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Use of Vaccines in Finfish Aquaculture[1]

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Introduction

Disease is one of the most important limiting factors in aquaculture. Optimal husbandry and general management—including biosecurity, nutrition, genetics, system management and water quality—are critical for maximizing aquatic animal health. However, all facilities are vulnerable to disease outbreaks because many pathogenic (disease-causing) organisms (e.g., bacteria, viruses, fungi, and parasites) are opportunistic and present in the environment, or may be found on some fish that are not showing signs of disease (carriers).

For most warmwater aquaculture facilities, disease prevention consists primarily of good husbandry techniques. When disease outbreaks occur, diagnostics are conducted to determine the cause, and then the fish are given an oral treatment, an immersion (a dip or a bath), or, in rare cases, an injection treatment. Costs incurred from delayed production and growth, treatment chemicals, mortalities, and labor can be significant. In many cases, when fish are no longer eating, treatment options become much more limited and treatment may no longer be effective. It makes sense, then, that prevention of disease is preferable to disease treatment (Grisez and Tan 2005).

Two approaches to disease prevention that historically have been used in other animal industries are vaccines and immunostimulants. Both approaches have been used successfully in some aquaculture industries, and should be considered fish health management options. This publication will address vaccine use; immunostimulants will be discussed in another publication.

What is a vaccine?

A vaccine is any biologically based preparation intended to establish or to improve immunity to a particular disease or group of diseases. Vaccines have been used for many years in humans, terrestrial livestock, and companion animals against a variety of diseases.

Vaccines work by exposing the immune system of an animal to an "antigen"—a piece of a pathogen or the entire pathogen—and then allowing time for the immune system to develop a response and a "memory" to accelerate this response in later infections by the targeted disease-causing organism.

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Vaccines are normally administered to healthy animals prior to a disease outbreak.

One analogy used for vaccines is that of an insurance policy (Komar et al 2004). A vaccine, if effective, can help prevent a future disaster from being a major economic drain. But vaccines, like insurance, have a premium, or cost. The producer must weigh the cost in materials and labor against the risk and cost of a disease outbreak to determine whether vaccination is warranted. When actual vaccine effectiveness is also unknown, this makes decision-making even more difficult. Consultation with a fish veterinarian or other fish health specialist will be helpful when examining cost vs. benefit of a particular vaccine.

What are the properties of the ideal vaccine?

The ideal vaccine:

1. is safe for the fish, the person(s) vaccinating the fish, and the consumer;
2. protects against a broad strain or pathogen type and gives 100% protection;
3. provides long-lasting protection, at least as long as the production cycle;
4. is easily applied;
5. is effective in a number of fish species;
6. is cost effective; and
7. is readily licensed and registered (Grisez and Tan 2005).

What are the different types of vaccines?

There are many different types of vaccines, and new kinds are continuously under development. Of the types currently in use, the most common are described below.

Bacterins are vaccines comprised of killed, formerly pathogenic bacteria. Bacterins stimulate the antibody-related portion of the immune response (i.e., the humoral immune response).

Live, attenuated vaccines are comprised of live micro-organisms (bacteria, viruses) that have been grown in culture and no longer have the properties that cause significant disease. Live attenuated vaccines will stimulate additional parts of the immune system (i.e., a cell-mediated, as well as a humoral [antibody] response).

Toxoids are vaccines comprised of toxic compounds that have been inactivated, so they no longer cause disease. An example, used in humans, is the tetanus toxoid vaccine.

Subunit vaccines are made from a small portion of a micro-organism (rather than the entire micro-organism) that ideally will stimulate an immune response to the entire organism.

Other types of vaccines in development use even more modern strategies. Examples include *recombinant vector vaccines*, which combine parts of disease-causing micro-organisms with those of weakened microorganisms, and DNA vaccines. Recombinant vector vaccines allow a weak pathogen to produce antigen. *DNA vaccines* are composed of a circular portion of genetic material that can, after being incorporated into the animal, produce a particular immune-stimulating portion of a pathogen (i.e., antigen) continuously, thus providing an "internal" source of vaccine material. Other vaccine strategies are also undergoing research and development.

