AquaVac ERM

AquaVac ERM Oral

AquaVac® ERM

Total Protection strategies against Enteric Redmouth Disease in Farmed Rainbow Trout

Intervet
Schering-Plough Animal Health

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Integrated Health Management Solutions

Intervet/Schering-Plough Animal Health Aquaculture is focused on the discovery, development, marketing and technical support of aquaculture products worldwide. Through its efforts, the company offers a broad line of products and services for Integrated Health Management to the aquaculture industry.

Intervet/Schering-Plough Animal Health is one of the world's leading animal health companies with a global presence. With subsidiaries in thirty countries, and distribution in an additional thirty countries, we can ensure that all national aquaculture industries will be closely supported. The company's research and development has generated many new animal health products over the years and has led the developments in aquaculture health products introducing:

- The first licensed fish antibiotic,
- The first licensed immersion and oral fish vaccines
- The first ever pan European licensed fish vaccine
- The first choice product for the control of sea lice in Atlantic salmon.

This expertise, coupled with a specialist aquaculture research group, will continue to bring forward new products for the prevention and control of diseases affecting the aquaculture industry around the world.

The company has invested and continues to invest, heavily in the aquaculture sector and has the vision, enthusiasm and commitment to maintain leadership in the aquaculture health industry in the future.

ERM Disease: Causative Agent and Epidemiology

Worldwide Distribution

Enteric Redmouth Disease (ERM) is caused by the bacterial pathogen Yersinia ruckeri.

Clinical outbreaks occurred in the UK in 1982 and in most other parts of Europe over the following few years.

It is now endemic in all trout-producing countries where it can cause severe economic losses.

It is also becoming a more significant pathogen of farmed salmon, primarily in freshwater, but it has been reported to cause losses in the sea as well.

Epidemiology

ERM is characteristically a disease of rising and falling water temperatures.

The highest risk of disease occurs between 8° and 16°C. However mortality can occur at temperatures as low as 4°C.

At the higher temperatures mortality can rise quickly. Levels of 15 - 20% have been reported even with antibiotic treatments.

Fish of all sizes are affected; however, fish of 50 - 200g are the most susceptible.

Stressful conditions such as high stocking densities and poor water quality will increase the level of susceptibility and mortality.

Symptoms

The most distinctive external and internal signs and symptoms of the disease are:

- Black, lethargic fish "hanging" in areas of low flow at the edges of ponds and raceways or against outlet screens.
- Bilateral exophthalmia. Abdominal distension as a result of fluid accumulation.
- Haemorrhages of the mouth and gills. The so-called "Redmouth" is rarely seen in acute infections but may be present in chronic infections.
- General septicaemia with inflammation of the gut. Petechial haemorrhage of the pyloric caecae.
- Diffuse haemorrhages within the swim bladder. These are the most diagnostic symptoms of ERM.
- The spleen is often enlarged and can be almost black in colour.

Isolation

The external symptoms mentioned above are also seen with other bacterial and parasitic infections, and so some further diagnosis must be carried out.

- *Yersinia ruckeri* is easily isolated from diseased fish on TSA plates or preferably upon a semi-selective media designed for the purpose.
- Following an incubation period of 24-48 hours shiny off-white colonies develop. Identity can be rapidly confirmed using specific antisera such as Mono YR test kits (available from Intervet/schering-Plough Aquaculture).

Husbandry

Good husbandry can play an important part in limiting the level of mortality caused by ERM. Reducing stocking densities at times of high risk and avoiding stressful procedures such as grading will help.

Treatment

Whilst antibiotic treatment can be effective in controlling mortality, it is preferable to avoid the need for it, because of the risk of residues in the flesh of the fish if improperly administered.

Resistance patterns should be determined prior to selecting antibiotic treatment.

Impact Of ERM on the European Trout Industry

According to estimates from within the British Trout Association in 1998, the cost of ERM in the UK trout industry alone was approximately £1.3-1.5 million per year.

