THE ISOLATION OF *Aeromonas hydrophila* AND *Escherichia coli* FROM LOU HAN *Cichlasoma synspilum* AND STUDIES OF THEIR HISTOPATHOLOGY CHANGES

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ABSTRAK

Penyebab kematian lou han *Cichlasoma synspilum* adalah *Aeromonas hydrophila* dan *Escherichia coli, d*engan ciri adanya pendarahan dan luka pada sirip dada. E. coli kemungkinan besar tidak menyebabkan kematian ikan sebab sampai sekarang belum pernah ada laporan bahwa bakteri tersebut menyebabkan sakit pada ikan di mana pun. Gejala klinis utama yang terlihat dari luar adalah pendarahan di sirip dada dan menimbulkan luka yang cukup dalam. Cairan *ascites m*enyebabkan membengkaknya perut, pembengkakan liver, dan kosongnya usus dari ikan yang sakit. Gambaran histopatologi menunjukkan nekrosis dan adanya *vacuole* pada *hepatocyte.* Sedangkan pada usus adalah rusaknya *mucosal ephitelium, lamina propia stratum compactum, stratum granulosum dan muscularis*. Ginjal mengalami kerusakan berat, hampir semua organela ginjal berantakan. Ikan ini dipelihara di aquarium sehingga menurunnya kualitas air atau kontaminasi lewat pakan dan penanganan yang buruk diduga sebagai *portal of entry* dari penyakit.

Kata kunci: lou han *Cichlasoma synspilum, Aeromonas hydrophila, Escherichia coli*

INTRODUCTION

Lou Han *Cichlasoma synspilum* is an expensive freshwater ornamental fish with optimal water temperature of 20–30° C. The disease of ornamental fish is often caused by the deterioration of water quality and competition of space. Intense physical contact will cause wound in the skin and will be primary portal of entry of the disease (Robert, 1988). Natural outbreak of *Aeromonas* sp. is due to stress mediated condition especially fluctuation of water temperature, decrease in water quality and overcrowding (Austin and Austin, 1987). The genus of *Aeromonas* is known to be pathogen to wild and cultured of freshwater fish. The carp *Cyprinus carpio*, gold fish *Carrasius auratus*, are among cultured fish infected by *Aeromonas* sp. and today golden shiners *Notemigonus chrysoleucas* and fathead minnows *Phimephales promelas* have also been infected (Plumb, 1994).

The pathogenicity of *Aeromonas* in fish was well documented especially in salmon. Although it primarily cause disease and extensive mortality among fish in freshwater, it has also been responsible for lost in saltwater. Two bacterial diseases that are most often associated with cyprinids are "carp erythrodermatitis (CE)" (Fijan, 1972) and "ulcer disease in goldfish (UDG)" (Elliot and Shotts, 1980). Both diseases are caused by the species of bacteria namely *Aeromonas salmonicida* subspecies *achromogens*. The distribution of the disease is worldwide from Europe, USA, Asia and Australia. The infection of the disease has been reported in Japan and Australia (Hamilton *et al*., 1981). The infected fish developed hemorrhage in the base of pectoral fin and other part of the body and displayed weakly movement.

The study was aim to understand the infected agent causing wound in pectoral fin and other body part of Lou Han rearing in aquarium because the Lou Han were lived for a week after infected disease.

MATERIALS AND METHODS

Fish

Lou han weighing 50 g was collected from diseased fish in Surabaya. The diseased fishes were characterized by hemorrhage in the body especially in the pectoral fin and other skin, the infection seem to be causing weakness of fish. The infected fish displayed no movement and they were lost of appetite. The stomach of the acute diseased fishes was empty because the fish did not eat during the sickness.

Isolation and Characterization of Bacteria

The bacteria were isolated from the wound in pectoral fin and kidney of fish. A piece of tissue was removed from the wound and mixed in serially ten fold dilution to 10^{-1}–10^{-4} in physiological saline (pH 7.0). One hundred micro liter of the solution were plated duplicate onto Nutrient Agar (NA). Using biochemical and physical parameters to

understand the species of bacteria carried out characterization of bacteria. The pure culture was then stored in -80° C with glycerol.

Histopathology

The wound organs were removed and immediately immersed into fixative solution. The tissue was dissected to small pieces in order the fixative solution (AFA's Davidson) enter the flesh rapidly. The others tissue processed according to the routine histopathological examination. The tissues were liver, intestine, and kidney, were fixed in Bouin's solution for at least 24 h, embedded in paraffin wax, and cut at 3–5 µm. Sectioned specimens were stained with Haematoxylin and Eosin (HE) or Giemsa.

