Immunization of Fishes: a Review

Author: SNIESZKO, S. F.

Source: Journal of Wildlife Diseases, 6(1) : 24-30

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-6.1.24

Immunization of Fishes: a Review

S. F. SNIESZKO
Bureau of Sport Fisheries and Wildlife
Eastern Fish Disease Laboratory
Kearneysville, West Virginia 25430

Received July 31, 1969

Abstract

Immune response in fishes, which are ectothermic animals, depends on temperature. The optimum response is at the optimum temperature for the species. It is, however, slower in coldwater fishes, such as salmonids, and faster in warmwater fishes.

Serum of fishes contains proteins very similar, but not identical, with those of mammals. Immune bodies are contained in gamma, beta, and alpha globulins. Gamma globulin is absent in some fishes. Fishes can be effectively immunized by injection of antigens, however, this is not a practical method. For this reason oral immunization has been attempted repeatedly but the effectiveness is variable.

Introduction

At present immunization is not used as a means of protecting fish populations from any of the common pathogenic bacteria. Recent developments in the study of the mechanism of fish immunization and suggestions for practical applications for the control of bacterial diseases of fish will be reviewed.

Immune Response in Fishes

The development and nature of the immune response has been reviewed by Smith et al.[29] and the specific nature of the response in fishes by Post.[25]

Ambrosius[2] immunized representatives of mammals, birds, reptiles, and fishes and analyzed the immunoglobulins by immunoelectrophoresis. He reported that immune reaction was similar in all vertebrates, but on the molecular level the greatest differences were between fishes and other vertebrates. Ambrosius et al.[4] immunized *Perca fluviatilis* and *Cyprinus carpio* with human gamma globulin and complete swine serum. Immunoelectrophoresis indicated the absence of gamma globulin in perch. Immune bodies were present in regions of beta-1 and alpha-2 globulins. The same was true in carp, but typical gamma globulin also was present in this species. Ambrosius and Lehmann[3] investimated the role of adjuvants and temperature on the quantity of immune globulins in *Ictalurus nebulosus*. They found a better response at 18-20°C than at 11°C. Aluminum hydroxide increased slightly, and Freund's adjuvant increased up to

90 times, the quantity of immunoglobulins, as compared with fish immunized without adjuvants. The effect of adjuvants was more pronounced with fish than with rabbits. Similar results were obtained earlier by Krantz et al.[21] who found that the antibody-stimulating effects of mineral oil adjuvant were more pronounced in brown trout *(Salmo trutta)* than in rabbits.

Post's[25] study of serum proteins of rainbow trout indicated that this species did not contain gamma globulin identical with that of endothermic vertebrates. Immune bodies found in serum fractions were similar to the slowest migrating beta and alpha globulins.

To identify the serum fractions containing antibodies, Summerfelt[33] immunized golden shiner *(Notemigonus crysoleucas)* with formalin-killed *Aeromonas liquefaciens (hydrophila).* Electrophoresis separated six serum components of which two were albumins, three pseudoglobulins, and the sixth, slowest migrating, was an euglobulin. In immunized fish this euglobulin increased from 6.6 to 8.8%. When the immune serum was absorbed with homologous antigen, the quantity of this component was lowered. The author did not claim that this component was homologous with gamma globulin of mammals. Watson et al.[35] observed in immunized golden shiners an increase of serum fractions designated as beta-2 and gamma globulins. In sera absorbed with homologous antigen a decrease was noticed in the gamma globulin fraction. Klontz[17] showed by immunoelectrophoresis that the immune globulin in rainbow trout *(Salmo gairdneri)* serum was in the beta-2 fraction.

