COLLEGE OF FISHERIES
KERALA AGRICULTURAL UNIVERSITY
Panangad, Kochi - 682 506

SILVER JUBILEE SOUVENIR
1979 - 2004

Released on the occasion of National Workshop on

Fish For All
Through Quality Fisheries Education

25-27 November 2004

Organised by :

Professional Fisheries Graduates' Forum (PFGF) &
College of Fisheries Panangad Alumni Association (COFPAA)

in association with

Kerala Agricultural University

CONTENTS

Preface 1
The Silver Lining 2
College of Fisheries, Panangad Celebrates Silver Jubilee
D. D. Nambudiri 3
Virtual University: Its RelevanceTo Agricultural Education
K. V. Peter *et al* 9
The Concept And Genesis Of A Fisheries College
M. J. Sebastian 17
Professional Fisheries Education In India And The Efforts of Professional Fisheries Graduates' Forum To Improve The Quality Of Education
M. C. Nandeesha 23
Recent Trends in Value Addition of Fishery Products
K. Devadasan 34
Marine Fisheries Of India : Concerns And Challenges
Mohan Joseph Modayil 44
Brackishwater Aquafarming – Prospects And Constraints
P. Ravichandran And S.M. Pillai 48
Statistical Tools In Fisheries Research
T.M. Sankaran 52
Oil Sardine Fishery Of India; Is There A Long Period Variability ?
K.K. Varma 55
Environment Friendly Aquaculture
Susheela Jose 58
Globalization In Indian Seas – Impact On Fisheries
K.S. Purushan 63
Sustainability Of Pokkali Farming System
C. G. Rajendran *et al* 68
Status Of Aquaculture Developmentin Kerala
T.D. Velayudhan 73
Drugs From The Sea
P.M. Sherief 87
Aquaculture In India – A N Overview Of The Present Scenario
C.M. Nair 95
Biodiversity Of Palaemonid Prawns Of India
K. V. Jayachandran 100
Indigenous Ornamental Fishes Of The Western Ghats Of India.
T.V. Anna Mercy 103

Industrial Fishing And Its Ecological Impact
J. Rajasekharan Nair 113
Technological Innovations In Drying Of Seafoods
S. Krishnakumar 117
Health Management In Aquaculture
K. Riji John And M. Rosalind George 120
Vaccines In Aquaculture
K. S. Sobhana 126
Environmental Impacts Of Aquaculture
S. Shyama 131
Role Of State Fisheries Resource Management Society (Firma) In Fisheries Development In Kerala
U. S. Sajeev 137
Gender Issues In Fisheries
Daisy C. Kappen 146
Awards, Prizes, Fellowships etc. Awarded To The Faculty i
Important Books Authored / Edited By Faculty ii
List Of Teaching Staff iii
Former Faculty Members iv
List Of BFSc. First Rank Holders v
Small Aim Is A Crime-
Dr. S. J. Kaushik Meets His Excellency, President Of India. ..... a
Proud Moments Of The BFGI Selection - 2003 ..... b
BFGI Selection - 2004 c
Developement Of Leadership Qualities
T. J. Varghese 149
Reaching The Goal
Swami Anupamanandaji 151
Value Based Professional Career
S.H.Somashekar 153
Inland Fishery Resource Management In Kerala
Challenges And Opportunities
K.G.Padmakumar 157
How To Be Successful As A Fisheries Professional -
Feed Back From Successful Professionals
M.C.Nandeesha 167
Quality Assurance In Fisheries Education
P.S.Bijulal 195
Obutuary To Late Mr. Debabrata Mohapatra 197
Impressions Of The Participants Of BFGI –2004 Selection 198

VACCINES IN AQUACULTURE

K. S. SOBHANA
Scientist (SS), PNP Division, CMFRI, Kochi-18

Prevention of infectious diseases is one of the major concerns in the rapidly growing aquaculture industry. Stress and disease that accompany intensive fish culture have led to increased treatment with antibiotics and chemicals. The use of drugs and antibiotics in aquaculture is highly regulated to avoid risks to public safety and to prevent the development of resistant strains of pathogens. Consequently, farmers are left with few resources other than the use of preventive measures such as early diagnosis, good husbandry techniques, vaccination, and the use of strains of fish and shellfish genetically resistant to diseases. The best alternative in this direction would be stimulating the immune system of the fish itself, thereby equipping it with desirable adaptive changes in the defence mechanisms against common pathogens.

