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Histopathological Studies on Parasitic Protozoan Diseases of the Channel Catfish in the United States

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With a greater emphasis on increased production in catfish culture, pathogens have increased among cultured fish. In this study, we confirmed histopathological changes of the channel catfish, *Ictalurus punctatus* infected with *Ichtyobodo necator* (Flagellata, Zoomastigia), *Ambiphrya ameiuri* (Ciliata, Peritricha), *Apiosoma micropteri* (Ciliata, Peritricha) and *Heteropolaria colisarum* (Ciliata, Peritricha). *I. necator* infected gills causing clubbing of filaments. *A. ameiuri* attached to the body surface showing a gold dust-appearance and skin degeneration. *A. micropteri* attached to gills evoking degeneration of respiratory epithelia. *H. colisarum* invaded the skin forming ulcerative lesions on the body surface including fins.

Key words : Ichtyobodo, Ambiphrya, Apiosoma, Heteropolaria

Farming of the channel catfish, *Ictalurus punctatus* is an important agricultural industry with more than 115,000 acres of water devoted to catfish production in the United States. With a greater emphasis on increased production in channel catfish culture, various kinds of diseases often occur among cultured fish and recognition of pathogens, including protozoan parasites, have increased (ROGERS 1979). However, there are few reports confirming histopathological changes which are evoked by protozoan parasites. From 1982 to 1983, diseased catfish, suffering from infections with *Ichtyobodo necator* (Flagellata, Zoomastigia), *Ambiphrya ameiuri* (Ciliata, Peritricha), *Apiosoma micropteri* (Ciliata, Peritricha) and *Heteropolaria colisarum* (Ciliata, Peritricha) were collected from commercial catfish ponds in Alabama and neighboring states. The authors studied the pathological changes caused by these protozoan parasites of channel catfish.

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Materials and Methods

Five fingerling catfish infected with *I. necator*, one fish (about 50cm in body length) with *A. ameiuri*, two fish (40-50cm in body lengths) with *A. micropteri* and five fingerlings with *H. colisarum* were collected from commercial catfish ponds in Alabama and neighboring states from 1982 to 1983. After gross observation of infected fish and wet mount preparations of parasites, skin lesions, gills and visceral organs were fixed in 10-15% formalin solution, and thin sections were stained with Gill's hematoxylin-eosin (H-E), Giemsa, PAS and Azan. Imprinted specimens were stained with Giemsa and PAS.

Results

1. *Ichtyobodo necator* (synonym : *Costia necatrix*)

In imprinted specimens of *I. necator* from gills, the free swimming forms had a flat, round or kidney-shaped body and from two to four unequal lengths of flagella extending outside the body. The body included one nucleus, one PAS-positive vacuole, a few vacuoles and many granules which were either basophilic or azurophilic (**Plate I-1**). The cytostome was usually invisible inside the body. The attached forms showed pyriform or triangular shapes. The cytostome stretched from the middle of the body to the tip of the process inside the host cell (**Plate I-2**). Flagella were invisible in this from. One to several parasites attacked single host cell and penetrated the disc or process of the pointed end of the body into host cells.

Infected fish showed listlessness, appeared starved, and had pale body coloration and swollen gill filaments.

On observation of histological sections, parasites heavily infected gill tissues. The infected gill filaments showed extensive hyperplasia and mitotic figures of epithelial cells in interlamellar spaces, followed by fusion of lamellae and clubbing of filaments (**Plate I-3, 4**). These changes usually developed from the distal ends of the gill filaments. Parasites inserted the tips of processes into the outermost cells of hyperplastic epithelia. Such affected cells were necrotic. Gill filaments which showed marked clubbing, included atrophied epithelial cells, neutrophils and macrophages within the hyperplastic epithelia. Lamellar capillaries of these filaments were flattened and resulted in circulatory disturbances such as oligemia. Some clubbing filaments were followed by proliferation of mucous cells over the hyperplastic epithelia, on which no parasite was found. Livers showed atrophic hepatic cells. Spleens displayed atrophic pulps and thickened trabecullae. Stomachs and intestines contained no food.