How are vaccines given to fish?

Vaccines are administered to fish in one of three ways: by mouth, by immersion, or by injection. Each has its advantages and disadvantages. The most effective method will depend upon the pathogen and its natural route of infection, the life stage of the fish, production techniques, and other logistical considerations. A specific route of administration or even multiple applications using different methods may be necessary for adequate protection.

Oral vaccination results in direct delivery of antigen via the digestive system of the fish. It is the easiest method logistically because feeding is a normal, ongoing part of the production schedule. Stress on the fish is minimal, and no major changes in

production are required. Prior to feeding, vaccine is mixed, top-dressed, or bioencapsulated into the feed. To reduce leaching into the water and/or to provide some protection against breakdown of the vaccine by the fish's digestive processes, a coating agent is often used. For small fish (e.g., 1-5 g or less), bioencapsulation may be a preferred method of oral delivery. Live food (rotifers, brine shrimp) is added to a concentrated vaccine solution, and allowed to take up vaccine. This live food is then fed to fry or small fingerlings. Although oral vaccine is the most preferred method, it conveys relatively short immunity (compared to the other methods) such that additional vaccination may be required. In addition, because of the problems involved with getting the vaccine intact through the intestine and adequately stimulating the immune system, there are few commercial oral vaccines available (Komar et al. 2004).

Immersion vaccination permits immune cells located in the fish's skin and gills to become directly exposed to antigens. These immune cells may then mount a response (e.g., antibody production), thus protecting the fish from future infection. Other types of immune cells in the skin and gills carry antigens internally, where a more systemic response will also develop. Immersion vaccination occurs by dip or by bath. Dips are short, typically 30 seconds, in a high concentration of vaccine. Baths are of longer duration—an hour or more—and in a much lower concentration of vaccine. In practice, dips are logistically more practical for large numbers of small (1- to 5-g) fish. Unfortunately, protection using immersion methods may not last long and a second vaccination may be required (Komar et al. 2004) because smaller, younger fish may have immature immune systems and because this is a more indirect route.

Injection vaccination allows direct delivery of a small volume of antigen into the muscle (intramuscular (IM) injection) or into the body cavity (intracoelomic [ICe= intraperitoneal or IP] injection), allowing for more direct stimulation of a systemic immune response. Injection vaccines normally include an oil-based or water-based compound, known as an adjuvant, that serves to further stimulate the immune system. Injection is effective for many pathogens that cause systemic disease; and protection—6 months to a year—is much longer than by other methods. Every fish in the population is injected, giving more assurance to the producer. Another advantage is that multiple antigens (for different diseases) can be delivered at the same time. However, vaccination by injection is logistically the most demanding of all three methods. Fish must be anesthetized to minimize stress. Injection requires more time, labor, and skilled personnel. The correct needle size is important. The vaccine may incite a more severe reaction if it is injected into the wrong portion of the fish. And finally, smaller-sized fish (under 10 g) may not respond well to this method (Komar et al 2004).

Have vaccines been used in fish?

Vaccines have been used in food fish, in particular the salmon industry, for approximately 30 years, and are believed to be one of the main reasons that salmon production has been so successful. Vaccination of salmon also dropped the industry's use of antibiotics to a mere fraction of its original use (Sommerset et al 2005). In Norway, for example, in 1987, before widespread use of vaccines, approximately 50,000 kg of antibiotics were used. By 1997, when vaccines had become more routine, antibiotic usage had dropped to less than 1000-2000 kg (Sommerset et al. 2005).

What fish vaccines are available in the United States?