Vaccination has emerged as an important immunoprophylactic fish health management technique in reducing fish mortalities in aquaculture systems. Although fish do not have bone marrow or lymph nodes, the thymus, kidney, and spleen constitute important organs of the immune system. It has been shown that specific antibodies and cell-mediated responses can be generated upon antigenic challenge. This adaptive immunity is specific to the challenging pathogen and persists for a relatively long period of time (immune memory). Specificity and memory are two of the key elements exploited by vaccination. Vaccines should be safe and potent. Though it is possible to achieve these characteristics using simple procedures like heat or chemical inactivation of cultures of pathogenic microorganisms, in most cases some degree of antigen purification is necessary. It is important to identify the relevant antigens which are important in stimulating a protective immune response.

The development of effective vaccines for the prevention of diseases caused by viruses, parasites, and intracellular bacteria in finfish has proven to be a difficult task. As a result, few commercial vaccines are available for inoculation against these kinds of diseases. Successful vaccines have been developed for bacterial diseases like Vibriosis, Enteric Red Mouth and Furunculosis that are now commercially available for salmonid fish. However, there are still no effective vaccines available for protection against several important bacterial pathogens of fish. Currently, several vaccine development programmes are under way for e.g. for Pasteurellosis, Proliferative Kidney Disease, Bacterial Kidney Disease, Rainbow Trout Fry Syndrome, *Aeromonas hydrophila* etc. Traditional vaccines

used to prevent fish diseases consist of either killed pathogens (called bacterins in the case of bacteria) or attenuated versions of the pathogen (live vaccines). Other alternatives being investigated include the use of purified or genetically engineered antigens from the pathogen (recombinant vaccines) and DNA vaccines.

Anti-bacterial vaccines

Efforts in the early 1970s lead to the development of vaccines against several bacterial strains, e.g. *Vibrio anguillarum*, *Aeromonas salmonicida*, *V. salmonicida*, and *V. ordalii*. The first of the vaccines licensed by the US Department of Agriculture were those against *Yersinia ruckeri* and *V. anguillarum*. The popularity of these initial vaccines was due to the immersion method as the intended means of administration. Injectable vaccines containing antigens against *A. salmonicida*, *A. salmonicida* plus *Y. ruckeri*, and *A. salmonicida* plus *V. anguillarum* were later made available. To enhance the performance against *A. salmonicida*, an adjuvant was incorporated in the formulation. Aluminum salts and glucan produce a moderate effect in improving the potency, while oil-based adjuvants (mineral oil) provide a higher level of protective immunity.

The acceptance of injectable vaccines in aquaculture was in part due to the heavy economic loss in the 1980s as a result of an outbreak of furunculosis infection in salmon. Currently, injectable polyvalent vaccines are in demand because salmonid fish are prone to infections by multiple bacterial strains. Almost all Atlantic salmon smolts are vaccinated with 'triple vaccines' against vibriosis, cold-water vibriosis and granulomatous furunculosis, 2–3 months prior to sea-transfer. It is well recognized that vaccination by injection yields the strongest immunity, followed by immersion and spraying methods with intermediate levels of protection, followed by oral vaccination.