2. *Ambiphrya ameiuri* (synonym : *Scyphidia macropodia*)

In the imprinted specimens from a skin lesion, *A. ameiuri* had a nearly cylindrical

body tapering toward a broad scopula. The anterior end of its body had a ciliated peristomal disk spiraling counter-clockwise to the cytostome. Its body surface was cross-striated and the ciliation was limited to one transverse row of cilia. The organism possessed a long, twisted ribbon-like macronucleus, an oval micronucleus and digestive vacuoles inside the body. Several specimens were observed to have lost their micronucleus and produced daughter cells inside the body (**Plate** Ⅱ- 2). The daughter cells were oval shaped, three to seven in number depending on size, and had an oval macronucleus, a rod-shaped micronucleus and the basophilic cytoplasm.

The infested fish displayed mucoid, patched lesions with descoloration and a gold dust appearance on both sides of the peduncle and caudal fin (**Plate** Ⅱ- 1).

The lesions on the body surface involved only the skin. Parasites attached with their broad scopula on the squamous cells of the superficial layer of epidermis. In epidermal lesions with slight infections, mucous secretion of the topmost layer was very slight and the underlying alarm substance cells were highly vacuolized (**Plate** Ⅱ- 3). In epidermal lesions with heavy infections, squamous epithelial cells of the topmost layer were necrotized and separated. The underlying alarm substance cells were highly vacuolized, necrotized and destroyed (**Plate** Ⅱ- 4). The epidermal bottom layer was extensively infiltrated by neutrophils and macrophages.

3 . *Apiosoma micropteri* (synonym : *Glossatella* sp.)

In the imprinted specimens from gills, *A. micropteri* showed an elongated pear-shaped body including a rounded macronucleus. The organism possessed a ciliated peristomal ring at the adoral end and a narrow disk at the distal end.

The infected fish showed no obvious external signs. Heavily infected gills displayed many parasites attaching on respiratory epithelia of lamellae (**Plate** Ⅲ- 1). These respiratory epithelia were separated,atrophied and slightly necrotized. Respiratory epithelia with slight infections showed no obvious change.

4 . *Heteropolaria colisarum* (synonym : *Epistylis* sp.)

In the imprinted specimens from skin lesions, *H. colisarum* formed colonies which were made up of many zooids on branching non-contractile stalks. Zooids had a cylindroid or bell-shaped body, a ciliated peristomal disc at the topmost end, and a horse-shoe-shaped macronucleus and many food vacuoles inside the cytoplasm.

The infected fish showed formation of small and large colonies of *H. colisarum* on the dorsal and pectoral fins, snout and on top of the head. The colonies had a whitish coat-like appearance with the colony stalks extending into the skin of fish. Such infected lesions showed ulceration involving fin rays and spines, pterygiophore, the skull and born of the jaws.

In slightly infected lesions of the epidermis, zooids attached with their short stalks on squamous epithelial cells of the topmost layer. The underlying alarm substance cells

were markedly vacuolized and necrotized. Heavily infected lesions showed necrosis and destruction of the skin, and developed into ulcerative lesions involving the skull, bone of the jaws and cartilage of fins (**Plate Ⅲ-2**). These ulcerative lesions extensively showed multiplication of rod-shaped and coccal bacteria (**Plate Ⅲ-3**) and necrosis of the subcutaneous and muscular tissues. Zooids extended their stalks to attach onto the bone tissues and fed on tissue debris and cells. Erythrocytes were observed inside their food vacuoles (**Plate Ⅲ-4**).

