Responsible use of vaccines and vaccination in fish production

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1. **Background.**

The Responsible Use of Medicines in Agriculture Alliance (RUMA) is a coalition of organisations including agricultural, veterinary, pharmaceutical and retail interests. This paper is one of a series of species-specific documents developed by RUMA. Initially RUMA came together to address issues of the use of antimicrobials in agriculture, and has published a summary and detailed guidance aimed at promoting responsible use of antimicrobials (available at [www.ruma.org.uk](http://www.ruma.org.uk)). This guidance advised that farmers should regard therapeutic antimicrobial products as complementing good management, vaccination, and site hygiene. It went on to repeatedly refer to the role of vaccination in reducing the need for antimicrobial medication. It is logical, therefore that we should, in this document, go on to consider vaccines and vaccination in more detail.

Initially aquaculture in the UK used antimicrobials to combat a range of bacterial diseases including Furunculosis and Vibriosis, as well as secondary bacterial infections subsequent to ectoparasites infestation caused by salmon lice. However improved husbandry combined with the widespread use of efficacious vaccines and efficient vaccination has had a major impact on the aquaculture industry, allowing economic and effective control of diseases which had previously caused major problems. Indeed the introduction of the oil-based bacterial vaccines in the late 1980’s and early 1990’s dramatically reduced the amount of antibiotics used in the salmon industry to a fraction of that previously used. However, vaccines do not provide the solutions to all of the disease challenges currently facing the aquaculture industry, but are very useful tools when used in conjunction with good management and hygiene practices.

2. **The Immune Response, in Particular the Differences from Mammals.**

All vertebrates have mechanisms for controlling pathogens – those organisms which are capable of causing disease. However fish are the most primitive organisms to have an adaptive immune system which is comparatively simple and undifferentiated compared with mammals. The immune system of fish has evolved with both non-specific (innate immunity) and acquired immune functions (humoral and cell mediated immunity) to eliminate invading foreign living and non-living agents.

2.1 Innate Mechanisms

Innate mechanisms require no previous exposure to the particular agent – this includes physical barriers such as skin and mucus layers, specialised cells such as macrophages and natural killer cells and particular soluble molecules such as complement and interferon.

The first line of defence which fish have against foreign agents, mucus and skin, contain immuno-reactive molecules (i.e., lysozyme, complement and immunoglobulin). Early research suggested that the immunoglobulin (Ig) in the
skin/mucus was non-specific in nature. However, recently specific antibody to parasites and bacteria were demonstrated in mucus. Apparently, this antibody is not produced in the serum but rather produced locally (i.e., by lymphocytes in the skin). Non-specific humoral molecules in fish include lectins (carbohydrate recognition), lytic enzymes, transferrin (iron binding protein) and components of the complement system. Non-specific cells of the fish immune system include monocytes or tissue macrophages, granulocytes (neutrophils) and cytotoxic cells. Macrophages function in phagocytosis and destruction of invading foreign agents and bacteria. Macrophage activation occurs through cytokines and immuostimulation (beta-glycan and other compounds) that increases the killing ability of these cells.

As far as the complement system is concerned, duplication and diversification of several complement components is a striking feature of bony fish complement systems. It gives an interesting insight into an evolutionary strategy for the possible enhancement of the repertoire of innate immunity. Recent studies have also confirmed the presence of functional homologues of mammalian cytokines in fish. Some of the elements of the innate defence mechanisms interact extensively with the adaptive mechanisms, which, though present in most vertebrates, are particularly well developed in mammals and birds.

2.2. The Adaptive Immune System

Acquired immunity in fish includes both humoral and cell mediated responses. Fish can display typical vertebrate adaptive immune responses characterized by immunoglobulins, T-cell receptors, cytokines, and major histocompatibility complex molecules (HSC). The cell-mediated response in fish is similar to that in mammals and relies on the presence of accessory cells (macrophages) to present antigen to T-cells. The correct presentation of antigen results in a cascade of events that includes cytokine production that regulates or enhances the cellular response.

One major difference between fish and other vertebrates is that fish lack bone marrow. The anterior portion of teleost fish kidney is most likely the source of HSCs that will later give rise to the B and T-cell lineages. T-cell development takes place in the thymus of all vertebrates based upon an assortment of criteria. In teleost fish, progenitor T-cells migrate from the kidney to the thymus for T-cell education (distinguishing self from non-self) and maturation (functional). The B-lymphocytes originate and mature within the kidney, therefore the anterior region of the fish kidney is considered to be the evolutionary equivalent of the bone marrow. B-cells of fish produce antibody when stimulated.

There are other areas in which the immune system of fish is quite different in its efficiency and complexity from that of higher vertebrates. One major difference is that fish in general are poikilothermic – in other words they adapt their body temperature to that of their surroundings – i.e the water temperature, and their metabolic rates and development of immune response are therefore directly dependent on the temperature of their aquatic environment.
Therefore, as with mammals, when a fish encounters an infectious agent, its response will depend on whether it has experienced this infectious agent previously. Aspects of both the innate response and the adaptive immune system will come into play depending on the type of infectious agent, route of infection and history of previous contact with this infectious agent. Other factors such as stress and nutrition status will also have a part to play in the quality of the response.

3. Objectives of Vaccination.

The objectives of vaccination are to prevent disease. In the aquaculture environment, when fish are kept in management methods such as in sea cages or in ponds and raceways supplied by environmental water, it is very difficult to prevent fish from coming in contact with either environmental pathogens or pathogens of wild fish origin, since contact with wild fish is often difficult or sometimes impossible to achieve. This is in contrast to many other intensive livestock keeping methods, which can by their nature isolate the livestock from wild members of the same species.

