

AN INVESTIGATION OF A DISEASE OF THE FRESH WATER FISH *CTENOPHARYNGODON IDELLUS* AND ITS CHEMOTHERAPEUTICAL CONTROL⁽¹⁾

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Abstract: Fourteen species of bacteria were isolated from diseased grass-carps, *Ctenopharyngodon idellus*. After identification and inoculation tests, one of these bacteria (A-2), *Aeromonas punctata* Zimmermann, was found to be a pathogen for the grass carp.

Aeromonas punctata is a gram-negative, rod-shaped bacteria, motile by means of a single polar flagellum. It is able to digest gelatin, casein and starch, and gives positive reactions for catalase, indole, hydrogen sulfide, methyl red and acetylmethylcarbinol tests. It ferments most carbohydrates with the production of acid and gas.

Aeromonas punctata is sensitive to many kinds of antibiotics and sulfonamides, but chloramphenicol was found to be the most efficient antibiotic in controlling this pathogen.

The raising of fresh-water fishes on Taiwan is an important industry. Deep-sea fishing and fishing in coastal waters has not been developed as rapidly as the need for fish has increased.

The grass-carp (*Ctenopharyngodon idellus*) is one of the principal fishes reared in fresh water ponds in Taiwan. It is susceptible to a disease and each year large numbers die thereby greatly reducing the profits to the raisers of these fish.

This disease of the grass-carp shows up with the following symptoms: Loss of appetite, swimming slowly near the surface of the water, a slight redness and swelling of the anus, some yellowish mucus in the abdomen around the intestines and the inflammation of the stomach with engorged blood vessels (Fig. 1).

The fish raisers feed rice bran ground bean cake, duckweed (*Lemna*) and night soil to the fish and so it was necessary to examine the water and feed in an effort to seek for the pathogen of the grass-carp.

This study was prompted by a desire to find the causal organism of this disease and methods of control.

MATERIALS AND METHODS

Diseased and healthy grass-carp were collected at the fisheries from the North Ward and from the An-nan Ward, Yung-kang District of Tainan County, and from the fisheries at Hu-wei District, Kaohsiung County. These fish were brought back to the Microbiology Laboratory of the National Taiwan University in Taipei, in clean plastic bags kept in thermos bottles with ice.

(1) This project was supported by a grant from the Rockefeller Foundation.

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Immediately upon arrival in the laboratory the healthy and the diseased fish were examined. Bacteria were isolated from the mucus in the abdomen, liver and blood and also from inside the intestines by sterile cotton swabs and streaked on different kinds of agar plates with a loop needle.

One of the isolates (A-2) was cultured in broth in an Erlenmeyer flask for 24 hours and then inoculated into healthy fish which had been brought back to Taipei and kept in large tanks.

The inoculation was carried out by two methods; First by putting a culture of the A-2 bacteria on rice bran which served as food for the healthy grass-carp. And second by intramuscular injection.

Water collected from the ponds in which the fish were being raised was cultured on agar plates by the dilution plate method, and by the Miles and Misra surface method in order to learn what bacteria were prevalent in the pond water.

The identification and classification of the bacteria isolated from both diseased and healthy fish were made according to the Bergey's Manual of Determinative Bacteriology and the Standard Descriptive Chart prepared by the Committee on Bacteriological Technique of the Society of American Bacteriologists.

RESULTS

(1) Fourteen species of bacteria were isolated and identified from the diseased fish as is shown in Table I. One species was always found in the diseased fish but never in the healthy fish. This was culture No. A-2, and this was selected as the possible causal organism and used in the inoculation tests.

(2) The result of inoculation test has been that by using the first method, i.e. of feeding the A-2 culture on rice bran to 34 grass-carps, four or five days after the inoculation with the bacteria, one of the healthy fish was found dead. Ten days after inoculation, three more were found dead and twenty others were sick, but the remaining ten seemed to still be healthy. A control of twenty five grass-carps were all in normal condition. By using the second method, i.e. intramuscular injection to 47 healthy grass-carps, the fish began to die one after another for twenty five days following the inoculation, and thirty six were found dead 45 days after inoculation; of the remaining eleven, five were sick and six appeared in normal condition.

(3) The result of the action of some of the antibiotics on the pathogen A-2 are shown in the following Table II.