Discussion

Infections with *I. necator*, *A. ameiuri*, *A. micropteri* and *H. colisarum* were common diseases in cultured channel catfish. The gill filaments which were heavily infected with *I. necator* showed hyperplasia of epithelial cells, follwed by clubbing of filaments and circulatory disturbances of the lamellar capillaries. These pathological changes would disturbe respiratory activity of gills and affect other metabolic processes. Atrophy of hepatic cells indicated long-term starvation. Imprinted specimens of parasitic forms of *I. necator* formed one cytostome which penetrated into host cells. This indicated that *I. necator* fed on the contents of host cells as shown by electron microscopic studies (SCHUBERT, 1966 ; JOYON and LOM, 1969). Such a stimulus might evoke a hyperplastic reaction of the epithelial cells of gills. On the contrary, the infection of *A. micropteri* did not cause a hyperplastic reaction of the epithelial cells of gills although they were abundant on gill epithelia. These findings indicated that *A. micropteri* caused lesser stimuli on gill epithelia than *I. necator*.

A. ameiuri caused necrosis and destruction of alarm substance cells of the epidermis. This change was also found in the epidermis infected with *H. colisarum*. These findings indicated that continued infections of these ciliata exhaused alarm substance and caused degeneration, necrosis and destruction of the alarm substance cells. In lesions caused by *H. colisarum*, separation of the epidermis due to destruction of the alarm substance cells allowed zooids to penetrate the skin with their stalks. When injured skin was invaded by bacteria and necrotized, zooids extended their stalks onto the subcutaneous bone or cartilage tissues and fed on degenerating host cells including erythrocytes. This demonstrated that *H. colisarum* damaged fish skin and acquired their nutrition by feeding on host cells.

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Explanation of Plate I

Fig. 1. *Ichtyobodo necator* free swimming-forms. They had unequal two or four flagella and a round body including one round nucleus and many azurophilic granules. Giemsa, X1000.

Fig. 2. *I. necator* patasitic forms. One or several parasites penetrated their pointed ends into one host cell (H). The cytostome (C) stretched from the middle of the body to the tip of the process inside a host cell. Giemsa, X1000.

Fig. 3. Gill filament heavily infected with *I. necator*. Affected filament showed hyperplasia of epithelial cells, fusion of lamellae and infiltration of infammatory cells. Many parasites attatched on epithelial cells of the topmost layer. H-E, X480.

Fig. 4. Gill filaments showing clubbing and heavy infection of *I. necator*. The filaments included atrophied epithelial cells. H-E, X80.

Explanation of Plate II

Fig. 1. Channel catfish infected with *Ambiphrya ameiuri*. The infected lesion was slimy and discolored and exhibited a gold dust appearance which extended allover the peduncle and tail.

Fig. 2. *A. ameiuri* including daughter cells inside the body. C : cytostome, D : daughter cells, M : macronucleus, P : peristomal disk, T : transverse ciliation, S : scopula, Giemsa, X1000 (enlarged) .

Fig. 3. Skin lesion slightly infected with *A. ameiuri*. Parasites attached on the outermost cells. Alarm substance cells were vacouolized in the epidermis. Arrows show parasites. A : alarm substance cells. H-E, X160.

Fig. 4. Skin lesion. The alarm substance cells vacuolized, necrotized and destroyed. The overlying layer was separated. H-E, X80.

Explanation of Plate Ⅲ

Fig. 1. Gill lamellae heavily infected with *Apiosoma micropteri*. They attached on respiratory epithelial cells which resulted in necrosis. H-E, X320.

Fig. 2. Skin lesion heavily infected with *Heteropolaria colisarum*. Zooids extended colony stalks (arrows) onto the cartilage (C) of skull destroying skin and subcutaneous tissues. Giemsa, X150.

Fig. 3. A detail of the ulcerative lesion. Many rod-shaped and coccal bacteria invaded and multiplied in the subcutaneous tissue. Giemsa, X300.

Fig. 4. A detail of *H. colisarum*. Zooids have cells and debris in their food vacuoles. Giemsa, X300.

