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FRESHWATER FISH DISEASES CAUSED BY BACTERIA BELONGING TO THE GENERA AEROMONAS AND PSEUDOMONAS

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INTRODUCTION

Bacterial fish diseases caused by pseudomonas-like bacteria which are now classified as belonging to the genus <u>Aeromonas</u> are very common among warmwater pond fishes. Here belong the economically most important pondfish diseases in Europe. There is increasing evidence that also in the United States and Canada fish diseases caused by bacteria belonging to this genus are of considerable economic significance. Fish diseases caused by bacteria belonging to the apparently related genus <u>Pseudomonas</u> are less common. Diseases caused by bacteria of this group have been described by various investigators as:

- 1. <u>Infectious abdominal dropsy</u> (Bauchwassersucht in Germany).
- 2. <u>Hemorrhagic</u> septicemia of warmwater fishes.
- 3. Red sore disease of pike.
- 4. Red mouth disease in rainbow trout.

5. Red leg disease in frogs.

Fish furunculosis should also be included but because of its importance it is described in a separate leaflet.

This outline contains more detailed information than other similar outlines because of paucity of reference material in the English language.

IDENTIFICATION

Most of the diseases listed above are caused by bacteria which are either identical with <u>Aeromonas liquefaciens</u> or related to it. In some instances fish diseases of similar nature may be caused by pseudomonads which produce fluorescent pigments in special media. In general, all these diseases are septicemic and therefore the causative bacteria are present in the blood and internal organs. In early stages of the disease, in resistant fish, or in fish recovering from infection, the pathogens may be present only in the lesions or in some of the organs without detectable pathological changes.

The most typical external sympton's are superficial shallow grayish or red circular or irregular ulcers. The mucous membranes and cartilaginous tissue in and around the mouth may

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be inflamed and even eroded as in the "red mouth" disease in rainbow trout. Fish may have distended abdomen filled with slightly opaque or bloody fluid. Some fish may have protruding eyes (exophthalmus) and behind the eyeball an opaque fluid may accumulate. Some fish, cyprinids in particular, may have furuncles similar to those in furunculosis, which may break out to the surface producing deep necrotic craters lined with red or grayish muscle tissue. In some instances these lesions can heal leaving scars. Fins may be inflamed.

Internally, in addition to the presence of opaque or purulent fluid in the abdominal cavity, the kidney may be swollen and soft, liver pale with or without necrotic areas and petechiae (small hemorrhages) may be present in the peritoneum and in the musculature. Lower intestine and vent are usually swollen, inflamed, and may contain bloody contents or discharge. The mortalities may be low or high and the epizootics may have a rapid or a slow course.

In frogs the main symptom of the disease is distended capillaries on the underside of legs and abdomen giving them a deep red color from which the name of the disease, "red leg," originated.

Outbreaks of this disease among the warmwater pond fishes and frogs usually occur in spring and coincide with the increase in the temperature of water. It is believed that at this time of the year fish have a decreased disease resistance owing to winter dormancy and starvation which usually results in some anemia and a decrease in the blood proteins.

CAUSE OF THE DISEASE

There is a considerable confusion regarding the taxonomy of bacteria involved in this disease or diseases. Apparently identical or very closely related cultures isolated from diseased fish and frogs and pond water were described under different generic and specific names. The writer's opinion is that bacteria causing most of the outbreaks of infectious dropsy, hemorrhagic septicemia, red mouth and red leg diseases should be classified as <u>Aeromonas liquefaciens 2</u>/. The following names are believed to be the most frequently used synonyms: Bacillus punctatus

Bacterium punctatum Pseudomonas punctata 3/ Pseudomonas hydrophila Proteus hydrophilus Bacillus ranicida Aeromonas punctata Aeromonas hydrophila

If a bacterium is isolated in pure culture from a lesion or blood of diseased fish, the following characteristics can be used for a presumptive test: gram-negative, motile, <u>single polar flagellum</u>, will grow well from 10 to 35° C (optimum about 30° C, 86° F), liquefies gelatin, acid and gas produced in dextrose, sucrose, and mannitol; in lactose no fermentation or slow fermentation after 2 to 4 weeks of incubation. With paper discs containing antibiotics and sulfonamides (medium concentration) and using petri plates with Trypticase Soy Agar (Baltimore Biological Laboratory), the following results can be anticipated:

Clear zones present	Clear zones absent
Chloromycetin +++	Carbomycin
Terramycin ++	Bacitracin
Aureomycin ++	Polymyxin
Tetracycline ++	Penicillin
Streptomycin +	Viomycin
Neomycin +	Magnamycin
Sulfonamides + or -	

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<u>Aeromonas liquefaciens</u> is a bacterium common in water containing organic matter. It is most likely a saprophytic bacterium which under certain conditions can be highly pathogenic to lower vertebrates. It is also pathogenic to mammals if injected into tissues or blood stream.