DISCUSSION

(1) The A-2 organism is *Aeromonas punctata* Zimmermann, which is a gram negative, short rod ($0.6-0.8 \times 0.9-1.4 \mu$) monotrichous bacteria (Fig. 3). It is the

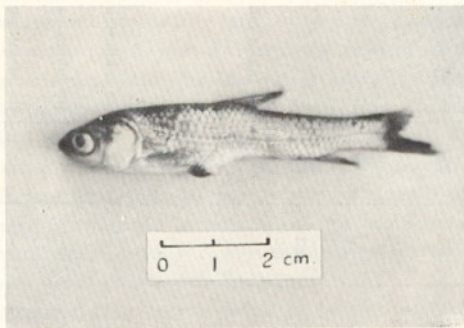


Fig. 1. Diseased fish of *Ctenopharyngodon idellus*

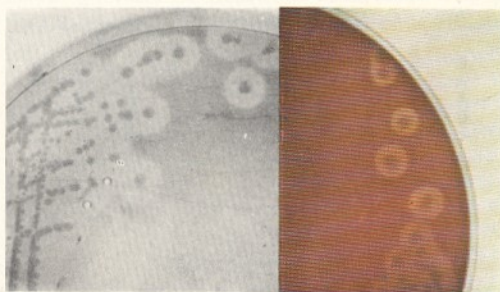


Fig. 2. Hemolysis of *Aeromonas punctata*

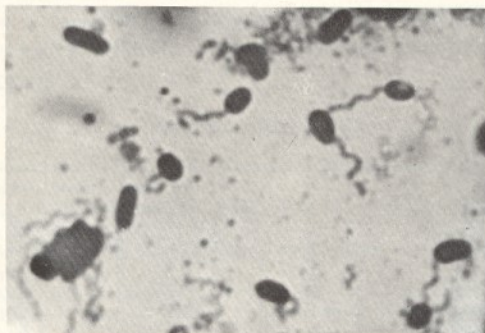


Fig. 3. Flagella stain of *Aeromonas punctata*

Table II

Chemotherapeutic agents	Inhibition zone (cm)			Note	Chemotherapeutic agents	Inhibition zone (cm)			Note
	Low conc.	Medium conc.	High conc.			Low conc.	Medium conc.	High conc.	
Sulfisoxazol	2.4	2.7	3.2		Auromycin	2.2	2.6	2.8	
Colistin	2.0	2.1	2.1		Terramycin	2.2	2.6	2.9	
Kanamycine	1.9	2.3	2.7		Bacitracin	x	1.1	1.2	
Dihydrostreptomycin	1.8	2.3	2.7		Declomycin	2.1	2.3	2.9	
Tetracycline	2.5	2.6	2.8		Ampicillin	x	x		No inhibition
Chloramphenicol	3.0	3.2	4.1		Cephalothin	-	-	2.8	
Leucomycin	x	1.3	2.0		Cephaloridine	-	-	2.8	
Oleandomycin	x	1.2	2.0		Nafcillin	x	-	-	No inhibition
Erythromycin	1.1	1.6	2.2		Lincomycin	x	-	-	No inhibition
Penicillin	x	x	x	No inhibition	Methicillin	x	-	-	No inhibition
Streptomycin	1.9	2.2	2.4		Sulfamerazine	1.6	1.9	2.1	
Trisulfapyrimidines	2.1	2.2	2.4						

pathogen that caused the edema in the grass-carps. Sometimes, a fish can first be infected by *Aeromonas* and secondarily infected by other pathogens⁽²⁾. The opinion of Goncarov, Tomasec *et al.*⁽⁴⁾ is that a virus is the primary cause of certain diseases, and the bacteria come into play only as secondary invaders.

(2) *Aeromonas punctata* was found in the water from the ponds in which the fish were raised. This bacteria was always associated with duckweed which is found in the ponds in which the carp, eel and other fishes occur.

(3) Penicillin interferes with the incorporation of a the N-acetyl-muramic acid peptide from a carrier within the bacteria cell to its position in the mucopeptide polymer⁽⁶⁾ structure that normally comprises the rigid bacterial cell wall. But *Aeromonas punctata* is not sensitive to penicillin.

(4) Chloramphenicol (Fig. 4) was found to be the most efficient antibiotic in this study. The inhibition zone of sensitivity disk test is the largest, as shown in Table II. The site of inhibition, of chloramphenicol blocks protein synthesis⁽⁵⁾, and has been shown to involve the transfer of amino acids from the transfer RNA component of RNA to the peptide-bond forming site in the ribosome^(11,18).

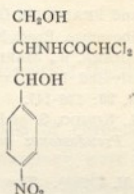


Fig. 4. Chloramphenicol

(5) *Aeromonas punctata* is also sensitive to heavy metal ions, such as copper. This phenomenon is known as the oligodynamic action.

This investigation has revealed that *Aeromonas punctata* is associated with the disease of grass-carps and that chloramphenicol is effective in inhibiting the growth of this pathogen, but since this an expensive antibiotic, there needs to be found a more economical method of control.

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