Plate I

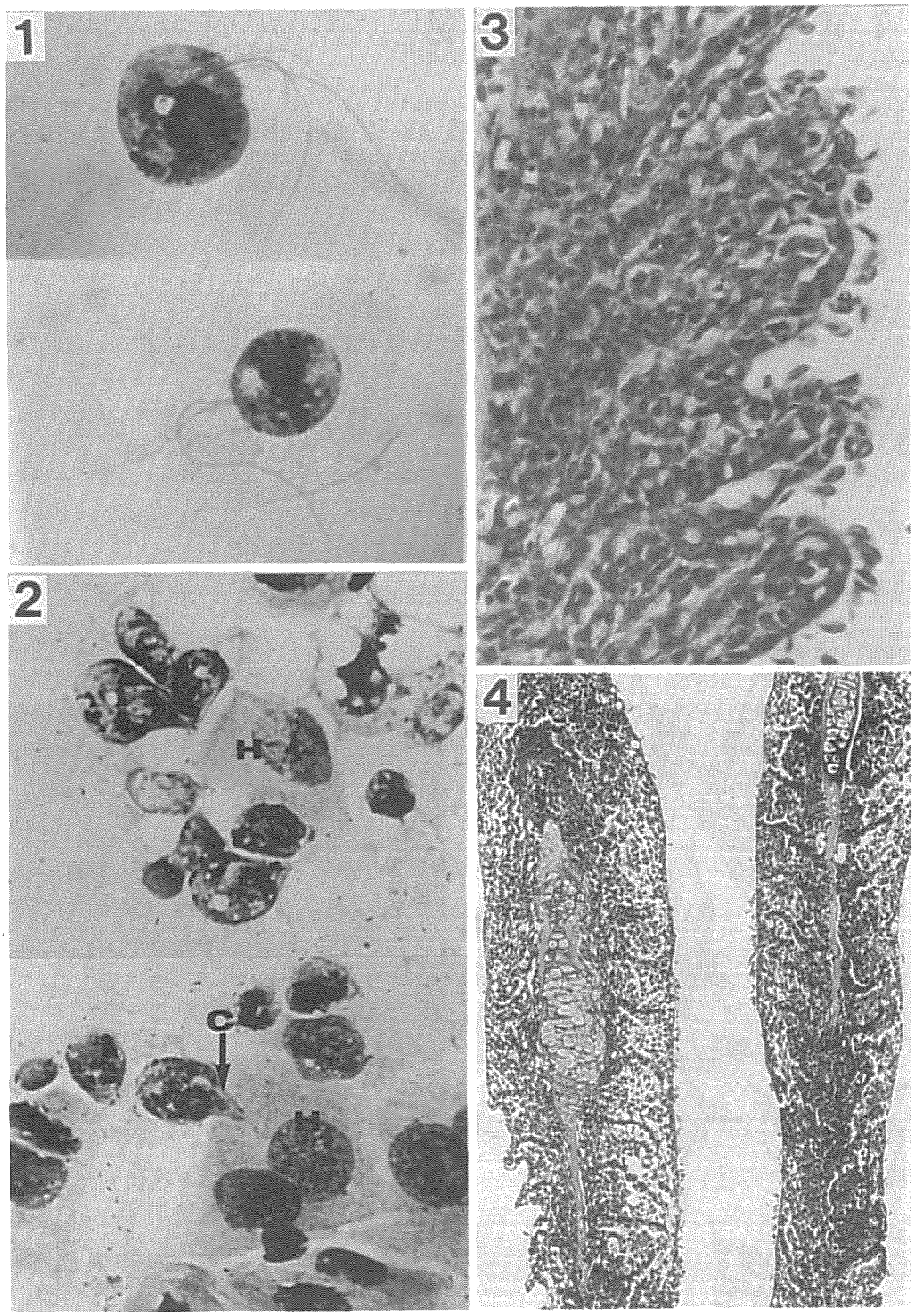

