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Systemic Granulomatosis in Guppies *Poecilia reticulata*

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ABSTRACT

In many guppy *Poecilia reticulata* farms in the vicinity of Bangkok, diseases have caused long-lasting fish death and consequently large cumulative mortality. Diseased guppies were collected and subjected to histopathological analysis. The analysis revealed systemic granulomatosis as a remarkable histological sign. Rod-shaped bacterial cells with strong acid-fastness were found in the granulomas. Therefore, this disease was diagnosed as acid-fastness bacterial infection. The acid-fastness bacterium infection seems to predispose various infections in the critical stage of the disease.

Key words: guppy, granuloma, acid-fastness bacterium, histopathology

INTRODUCTION

Ornamental fish business has slowly but steadily grown up and become an industry in Thailand. Many species are being spawned and raised in commercial scale in farms all around the country such as Siamese fighting fish, goldfish and discus. Harvests are distributed within the country and many species are highly demanded from many countries all around the world. Guppy *Poecilia reticulata* is the most popular fish on the commercial basis farming nation-wide. In many guppy farms in the vicinity of Bangkok, a disease has caused long-lasting fish death and consequently large cumulative mortalities, particularly during the rainy and/or cold periods. The affected fish show off-feeding, sluggish swimming, fin erosion, and skin ulceration which reaches to vertebral bone on some occasions (Figure 1). Antibiotic and disinfectant treatments have been applied for disease control in the farming and marketing processes. The pathogenic bacteria like *Aeromonas hydrophila* and *Flexibacter columnaris* and protozoan *Tetrahymena* and *Epistylis* are isolated from the diseased fish in most cases. However, the treatments usually end up in unsuccessful result where mortality continues causing severe loss in each crop. This histopathological study revealed the systemic granulomatosis as a remarkable histological sign from the diseased guppies. Suggestion on the etiology and control method of this disease was also discussed.

MATERIALS AND METHODS

Fifty diseased guppies *Poecilia reticulata* (20 to 40 mm in body length), showing the gross signs in various extents, were collected from several farms in the vicinity of Bangkok in March, 2001. The fish were fixed in 10% formalin and processed

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using a conventional paraffin method. Tissue sections were cut at 5 μm, stained with H & E, PAS, Giemsa, and Ziehl's acid-fastness reaction and then examined under a light microscope.

RESULTS

If was found that granulomas multifocally distributed in the dermal and subdermal tissues, visceral and parietal peritonea, and visceral organs of all the diseased fish (Figure 2), although they varied from large to small in the frequency depending on the specimens. The granulomas consisted of central macrophage aggregations and surrounding lymphocytes and fibroblasts. Rod-shaped bacterial cells, strongly positive to Ziehl's acid-fastness reaction, were detected in the macrophage aggregations (Figure 3). Extensive necrotic dermatitis and peritonitis with leukocyte infiltration and fibroplasia stood out in the areas adjacent to the granulomas. The epidermal necrosis was serious in affected areas, sloughing off to make erosion or ulceration (Figure 4). The necrotic dermatitis also extended to the underlying tissues in some of the diseased fish, causing necrotic myositis and perimyositis.

Heavy *Tetrahymena* infection was observed in several diseased fish (Figure 5). Serious fat degeneration was found in the liver of all the diseased fish (Figure 6). There was no lesion in the intestinal mucosa in all fish subjected to this histopathological study.

DISCUSSION

Notable histological sign in all diseased guppies was the systemic granulomatosis, accompanying peritonitis and dermatitis. These host responses are diagnostic signs in acid-fastness bacterial infection (Richards and Roberts, 1978; Ferguson, 1989). Besides, bacterial cells in central macrophage aggregations of granulomas show strong acid-fastness. Therefore, the acid-fastness bacterium seems to be the most suspicious agent as the primary cause for the systemic granulomatosis in the diseased fish. Judging from the rod-shaped form, the bacterium probably belongs to *Mycobacterium*. *Mycobacterium* infections have been reported in guppy and other ornamental fish from few countries. Conroy and Conroy (1999) isolated acid-fast bacteria identified as *Mycobacterium* sp. from fancy veiltail guppy *Lebistes reticulatus* raised on farm in Venezuela. Clinical signs of the infected fish included listlessness, emaciation, spinal curvature, sunken eyes and loss of color. The bacteria were isolated from smears of kidneys, liver, mesentery, spleen, fresh faecal materials and unborn embryos of infected gravid females. They also recommended the effective treatment of 50 ppm kanamycin sulphate at four occasions with 48 hours between each dose. Bragg *et al*. (1990) reported the isolation of *Mycobacterium fortuitum* from oscars, guppies and discus for the first time in South Africa. Heavy mortalities were reported from guppy farms in this country associated with the acid-fast bacteria and development of multi-resistance against many anti-microbial substances.

In the past bacteriological surveys on this guppy disease, *Aeromonas hydrophila* and/or *Flexibacter columnaris* have been incriminated as the primary agent, because of their isolations from most of the affected fish. However, such the bacteria never bring about the granulomatous inflammation (Ferguson, 1989). In addition, this disease has repeatedly broken out in the farms despite antibiotic treatments. The *Aeromonas* and *Flexibacter* infections, therefore, seem to overlap on the predisposing acid-fastness bacterium infection in the critical stage of this disease. In this study, heavy *Tetrahymena* infection was also recognized in the several diseased guppies. Thus, various infections are co-incident in the critical stage of this disease which can accelerate the fish mortality.