A current list of U.S. Department of Agriculture (USDA)-licensed vaccines for fish and their manufacturers can be found at the following USDA Web site:
http://www.aphis.usda.gov/animal_health/vet_biologics/publications/aquaproducts.pdf

In the U.S., most of the vaccines available commercially have been developed for use in salmonids. For salmonid bacterial diseases, bacterins are available for furunculosis (caused by *Aeromonas salmonicida*); vibriosis (*Listonella anguillarum* and *V. ordalii*); enteric redmouth (*Yersinia ruckeri*); winter ulcer disease (*Moritella viscosa*); bacterial kidney disease (*Renibacterium salmoninarum*); and

salmonid rickettsial septicemia (*Piscirickettsia salmonis*). In addition to separate bacterins, a number of vaccines are available for salmonids that target multiple diseases. A live attenuated vaccine is available for the salmonid viral disease infectious salmon anemia (USDA-APHIS-Center for Veterinary Biologics [CVB].

Are commercial vaccines available in the United States for warmwater fish?

Currently, two live attenuated vaccines have been developed and are commercially available in the U.S. for enteric septicemia of catfish (caused by the bacterium Edwardsiella ictaluri) and columnaris disease (caused by the bacterium *Flavobacterium columnare*) (USDA-APHIS-CVB).

Are vaccines available in other countries and can we use them in the United States?

Knowledge of what vaccines are available in other countries allows U.S. producers to understand how their competitors manage specific diseases and whether or not access to these vaccines through discussions with manufacturers will be worthwhile. There are a number of vaccines commercially available in other countries (Biering et al. 2005; Hastein et al. 2005).

Ultimately, commercial vaccine availability is a matter of economics. Manufacturers of these vaccines must first decide whether or not the U.S. market will be economically beneficial to them. Next, they must submit their vaccine(s) to the USDA-APHIS-CVB for official licensing, and this can be a lengthy process. After all licensing, testing, and other requirements are completed, the vaccine would be available for U.S. producers.

A number of vaccines are not currently available in the U.S. but have been, or are registered and commercially available in other countries. For the prevention of bacterial diseases, vaccines include those for streptoccocosis (caused by *Streptococcus iniae* and related bacteria); pasteurellosis (*Photobacterium damsela* subsp. *piscicida*); warmwater vibriosis (*V. alginolyticus*, *V. harveyi*, *V. vulnificus*, and *V. parahaemolyticus*); and lactococcosis (*Lactococcus garvieae*). Vaccines for the prevention of viral diseases include those for koi herpesvirus (CyHV-3); infectious pancreatic necrosis (IPN); infectious hematopoietic necrosis (IHNV); viral hemorrhagic septicemia (VHS); salmon pancreatic disease (SPD); grass carp aquareovirus (GCR); and iridovirus in *Seriola spp*. (Grisez and Tan 2005; Hastein *et al*. 2005).

What are the differences between autogenous vaccines and licensed, commercial vaccines?

Licensed, commercial vaccines are produced by a manufacturer for a specific fish species or species group and for a specific disease. Commercial vaccines (and other products considered under the umbrella term, "biologics," meaning of biological origin) are regulated and licensed by the USDA-APHIS Center for Veterinary Biologics (CVB), http://www.aphis.usda.gov/animal_health/vet_biologics/, under the Virus-Serum Toxin Act, http://www.aphis.usda.gov/animal_health/vet_biologics/publications/VSTA.pdf, and go through rigorous testing for safety and effectiveness.

Autogenous vaccines are made from a particular pathogen (commonly a specific bacteria) that has been isolated from diseased fish at a specific facility. Although autogenous vaccine production also must follow strict guidelines, there is more flexibility with use of these products. Autogenous vaccines are restricted to use by or under the direction of a licensed veterinarian within the context of a veterinarian-client-patient relationship (VCPR--see the description in the box below). There are situations when a USDA-licensed commercial vaccine is not available, and autogenous vaccines are an excellent solution in these cases. For more information on autogenous vaccine regulations, see 9CFR113.113 (Code of Federal Regulations), http://edocket.access.gpo.gov/cfr_2003/9cfr113.113.htm, and V.S. Memos 800.69 and 800.103 (USDA-APHIS Veterinary Services guidance documents, available on line in numerical order beginning at:

http://www.aphis.usda.gov/animal_health/vet_biologics/vb_vs_memos.shtml).