Plate II

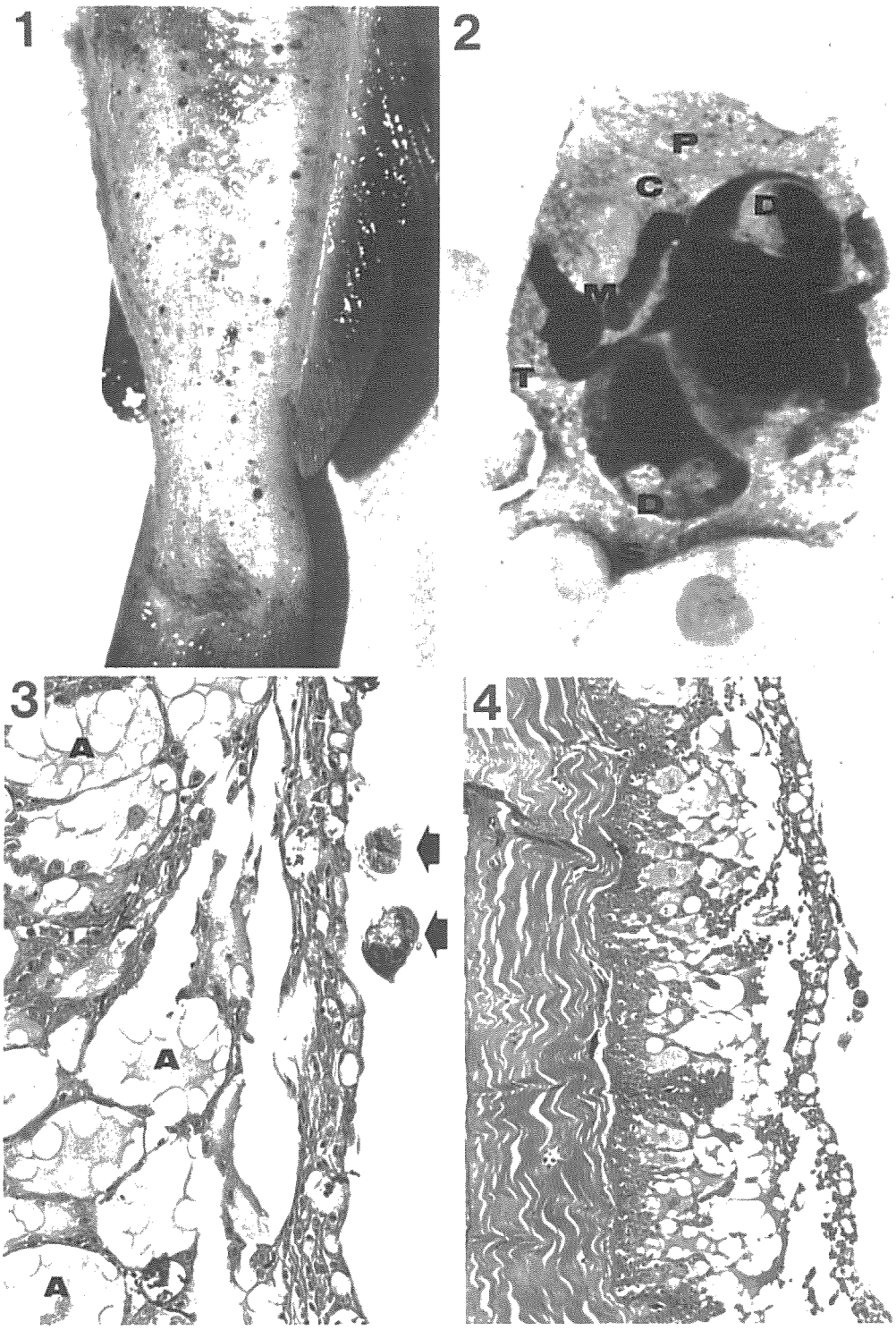

Plate Ⅲ