Treatment of fish following infection can also present challenges – in general, medications in the commercial farming situation are administered either in-feed or by immersion. In the case of administration of medicinal products such as antibiotics the injection route is reserved for small numbers of individual high value subjects such as broodstock.

Bathing or immersion of fish in medicinal products for large numbers of fish of significant size in a marine situation such as salmon in sea cages also can be technically a very demanding operation.

Therefore in-feed medication is the commonest route of administration. However, there are also some problems associated with this route. Not only can some products, such as some anti-bacterials have palatability issues, there are other factors to consider such as bullying where the larger stronger fish will eat more of the medicated feed, to the really major issue in all of this that the first thing which happens when a fish becomes unwell is that its appetite is reduced, so the chances of treating sick fish are therefore much reduced. Therefore in-feed medication will only work on fish which are in the very earliest stages of succumbing to a particular disease.

The other problem in treating fish is that it can be logistically difficult to observe signs – a significant amount of diagnosis is done by post-mortem which can make it difficult to ensure medication sufficiently early in an acute outbreak.

Finally, although there are a number of viruses which cause problems in aquaculture, there is no medication available in aquaculture for the treatment of viruses, only the secondary opportunistic bacteria.

Since the main objective of vaccination is to increase the specific immunity to infections to which the vaccinated fish are likely to be exposed this general objective can be applied to the individuals of a vaccinated population, just as it
does to whole populations, tanks, netpens, sea sites, farms, companies. The associated economic objective is to ensure that, on average, the cost of the vaccines purchased, their application, and any loss of productivity caused by their application, is less than the cost of the disease if vaccines are not used.

Unlike any other class of livestock, there are limited opportunities during the lifetime of farmed fish when vaccines can be administered. The earliest point in the life of the fish when it can be vaccinated is after it becomes immunocompetent (some time subsequent to when the yolk sac has been absorbed). However, vaccine administration is then limited by the physical size of the fry and may only be possible by the immersion or oral routes which are limited in their duration of immunity.

There are minimum sizes of fish for injection vaccination, and these sizes are determined by the companies developing the vaccines and the size of fish in which they were developed. Also in the case of salmon vaccination is generally carried out in fresh water in the hatchery stage, before smoltification (which is the normal adaptive process in salmon for them to move to the seawater stage of their life). This for a variety of reasons which include ease of access – since fish must be sedated or anaesthetised in order to be injection vaccinated, it is more practical to carry this out on land in contained facilities. Also since the seawater phase is the time of rapid growth, the logistics of vaccinating at sea can be tricky as the population of each individual sea cage will be measured in tonnes.

In addition, the immune response in salmon can be significantly affected by smoltification, and vaccination should be avoided during this time in order to ensure the maximum immune response.

The geographical location of some hatcheries combined with the availability of suitably trained staff can also limit the window of opportunity for vaccination. Also since fish have to be starved prior to the administration of the anaesthetic, and may take a few days to return to feeding subsequent to vaccination, it makes good husbandry practice to limit this to a once only event if possible.

4. Responsible Use of Vaccines in Aquaculture Production.

4.1 Responsible Use – Veterinary Surgeons.

1. The Royal College of Veterinary Surgeons Guide to Professional Conduct makes specific and detailed reference to the use of veterinary medicinal and related products. In 1998 the British Veterinary Association published their Code of Practice on Medicines. The challenge to the veterinary profession is to ensure that these codes of practice are effective and properly implemented.
2. To accord with these guidelines POM-V products may only be prescribed and used under the direction of a veterinary surgeon when:
   a. the veterinarian has been given the responsibility for the health of the fish in question by the owner or the owner’s agent.
   b. the care of the fish by the veterinarian is real and not merely nominal. 

The Veterinary Medicines Regulations 2006 require that such products may only be prescribed by a veterinary surgeon following a clinical assessment of the animals and the animals must be under the care of that veterinary surgeon. However, it is for each person to decide when they consider that the animals are under their care and they have carried out a clinical assessment and defend that decision if required.

3. Although circumstances will vary enormously the veterinary surgeon may consider that the requirements are met if they have at least: (a) either seen the fish for the purposes of diagnosis or prescription; or (b) have visited the farm or other premises where the fish are kept sufficiently often and recently enough to have acquired from personal knowledge and inspection an accurate and up-to-date picture of the current health status on that farm sufficient to enable him or her to formulate a veterinary health plan for disease prevention and prescribe for the fish in question.

4. Any suspicion of adverse reactions including lack of efficacy should be thoroughly investigated. Suspected adverse reactions should be reported to the Veterinary Medicines Directorate through the Suspected Adverse Reactions Surveillance Scheme (SARS) using the “Yellow form” MLA252A. This form can be downloaded from the VMD website www.vmd.gov.uk

4.2 Responsible Use – Fish Farmers.

Fish farmers have a responsibility to safeguard the health of the fish on their farm. Where appropriate, farmers may ask their veterinary surgeon to help them discharge this responsibility. Farmers and fish keepers can play a major role in ensuring the responsible use of vaccines on fish farms by following the guidelines below. Similar guidelines form part of all farm assurance schemes.

1. Regard vaccines and vaccination as complementary to good management, and general site and farm hygiene.

2. A site and farm health plan should be drawn up that outlines routine preventative treatments (for example in addition to vaccination, fungus control, salmon lice control etc.).

3. Ensure that accurate information is given to the attending veterinary surgeon in order that the correct volumes of vaccines can be prescribed for the fish concerned, and ensure that clear instructions for dosage and administration are obtained and passed on where necessary to the staff responsible.
4. Maintain a fish medicines record book on farm together with copies of relevant regulations and Codes of Practice.

5. Accurately maintain the records required by the legislation including the identity of the fish vaccinated and the batch number, amount and expiry date of the vaccine used.

6. For all vaccines used, appropriate information should be kept on file – for example, product data sheets, package inserts or safety data sheets as available.