In higher vertebrates, antibodies are formed in lymphoid organs. In red snapper *(Lutianus griseus)* Ortiz-Muniz and Sigel[24] found that antibodies were produced *in vitro* in organ cultures of the spleen, anterior kidney and thymus. In larval bullfrogs *(Rana catesbiana)* with lymph glands removed, although antibodies were not produced, allografts were rejected.[5]

In warmwater pondfishes such as carp the immune response was rapid at temperatures from 15° to 25°C. At temperatures of 10°C or below there was no immune response.[25] Smith[30] reported that at 10°C the response of brown and rainbow trout to immunization was poor. Recent investigations by Krantz et al.[21] have shown that trout are capable of a good immune response. In brook *(Salvelinus fontinalis)* and brown trout maintained in water at 11°C, a measurable immune response to *A. salmonicida* antigen was noted one month after intraperitoneal injection and the peak was reached after 3 months. When adjuvant was used, the response was more rapid and the titer was higher. Antibodies persisted in the circulation for a year or longer. When immunized and control trout were challenged by injection with an LD_{50} dose of *A. salmonicida,* those immunized with killed bacteria and adjuvant were protected from the disease. Control trout and trout immunized without adjuvant sustained considerable losses.

These results indicate that trout can be immunized by parenteral introduction of bacterial antigen with adjuvant. Practical application of such immunization on a large scale is at this time not possible because the cost of injecting individual fishes in a large fish cultural establishment is economically prohibitive. Also the handling necessary during immunization would probably do more harm to the fish than any benefit resulting from immunization. For this reason oral immunization of fishes has been tried at different times. Observations made on oral immunization of higher vertebrates including man were not encouraging because all attempts of such immunization with killed pathogens gave a low or questionable degree of immunity.[34] Exceptions are BCG immunization against tuberculosis and oral immunization against poliomyelitis. In these cases living attenuated disease agents are used and immunity is obtained by asymptomatic infection.

Recent developments in oral immunization of the chicken with killed *Pasteurella multocida*[15] and with killed Newcastle virus[32] indicate that protective immunization with killed antigen administered by the intestinal route may be practical.

Oral Immunization of Fishes

Freshwater fishes do not harbor specific bacterial flora in their intestinal tract. The intestinal flora of fishes reflects the flora of the environment. Janssen and Meyers[16] detected specific antibodies to several human pathogens in perch from surface waters adjacent to heavily populated areas. Perch from sparsely populated areas did not contain such antibodies. According to Geldreich and Clarke,[12] the intestinal flora of fishes was related to the level of bacterial contamination of the water and the fish food. Serotypes of coliforms isolated from trout reflected the serotypes introduced into the water by sewage pollution.

An illustration in Andrew's[1] textbook shows macrophages containing diatom fragments and shells in ice fishes *(Chaenocephalus aceratus)*. Feng[10] noticed in oysters *(Crassostrea virginica)* that leucocytes containing phagocytized bacteria passed through the intestinal wall to the lumen. According to Avetikyan[3] leucocytes play an important role in the process of intestinal digestion in fishes. Thus it is possible that particles of bacterial antigen may be transported by white blood cells to the tissues stimulating the production of low titer antibodies which are released into the circulation. This may explain why, under favorable conditions, fish gain some level of immunization by the intestinal route.

Guelin and Lablin[14] reported that bacteria introduced to trout less than 49 hours old disappeared rapidly. This was attributed to massive penetration of leucocytes into the digestive tract during the digestive process.

Recent investigations have shown that fish sera contain antibodies to species of aquatic bacteria which at times may become pathogenic. Luklyanenko[24] found antibodies to *Aeromonas punctata* and *Pseudomonas fluorescens* in sera of 10 species of fishes. Bullock and McDaniel[9] found antibodies to myxobacteria associated with gill disease of salmonid fishes to be very common, probably because fingerling salmonids often suffer from gill disease. Trout in populations resistant to furunculosis, but having endemic asymptomatic furunculosis, have low titer antibodies to *A. salmonicida*.[22] The presence in fish sera of low titer antibodies to some of the bacteria pathogenic to fish may be considered as evidence that these fish had, or have, asymptomatic infections.

The first promising oral immunization of fish with killed bacteria was reported by Duff.[9] He prepared antigen from four-day-old virulent cultures of *A. salmonicida* killed with chloroform. Bacteria harvested from one Roux flask were used to treat 1.5 to 2.5 kg of soft fish feed. Yearling cutthroat trout *(Salmo clarki)* were fed continuously with antigen for 40 to 70 days at mean water temperature of 7° to 8.6°C. Following immunization, trout were adapted to 19°C at which temperature they were more susceptible to furunculosis. They were then challenged by the introduction of virulent cultures of *A. salmonicida* to the water, or by injection. A significant degree of protection was provided by oral immunization if trout were challenged by external exposure to infection, and a low degree of protection was noted if the challenge was made by injection. Serum from trout which were immunized orally contained a higher titer of specific agglutinins than did serum from control trout.