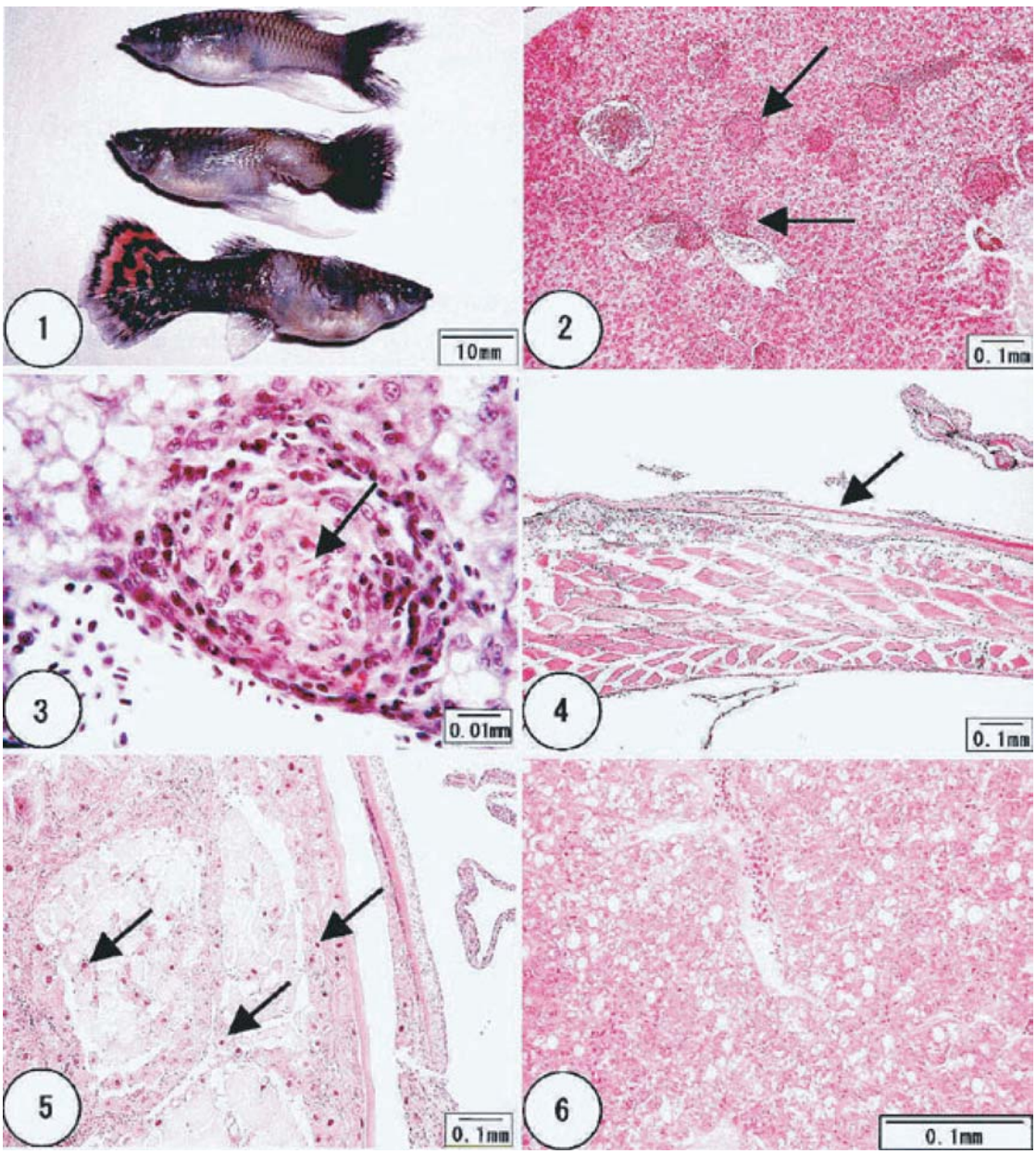

Figure 1 Diseased guppies *Poecilia reticulata* showing fin erosion and skin ulceration

Figure 2 Multifocal granulomas (arrows) in the liver of diseased guppy, H&E.

Figure 3 Granuloma consisted of a central macrophage aggregation and surrounding lymphocytes and fibroblasts. Rod-shaped bacterial cells (arrow) with strongly positive to Ziehl's acid-fastness reaction detected in the macrophage aggregation.

Figure 4 Epidermal necrosis, sloughing off to cause erosion or ulceration, H&E.

Figure 5 Heavy *Tetrahymena* infection observed in the diseased fish, H&E.

Figure 6 Fat degeneration in the liver of diseased guppy, H&E.

Removal and burning of diseased fish and disinfection of ponds, equipments, hands and feet of workers by appropriate disinfectants are encouraged as the control for the acid-fastness bacterium infection. Besides, the serious fat degeneration found in all the affected fish also suggests nutritional improvement in the guppy farming.

CONCLUSION

The histopathological study from the diseased guppy *Poecilia reticulata* sampled from the farms in the vicinity of Bangkok clearly indicated the acid-fastness bacterium infection with systemic granulomatosis in the liver. This bacterial infection was probably caused by *Mycobacterium* sp. which predisposed the fish to other pathogens commonly found in the guppy farms, such as bacteria *Aeromonas hydrophila* and *Flexibacter columnaris* and protozoans. Prevention and treatment methods for diseases in guppy farms should be furtherly investigated judging from the result of this study.

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