7. Report to the veterinary surgeon, (or in the case of non-prescription medicines, the supplier, or direct to the Veterinary Medicines Directorate) any suspected adverse reaction to a vaccine in either the treated fish or farm staff having contact with the vaccine. A record of the adverse reaction should also be kept on the farm: either a copy of the VMD adverse reaction form or a note in the appropriate record book.

8. Farmers and fish keepers have responsibilities for the safe use, storage and disposal of vaccines. This must be backed up by recording systems which are essential in providing a framework for identifying disease problems and allowing appropriate changes to management practice.

4.3 Disease Prevention.

The best way to prevent disease is to prevent it from entering the farm. It is important to develop a preventative programme and consulting with those who have additional expertise and experience in the use of suitable products to prevent disease may assist this.

4.4 Biosecurity.

1. Biosecurity is a management strategy designed to minimise the potential for introducing disease-causing organisms onto the farm. People, fish, animals or wildlife may transport diseases from outside the site.

2. Visitors and vehicles from outside the premises should be cleaned and disinfected before going on to the site. Keep disinfectants available for those who must come on to the site.

3. Vaccination teams must observe hygiene between farm sites and between farms.

4.5 Routine Health Procedures.

1. Attention must be given to good management as part of disease control.

2. A programme should be prepared for routine treatments to control external parasites and gill problems.
3. Water quality should be maintained at the optimum for the species kept.

4.6 Cleaning and Disinfection.

1. Cleaning and disinfection are the most basic and the most important of all the disease control measures. Prompt and proper removal of wastes, and cleaning and disinfection of equipment is central to disease control. Effective disinfection requires cleanliness first because the disinfectants have little or no action on dirty surfaces. Organic material inactivates chemical disinfectants. Also organic material provides protection for disease organisms and the chemical solution is unable to penetrate and reach them.

2. Cold temperatures can reduce the effectiveness of most disinfectants. Note that the chemical agents commonly used may require several minutes contact time with the disease producing agents to be effective.

3. Care should be taken to ensure that the chosen disinfectant is compatible with the aquatic environment – this may be especially important on marine sites. Care should also be taken in the disposal of the chemicals after use.

4.7 Responsible Use – Farm Assurance Schemes.

1. Farm assurance schemes have a very important role to play in promoting the responsible use of vaccines on fish farms. Credible farm assurance schemes with a credible inspectorate are essential if the industry is to reassure consumers. Farm assurance scheme assessments and audit trails should be consistent.

2. Farm assurance schemes often require farmers to nominate a veterinary surgeon or veterinary practice. Veterinary surgeons prescribing POM-V products are in a position to certify compliance with standards of the farm assurance scheme providing the fish were actually under his care and he was aware of all products used on the farm.

3. Veterinary surgeons should play an important role in assurance schemes while recognising the expertise of the farmer in managing his own fish stock. A Farm Health Plan should be developed with the assistance of a nominated veterinary surgeon where necessary. Regular and frequent review of this Health Plan is recommended. It is recognised that the frequency of the review will vary according to the situation and the requirements of the particular farm assurance scheme.

4.8 Summary.

There should be consultation with a veterinary surgeon for help with disease prevention, control, diagnosis and treatment. A farm health plan including vaccinations and where appropriate, parasite control, should be developed and reviewed and updated often. Basic disease prevention and control methods should be used to the greatest degree possible, and the farmer must not simply rely on vaccination to prevent disease.
5. Main Diseases Controlled with Vaccination.

The main groups of farmed fish which are routinely vaccinated are Atlantic salmon (*Salmo salar*), Rainbow trout (*Onchorhychus mykiss*), and now, increasingly, Atlantic cod (*Gadus morhua*).

The main diseases in these groups of fish controlled by vaccination and their associated infectious agents are as follows-

- Furunculosis associated with *Aeromonas salmonicida*.
- Vibriosis caused by *Vibrio anguillarum* serotypes 01 and 02
- Enteric redmouth (ERM) – *Yersinia ruckeri*
- Infectious pancreatic necrosis caused by IPN virus.
- Salmon pancreas disease caused by SPD virus.

There are a number of other infectious agents with potential vaccines under current development.

5.1 Furunculosis

Furunculosis has been recognised as a major bacterial problem in a range of fish species for over 100 years. It is caused by *Aeromonas salmonicida*, and although it was previously considered as a problem associated with salmonids, it has been shown in a variety of fish species in a range of habitats from fresh water right through to sea water. It also can affect fish from fry right through to broodstock, and the disease is often triggered by sharp rises in water temperatures combined with changes in fish physiology such as smoltification or spawning.

The vaccines available to prevent this disease are very effective, and have made a major difference to the management and control of this disease in farmed salmonids in the UK.

5.2 Vibriosis

Vibriosis can be caused by one of the two serotypes of *Vibrio anguillarum*. It is a disease of the sea water phase of farmed fish production, and can affect the range of fish species cultivated in sea water conditions – including Atlantic salmon and Atlantic cod. Pre-disposing factors for the development of the clinical disease include stress, water quality, temperature and the virulence of the *Vibrio* strain.

As with furunculosis, the development and use of effective vaccines has significantly reduced the impact of this disease.

5.3 Enteric Redmouth

Enteric redmouth, caused by *Yersinia ruckeri*, is mainly a fresh-water disease of Rainbow trout, although it can affect other fish species such as Atlantic salmon in the freshwater phase and occasionally even at sea. Outbreaks can be triggered by fluctuations in water temperatures combined with other factors such as stress. It
can affect fish of a wide size range and is virtually endemic throughout the Rainbow trout farming areas of Europe.

It can cause significant economic losses, and management practices such as avoidance of high stocking densities and attention to water quality can reduce losses.

