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IMMUNIZATION WITH VACCINES

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World demand for high quality protein foods has stimulated a rapid development of intensive iish culture techniques. Inherent with the intensive rearing of fish are related problems of high loading densities, declining water quality, adequate diets, handling, and disease control. Man has only recently recognized the threat imposed by disease and its limitation on economic development of the aquaculture industry (Roberts 1978).

When confronted with a disease problem, the producer has had essentially only two expensive and potentially devastating options at bis disposal: a) antibiotic prophylaxis and treatment of fish (which could affect palatability of food and may result in the development of resistant strains of bacteria); and b) destruction of all fish at the station followed by thorough hatchery disinfection and reintroduction of disease-free stock. Both the producer and the fish health specialist appreciate that while some chemotherapeutants for controlling disease have proved very successful, the availability of registered drugs and chemotherapeutics is decreasing.

In recent years, a new technique for the prevention of fish diseases is rapidly emerging as a result of research into the development of fish **vaccines.** Fish immunology has a more recent history than human and veterinary immunology but the techniques used are similar. However, methods of administering vaccines to fish differ and are dependent upon species, pathogen, temperature, and environment (Anderson 1974).

Immunity is an important physiological mechanism in animals for protection against infectious disease agents and the maintenance of internal homeostasis (Ingram 1979). Work by Duff (1942) involving the oral immunization of cutthroat trout against furunculosis provided the first evidence that fish possess an immune

response system. Subsequent oral immunizatton studies by Post (1963), Krantz et al. (1963), Ross and Klontz (1965). Spence et al. (1965) and Klontz and Anderson (1970), using various salmonid species, substantiated Duffs earlier work.

In very general terms, fish are protected from infectious diseases by non-specific barriers such as the mucus, scales and epidermis (Anderson 1974); by non-specific factors which include complement, interferon, and lysozymes (Anderson 1974; Ellis 1978; Ingram 1979); and by specific defense mechanisms such as antibody production (Anderson 1974; Ellis 1978; Ingram 1979). An antibody is a specific immunoglobulin (modified protein) produced in response to and that reacts specifically with an antigen. An antigen is any foreign substance which is capable, under appropriate conditions, of stimulating the formation of antibodies and reacting with the produced antibodies, in a detectable manner (Davis et al. 1967). Vaccines or bacterins contain antigens that are generally attenuated or killed disease agents which, when administered to a host, stimulate the production of specific antibodies or non-specific resistance to that particular disease agent. The protection conferred to an immunized animal by the production of antibodies and other factors will enhance its chances of survival when subjected to a natural challenge by the pathogen.

Intensive research into vaccine development commenced in the early 1970's and has resulted in production of bacterins for three salmonid diseases: enteric redmouth (Yersinia ruckeri), vibriosis (*Vibrio* anguillarum) and furunculosis (Aeromonas salmonicida). All three bacterins have been licensed by the United States Department of Agriculture/Animal and Plant Health Inspection Service (U.S.D.A./A.P.H.I.S.) and are commercially available. Vaccines for the salmonid viruses (IPN, IHN, VHS), and a bacterin for bacterial kidney disease (*Renibacterium* salmoninarum) are still in the developmental stages. Biologics for diseases of warmwater fishes, as well as other vertebrates and invertebrates raised in aquaculture, are also being planned (G. Tebbit 1981, Wildlife Vaccines, Wheatridge, CO, personal communication). Ambient water temperature, size and species have a direct effect on the immune response in fish and should always be considered at the time of immunization. Research has demonstrated that fish respond faster immunologically and retain immunity longer in a manner directly proportional to increasing water temperature and fish size (Fender and Amend 1978; Amend and Eshenour 1980).

DELIVERY SYSTEMS

Fish can be vaccinated by a variety of methods. Two delivery systems that have been used successfully to immunize fish include: (a) immersion/spray-shower vaccination and b) vaccine injection.

Immersion/Spray-Shower Vaccination

The immersion method is a fast, efficient and economical way to vaccinate. It is particularly suited to small fish (1-4 g) (Antipa and Amend 1977; Egidius and Anderson 1979; Amend and Eshenour 1980). Spray-shower vaccination is a variation of immersion vaccination designed and recommended for fish larger than 4 g. An added advantage of this system over the immersion method is the

Vaccinating large rainbow trout (10-15 cms) in the field (British Columbia Min. of Env.)

fact that greater weights of fish can be vaccinated more economically with less effort (Amend and Eshenour 1980). Under optimum conditions, following antigenie stimulation, 2-4 wk are required before protective immunity develops, Therefore, the producer should allow sufficient time for immunization before the first expected outbreak of disease.

PROCEDURES

Commercially available fish vaccines or bacterins should always be used according to the recommendations included with the product. The procedures have been substantiated by the manufacturer and approved by the U.S.D.A./ A.P.H.I.S.