On occasions typical water-inhabiting pseudomonads, as for example <u>Pseudomonas</u> <u>fluorescens</u>, can cause epizootics among pond fishes. Pseudomonas fluorescens in contrast to

2/ See the 7th Edition of the Bergey's Manual of Determinative Bacteriology, pp. 189-193, 1957.

 $\frac{3}{20}$ Name used most frequently during the past $\frac{3}{20}$ years.

A. <u>liquefaciens</u> slightly ferment some sugars with production of some acid but no gas. It produces brilliant fluorescence if grown on tryptose phosphate agar and exposed to ultraviolet illumination. It also produces green pigment in media containing glycerol.

SOURCE AND RESERVOIR OF INFECTION

Pond water, diseased fish, diseased frogs, as well as fish which have recovered from the disease and are immune or resistant carriers (fish and frogs).

MODE OF TRANSMISSION

Through intestinal tract and external lesions. External parasites as trematodes (Gyrodactylus), copepods and leeches which fed on infected fish were found to contain this pathogen in their intestinal tract. Therefore external parasites may be responsible at least for some outbreaks of this disease.

INCUBATION PERIOD

Depends on the species of fish, their resistance, external conditions and the season of the year.

PERIOD OF COMMUNICABILITY

Adult resistant fish and frogs as well as animals which recovered may serve as carriers for an indefinite period of time.

SUSCEPTIBILITY AND RESISTANCE

In general the warmwater fish are most susceptible in the spring in the climatic zones in which fish are inactive during winter. It is believed that infection frequently occurs during winter with the disease breaking out in the spring. Aquarium fishes can develop this disease at any time of the year. Of salmonid fishes, the rainbow trout seems to be most susceptible to infections with these bacteria. German investigators were able to breed strains of carp having increased resistance to infectious abdominal dropsy (hemorrhagic septicaemia). Handling and crowding of fish can cause severe outbreaks.

RANGE

Apparently all over the world.

OCCURRENCE

The greatest epizootics were described in Europe. Disease is common in warmwater pond fish in the United States, in rainbow trout in the western states and Europe, in northern pike in Canada, and in aquarium fishes.

METHODS OF CONTROL

- A. Preventive measures.
 - a. Avoidance of transfer of fish from ponds or hatcheries in which fish have or recently had this disease to hatcheries free from it.
 - b. Wherever this disease breaks out frequently the pond fish should not be handled and transferred in spring of the year until fish become active and the temperature of water is high enough for fish to feed normally.
 - c. Very substantial reductions of mortalities amounting to from 80 to 90 percent were obtained in Europe if fish at the time of spring transfer were given intraperitoneal injections of 2 to 5 milligrams of Chloromycetin dissolved in water per 100 grams of fish (10 to 25 milligrams per pound). This is a practice which is becoming a standard procedure in Germany (Schäperclaus 1956).
 - d. If warmwater fish are held in tanks, shipped in trucks or in plastic bags the value of addition of antibiotics or disinfectants should be tested. The most promising substances are chloramphenicol, Terramycin, Aureomycin and a mixture of penicillin and streptomycin added to water at a rate of 10 to 50 milligrams per liter (40 to 200 milligrams per gallon). van Duijn (1956) recommends addition of acriflavine and phenoxyethanol to aquarium water. According to his recommentations, acriflavine should be used as a stock

solution containing 10 p.p.m. This should be added at a rate of 8 milliliters per gallon. Phenoxyethanol should be used as a 1 percent stock solution; 38-75 milliliters of this solution should be used per gallon. Other authors recommend use of much stronger concentrations of acriflavine, namely 10 p.p.m. in water containing fish.

- e. Selective breeding of stocks of fish resistant to this disease. This is one of the most promising longrange control measures.
- B. Therapy.
 - a. If fish are regularly being fed any diet which is taken promptly so that the drugs cannot be leached out completely, drugs can be incorporated with the diet. This is particularly true with salmonid fishes raised in hatcheries, minnows and aquarium fishes raised in ponds, and aquarium fishes. In such cases the drugs of choice are Chloromycetin (chloramphenicol) and Terramycin (oxytetracycline). They should be used at a rate of 50 to 75 milligrams of pure antibiotic activity per kilogram of fish per day (2.5 to 3.5 grams per 100 pounds). Sulfonamides, such as sulfamerazine, sulfamethazine, sulfadiazine, or sulfisoxazole, can be also used but they are less likely to be effective. They should be incorporated in the diet and given at a rate of 10 grams per 100 pounds of fish per day. Nitrofurans should be tried as very promising.
 - b. Addition of drugs to water is less effective in treatment than in prophylaxis. If drugs cannot be given orally they should be used as described under Preventive Measures in paragraphs c and d.

ANNOTATED BIBLIOGRAPHY

Bibliography of these diseases contain many papers which were published in German, Russian and French. The reference material available in the English language is comparatively small. Therefore some foreign language papers of highest importance are listed for persons who can use them. <u>None of the foreign</u> papers which were reviewed and listed by Griffin and Snieszko in the "Symposium" on fish diseases (1954) are here included.

