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Source: Journal of Wildlife Diseases, 17(4) : 505-510

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-17.4.505

A BACTERIAL DISEASE OF PERCH *(Perca fluviatilis L.)* IN AN ALPINE LAKE: ISOLATION AND PRELIMINARY STUDY OF THE CAUSATIVE ORGANISM

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Abstract: An epizootic among perch *(Perca fluviatilis L.)* occurred during the summer and fall of 1979 in an alpine lake (Lake Annecy, Haute-Savoie) in France. Hemorrhagic and ulcerative clinical signs were associated with the height of the mortality. A Gram-negative, non-motile, slow-growing bacterium was isolated from skin lesions in diseased fish. *Aeromonas hydrophila* often was present. Since the non-motile bacterium was suspected to be the etiological agent, its characteristics and pathogenicity were determined. The bacterium was pathogenic to perch, possibly pathogenic to rainbow trout, and non-pathogenic to carp. It could be reisolated from infected fish, but its physiologic and biochemical properties have not been assessed to determine the taxonomic position.

INTRODUCTION

During the summer of 1979, mass mortalities of perch *(Perca fluviatilis* L.) occurred in Lake Annecy (Haute-Savoie, France). Losses were primarily noticed in June, when 250 kg of large perch, each weighing up to 1.5 kg were found stranded on the banks of the lake. The mortality continued through fall 1979. About 33% of the perch netted by the professional fishermen had hemorrhagic and ulcerative lesions, mainly localized on the abdominal area, and numerous smaller perch, 5 to 7 cm in length, were found entangled in the vegetation between 4 and 7 m beneath the surface. Unfortunately, the laboratory was not advised during the peak of the epizootic; therefore, samples of diseased, but live fish were not examined before October, 1979.

MATERIAL AND METHODS

Bacteriologic studies. Live, diseased perch were received on three occasions. They were killed in 0.2% 2-phenoxyethanol (Eastman) and inspected externally and internally for signs of disease and the presence of parasites. Bacteriologic examination was performed on smears of kidney, liver and spleen, stained by toluidine blue. Materials obtained from kidney or external lesions were inoculated on trypticase-soy-agar (TSA), and TSA supplemented with 10% of horse serum, and incubated at 22 C. Blood samples were collected from perch and the sera were used in serologic tests with the carp erythrodermatitis agent in a passive hemagglutination assay. Direct agglutination was also performed, using an *Aeromonas salmonicida* bacterin.

Aeromonas hydrophila, and another bacterium which produced small colonies after 4 to 5 days incubation, were isolated from external lesions. This slow-growing bacterium, suspected as the cause of disease, was examined in detail. TSA with 10% horse serum was used in most cases. Occasionally, media were enriched with horse blood (5%), human ascitic fluid (5%), V and X factors (0.2%) and yeast extract (0.5%/ Utilization of carbohydrates was studied in peptone water with 0.1% yeast extract. Fermentable materials were added in 1% concen-

tration and bromthymol blue was used as an indicator. Lysine, arginine and ornithine media were prepared according to Richard.[7] Other tests were carried out in the routine manner, using stains and media from the Pasteur Institute. All media were enriched with 0.1% yeast extract. Duplicate tests were performed at the Pasteur Institute to confirm our own results.

Sensitivity to antimicrobial drugs was determined in liquid media. Agar diffusion method was not used because of slow growth of the tested organism. Drugs were purchased from Sigma, except for the flumequine, directly supplied by Riker Laboratories (Pithiviers, France). Stock solutions containing 2 mg/ml of antibiotics were prepared in water or methanol according to their solubility. Two-fold dilutions were prepared in the same basal medium as used for the carbohydrate utilization test, containing 1% glucose. Preinoculated medium was added to the medium with antibiotics, giving a final drug concentration of 0.125 to 128 μg/ml. Cultures were incubated for 4 days at 22 C. The minimal inhibitory concentration was in tubes with the highest dilution of antibiotic and no acid, indicating absence of growth.