1. Immersion Vaccination

   This procedure involves the following steps:

   a. Determine the amount of vaccine required by referring to the manufacturer's reference chart or labels.

   b. Prepare the vaccine solution based on the total weight of fish to be immunized.

   c. Crowd the fish into a confined area.

   d. Weigh the fish or inventory by displacement, if necessary.

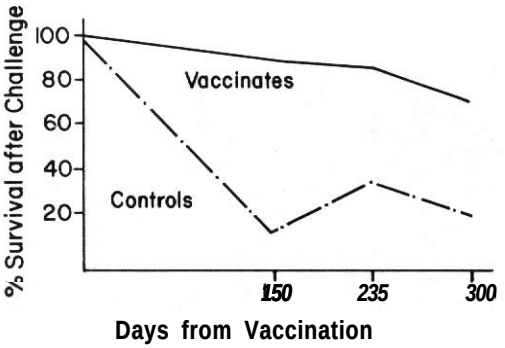
   e. Immerse fish in the vaccine solution,

   f. Expose the fish for the proper length of time.

   g. Return fish to the rearing area.

To insure immunization efficacy, do not exceed the manufacturer's recommended number of immersed lots of fish. When this number has been reached discard the vaccine and prepare a fresh solution.

Fig. I. Graph showing high levels of protective immunity in salmon after vaccination with Wildlife Vaccines' Vibrio anguillurum bacterin.*



* Reprinted *with permission from* Wildlife *Vaccines Inc. ERM and Vibrio Bacterin* Product Literature.

2. Spray-shower Vaccination

This involves the following steps:

a. Prepare the bacterin as recommended by the manufacturer.

b. Place the bacteria solution in the reservoir of a system calibrated to deliver a prescribed volume of vaccine per minute through a fine apray-shower nozzle.

c. Place fish in the immersion vaccination unit and allow a contact exposure time of 2-5 s from a distance of 30-50 cm.

d. Return fish to the holding area.

e. Discard bacterin solution after recommended use.

DEGREE OF Success

Field and laboratory studies have reported survival rates of 80-97% in terms of relative percent survival (Table 1, Fig. 1) (Antipa and Amend 1977; Croy and Amend 1977; Sawyer and Stout 1977; Lannan 1978; Gould et al. 1979; Antipa et al. 1980; Amend and Eshenour 1980; and G. Tebbit 1981, Wildlife Vaccines, Wheatridge, CO, personal communication).

Table 1. Documented results from field vaccinations conducted from February 1978 to August 1980. *

	Starting Population	ERM Mortality	Medication Fed-(kg)	Conversion Rates(a)
Vaccinated	22959.329	299,815 (1.3%)	91,295	1.9
Non-vaccinated	4,272,728	342,642 (8.0%)	74,600	2.2

a) Kg of food fed per kg of fish gained.

*Reprinted with permission from Wildlife Vaccines Inc. ERM and Vibrio Bacterin Product Literature.

Under optimal conditions, fish can retain their immunity for well in excess of 300 days if temperatures are favourable (Amend and Eshenour 1980).