- This figure is based on the following
  - Costs of mortality,
  - Growth penalties and
  - Subsequent reduction in feed conversion rate,
  - Grading problems,
  - Withdrawal periods due to antibiotic treatments and
  - Harvesting delays as a result of the disease.

At the time this could be calculated as 10% of the production cost of the industry.

If this figure of 10% is applied to the European trout industry that produces 200,000 tons per year, then it can be assumed that;

ERM causes a potential economic loss of £20 million each year.

ERM causes a potential economic loss £20 million *each year*

The AquaVac ERM Solution

Vaccination can prevent ERM from having a significant impact at any stage of the farming cycle of rainbow trout. This vaccination strategy has been developed to provide and maintain optimum protection against ERM throughout the production cycle.

The recommended vaccination programme involves:

AquaVac* ERM vaccine and AquaVac ERM Oral vaccine.

1. Immersion prime vaccination of fry of 2-5 grams
2. Oral booster vaccination 4-6 months after the immersion vaccination.

Primary Vaccination with AquaVac ERM

Immersion vaccination against ERM was first established in the early 1980's, and AquaVac ERM has now become the first ever fish vaccine to be approved through the European mutual recognition.

AquaVac ERM immersion vaccine has been shown to reduce mortality in farmed rainbow trout during the first few months of the production cycle. The level and duration of protection afforded by immersion vaccination are sufficient for at least six months.

A booster vaccination with AquaVac ERM Oral is recommended to extend protection beyond this time.

Booster Vaccination with AquaVac ERM Oral

AquaVac ERM Oral is the first oral fish vaccine to ever be approved through the EU's mutual recognition procedure.

AquaVac ERM Oral is an oral vaccine administered in the feed and is used to boost the immunity provided by the initial vaccine protection of AquaVac ERM.

AquaVac Ergosan*

AquaVac Ergosan, a natural feed ingredient based on sea weed that helps ensure good nutrition, enables the fish to mount an optimal immune response.

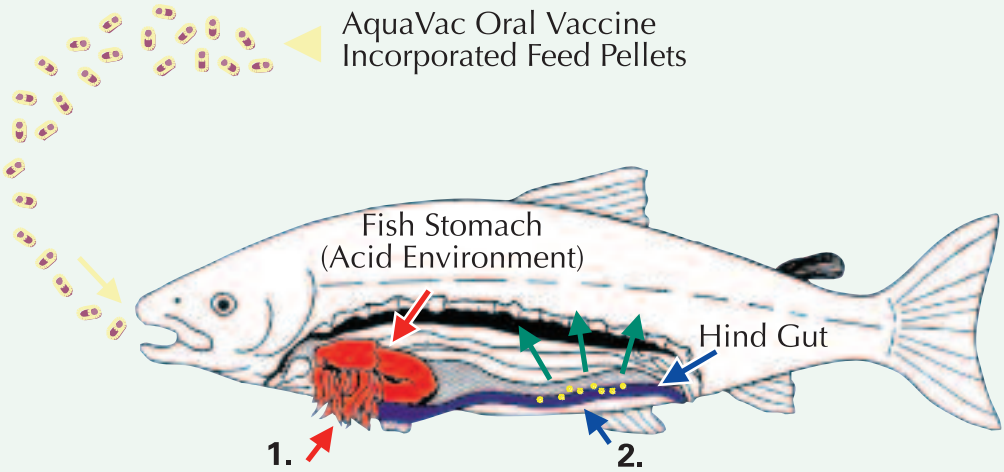
The oral booster should be incorporated into a high quality diet and can be supplemented with the complementary feeding stuff AquaVac Ergosan.

Oral Vaccine Technology

The development of effective oral vaccines has been one of the major goals for fish pathologists for many years, but proved particularly difficult due to antigen degradation in the acid environment of the fish stomach.

Intervet/Schering-Plough Animal Health has overcome this problem by the development of a reliable Antigen Protection Vehicle (APV) which protects the antigens through the stomach and delivers them intact to the hindgut where an immune response is initiated. AquaVac* ERM Oral has been extensively field tested under different farming conditions and is the first ever effective oral vaccine for trout.