Tests for pathogenicity. Infection experiments were performed with perch, rainbow trout *(Salmo gairdneri* Richardson) and carp *(Cyprinus carpio* L.). All fish were kept at 15 C in 12 l aquaria supplied with dechlorinated tap water. Three separate tests were performed with perch. These included 20 young perch of 10 to 12 cm in length, and also 3 large perch weighing 250 g each. Cultures in a liquid trypticase-soy-broth (TSB) medium with 0.5% yeast extract were used for infection. Cultures were agitated while incubated for 48 to 72 h in flasks containing 100 ml of medium. Perch were inoculated intramuscularly with 0.1 ml undiluted culture, or diluted 1:10 and 1:100. Groups of 10 trout or carp received IM or intraperitoneal injections. In addition some fish were scarified with a scalpel and a drop of culture was deposited on the scarified area.

Infected fish were observed for several weeks. Dead fish were examined for bacteria and the isolates were identified.

RESULTS

Clinical examination. Mortalities of perch in Lake Annecy started in June, 1979, but the first specimens for examination were not available until October. In the early period of the epizootic, signs of the disease, reported by fishermen, were apparently hemorrhagic. Fish examined in October had congestion at the base of fins and external ulcers, with exposed muscle tissue. Internal organs were normal. Larvae, presumably of *Triaenophorus* sp., were found in the liver of some fish.

Bacteriological examination. Only living fish were examined. Different bacteria, but especially *A. hydrophila* predominated on media inoculated from superficial lesions. After 5 days of incubation, numerous small colonies appeared and were subsequently isolated. As they had been isolated from several fish in a time span of 5 weeks they were suspected to be involved in the etiology of the disease. No bacteria were found in the internal organs.

The small, slow-growing colonies were beaded and so compact that entire colonies could be moved with an inoculating loop. This bacterium produced granular sediments in liquid media. Of the different nutrients tested to improve the *in vitro* growth, yeast extract gave the best results. The studied bacteria were gram-negative, non-motile, non-spore forming, straight rods 2 to 2.5 $\times$ 1 to 1.5 μm with a tendency of polar staining, and were non-encapsulated. Other characteristics are presented in Table 1. In deep agar cultures growth was at the top of the medium indicating aerobic requirements, but on primary isolation a few colonies were in the anaerobic part of the medium. Production of acetoin also

TABLE 1. Physiological, biochemical and cultural characteristics of the perch bacterial strain TG 81/79.

General characteristics		**Protein hydrolysis:**	
Gram staining	-	Photographic film, emulsion.	-
Motility	-	Coagulated serum	-
Metabolism	Aerobic ? (a)	Frazier's gelatin	-
Oxidase	+	Casein	-
Catalase	+	LDC	-
Gas production (in glucose)	-	ODC	-
H_2S (in Kligler-Hajna)	-	ADH	-
Nitrate reduction	-	Urease	-
Voges Proskauer	+	TDA	-
		PhDA	-
		Indol	-
Carbohydrates metabolism:			
		DNAase	-
Glucose	+	Simon's citrate	-
Mannitol	-	0/129	resistant
Lactose	-		
Sucrose	-	**NaCl tolerance:**	
Arabinose	-	0%	+
Adonitol	-	2.5%	-
Rhamnose	-	5%	-
Inositol	-	7.5%	-
Glycerol	-	10%	-
Starch hydrolysis	+	**Temperature range:**	
ONPG	-	4 C	+
Tween 80 hydrolysis	+	22 C	+ (optimum)
		30 C	+
		37 C	-

(a) See the text.

indicated that facultative anaerobic growth was possible.

The bacteria was sensitive to streptomycin, kanamycin, chloramphenicol, oxytetracycline, erythromycin, colistin, trimethoprim and flumequine. It was resistant to ampicillin and sulfathiazole.

Since it was suspected that the isolated bacterium may be identical or similar to the *A. salmonicida nova* isolated from carp with erythrodermatitis, serologic testing was performed but cross reactions did not occur. Agglutination of perch sera with typical *A. salmonicida* was also negative.