Problems and Precautions

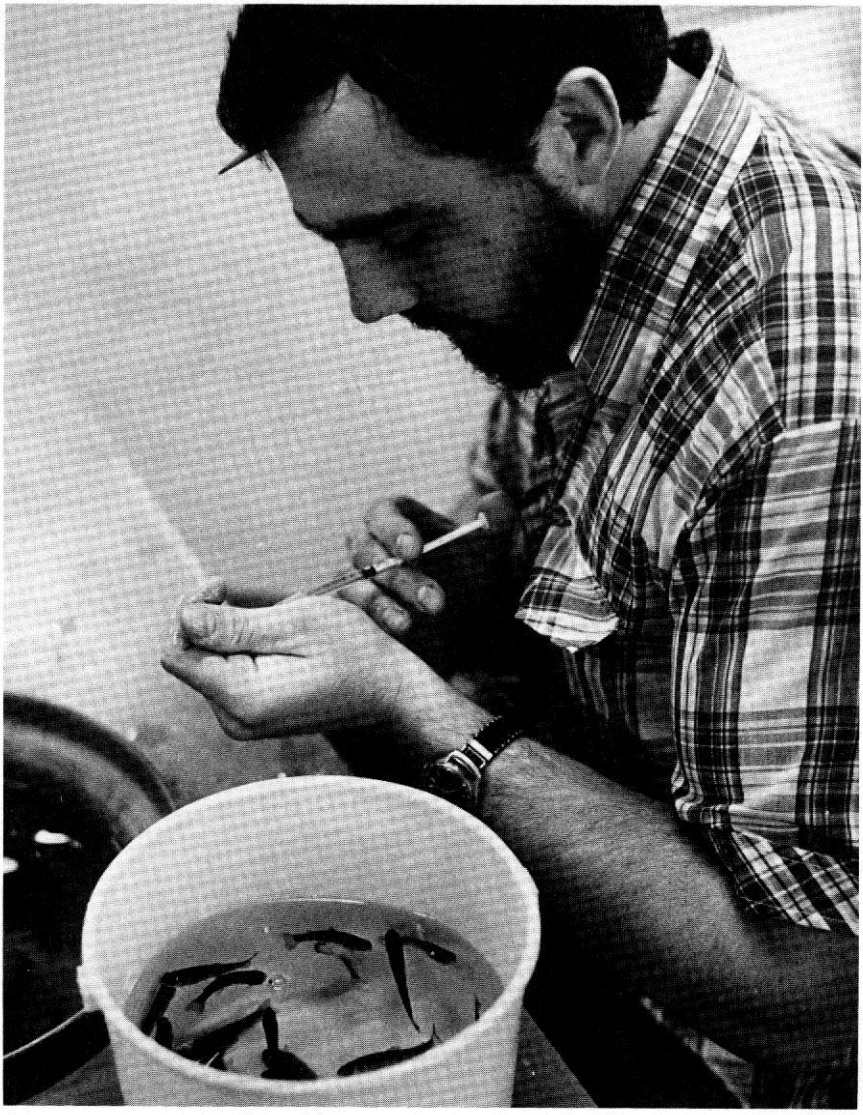
To date, few problems have been encountered if immersion and spray-shower vaccination are administered according to the manufacturer's recommended procedures. Potential problem areas of which the producer should be aware include: treatment stress, especially when vaccinating fish less than 1 gin weight (greater than 450/lb); improper vaccine dilution; and exceeding the prescribed number of immersed lots resulting in a reduction of vaccination efficacy. Precautions to be taken include: not vaccinating fish during an epizootic; not feeding fish 12 h prior to handling and treatment; not storing the diluted vaccine solution; and not vaccinating fish within 21 d of slaughter because the bacterins contain oxytetracycline hydrochloride as a preservative (Tavolek Inc. 1978, and Wildlife Vaccines Inc., 1981).

Useful Features

Immersion and spray-shower vaccinations provide rapid administration, easy adaptation to different fish culture situations, cost effective treatment, reduced treatment stress, less dependence on fish size at vaccination, and allow the administration of several vaccines in combinations.

Injection

Vaccines also can be administered by injection. Subcutaneous, intraperitoneal, and intramuscular inoculations can be used but intraperitoneal injection is preferred because of the rapid development of protection and ease of administration (Anderson 1974).



Injecting individual fish with vaccine (U.S. Fish and Wildl. Serv.)

Equipment

- Vaccine and diluent (sterile 0.85% saline)
- Automatic Cornwall-type repeating syringe with stainless steel needles
- Nets and seines
- Plastic containers
- Anesthetic
- Balance or scale
- Pencil and paper

Procedure

Vaccines for injection purposes are prepared and standardized to deliver a prescribed amount of antigen per unit-weight of fish. Experienced personnel in this area should be consulted prior to the use of this technique.

Degree of Success

Factors such as handling costs, treatment stresses, processing time. and fish size, limit injection as a method of choice for vaccine administration. However, use of an automatic repeating syringe allows an experienced individual to inject up to 1,000 fish per hour at minimal cost to the producer (Antipa 1976). Studies have shown that intraperitoneal injection provided protection slightly superior to immersion vaccination (W. Paterson 1981, Connaught Labs, Toronto, Ont., personal communication).

Problems and Precautions

Vaccine injection is now recognized as a safe, effective, economical means of vaccinating fish. Some potential problem areas of which the producer should be aware include: excessive treatment stresses which can result in mortalities or predisposition of the fish to infection by ubiquitous, water-borne, opportunistic pathogens; poor inoculation technique which can cause excessive tissue damage at the inoculation site and result in necrosis, infection, and/or internal organ damage and death; and improper vaccine preparation or failure to maintain vaccine in constant suspension resulting in a reduced introduction of antigen and a concomitant low level of protection (Anderson 1974). Precautions to be taken are similar to those for immersion and spray vaccination.

Useful Features

The advantages of injection include the incorporation of adjuvants into the vaccine which enhance the immune response and the option to include multiple antigens that can be injected in combination. Injectable vaccines have been used primarily to protect valuable broodstocks or genetic strains that are limited in numbers. In these cases, injection may prove more feasible than immersion vaccination. Furthermore, intraperitoneal injection may be required for vaccines developed in the future and, as such, the development of advanced methods will overcome disadvantages of the injection delivery system.

A number of other significant benefits have been demonstrated as a direct result of vaccination, including reduced need for antibiotic prophylaxis, faster growth rates, and improved feed conversion (C. Tebbit, Wildlife Vaccines, Wheatridge, CO, personal communication). Although the benefits of fish vaccination are substantial, the hatchery operator should not view vaccination as a panacea or as a substitute for effective husbandry practices, but rather as one of many disease preventative measures available for controlling fish diseases and ultimately maximizing fish survival and monetary return.

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