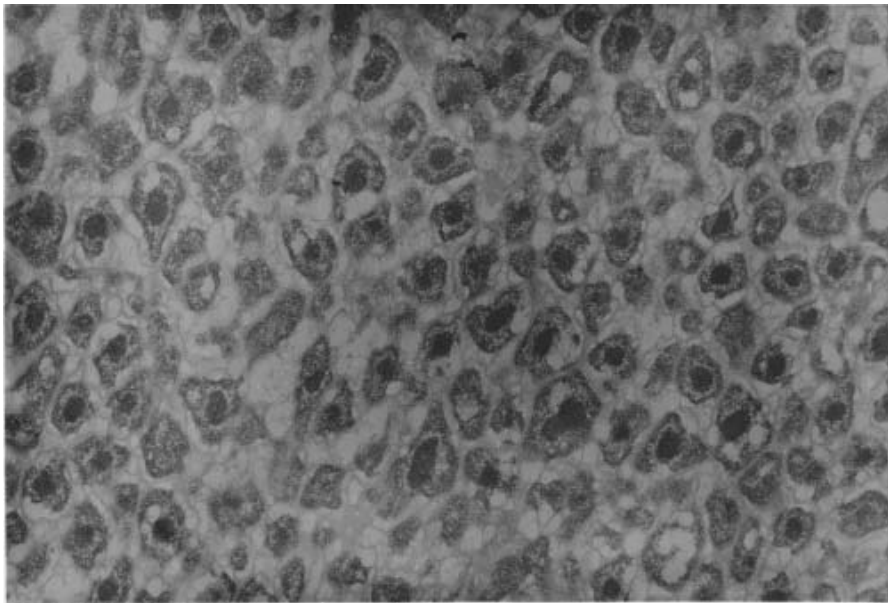


Fig. 2. Chlamydia-like causative agent shown by transmission electron microscopy in the gill filament of epitheliocystis. Cyst was filled with polymorphic cells, 0.2 to 0.4 μm in diameter ($\times 28,000$).