Tests for pathogenicity. Infection experiments indicated that carp were resistant. Only 2 of 10 rainbow trout inoculated intramuscularly died, and the bacterium could be reisolated from muscular lesions resembling "furuncles", but this condition failed to be reproduced in two subsequent experiments. Perch provided more consistent results (Table 2). In two different experiments, young fish died in 7 to 12 days, with signs of a systemic infection: external hemorrhages and congestion of the internal organs. The bacterium, in pure culture, was reisolated from the kidneys of 6 dead fish (about half of the attempts). The 3 large perch developed a slow and chronic ulcerative process at the site of injection, but the bacteriologic examination was not carried out until the third month and without any success.

DISCUSSION

At two different times the slow growing gram-negative bacterium was isolated from diseased wild perch. In the tests for pathogenicity it produced lethal infections with hemorrhaging. It was reisolated in pure culture from experimentally infected perch. This observation indicated that it fulfilled Koch's postulates. Therefore it seems justified to consider it as pathogenic to perch.

The organism appeared to be specific to perch because carp and rainbow trout infected experimentally remained healthy. Its actual role in the mortalities of perch in Lake Annecy is not entirely clear because we did not have the opportunity to observe the diseased fish at the peak of the epizootic, and *A. hydrophila* was sometimes present in the lesions. But a culture of *A. hydrophila* isolated from the diseased perch was not pathogenic to perch. Therefore, it was believed to occur as a secondary invader.

The bacteriologic study indicated that the described bacterium was presumably a new pathogen of perch. It was not possible to assign it to any genus or family on the basis of biochemical characteristics, and serologic testing did not indicate any similarity with *A. salmonicida* or *A. salmonicida nova* isolated from carp with erythrodermatitis.[4] Further investigations will be required, including GC ratios and improvement of serological techniques,

TABLE 2. Results of 3 experimental infections of perch with the bacterial strain TG 81/79 (temperature of water: 15 C).

	1st trial	2nd trial		3d trial
Mean weight (g)	14	14	14	250
Number of perch	10	5	5	3
Culture dilution (doses = 0.1 ml IM)	10^{-1}	10^{-1}	10^{-2}	0
Dead perch	9	4	0	2
Lesions	hemorrhages	hemorrhages	0	hemorrhages or ulcers
Isolation of the organism	+ (5/7)	+ (1/4)	-	-

presently precluded by its auto-agglutinating properties, before solving the question of the taxonomic status of the organism.

Few reports are available on the diseases of perch. Typical or atypical strains of *A. salmonicida* have been reported to cause disease in perch.[3,5] Ross *et al.*[8] have described an epizootic among yellow perch *(Perca flavescens* Mitchill) in Montana. The isolated bacteria differed markedly from that isolated from Lake Annecy perch. More interesting would be the comparison of this bacterium with the *Pasteurella* sp. found by Snieszko *et al.*[9] in Chesapeake Bay. Both organisms seem to share a large number of characters. However, Snieszko's isolate affected white perch *(Roccus americanus)* and when Allen and Pelczar[1] experimentally reproduced the infection, they did not test its pathogenicity for perch. In 1924 Plehn[6] reported on a perch disease of Lake Geneva which resembles the Lake Annecy infection: similar geographical and ecological conditions, summer occurrence, hemorrhages and grey epidermal lesions. Unfortunately, the report lacks details on the pathogen itself. In a recent review, Bucke *et al.*[2] pointed out the importance of perch mortalities associated with ulcerative lesions in lakes and reservoirs of the United Kingdom, but failed to detect a cause. Lastly, in France, perch mortalities were recorded in lakes of Auvergne in the summer of 1979. Results here presented do not give information on the distribution and frequency of the disease identified in Lake Annecy. Therefore further studies are needed, and it will be necessary to improve diagnostic methods and serologic tests to assess the significance of this new etiologic agent of perch disease.

Acknowledgements

This work was supported with a grant of the Conseil Supérieur de la Pêche (Agreement 651 278). The serological tests were performed at the Laboratoire Central de Recherches Vétérinaires de Maisons-Alfort. We are indebted to Mr. J. Debruille, Fisheries officer, Mr. J. Hennequart, fishculturist, and Mr. R. Rigaud, Director of the Aquarium of Aix-Les-Bains, for providing the perch used in this study.

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Received for publication 16 December 1980