AquaVac - Antigen Protection System (APV)



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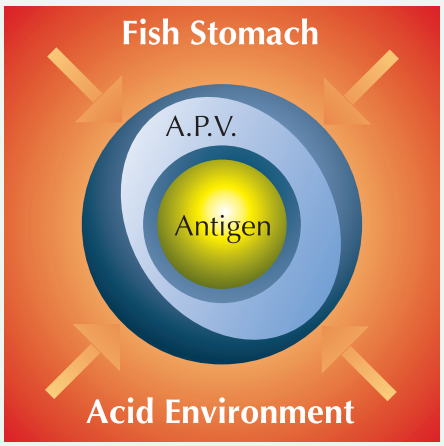

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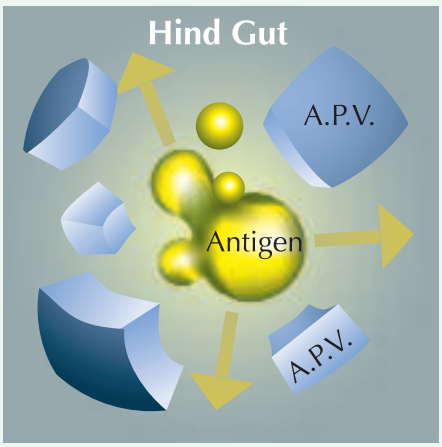


1. In the acid environment of the fish stomach, the feed pellets are digested. The antigens themselves are protected by the APV and pass through intact.

2. Antigens are delivered to the area of the hind gut where they are absorbed and activate an effective immune response in the fish.

ERM Disease Dynamics

Risks of clinical ERM

Infection with Yersinia ruckeri will lead to clinical disease and subsequent mortality in farmed rainbow trout when bacterial loading rises sufficiently and coincides with environmental or production stresses.

Mortality can be severe despite antibiotic treatments. Infected populations contain carrier fish which will be a source of subsequent outbreaks of disease. In fact unvaccinated infected populations often require repeated antibiotic treatments during the production cycle.

- Infection and repeated disease and treatment cycles result in poor growth, a wide size variation in batches and poor quality fish as well as an increased risk of antibiotic residues.
- Vaccination by immersion with AquaVac* ERM vaccine followed by booster vaccination with AquaVac ERM Oral vaccine can eliminate the need for antibiotic treatments by significantly reducing infection levels in the vaccinated population and preventing clinical ERM outbreaks.