A valid veterinarian-client-patient relationship is one in which:
(1) A veterinarian has assumed the responsibility for making medical judgments regarding the health of (an) animal(s) and the need for medical treatment, and the client (the owner of the animal or animals or other caretaker) has agreed to follow the instructions of the veterinarian;
(2) There is sufficient knowledge of the animal(s) by the veterinarian to initiate at least a general or preliminary diagnosis of the medical condition of the animal(s); and
(3) The practicing veterinarian is readily available for follow-up in case of adverse reactions or failure of the regimen of therapy. Such a relationship can exist only when the veterinarian has recently seen and is personally acquainted with the keeping and care of the animal(s) by virtue of examination of the animal(s) and/or by medically appropriate and timely visits to the premises where the animal(s) are kept.

Within the context of a veterinarian-client-patient-relationship, USDA-licensed, commercial vaccines can be used for other species or other indications—for example, a related bacterial or viral disease other than those for which the vaccines were originally manufactured. For example, the commercially available, USDA-licensed catfish vaccine for columnaris disease can be used against columnaris in other species, under the direction of a veterinarian. Biotests should be run on small numbers of fish before large-scale use to determine safety and efficacy.

Under specified conditions, autogenous vaccines may also be used for animal populations that are close to the facility for which the autogenous vaccine was originally manufactured. Autogenous vaccines have been developed and used successfully in ornamental fish aquaculture (Russo et al. 2006a; Russo et al. 2006b).

What is required to develop a vaccine for a specific disease?

A number of factors and a certain amount of information are necessary to facilitate vaccine development and use. The specific farming and husbandry methods should be well understood, and relatively intensive culture of a fish with good management is ideal. There should be a good understanding of what organism causes the disease under study, and under what conditions. In some cases, if scientific resources are available, the biological structure of relevant antigens (parts of the pathogen that incite the best immune response) can be determined and used. The vaccine should be administered in a way that will stimulate the proper immune response and should mimic how the pathogen infects the fish naturally. Knowedge of the fish species' immune system development will also help identify how early the fish can be vaccinated and when during the production cycle the vaccine should be administered (Grisez and Tan 2005).

Manufacturers and researchers must run numerous experimental trials when developing and testing vaccines for commercial production. During these trials, vaccinated and unvaccinated groups of fish are challenged (infected in the laboratory with a given pathogen) to determine the effectiveness of a given vaccine. Researchers must have a good understanding of the disease and the fish species, and should be able to grow the disease organism easily.

An autogenous vaccine or bacterin for a facility with a specific disease problem can be manufactured much more rapidly than a commercial vaccine or bacterin can be developed and licensed. Autogenous vaccines for a specific pathogen causing disease in a specific facility can be developed based on the guidelines identified above (9CFR113.113 and V.S. Memos 800.69 and 800.103). Briefly, the facility must work with a veterinarian or a qualified fish health specialist; the pathogen of concern (in most cases, bacteria) must be identified and cultured from diseased fish at the facility; and then, using that particular isolate/strain of pathogen, the autogenous product can be produced by the veterinarian or by a USDA-licensed vaccine production facility, following appropriate guidelines. This process may

take several months or longer, depending upon the situation. Additional rules regarding use of this autogenous product, including length of time for use and how many times the product can be reissued, must also be followed.

Bottom line, as much information as possible is needed to ensure that other factors that contribute to disease are minimized and that vaccination occurs at the best time during production for logistical reasons and effectiveness.

Can I use vaccines after a disease outbreak has begun?

Vaccines are not the same as antibiotics and generally will not be effective for stopping a disease outbreak once it has begun. Vaccines are used to prevent a specific disease outbreak from occurring and are not a therapy. Vaccines rely upon a healthy immune system to work properly.

What factors determine how well a vaccine will work?