Anti-viral vaccines

Important fish viral diseases include infectious pancreatic necrosis (IPN), viral hemorrhagic septicemia (VHS) and infectious hematopoietic necrosis (IHN). The development of vaccines for these diseases was rather unsuccessful until recently. The difficulty in developing anti-viral vaccines lies in the fact that these diseases occur primarily at the fry age of fish, making it difficult to use injection as the means of vaccine administration. Early prototype vaccines containing inactivated viruses developed for immersion all resulted in insufficient protection. The use of attenuated or avirulent forms of the viruses is regarded as unacceptable due to the residual virulence in targeted species and virulence in non-target species. A major advancement in the area of anti-viral vaccine research was the result of recombinant DNA technology. An injectable anti-IPN vaccine based on viral protein VP2 was formulated for use in Norway. It is added to a polyvalent vaccine as a component in a commercial oil / glucan-based formula.

Future possibilities for aquaculture vaccine delivery systems

The current art of aquaculture vaccine preparation still relies on the approach of whole broth culture, in which the bacteria are inactivated with formalin and emulsified with mineral oil or other oils. The most effective delivery route is by intraperitoneal injection. Although injectable oil-adjuvanted vaccines have been accepted by the industry for use in fish > 15g, there are still major concerns over their side-effects. Intraperitoneal injection of these vaccines leads to the formation of unsightly granulomatous lesions adhering to the viscera. In addition, mineral oil adjuvants are known to result in a reduction in weight gain of fish. Other routes of vaccine delivery have been explored, e.g. topical by immersion, by bath, or by hyperosmotic infiltration, oral and anal. The feasibility of the oral immunization method was in fact demonstrated as early as 1942 by Duff. The advantages of oral vaccine delivery in fish have been exemplified by Horne (1997) to include improved performance, easy application, increased flexibility and improved safety. The success of the anal intubation method (Johnson and Amend, 1983) further supports the hypothesis that enteral administration of vaccines is feasible provided that the antigens be protected through the anterior gut.

Different approaches have been employed in recent years to formulate oral vaccine delivery systems with certain advanced features, e.g. bio-degradable polymer as matrix (Lavelle et al., 1997). Poly (DL-lactide-co-glycolide) microspheres containing human gamma globulin (HGG) were prepared using the water-in-oil-in-water (w/o/w) emulsion / solvent evaporation method and delivered orally to rainbow trout. It was found that microencapsulated HGG was retained in the stomach for a longer duration of time compared to soluble HGG. As a result, proteolysis of surface-adsorbed antigen was detected. Nevertheless, a significant amount of intact antigen was found in the posterior intestine and in the bloodstream. This provides evidence that poly-DL-lactide-co-glycolide protects the antigen, at least partially. Unlike the mammalian intestine, which absorbs the microspheres through the microfold cells of Peyer's patches, teleosts do not appear to possess such patches. The mechanism of increased antigen absorption is thus largely unexplained.

The use of an enteric coating in protecting orally delivered vibrio vaccine was explored by Lillehaug (1989) and Wong *et al.* (1992). In 1989, Lillehaug formulated two oral vaccines against vibriosis, one of which comprised a slow-release matrix and the other consisted of lyophilized whole-cell granules coated with 10% Eugragit L 100-55 for enteric protection. The slow-release matrix was formulated with saturated long-chain fatty acids, oleic acid, lecithin, glycerol, and the like. In vivo challenge with live bacteria resulted in higher mortality rates in fish given either the slow-release or enteric-coated vaccines, compared to those dosed with unprotected vaccine. It was postulated that the enteric coat

or the slow-release matrix had prevented antigen uptake by the gut epithelial cells. The lipopolysaccharide component in the vaccines may, in fact, be sufficiently stable in the gastric fluid to allow absorption of unprotected vaccine to occur in the intestinal tract. Wong *et al.* (1992) lyophilized formalin- denatured *Vibrio anguillarum* cells and spray-coated them onto sugar beads. The antigen-loaded beads were further coated with Eudragit L-30D, an enteric coating material. The vaccine was then dosed to Coho salmon in feed. The result from the study indicated that enteric coating can be a viable approach for aquaculture vaccine delivery.