Fig.1: ERM Immersion Vaccine Yersinia ruckeri spleen infection

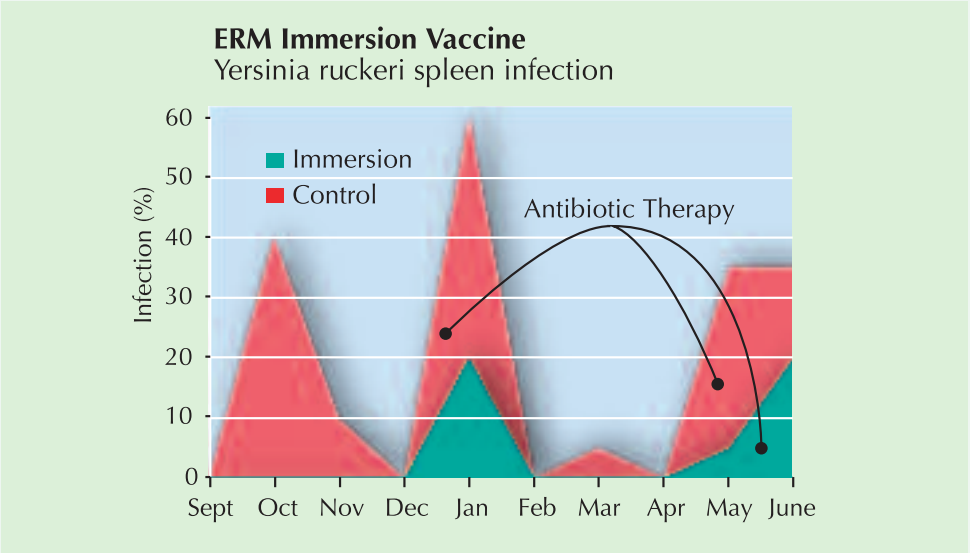


Figure 1. illustrates the fluctuations in infection levels in 2 groups of fish on a commercial trout farm. The control (red) group was unvaccinated and became infected immediately on transfer from the hatchery to the farm. The infection develops severly enough to require treatment in December and the following June coinciding with stressful temperature fluctuations. A second group (green) vaccinated with AquaVac ERM by immersion in the hatchery resist infection and disease for eight months until June.

Proven Protection against ERM

Laboratory Trials

The following studies were performed as a part of the development programme for the AquaVac ERM vaccination programme.

Protecting Small Fry -- Study 1

Healthy disease-free fry were vaccinated under controlled conditions using AquaVac ERM immersion vaccine at 2 grams.

- Controlled Conditions includes:

  Water Temperature : 12°C

  Water quality : Infection free

  Fish : Clinically healthy

The immersion vaccinated group was subsequently divided into 2 groups with one boosted using AquaVac ERM Oral. A third group of unvaccinated fish was kept as a control.

Group 1: Immersion prime vaccinated fish.

Group 2: Immersion prime vaccinated fish and orally boosted.

Group 3: Unvaccinated control groups.

These fish were then challenged 90 days after vaccination. The results can be seen in Table 1

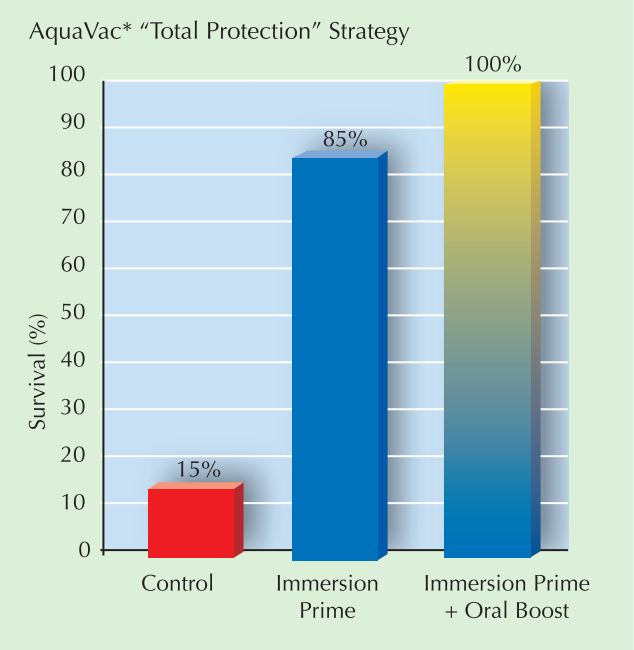
Table 1: Protection in laboratory challenge 90 days after vaccination

Vaccination Strategy	Mortality	RPS
1) AquaVac ERM Immersion 2g fish	14%	79%
2) AquaVac ERM Immersion + AquaVac ERM Oral boost	8%	88%
3) Unvaccinated controls	66%	–

Recommended AquaVac ERM Total Protection Programme - Study 2

This study was designed in a similar fashion to the first to confirm the efficiency of the vaccination schedule. Rainbow trout fry were immersion vaccinated using AquaVac ERM at 4 grams and booster vaccinated 45 days later using AquaVac ERM Oral.

Fig.2: ERM Vaccination Strategies: Lab Results



This study was designed in a similar fashion to the first to confirm the efficiency of the vaccination schedule. Rainbow trout fry were immersion vaccinated using AquaVac ERM at 4 grams and booster vaccinated 45 days later using AquaVac ERM Oral.

$$^{\dagger}\text{Relative Percent Survival (RPS)} = \left[1 - \left[\frac{\%\ \text{mortality in vaccinated fish}}{\%\ \text{mortality in control}}\right]\right] \times 100$$

Field Trials

AquaVac ERM and AquaVac ERM Oral vaccines have been extensively field- tested.