Just as with any other fish health management tool or technique, vaccines are not a universal preventative. Excellent husbandry, including good biosecurity and genetics, optimal nutrition, appropriate densities, life stage considerations, and system management (including water quality/chemistry) are all important for minimizing disease in general, as well as for maximizing vaccine effectiveness. Good biosecurity, including appropriate quarantine and adequate sanitation and disinfection, will minimize the potential for introduction of unwanted pathogens and reduce loading of opportunistic pathogens. Making sure the fish are as healthy as possible, given production parameter constraints, will go far toward preparing fish for vaccination and boosting their immune systems.

Proper diagnosis of a disease is also critical. Producers should work with an aquatic veterinarian or other fish health professional, and with a fish disease diagnostic laboratory to determine the major contributing factors to a disease outbreak. This will facilitate vaccine development and feasibility. Some diseases will have primarily environmental (water quality, handling) components that may not require a vaccine. Some diseases will have a specific pathogen, such as a specific strain of bacteria, for which a legal, commercial vaccine may or may not be available.

Determining when in the production cycle fish get sick (for ongoing diseases) and at what stage fish can be successfully vaccinated may require some additional diagnostics and experiments. This step is also necessary for proper vaccine use.

The various methods of administration (injection, immersion, or oral) have different advantages and disadvantages; the choice of which one to use will depend upon the situation. Water temperature during the immunization process will determine how quickly immunity will develop. Any stress during this time period will also hinder development of immunity. For warmwater species, 1-3 weeks may be required.

Commercial vaccines may or may not be adequate, and autogenous vaccines may be required for a given situation. However, since less research will have been carried out with autogenous vaccines, their effectiveness will be more variable and, ideally, small-scale trials should be run at the facility before these vaccines are put into widespread use.

Summary

In addition to optimizing husbandry and general management practices, vaccine use, although still limited, is becoming more widespread in certain sectors of aquaculture for disease prevention. A number of vaccines have been in use by the salmonid industry for decades. However, commercial vaccine development for other aquaculture sectors, including producers of warmwater fish, is still quite limited in the U.S. Greater demand by producers and increased levels of research and interest by manufacturers is helping to make vaccination a more viable option. Currently, vaccines are available for some economically important bacterial and viral diseases. Vaccines for protection against parasitic and fungal diseases have not yet been developed.

Vaccination should be considered part of a comprehensive fish health management scheme, and not the only solution for a disease problem.

Vaccination is intended for disease prevention, and not for disease treatment, and can be thought of as "insurance." To ensure that vaccination will work, producers must weigh carefully the many factors that determine whether a given vaccine will be effective in a given situation (the particular pathogen and disease, the fish species and age, fish production methods, vaccine route of administration, and economics).

Autogenous vaccines are manufactured from specific pathogens (e.g., bacteria or viruses) isolated from sick fish at a particular facility. This type of vaccine can help prevent recurrent disease problems, and should be considered a management option. However, as with commercial, licensed vaccines, strict USDA guidelines must be followed. Producers considering incorporating vaccines into their management schemes should work closely with a fish veterinarian or other fish health specialist.

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USDA-APHIS Center for Veterinary Biologics websites (November 2008):

Licensed veterinary biological products list:

http://www.aphis.usda.gov/animal_health/vet_biologics/vb_licensed_products.shtml

Currently available biologics for fish (incuding list of vaccines):

http://www.aphis.usda.gov/animal_health/vet_biologics/publications/aquaproducts.pdf

Virus-Serum Toxin Act (21USC151-159 et seq):

http://www.aphis.usda.gov/animal_health/vet_biologics/publications/VSTA.pdf

USDA-APHIS Veterinary Services autogenous vaccine guidance documents:

http://www.aphis.usda.gov/animal_health/vet_biologics/vb_vs_memos.shtml

For more information:

To find veterinarians who work with fish, go to http://www.aquavetmed.info/ or contact your state veterinary medical association.

For more information on vaccines, see the USDA-APHIS Center for Veterinary Biologics websites listed above, or contact:

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