Natural bacterial populations tend to occur as assemblages enmeshed in a polymeric glycocalyx matrix called biofilm to take advantage of the nutrient concentrating effect and to gain protection against predators and toxic agents. This protective nature of bacterial biofilms was exploited for the development of an effective oral vaccine that can resist gastric destruction of epitopes, facilitating improved antigen delivery (Azad et al., 1999). Oral vaccination with biofilm cells of *Aeromonas hydrophila*, elicited a significantly higher immune response and protection in carps. The better performance of the biofilm vaccine was attributed to superior antigen delivery to the lymphoid tissues as demonstrated by antigen localisation using monoclonal antibodies (Azad et al., 2000).

Future efforts should focus on the absorption mechanism(s) of large proteins in the fish intestine. With a full understanding of such mechanisms, proper pharmaceutical delivery systems can be designed through a combination of available approaches, i.e. protection from proteolysis, improving permeability of the antigen and controlled / sustained release patterns for better immune response.

Recombinant vaccines

Recombinant DNA technology was considered to be the most suitable solution for development of vaccines against diseases in fish where traditional technologies had failed. This includes vaccines against many diseases of viral or parasite origin as well as some bacterially induced disorders. However, so far only one vaccine containing recombinant products is commercially available for use in aquaculture i.e., against IPN virus (Christie, 1997). The potential of the technology is nevertheless steadily increasing, and it is probably a question of when, rather than whether, recombinant vaccines will appear as an efficient tool for prevention of several important diseases in aquacultured fish. Recombinant DNA technology allows construction of multivalent vaccines inducing protection against two or more pathogens simultaneously, and also makes it possible to build in adjuvant and/or targeting components. Research on recombinant vaccines for fish in the sense of products fully or partly based on cloned pathogen genes has so far included : recombinant proteins/ antigens expressed in prokaryotic or eukaryotic cells by fermentation under strictly controlled

laboratory conditions; genetically attenuated pathogens; live but nonpathogenic recombinant microorganisms carrying foreign pathogen genes and vaccines based on naked DNA. These vaccine development strategies have all been demonstrated to work in fish to a certain extent, under experimental conditions.

DNA vaccines

DNA vaccines are considered the "third revolution" in vaccine development. In DNA vaccines, a gene coding for a protein from the pathogen is inserted into a bacterial plasmid. These plasmids are replicated in bacterial culture and the DNA is purified and transferred to live fish by intramuscular injection. Some cells in the fish pick up the DNA and express the protein. The immune system recognizes the protein as foreign and mounts a response against the antigen, which protects the fish when they eventually become infected with the pathogen. The main advantages of DNA vaccines are that they are simple to prepare and that DNA can be produced in large quantities with high purity. Furthermore, DNA is highly stable and resistant to temperature extremes, which facilitates the storage, transport, and distribution of vaccines. In addition to these commercial considerations for vaccine production and distribution, DNA vaccines also have immunological advantages. Since the immune system sees the expressed antigen in a way that is similar to how it sees virus and intracellular bacteria, DNA vaccines are especially useful in the prevention of diseases caused by these pathogens. In contrast to conventional vaccines, a DNA vaccine introduces only the DNA coding for a specific component of the disease causing organism into the subject. When compared to conventional vaccines they are remarkably stable over time, do not require refrigeration, and provide life-long protection against the intended disease-causing organism without the need for multiple injections. Nucleic acid vaccines have proven to be very effective in mammals, and these could provide a good alternative for piscine diseases where classical antigen-based products have failed.

DNA vaccines have been tested in fish by several research groups and have been shown to protect rainbow trout against infectious hematopoietic necrosis virus, viral hemorrhagic septicemia, and bacterial kidney disease, diseases that have a serious impact on salmonid aquaculture throughout the world. However, several problems remain to be solved before these vaccines can be used in the farms. There are safety issues that need to be addressed before vaccine companies can commercialize these vaccines. Research is on in the western countries in developing, safe plasmid constructs specially designed for the immunization of fish with DNA. These plasmid constructs should satisfy regulatory guidelines from the Food and Drug Administration, the U.S. Department of Agriculture, and the European Union.