Klontz[17] noted that saline extract from sonically disrupted cells of *A. salmonicida* contained fish toxin. This probably was an endotoxin commonly present in gram-negative rod-shaped bacteria. Toxoid was prepared in the form of an alum precipitate. Brook trout immunized by oral administration for over 30 days by feeding food containing 0.02% of this antigen had antibodies in lymphoid cells which were demonstrated by immunofluorescence.

Later about 700,000 fingerling coho salmon *(Oncorhynchus kisutch)* at Issaquah hatchery in Washington were divided among 12 ponds. One-half was immunized by feeding a total of 33 mg of antigen per fish with food over a 49 day period.[18,19] During natural outbreaks of furunculosis in May and June 5.6%

died in the immunized lots and 11.4% in controls.

In a test performed at Siletz hatchery in Oregon, coho salmon were immunized with antigen prepared from the local strain of *A. salmonicida*. When furunculosis occurred, 22 and 37% of the fish died in control groups and less than 1% in the immunized group.[18]

In 1967[19] this antigen was prepared commercially and administered orally to fingerling coho salmon at three hatcheries in Washington state. This time oral immunization "was without benefit in controlling the incidence of furunculosis." To explain this failure, a laboratory test was carried out to compare the laboratory-prepared antigen with one prepared commercially. The laboratory batch conferred better protection against this disease than did the commercial one. Circulating antibodies were higher in cohos orally immunized with a laboratory batch of antigen. Klontz[19] suggested that the difference between the two antigens was probably due to particle size.

In 1968 a large scale test was carried out at selected salmon and trout hatcheries in different parts of the United States. Antigen for this oral immunization was commercially prepared. Unfortunately the results were inconclusive.[20]

Early in 1969[20] seven antigens prepared by different methods were used in parenteral immunization of brook trout. Trout immunized with heat-killed *A. salmonicida* had the highest degree of precipitating antibody, but none of the trout were effectively protected against furunculosis.

Krantz et al.[22] attempted to immunize 2-year-old brown trout by oral administration of living and chloroform-killed *A. salmonicida* and by intraperitoneal injection of formalin-killed cells with adjuvants. The feeding of living and chloroform-killed bacteria did not stimulate an antibody titer above the level occurring in control trout which were exposed to *A. salmonicida* in the environment. The authors concluded that oral immunization with a living or killed cell of *A. salmonicida* was not satisfactory when compared with parenteral immunization. Similarly unsatisfactory results were obtained by Spence et al.[31] who attempted to immunize coho salmon against furunculosis by the oral route. Passive immunization with serum of actively immunized rainbow trout conferred a temporary protection and resulted in delayed mortality as compared with controls.

Redmouth is a bacterial disease of rainbow trout, and is endemic in the western mountain states, particularly in Idaho. It is caused by an enteric bacterium so far unnamed.[25] The disease starts with inflammation and necrosis of the mouth and terminates as a systemic infection. Oral immunization was attempted with 100 mg of alum-precipitated endotoxin per fish, given over a period of 80 days.[10] In a June outbreak of the disease losses in the immunized group were about 1%, and very heavy in the controls. Somewhat later, however, the disease caused heavy losses in the orally immunized group, showing that the duration of immunity was short.

Oral administration of phenol-killed redmouth (RM) enteric organisms to yearling rainbow trout resulted in an effective protection against intraperitoneal challenge with an LD_{50} dose of the pathogen. In one of the tests, 90% of trout survived in the immunized and 20% in the control group.[25]

Comparison of oral with parenteral immunization of 2-year-old rainbow trout with *Aeromonas hydrophila* has shown that prolonged oral immunization with heat-killed bacteria produced a detectable level of antibodies in only 50% of the trout. After parenteral challenge with an LD_{50} dose of live bacteria 10 to 30% died in the parenterally immunized group, 60 to 80% died in the orally immunized group, and 80 to 90% died in the controls.[25,26]