There are a variety of vaccination regimes to control this disease in Rainbow trout. There is no current ERM vaccine authorised for use in salmon.

**5.4 Infectious Pancreatic Necrosis**

Infectious pancreatic necrosis is a viral disease caused by an aquatic birnavirus, IPNV. This virus is related to infectious bursal disease (IBD) of poultry, and in some studies the two viruses were morphologically indistinguishable.

The virus can cause problems in both fresh water and in the sea water phase of fish rearing. It tends to be a disease of younger fish, but the carrier status can exist which can give challenges in the control of the disease, especially in deciding where to transfer fish. It also is reported to be immunosuppressive, and therefore can have an effect on the immune response either in association with other disease challenges or in the response to vaccination.

In the salmon industry it can cause serious problems in the hatchery for young salmonids, but one of the major triggering points for outbreaks is just after sea water transfer for Atlantic salmon. During smoltification and immediately subsequent to smoltification young salmon can have increased susceptibility to disease, and IPN can be a major cause of not only mortalities but also sub-optimal growth and performance in smolts just after transfer – ‘fading smolt syndrome’.

The disease can be transmitted horizontally directly from fish to fish, or may also be transmitted vertically from the broodstock. It can also be transmitted in contaminated equipment, and it is thought that even some birds may play a role in its transfer.

There is a vaccine available for Atlantic salmon in the UK under a Provisional Marketing Authorisation (PMA).

**5.5 Pancreas Disease**

Pancreas disease is caused by an alphavirus, salmon pancreas disease virus, which is very closely related to the virus causing sleeping disease of Rainbow trout. In Atlantic salmon, pancreas disease is a major economic problem since it affects large fish, near market weight (just before harvest) causing up to 50% mortality as well as reduced appetite, growth and hence performance.

Sleeping disease of Rainbow trout was first recorded as a problem for farmed Rainbow trout in 2002, and has potential as a major problem for the farmed Rainbow trout Industry. Although the disease is being controlled by bio-security, it is still a risk for trout growers.
There is a salmon pancreas disease vaccine available under a PMA, but unlike all the other combination salmon vaccines designed for administration in a single injection this has to be given separately from any other injectable vaccine. To date there is not yet any vaccine available for trout.

6. Types of Vaccines.

Vaccine types used in farmed fish can be broadly divided as follows:-

6.1 Inactivated bacterial
6.2 Inactivated viral
6.3 Subunit (derived from recombinant technology)
6.4 DNA

Because farmed fish are not usually kept in isolation from their wild counterparts, the use of live modified or attenuated products has not been approved in UK aquaculture.

6.1 Inactivated Bacterial Vaccines

These are produced by fermenting the pathogen in large quantities and then introducing inactivating agents such as formalin which kill all of the bacterial organisms. These inactivated organisms still retain the original antigenic characteristics of the live bacteria since their basic shape and structure has not been altered, simply their ability to grow and reproduce (and cause infection in the host). Manufacture of vaccines is carried out under strictly controlled conditions, with Quality Control tests being carried out at every step in the production process. One of the important quality control tests for an inactivated vaccine is to confirm that the bacteria have indeed been killed, and this is carried out by the sampling laboratory.

However, simply injecting a dose of the inactivated bacteria will not produce a long-term immunological response, and hence will not induce long-term protection to the disease. Therefore the inactivated bacteria must be combined with a compound which not only improves the presentation of the antigen to the immune cells of the fish, but also encourages the antigens to persist within the body cavity of the fish to improve the duration of protection. Thus the history of fish vaccine development is closely tied with adjuvant development. At the time of writing the adjuvants in use in the intra-peritoneal (i.p.) injection vaccines as part of their functional response will encourage the development of a peritoneal reaction. This is turn will take the form of immune cells migrating to the vaccination site. These cells will include melanocytes which will in some cases produce melanisation of associated tissues. Another sequel can be the formation of adhesions in the peritoneal cavity. The salmon industry has recognised this phenomenon for some years, and has triad a number of different approaches to minimise these effects on the growing fish.
6.2 Inactivated Viral Vaccines

These are produced by growing the virus in significant quantities in media such as tissue culture. As with the inactivated bacterial vaccines the culture is then treated with special chemical agents which kill the virus particles without altering their basic shape and structure and hence antigenicity. Also as with the inactivated bacterial products, extensive testing is carried out by the vaccine manufacturer during the manufacturing process to ensure that all of the virus particles have in fact been inactivated. There is also extensive testing of the seed materials to ensure they are not contaminated with other disease agents.

One of the difficulties associated with commercial inactivated viral vaccine production is in producing sufficient virus particles at a commercially feasible rate. This is because the current methods of commercial virus production are of necessity not able to produce the volumes of material which can be produced by bacterial fermentation.

Again, as with the inactivated bacterial vaccines, an adjuvant will be required to ensure adequate presentation of the antigen to the immune response cells and also to ensure persistence of the antigen.

6.3 Subunit Vaccines

Subunit vaccines are produced by implanting the part of the DNA which encodes for the production of the specific antigens to trigger an adequate immune response into another type of organism.

The recipient organism is chosen for characteristics such as the ability to be easily produced on a large commercial scale and also to secrete the donor protein antigens in a format which can be readily commercially harvested.

Although there are a number of advantages to this technology for vaccine production, it also requires a lot of background testing information to indicate the “vehicle” used to product the subunit has to be shown to be safe and that any genetic material is not transferred to the subunit protein i.e. there are a number of regulatory requirements to be satisfied.

Subunit vaccines are safe for the animal or fish which receives the vaccine since there is no possible risk of giving the recipient the disease. The fish is simply receiving a protein – the antigen which will stimulate the specific immune response. This also applies to any humans which consume the recipient animal or fish. There is also little risk of contamination of the vaccine by other microorganisms.