- Ambrus, J.L., C.M. Ambrus and J.W.E. Harrison
 - 1951. Prevention of <u>Proteus hydrophilus</u> infections (red leg disease) in frog colonies. Amer. Journ. Pharm., Vol. 123, p. 129.

Addition of 50 milligrams of chloramphenicol to water in which frogs were kept protected frog colonies from red leg disease.

Besse, P.

1954. L'hydropisie des truites arc-enciel ou exophthalmie epizootique.
Bull. Franc. de Pisciculture, No. 172, pp. 113-115.

A bacterial infection of rainbow trout in Switzerland. Infected fish have exophthalmia, photophobia, lack of appetite, pale gills, distention of abdomen with accumulation of abdominal fluid, gut filled with yellow gelatinous mass.

- Curran, H. Wesley
 - 1947. A biological survey of Lake Opinicon. Progress Report, Queens University Biological Station, Ontario. pp. 47-48.

Infection of sunfish with <u>Aeromonas lique-faciens</u> (<u>Proteus hydrophilus</u>). The disease is characterized externally by infected areas which range from small red spots to open necrotic lesions of the skin or any part of the body or fins. In advanced cases in the red, slimy necrotic areas scales are displaced and the lesion penetrates the skin.

van Duijn, C., Jr.

1956. Diseases of Fishes. Dorset House, London. Fish Dropsy is described on pp. 97-101.

Symptoms, cause and treatment of aquarium fishes are described.

- *Griffin, P.J. and S.F. Snieszko
 - 1951. A unique bacterium pathogenic for warm-blooded and coldblooded animals. U.S. Fish and Wildlife Service, Fish. Bull. 68, pp. 187-190.

A bacterium which caused infection and death in several species of aquarium fishes was isolated. It is identical with or very similar to <u>Aeromonas lique</u>faciens. Characteristics of the bacterium are described in detail.

*Reed, G.B. and G.C. Toner

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1941. Red sore disease of pike. Canad. Journ. Res., Sect. D. Zool. Sci., Vol. 19 (5), pp. 139-143.
This disease is common in northern pike (Esox lucius). It is characterized by

shallow ulcers, gray in appearance. It is caused by <u>Proteus hydrophilus</u> (Aeromonas liquefaciens).

- 1942. <u>Proteus hydrophilus infections of pike, trout and frogs. Canad.</u> Journ. Res., Vol. 20, Sect. D, pp. 161-166.
 - The "red sore" disease in trout and pike usually coincided with the outbreaks of "red leg" disease in frogs. <u>Proteus</u> <u>hydrophilus (Aeromonas liquefaciens)</u> was regularly isolated from the lesions and frequently from the internal organs of diseased fish or frogs. Since methods for the isolation of <u>Hemophilus piscium</u> were not known at that time, it is impossible to determine now if the disease in brook trout was not the typical ulcer disease with <u>A</u>. <u>liquefaciens</u> present as the secondary invader.

Schäperclaus, W.

- 1954. Fischkrankheiten. Berlin, Germany.
 Infectious dropsy and similar diseases are excellently described. Many excellent photographs, pp. 447-524.
 - 1956. Bekämphfung der infektiösen Bauchwassersucht der Karpfens durch Antibiotika. Zeitschr. f. Fischerei, Vol. 5(1-2), pp. 3-59. Results of extensive experiments on the prophylaxis of infectious abdominal dropsy in carp by single intraperitoneal injection of antibiotics. Chloramphenicol and streptomycin are best. The former last longer in fish and in a solution and can be used in smaller quantities. Single injection of about 2 milligrams per 100 grams of carp during the spring transfer increased the fish survival manyfold for the entire growth period in summer. Author gives extensive review of German and Russian bibliography. American publications were not listed.

Seaman, W.R.

1951. Notes on a bacterial disease of rainbow trout in a Colorado hatchery. <u>Progr. Fish-Cult.</u>, Vol. 13(3), 139-141.

> An outbreak, symptoms and successful treatment with sulfonamides of a bacterial disease which is probably identical with the "red mouth" disease. Treatment with 15 grams of sulfamerazine given orally with food per 100 pounds of trout per day gave excellent results. The identity of the pathogen was not definitely established except that it was most likely a pseudomonad.

- *Snieszko, S.F.
 - 1940. A bacterial disease of carp in central Europe. Progr. Fish-Cult., No. 52, pp. 12-15.
 A review of the problem.

*Symposium: Research on Fish Diseases 1954. Trans. Am. Fish. Soc., Vol. 83 (for 1935). See the papers by Griffin, pp. 246-248 and by Snieszko pp. 317-318. *Wagner, E.D. and C.L. Perkins

Pseudomonas hydrophila, the cause of "red mouth" disease in rainbow trout. Progr. Fish-Cult., Vol. 14(3), pp. 127-128.

Pseudomonas hydrophila (Aeromonas liquefaciens) has been found to be the cause of "red mouth" disease in rainbow trout and "red leg" disease in frogs.

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* Papers indicated by an asterisk are of special importance to fish culturists.