OBSERVATIONS ON THE PRESENCE OF AEROBIC BACTERIA IN CARP *(CYPRINUS CARPIO)* INTERNAL ORGANS

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In some instances, from appearently healthy fish internal organs various bacteria were isolated. Schäperclaus and Mann (22) found that at suboptimal temperatures (0—10°C) carp had asymptomatic infections with bacteria belonging to the genera *Aeromonas* and *Pseudomonas*. Bisset (5) was the first to investigate the effect of temperature on the presence of saprophytic water bacteria in the fish tissues. He found that at 10°C, or below, fish frequently contained bacteria in their tissues without becaming diseased. According to Evelyn and Mc Dermott (10) bacteria representing genera *Pseudomonas, Aeromonas, Achromobacter, Alcaligenes, Flavobacterium, Aerobacter, Escherichia, Paracolobacterium, Lactobacillus, Micrococcus, Bacillus* and *Brevibacterium* were isolated from various fresh water fish internal organs. Similar bacteria were also isolated from white perch (*Roccus americanus*) in an estuarine environment (Allen and Pelzar, 1). Bullock and Snieszko (7) isolated from blood

and kidney of healthy trout low numbers of bacteria representing the genera *Pseudomonas, Aeromonas* and *Flavobacterium.* In adition, sera from these trout had agglutinins against some of the isolated bacteria, and bacterial types found in trout were the same as those cultured from race way water in which fish were raised.

To further extent these observations to Poland, bacteriological examinations of the kidney, spleen and liver of appearently healthy carp *(Cyprinus carpio)* were undertaken with a purpose to determine the kind and number of live bacteria present in these organs. The results were to enlighten the fish bacterial disease ecology.

Material and Methods

The observations were performed in autumn and spring of 1969 and 1970. A total of 103 normally appearing yearling and two years old carp from fish pond farms of the Lublin district were used for bacteriological examinations of the kidney, liver and spleen. The fish were caught and delivered to the laboratory at temperatures between 10°C and 15°C, they were immediately killed, dried, and disinfected with iodine and 70% isopropyl alcohol. The body cavity were cut and opened, then pea size samples of the kidney, liver and spleen were placed into 1-ml syringes and pressed out. 0.1-ml samples of each homogenized organ was placed separately in tubes containing 0.4 ml of physiological salt solution. The tubes were vigorously shaken, and dilutions 1:10, 1:100, 1:1000 were made. 0.05 ml of each dilution were placed on nutrient agar plates and thoroughly spread. After 24- and 48-hr incubation at 25°C and 30°C, colonies were counted and two representative of each kind were inoculated on soya agar slants. Identifications of cultures was accomplished using previously described determinative schemes and media (2, 3, 4, 6, 8, 9, 11, 12, 13, 14, 15, 17, 18, 20, 21, 23, 24, 25).

Results

A total of 556 bacterial cultures were isolated from the liver, kidney and spleen of 64 out of the 103 carp examined. Thirty-six carp internal organs proved to be sterile. Forty-three cultures were lost through death being too fastidious to survive the routine culture methods employed. Sixty-four cultures were unidentified owing to shortcoming of the present system of classification. The remaining bacteria presented in Table 1 were classified to the rank of the genus as follows: 206 Gram--negative, cytochromooxidase positive, motile rods of various length showing vigorous glucose, mannitol and arginine decomposition both aerobically and anaerobically accompanied in the majority of cases by marked gas production, liquefying gelatin — appeared to be *Aeromonas*; 134 Gram-negative short, even coccoid, nonmotile rods, biochemically inactive fited into genus *Achromobacter;* 50 Gram-negative, cytochromooxidase positive, motile rods decomposing glucose and arginine only aerobically — were classified as *Pseudomonas; 30* Gram-negative rods, in majority nonmotile, cytochromooxidase positive, producing yellow pigment on nutrient agar, oxidasing glucose — were identified as *Flavobacterium;* 14 yellow, Gram-negative, cytochromooxidase positive, motile rods, oxidasing glucose — were described as *Xanthomonas;* 8 Gram-negative, cytochromooxidase negative, motile rods showing vigorous fermentative ability in Hugh-Leifson's medium with glucose, not liquefying gelatin and showing no activity in lactose medium — were