It confers the ability to the vaccine producer of manufacturing large quantities of the chosen antigen or antigens at relatively lower cost than growing the original organism, particularly in the case of viruses.
It opens the possibility of vaccination against larger more complex organisms such as ectoparasites by selecting the appropriate antigens from these organisms and manufacturing them by this subunit technology.

6.4 DNA Vaccines

The principle of DNA vaccination is to make the host animal receiving the vaccine responsible for the production of the antigen and then the immune response to the antigen. It is also known as Genetic immunisation. Basically DNA vaccination or nucleic acid immunisation entails the delivery of DNA (or RNA) encoding a vaccine antigen to the recipient. The DNA is taken up by host cells and transcribed to mRNA, from which the vaccine proteins are then translated. The expressed proteins are recognised as foreign by the host immune system and elicit an immune response, which may have both cell-mediated and humoral components. DNA vaccines offer a number of advantages over conventional vaccines, including ease of production, stability and cost. They also allow the production of vaccines against organisms which are difficult or dangerous to culture in the laboratory. At the time of writing no DNA vaccines have received Marketing Authorisation for use in fish in the UK.


The early fish vaccines were very basic formalin inactivated bacterial cultures, which were administered initially by immersion and subsequently by injection.

The immersion technique has endured for some bacterial antigens since it is still the most effective method of ensuring short-term immunity to small fish which for a number of reasons including size, individual value and reaction to the general stress of handling make ideal candidates for this approach.

Although the immersion technique certainly produced some levels of immunity to certain bacteria, further research was directed at the injectable vaccines. Although a degree of protection can be achieved by simply injecting the killed bacterial suspension i.p., improved levels and duration of protection were dependent on the development of suitable adjuvants (these are constituents of vaccines which enhance the degree and the duration of the immune response). However the early oil-based adjuvants had the undesirable effect of producing lesions within the peritoneal cavity post-vaccination. These lesions have been the subject of much intensive research within the farmed salmon industry since although the development of efficacious injectable vaccines against Furunculosis revolutionised the salmon industry, by not only increasing the survivability of the salmon, but also reducing antibiotic usage to a fraction of the levels used prior to the development of these efficacious vaccines. Since then there has been a major effort by the vaccine producers to develop adjuvants which confer long-term protection without the consequent growth and down-grading penalties associated with post-vaccination intra-abdominal lesions.

One of the other issues associated with the development of injection vaccines is the dose volume. Injection vaccines are designed in the main to be administered into the peritoneal cavity (although in some cases for the vaccination of potential
broodstock in order to avoid the potential issues surrounding post-vaccinal reactions and their consequent effects on the recovery of viable eggs the vaccination can be administered into the dorsal median sinus) and the original dose volume in the early vaccines was established at 0.2ml. However newer technology adjuvants can confer adequate protection using a dose of 0.1ml, which significantly reduces the adhesion and melanisation situation. However although the multi-antigen products currently available have physical space within the vaccine dose for all of the required antigenic content, future addition of other antigens as they are developed may become an issue for this smaller dose volume.

Frequency of dosing is another area where the aquaculture vaccination protocols differ from terrestrial animals. As far as injection vaccination of salmon is concerned, vaccination is routinely carried out in the fresh water stage at the hatchery where the fish are kept in tanks on dry land This procedure has been well refined over the years, but still is potentially stressful for the fish which in some cases can take a few days to return to normal feeding post-vaccination. Anaesthetic risks are small but present a potential area for loss of fish and in addition the overall stress of the procedure can in some cases trigger other diseases such as fungal infections which are opportunistic pathogens and are ubiquitous in the fresh water aquatic environment.

Since as part of the immune response there is a reaction in the peritoneal cavity, most of the required antigens for the life of the salmon are administered in this single injection apart from one involving a new antigen. No other subsequent injections are carried out into the peritoneal cavity to avoid potentially serious peritoneal reactions.

Also injection vaccination in salmon is carried out in freshwater, prior to sea transfer since the logistics of anaesthetising and handling the fish at this stage are very much simpler than when they go to sea. It is during the seawater phase that the salmon grow dramatically, and since each sea cage on a raft of cages on a sea site will contain many thousands of salmon, the logistics preclude the use of injection vaccines during the seawater grow-out phase. In practice it has been done, but only rarely in extreme circumstances where there was no option for the welfare of the fish.

Thus the only potential for routine booster vaccination of salmon at sea would be by the oral route. Oral vaccine technology has been seen over the years as the ideal method of administration of vaccines to fish. There has been considerable effort devoted to the research and development of oral vaccination but there are again some major limiting factors. These are basically presentation of the vaccine in a suitable form, ensuring even distribution of the dosage form throughout the population of fish to be vaccinated, duration of immunity and presentation of the appropriate range of antigens.

Since oral vaccines are designed to be administered with the feed, this can itself present some challenges. Either the vaccine can be top-dressed on to the feed using an adhesive agent such as edible oil to ensure that the vaccine sticks to the feed particles, or in the case of liquid oral vaccines they can be sprayed on to the feed, or the vaccines can be mixed with the feed as part of the processing
techniques. However, although feed incorporation appears to be a superior method, the vaccine antigens have to be presented in a way which enables them to withstand the very high temperatures and pressures associated with the feed manufacturing and extrusion process, and currently there are no oral vaccines licensed in the UK which can be included during the manufacture of the feed.

The foregut of fish is highly acidic, and this can present a problem in the presentation of the antigens in oral vaccination. They have to be protected in some way to ensure their passage through to the hindgut where they can be processed by the immune cells. This can present some challenges in the encapsulation technology employed.