RESULTS AND DISCUSSIONS

Nord *et al.* (1975) showed that *A. hydrophila* produces gelatinase, caseinase, elastase, lipase, lecithinase, and deoxyribonuclease in addition to the hemolytic, cytotoxin and enterotoxin. The bacteria present in all water except the most saline, and they are more abundant in water contain high organic load (Kaper *et al*., 1981). *E. coli* has not yet been reported as fish pathogen in everywhere, but the present bacteria in the fish due to tap water contaminated by bacteria. The fish died after 7 days post infection and the prominent clinical signs are hemorrhage in the base of pectoral fins.

Motile aeromonads are found in fresh water, sewage, relatively unpolluted water and they are much more abundant in water with a high organic load. The hemorrhage of the pectoral fin is prominent and seems the fish very irritated because displayed no movement. Even though the disease is not caused high mortality, but without proper treatment fish would be death a weeks later. The isolation of bacteria from dead fish is presented in Table 1. It was alarming that a significant number of *A. hydrophila* in the aquarium be potential of source infection in freshwater ornamental fish. Tentatively the source of bacteria could be from the water contaminated by bacteria when handling or feeding fish. The external signs are develop ulcer in the base of pectoral fins and some part of the body.

Histopathology of disease fish is presented in Table 2. The hepatocyte of diseased fish undergoes necrosis, and the damage of parenchymal hepatocyte was not remarkable. The arrangement of hepatocyte cells was disturbed although some cells showed vacuolation. Vacuoles were observed within nucleus of parenchymal hepatocyte. Severe damaged of parenchymal hepatocyte was reported by Suprapto (1997) in Japanese eel injected with extra cellular products of *E. tarda* NUF 251, because liver has a function to neutralize the toxin in the blood of animal. Blood congestion in the sinusoid or small veins in the liver sometimes occurred.

The intestines of disease fish were severely damaged, the Goblet cells and other organelle disappeared from mucosal epithelium. All the intestine cells such as mucosal epithelium, lamina propia, stratum compactum, stratum granulosum, muscularis, tela subserosa and serous membrane were undergo severely damaged. The infection may have occurred in a long time and the disease marked

Table 1. Characteristics of *A. hydrophila* and *E. coli* isolated from lou han

Characters	A. hydrophila	E. Coli
Aesculin hydrolysis	+	
Motility	+	+
Indole production	+	+
Voges-Proskauer	+	-
Citrate, Simmons	d	-
Methyl red		+
H_2S production	+	-
Urea hydrolysis	-	-
Lysine decarboxylase	D	
Gelatin hydrolysis	+	-
KCN growth	+	-
L-Histidine & Arginine utilization	+	
Malonate utilization	-	-
ONPG	+	+
Oxidase	+	-
Nitrate reduction	+	+
Sucrose	+	d
L-Arabinose utilization	+	
Gas from glucose	+	
0% NaCl growth	+	
1% NaCl growth	+	
6% NaCl growth	-	
0/129 sensitivity	-	

Table 2. Pathological changes of internal organs of lou han naturally infected by *A. hydrophila* and *E.coli*.

Histopathology Changes	Liver	Intestine	Kidney
Necrosis	+	+	+
Severely damaged	-	+	+
Cirrhosis	+		
Vacuole degeneration	+	-	+
Light damaged	-		-
Melanine pigment Hyaline droplet	-	-	+
Melanine pigment Hyaline droplet	-	-	+

to be chronic. The damaged mucosal epithelium disturbed the all metabolism system causing the fish died. The connective tissue rich in capillaries from lamina propia were also ruptured.

The kidney of diseased fish were severely damaged, glomelular cells, proximal convoluted segment and distal convoluted segment were ruptured. The histopathology of lou han kidney showed that the bacteria produced the toxin because the damage were resembled to eel injected with extra cellular product of the bacteria. Hyaline droplet degeneration showed in the tubular epithelial cells in the proximal convoluted segment of renal tubule. In the salmon, motile aeromonad are causing rupture of minor blood vessels. The hemorrhage caused by a soluble hemolytic associated with ulcerative skin and may be on the surface of organ or deep within tissue (Aoki and Hirono, 1991). External lesion may vary from an extensive superficial reddening of the surface of a large area of the body, often with necrosis of fin or tail.

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