Oral immunization of juvenile coho salmon with heat-killed myxobacterium, *Chondrococcus columnaris*, resulted in immunity sufficient to reduce losses due to natural infection. In an outbreak of columnaris disease, 8% died in the immunized and 48% in the control fish.[11]

Conclusions

I have presented above a review on immunization of fishes with particular emphasis on bacterial diseases. It is now well established that several factors are involved in diseases caused by infective agents. They are the nature of the host, the nature of the pathogen, and conditions of the environment. The susceptibility of the host to a particular disease and the virulence of a particular pathogen are influenced by phenotypic and genetic characteristics of both. Growth, disease resistance, and adaptability to changing environmental conditions usually do not have a one-to-one relationship between genetic and phenotypic characteristics.[7] In other words the disease resistance depends on the interaction of hosts, pathogens, and environment.

The same is true with acquired immunity in fishes. Fishes which have a low level of antibody due to exposure to certain bacteria, as for example by endemic disease or by oral immunization, may have a low degree of protection which will suffice to give disease protection to fishes after a light challenge and under favorable conditions. Under less favorable conditions, or stress, this low degree of protection may not be sufficient. This may explain why, according to some published reports, oral immunization is significantly better than no immunization at all, while according to others it has no value whatsoever. This also may explain why, in experiments performed under well controlled laboratory conditions, oral immunization often was reported to be beneficial, while under larger scale field tests the results were inconclusive.

With the present state of knowledge only a guarded optimism is justified towards the effectiveness of oral immunization of fishes. Nevertheless, the experimental evidence is sufficient to justify further carefully designed experiments to learn if this very convenient way of fish immunization has real merit in fish culture.

Literature Cited

1. ANDREW, W. 1965. Comparative Hematology. Grune and Stratton, New York. 188 pp.
2. AMBROSIUS, H. 1967. Untersuchungen über die Immunglobuline niederer Wirbeltiere. Allergie und Asthma 13(2/3): 111-119.
3. ———— and LEHMANN, R. 1965. Beiträge zur Immunbiologie pcikilothermer Wirbeltiere. III. Der Einfluss von Ajuvanten auf die Antikörpwer-produktion. Acta biol. med. German., Band 14: 830-844.
4. ————, RICHTER, R., and KONIG, I. 1967. Ueber die Immunglobuline von Knochenfischen. Acta biol. med. German., 19: 599-601.
5. AVETIKYAN, B. G. 1958. Immunological reaction in fishes (translated title). Akad. Nauk SSSR, Ikht. Kom. No. 8, 387-392.
6. BULLOCK, G. L., and McDANIEL, D. W. 1968. Bacterial diseases. U.S. Fish and Wildl. Ser., Progress in Sport Fishery Research, Resource Publ. 64: 18-20.
7. BUTLER, L. 1968. The potential use of selective breeding in the face of a changing environment. A Symposium on Introductions of Exotic Species. Ontario Dept. Lands and Forests, Res. Rept. 82: 54-72.
8. COOPER, E. L. 1968. Lymphomyeloid organs of amphibia. III. Antibody synthesis and lymph glands in larval bullfrogs. The Anatomical Record 162(4): 453-458.

9. DUFF, D. C. B. 1942. The oral immunization of trout against *Bacterium salmonicida*. J. Immunol. 44: 87-94.

10. FENG, S. Y. 1966. Experimental bacterial infections in the oyster *Crassostrea virginica*. J. Invert. Pathol. 8(4): 505-511.

11. FUJIHARA, M. P. 1969. Oral immunization of juvenile coho salmon against *Chondrococcus columnaris* disease. Bacterial. Proceed. M164, p. 92.

12. GELDREICH, E. E., and CLARKE, N. A. 1966. Bacterial pollution indicators in the intestinal tract of freshwater fish. Appl. Microbiol. 14(3): 429-437.

13. GLANTZ, P. J., and KRANTZ, G. E. 1965. *Escherichia coli* serotypes isolated from fish and their environment. Health Lab. Sci. 2(1): 54-63.

14. GUELIN, A., and LABLIN, D. 1964. The survival of bacteria and psychrotropic bacteriophages in the digestive tubes of trout. Ann. Inst. Pasteur Lille 15: 207-211.