One of the other basic challenges with oral vaccination is to ensure that each fish receives an adequate amount of the antigens. Since fish are fed in large groups, with behavioural patterns within the group having an influence on feed uptake, it can be difficult to ensure that all of the target fish receive sufficient vaccine. Also from a commercial standpoint, the volumes of antigen require for this method are by necessity much larger than those required for individual injection vaccination, especially if they are targeted at larger body weight fish such as salmon in the seawater phase of their cycle, and so production of high quality large volumes of antigen may present a fairly major commercial manufacturing challenge.

Duration of immunity is another issue which can create areas of uncertainty in oral vaccination regimes. Since there is no adjuvant in oral vaccines to prolong the presentation of the antigens, the immune response can be very short-lived, and the longer responses will be almost entirely dependent on memory cells. Therefore timing of booster vaccination can present some problems.

Despite the problems, research work continues and currently there are oral enteric redmouth and vibriosis vaccines available.

In summary, a great deal of development is required to produce a safe and efficacious vaccine. The detail and course of this varies greatly depending on the type of disease, and the nature of the vaccine and target stock. In essence there are a number of identifiable steps, which will be slightly different for fish than for other terrestrial species since there are no live aquaculture vaccines in the UK.

- Isolation and identification of the causal micro-organism
- Culture of the micro-organism itself or of the target antigen in some other way
- For inactivated vaccines – inactivation to kill the micro-organisms
- Confirmation that the vaccine is free of extraneous agents
- Confirmation that the vaccine is safe for the target species
- Confirmation that it is effective in preventing or at least reducing the effects of the target disease.
- Formulation of the vaccine in an appropriate diluent, carrier, with adjuvant or without, and in a package to facilitate storage

The time taken to get through all of these steps will vary, but for a fully-licensed commercial product this is unlikely to be less than five years. In order for a product to fully meet all the regulatory requirements for safety, quality and
efficacy, the development process needs to generate a complete dossier to satisfy the assessor, and in the case of vaccines developed as a result of recombinant technology the dossier will have to go through the Centralised Procedure.

8. Registration of Vaccines, Regulation of Distribution and Use.

The registration of all veterinary medicines is regulated under common EU Directives which are implemented in the UK by the Veterinary Medicines Regulations. Registration and other aspects of the regulation of veterinary medicines are the responsibility of the Veterinary Medicines Directorate, an executive agency of DEFRA. The definition of a veterinary medicine, including a vaccine is “any substance or combination of substances presented as having properties for treating or preventing disease in animals.”

There are some regulatory requirements specific to the licensing of vaccines – from full information on the original organism used as the Master Seed through various aspects of in-process controls, to the final batch release tests of which one the batch safety test is performed on all batches of vaccine produced for sale and must be carried out on the target species for the first ten batches of vaccine produced. These control checks are all designed to ensure batch-to-batch consistency in the production of vaccines since it is also recognised that since vaccines begin as some development from a living micro-organism, slight variations in how this organism is grown and replicated make each vaccine a unique development.

The Veterinary Medicines Regulations also control the distribution, and use of all medicines, as well as record keeping requirements and fees levied by VMD for various approvals and other activities. Vaccines approved for use in fish are in one of two distribution categories:

(a) Prescription Only Medicine–Veterinarian (abbreviated to POM-V);
(b) Prescription Only Medicine–Veterinarian, Pharmacist, Suitably Qualified Person (abbreviated to POM-VPS);

Each category affects how the products may be supplied and purchased. Products in the first category may only be supplied by veterinarians or pharmacists against a prescription issued by a veterinary surgeon. Veterinarians are only permitted to issue prescriptions for animals under their own care after a clinical assessment in this case.

Products in the second category (broadly equivalent to the previous PML category for food-producing animals) are not subject to the same degree of control – they may be supplied by any of the people mentioned, to a prescription issued by such person provided that certain duties are carried out.
9. Methods of Vaccine Administration.

9.1 Immersion/Spray
9.2 Oral
9.3 Injection

9.1 Immersion/Spray

The biomass of the fish to be vaccinated is calculated since the vaccine is administered on a combined body-weight basis. Also the minimum size of the fish is checked since there will be a minimum size below which fish should not be vaccinated – the vaccine data sheet and package insert will give information on the minimum size of fish for vaccination with the particular vaccine.

The vaccine is diluted according to specific instructions using some of the water in which the fish are kept, and the fish are immersed in batches in the diluted vaccine for the recommended time – usually around 30 seconds. Each bottle of concentrated vaccine will be sufficient to vaccinate a designated weight of fish.

During immersion care must be taken to aerate the diluted vaccine whilst the fish are in it. Also follow the vaccine instructions in respect of minimum temperatures below which fish should not be vaccinated. This is because the fish immune response will depend on the temperature of the water in which the fish are kept, and below temperatures such as 4 - 5ºC the response will be insufficient to ensure adequate protection. All fish vaccines will carry the recommendation that only healthy fish should be vaccinated. Also individual vaccines should not be mixed.

9.2 Oral

The method of oral administration can vary according to the vaccine. The three methods are top-dressing the finished feed with the vaccine powder using an adhesive agent such as edible oil or even gelatine, spray-dressing the finished feed if the vaccine is in liquid form, or incorporating the vaccine into the feed during the feed manufacturing process.

The biomass of the fish to be vaccinated should be estimated and the vaccine mixed with the feed according to the manufacturer’s instructions.

With liquid vaccines, bring the vaccine to room temperature (20ºC) for 1 hour before use to allow the vaccine to become more liquid. If any separation occurs, shake the bottle vigorously until the separated layers are completely dispersed. Turn the required weight of feed pellets in a mixer, e.g. a concrete mixer, and slowly pour or spray the vaccine directly onto the pellets. If a sprayer is used, it should be set to deliver a coarse spray without risk of aerosol particle generation and the spray container must be completely emptied during the mixing operation. Mix the pellets for at least 2 minutes after all the vaccine has been added. Keep the prepared feed for 1 hour before feeding, to allow the vaccine to impregnate the pellets completely.
The vaccine-incorporated feed should then be fed according to the vaccine manufacturer’s instructions, as a course of vaccine may be required to induce an adequate immune response. The vaccine manufacturer’s guidance on storage of feed containing vaccine should be observed, as well as the minimum size of fish which can be vaccinated with any particular vaccine.