Table 1

Biochemical properties of bacteria isolated from carp internal organs

Genus and number of bacterial strains isolated	Motility		Glocose utilization		Mannitol utilization	Lactose utilization	Gelatine utilization		Arginine utilization		Cytochromo-oxidase production	
	0.25% agar	Ball-Sellers' medium	Hugh-Leifson's medium aerobically	Hugh-Leifson's medium anaerobically			14% gelatine	Ball-Sellers' medium	Thornley's medium aerobically	Thornley's medium anaerobically	Gaby-Hadley's method	Kowacs' method
Aeromonas, 206	+	+	+	+	+	11 + 195 —	+	+	+	+	+	+
Achromobacter, 134	—	—	57 + 77 —	—	—	—	—	—	—	—	—	—
Pseudomonas, 50	+	+	42 + 8 —	—	—	—	+	+	+	—	+	+
Flavobacterium, 30	4 + 26 —	8 + 22 —	+	—	—	—	+	+	12 + 18 —	—	+	+
Xanthomonas, 14	+	+	+	—	+	—	+	+	—	—	+	+
Paracolobacterium, 8	+	+	+	+	+	—	—	—	—	—	—	—

+ positive reaction, — negative reaction

identified as *Paracolobacterium;* finally, 7 Gram-positive, biochemically inactive cocci were also found.

Table 2 presents the amount of the inoculations which yielded bacteria of every described genus and the largest number of various bacteria in cc. of carp internal organs.

Table 2
Results of bacteriological examinations of carp internal organs

Genus of isolated bacteria	Organ	The largest number of the bacteria in 1 cc.	Percentage of positive inoculations
Aeromonas	liver	2400	27.2
	spleen	400	20.4
	kidney	1800	23.3
Achromobacter	liver	2100	25.3
	spleen	4200	22.3
	kidney	800	10.7
Pseudomonas	liver	400	4.8
	spleen	300	1.0
	kidney	200	1.9
Flavobacterium	liver	300	9.6
	spleen	200	8.7
	kidney	400	6.8
Xanthomonas	liver	300	3.8
	spleen	200	2.9
	kidney	—	—
Paracolobacterium	liver	200	1.0
	spleen	400	1.9
	kidney	—	—

Discussion

The presence of saprophytic water bacteria in internal organs of healthy fish agree with the observations of other authors (1, 17, 10). The predominance of *Aeromonas, Achromobacter, Peseudomonas* and *Flavobacterium* genera has usually been found to be the case by other investigators (7, 19). This phenomenon probably reflects the ubiquitous nature of these aquatic bacteria. Bullock and Snieszko (7) found that under normal conditions, the presence of a few cells of various aquatic bacteria, including fish pathogens, should not be interpreted as a disease state.

The new method of inoculations preceeded by homogenization of the fish internal organs by means of sterile syringes enabled to assess the

number of bacteria in the kidney, spleen and liver. It is interesting to note that these organisms were not doing appearently any harm in numbers detected, since no lesions were found. Their presence shows that potential hazard of fish disease exists if fish would be exposed to undue stress. The intensive level at which warm and cold water fishes are cultured, poor environmental conditions such as low oxygen content, crowding and the bacterial load in the water may weaken fish and allow bacteria present in tissues to multiply and produce bacteriaemia. For example, low level of dissolved oxygen triggered outbreaks of furunculosis in hatchery trout (19), hemorrhagic septicemia in American shad (*Alosa sapidissima*) and threadfin shad (*Drosoma petense*) (16), gill disease in fingerling trouts (7).

According to Schäperclaus and Mann (22), carp with asymptomatic infections that had been acquired in lower temperature, at temperatures 20° would succumb to the infections or produce specific antibodies and eliminate bacteriemia.

Although certain types of *Pseudomonas fluorescens* and *Aeromonas* sp. are apparently more capable to produce a disease than other aquatic types, epizootics induced by other water bacteria do occur (7), e. .g *Achromobacter* infection in Australian brown trout (*Salmo trutta*). Allan and Pelczar (1) found that a number of bacteria representing normal flora of white perch could cause infections when inoculated into this fish, and they concluded that various microorganisms found in healthy fish could initiate epizootics.

The presented work stressed the importance of examining not only a kind but also quantity of bacteria in fish internal organs for purpose of proper assessing the possibility of disease outbreak and for applying proper preventive measures. Although it ist not feasible to eliminate all microorganisms from water in which fish are raised, proper therapy and conditions favorable to the health of fish could help in maintaining a balance between fish and bacteria and thus reduce the number of bacterial epizootics.

Conclusions

1. The healthy carp (*Cyprinus carpio*) internal organs proved to be infected with various bacteria in 62% cases.
2. *Aeromonas, Achromobacter, Pseudomonas* and *Flavobacterium* sp. were the predominating bacteria in the kidney, spleen and liver.
3. The new method of inoculation preceded by tissue homogenization, was found to be useful for assessing of the number of microorganism in fish.
4. A small number of *Pseudomonas* bacteria detected, comparing with finding of the authors, are supposed to be due to difference in milieu from which fishes originated.

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