15. HEDDLESTON, K. L. and REBERS, P. A. 1968. Fowl cholera: active immunity induced in chickens and turkeys by oral administration of killed *Pasteurella multocida*. Avian Diseases 12: 129-134.

16. JANSSEN, W. A. and MEYERS, C. D. 1968. Fish: serologic evidence of infection with human pathogens. Science 159(3814): 547-548.

17. KLONTZ, G. W. 1966. Immunopathology. U.S. Fish and Wildl. Ser., Progress in Sport Fishery Research, Resource Publ. 17: 11-12.

18. ————. 1967. Immunopathology. U.S. Fish and Wildl. Ser., Progress in Sport Fishery Research, Resource Publ. 39: 81-82.

19. ————. 1968. Immunopathology. U.S. Fish and Wildl. Ser., Progress in Sport Fishery Research, Resource Publ. 64: 8-12.

20. ————. 1969. Immunopathology. U.S. Fish and Wildl. Ser., Progress in Sport Fishery Research. In press.

21. KRANTZ, G. E., REDDECLIFF, J. M., and HEIST, C. E. 1963. Development of antibodies against *A. salmonicida* in trout. J. Immunol. 91(6): 757-760.

22. ————. 1964. Immune response of trout to *Aeromonas salmonicida*. II. Evaluation of feeding techniques. Prog. Fish-Cult. 26(2): 65-69.

23. LUK'YANENKO, V. I. 1965. Natural antibodies in fish. Zool. Zh. 44(2): 300-304. (Engl. summ. Biological Abstracts 47(5): Abst. No. 23138, 1966).

24. ORTIZ-MUNIZ, G., and SIGEL, M. M. 1968. *In Vitro* synthesis of anti-BSA antibodies by fish lymphoid organs. Bact. Proceed. M8, p. 66.

25. POST, G. 1963. The immune response of rainbow trout *(Salmo gairdneri)* to *Aeromonas hydrophila*. Utah State Dept. Fish and Game Pub. No. 63-7. 82 pp.

26. ————. 1966. Response of rainbow trout *(Salmo gairdneri)* to antigens of *Aeromonas hydrophila*. J. Fish. Res. Bd. Can. 23(10): 1487-1494.

27. ROSS, A. J., and KLONTZ, G. W. 1965. Oral immunization of rainbow trout *(Salmo gairdneri)* against an etiologic agent of "redmouth disease". J. Fish. Res. Bd. Can. 22(3): 713-719.

28. ————, RUCKER, R. R., and EWING, W. H. 1966. Description of a bacterium associated with redmouth disease of rainbow trout *(Salmo gairdneri)*. Can. J. Microbiol. 12(4): 763-770.

29. SMITH, R. F., MIESCHER, P. A., and GOOD, R. A. 1966. Phylogeny of Immunity. Univ. of Florida Press, Gainesville, Fla. 276 pp.

30. SMITH, W. W. 1940. Production of anti-bacterial agglutinins by carp and trout at 10°C. Proc. Soc. Expt. Biol. Med. 45: 726-729.

31. SPENCE, K. D., FRYER, J. L., and PILCHER, K. S. 1965. Active and passive immunization of certain salmonid fishes against *Aeromonas salmonicida*. Can. J. Microbiol. 11(3): 397-405.

32. STONE, H. D., RITCHIE, A. E., and BONEY, W. A. 1969. Immunization of chickens against Newcastle disease with beta-propiolactone-killed virus antigen administered in drinking water. Avian Diseases 13(3): 568-578.

33. SUMMERFELT, R. C. 1966. Identification of fish serum protein with antibody activity. Bull. Wildl. Dis. Assoc. 2: 23-30.

34. THOMSON, D., THOMSON, R., and MORRISON, J. T. 1948. Oral vaccines. Williams and Wilkins Co., Baltimore, Md. 329 pp.

35. WATSON, L. J., PAULISSEN, L. J., and YEN-WATSON, B. 1968. Serum protein changes and C-reactive protein presence in fish after injection of *Aeromonas liquefaciens* and *Streptococcus* OX39. Bact. Proceed. M6.