9.3 Injection

Fish require to be sedated or anaesthetised in order to be easily and safely handled to be injected, whether by automatic vaccinating machine or by hand. They will have to be starved prior to this procedure, and may take a few days to return to normal feeding after the procedure. This has a consequence in terms of growth and production. Also the use of anaesthetics, whilst now very much routine in some sectors of aquaculture, will always carry some risk – although a very small percentage of fish die subsequent to the administration of an anaesthetic, this is still a potential loss of stock to the farmer. Also the injection of individual fish is a skill. In some sectors there are dedicated vaccinating teams but there are other issues associated with this – timing of vaccination in relation to the availability of the team is one factor, as well as general biosecurity.

Although there has been considerable work in the development of automatic vaccinating machines, these will not totally replace hand vaccination. Although the principal of using a machine for such a labour intensive procedure is desirable, human involvement at some stage in the process is absolutely necessary. Fish still require some examination or inspection at this time to sort out abnormalities, which can only be done by trained personnel, and even relatively simple issues as ensuring the fish are fed into the vaccinating machine correctly so that the injection is placed where expected, and not into another part of the fish’s body such as the dorsal muscle still require human input.

However, injection vaccination of large numbers of fish of a variety of species has been carried out very successfully for a number of years, and this route of administration, with the general principle of one injection to protect for the life of the fish through to harvest remains still the most efficient method of protection against a variety of diseases. Also the very small mark left on the abdominal body wall by the needle serves as a definite indicator that the fish has been vaccinated, which is not possible with the other methods of administration. This can be important in the positive aspects of quality assurance and health assurance, but also be of use in investigating adverse post-vaccinal events.

However there are also some safety matters which require attention, not only for the fish receiving the injection vaccination but also the personnel involved directly in the injection technique.

Vaccination methods must be arranged to minimise handling stress to the fish, in terms of transporting fish to the vaccinating tables, handling during the anaesthetisation and vaccination procedures and during the return to the holding tanks to minimise scale damage.
As far as the fish are concerned, the skill of the vaccinator can have a major effect on the fish – repeat high speed ip injection is a very skilful task, and there is always the potential for human error – great care must be taken to ensure that the needle length is appropriate for the size of fish – even to the extent of slaughtering a few fish at the start of a vaccinating programme to ensure that the vaccine is being placed in the part of the abdominal cavity expected, and not in any of the internal organs such as the swim bladder or kidney. Also since needles are used for a number of fish during a vaccinating session, care must be taken not to use a blunt needle which will damage the skin of the fish and give the opportunity for secondary infectious organisms such as anaerobic bacteria or fungi to infect the injection site. The mechanics of vaccinating fish will inevitably lead to scale and mucus build-up on the needles – again something which must be addressed for the welfare of the fish. The actual size of the needles is also important – too large a needle bore will lead to the vaccine leaching out of the vaccination site of the fish, resulting in a sub-optimal dose, and a too-fine needle bore may give difficulties with the physical passage of the vaccine down the needle, especially with vaccines with a fairly high oil content in cold weather situations.

The other major issue is the safety of the vaccinating personnel. A number of fish vaccines in current usage have an adjuvant which has a relatively high mineral oil content. Accidental self-injection with this type of vaccine can lead to potentially serious consequences. All oil-based vaccines must carry a warning in the packaging and if the operator does receive an accidental injection of the fish vaccine, medical help must be obtained immediately. The consequences may range from excision of the affected area to potential loss of the digit injected, through to a systemic anaphylactic reaction.

Since in a number of cases fish vaccination is carried out in fairly geographically remote areas, suitable emergency procedures must be in place with the appropriate drug treatments for every vaccinating procedure.

Also, if accidental self-injection has occurred in an individual, they must not be permitted to vaccinate fish again with this type of product.


10.1 Vaccines administered by injection

(Acknowledgements to Pharmaq Ltd for the Injection Vaccination protocol)

Two weeks prior to vaccination

1. Check that the vaccination protocol is appropriate for the vaccine being used
2. Check the health of the fish
3. Check the fish are / will be a suitable size for vaccination
4. Ensure that the vaccination team is aware of the planned vaccination date
5. Ensure that the fish will not be stressed prior to the vaccination date
One week prior to vaccination

1. Check that the correct vaccine, quantity and giving sets are refrigerated (2 – 8 °C)
2. Check the vaccine expiry date and quality
3. Check detergents, disinfectants and anaesthetic are on-site (brand/quantity)
4. Vaccination guns/tables/machine, grading and support equipment is on site and functional
5. Check sufficient numbers of needles of the correct diameter and lengths are on site
6. Check that protectors against self-injection and injectable adrenaline (NB expiry date!) are on site
7. Inform the nearest doctor/medical centre of the planned vaccination dates
8. Check the vaccine SPC (Data Sheet) is available for reference

Days prior to vaccination

1. Check the health of the fish – behaviour, appetite and mortality
2. Check the fish are ready for vaccination – average size.
3. Take the fish off feed (48 – 72 hours) prior to vaccination.

The day before vaccination

1. Check that the vaccination and support equipment has been cleaned and disinfected ready for use
2. Tanks/cages to receive the vaccinated fish should have been cleaned and disinfected

General pre-vaccination points

1. Only vaccinate healthy fish
2. Weigh fish and record minimum, maximum and average weights
3. Measure body wall thickness and record minimum, maximum and average
4. Check that the correct needle lengths are being used to allow 1-2 mm penetration into the abdominal cavity.
5. Vaccines and injection devices to be connected in a sterile manner
6. Vaccination devices to be fitted with protection and the dose calibrated as per the SPC (Data Sheet) instruction
7. Prepare anaesthetic and check that air stones are in place.
8. Adjust anaesthetic concentration – use some test fish (observe for 45 – 60 seconds)
9. Complete vaccination record sheet apart from fish numbers and signatures.

During Vaccination

1. Adjust speed of sedation to vaccination rate
2. Continuously monitor time taken to achieve sedation and the time in the anaesthetic solution. If fish are in the anaesthetic for more than 1 –2 minutes – Alarm! Also if the time taken to achieve sedation is more than 1 – 2 minutes alarm also.
3. Each fish should be handled in a gentle manner to assure minimal stress, mucus and scale loss.
4. Check each fish for any obvious abnormalities.
5. Each fish is injected in the midline, 1 – 1.5 pelvic fin lengths anterior to the base of the pelvic fins.
6. Check that the entire vaccine dose is deposited in the abdominal cavity and record.
7. Remove scales from needles frequently
8. Replace needles as soon as they become blunt (manual vaccination)
9. Replace needles according to manufacturers’ instructions for vaccinating machines.
10. Monitor regularly the time for recovery from anaesthetic and any vaccine leach back (seen on the surface of the recovery tanks)
11. Regularly clean and disinfect all surfaces in contact with the fish
12. Place the vaccinated fish into clean disinfected tanks
13. Record the number of fish in each tank on the Vaccination Record
14. Complete and sign the Vaccination Record
15. Ensure the in use shelf-life is respected

16. IN THE EVENT OF ACCIDENTAL SELF-INJECTION the injured person must be taken immediately to a medical centre. The Safety data Sheet must be taken with them for the Doctor’s information.

After Vaccination

1. Inspect for vaccination quality and record results from a random representative number of fish
2. Do not handle, grade or transfer the fish for four weeks after vaccination
3. The Vaccination Record sheet, initialled by the recording person, should be completed and signed by the vaccinating team supervisor and the site manager.

General notes

1. Vaccines must be stored and administered according to label directions if they are to be effective. In most cases vaccines will need to be stored in a refrigerator. Refrigerator temperatures should be monitored.
2. Vaccination teams must observe hygiene between farm sites and between farms.
3. Vaccination methods must be arranged to minimise handling stress to the fish, in terms of transporting fish to the vaccinating tables, handling during the anaesthetisation and vaccination procedures and during the return to the holding tanks to minimise scale damage.
4. Vaccination needles should be changed frequently to avoid damage to the fish and the passage of contamination from fish to fish.
5. Once vaccine containers have been broached, they must be used up during that day’s vaccination session or discarded according to the manufacturers’ instructions.
10.2 Vaccines administered by immersion

One week prior to vaccination

1. Check that the vaccination protocol is appropriate for the vaccine being used
2. Check the health of the fish – behaviour, appetite and mortality
3. Check the fish are / will be a suitable size for vaccination
4. Check that the correct vaccine and quantity are refrigerated (2 – 8 °C)

The day before vaccination

1. Check that the vaccination and support equipment has been cleaned and disinfected ready for use
2. Tanks/cages to receive the vaccinated fish should have been cleaned and disinfected
3. Weigh fish and record minimum, maximum and average weights
4. Complete vaccination record sheet apart from fish numbers and signatures.
5. Check the vaccine SPC (Data Sheet) is available for reference

During vaccination

1. Shake the vaccine bottle well before use to ensure contents are evenly mixed
2. Dilute the contents immediately after opening.
3. Dilute the vaccine using hatchery water according to the SPC (Data sheet).
4. Do not mix immersion vaccines with any other products.
5. Ensure the temperature of the diluted vaccine does not differ from the temperature of the holding area by more than 5°C.
6. Vaccinate the fish in batches according to the size of the fish and the volume of the diluted vaccine.
7. Follow the SPC/Data Sheet for the timing of the immersion of the fish
8. Ensure all personnel involved in handling either the vaccine or vaccinated fish wear suitable protection such as rubber gloves.
9. Complete and sign the Vaccination Record
10. Discard any used vaccine containers according to the manufacturers instructions.

Further Reading:-

NOAH Compendium of Data Sheets for Animal Medicines, published by NOAH and online at http://www.noahcompendium.co.uk
The Responsible Use of Medicines in Agriculture Alliance (RUMA) was established in November 1997 to promote the highest standards of food safety, animal health and animal welfare in British livestock farming.

A unique initiative involving organisations representing every stage of the food chain, RUMA aims to promote a co-ordinated and integrated approach to best practice in the use of animal medicines.

RUMA membership spans the food chain and includes organisations representing interests in agriculture, veterinary practice, the pharmaceutical industry, farm assurance, training, retailers, consumers and animal welfare interests.

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RUMA is made up of the following organisations:

- Agricultural Industries Confederation (AIC)
- Animal Health Distributors Association (AHDA)
- Animal Medicines Training Regulatory Authority (AMTRA)
- Assured Food Standards (AFS)
- British Poultry Council (BPC)
- British Retail Consortium (BRC)
- British Veterinary Association (BVA)
- Linking Environment and Farming (LEAF)
- Meat and Livestock Commission (MLC)
- National Beef Association (NBA)
- National Consumer Council (NCC)
- National Farmers Union (NFU)
- National Office of Animal Health (NOAH)
- National Pig Association (NPA)
- National Proficiency Test Council (NPTC)
- National Sheep Association (NSA)
- The Royal Association of British Dairy Farmers (RABDF)
- Royal Pharmaceutical Society of Great Britain (RPSGB)
- Royal Society for the Prevention of Cruelty to Animals (RSPCA)


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