Since 1983 when AquaVac ERM vaccine was first licensed in Europe over 1 billion rainbow trout have been vaccinated.

The overall conclusion from this vast experience is that

- Effective immersion vaccination at 5 grams will protect rainbow trout for at least 6 months.
- The duration of protection is dependent upon
  - the size of fish at immersion vaccination,
  - the temperature when they were vaccinated and
  - the season (month during which they are introduced to the on growing site relative to the "high risk" period).

On-Farm Experience:

Determining the need for booster vaccination

Evaluation of duration of protection with AquaVac ERM by immersion.

N° of Batches: 17	Time to First Antibiotic Treatment
1	5 months
8	7 months
4	8 months
3	9 months
1	Harvested without treatment

The data illustrated above were collected from an intensive trout farm. The time of first antibiotic treatment indicates when the protection provided by immersion vaccination was inadequate to prevent disease. The experience is typical of most fish farms showing that the vast majority of trout (95%) require booster vaccination.

Same large table farm with oral booster use

N° of Batches of 5-10g fry onto farm	Time from immersion to Oral Booster Vaccination	Time from immersion to First Antibiotic Treatment
15	3-6 months	Harvested without treatment
1	7 months	9 months

Oral booster has (virtually) eliminated clinical ERM outbreaks with only 1 batch requiring antibiotic therapy.

These controlled field trials show that during an ERM outbreak protection rates will vary between 100% and 60%. Where the fish are fully protected (i.e., during the first 6 months post vaccination), no treatments are required.

However, although there is still good protection relative to unvaccinated fish after this time, there is a risk that antibiotics will be required with the associated costs and problems this entails. This has led to the development of the Total Protection strategies to help provide full lifecycle protection from ERM.

AquaVac ERM Oral Booster Trial

The extensive field trials have been used to develop the most effective vaccination program possible.

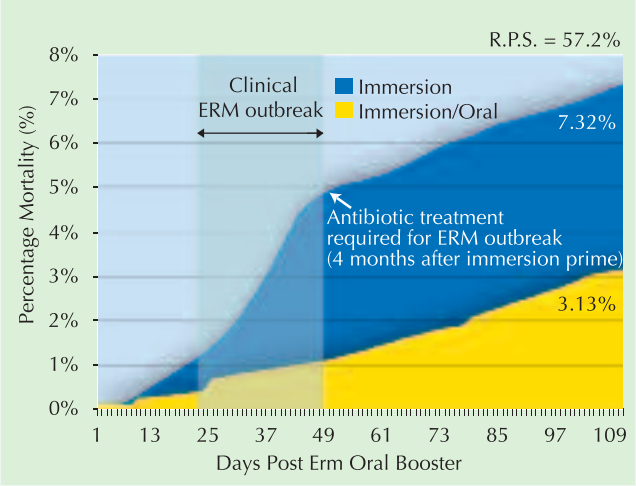
The trial illustrated in figure 3. was conducted in an intensive trout farm in the UK.

AquaVac ERM Oral was incorporated into the farms normal feed and fed under the 5/5/5 feeding regime (see instruction - page 8).

The vaccine was administered 3 months after primary immersion vaccination with AquaVac ERM.

This coincided with the start of the major disease season on the farm. Total losses rose to 7.32% during the period of the outbreak and an antibiotic treatment was administered to control the disease. The group of fish booster vaccinated with AquaVac ERM Oral did not require treatment, and the losses were maintained at background levels.

Fig.3: AquaVac ERM Oral Booster Trial



Actions resulting form the successful trial

Following completion of the development trials and full training of AquaVac ERM, the trout farm described above adopted a full tailor-made Total Protection vaccination Strategy for control of ERM.

- Each batch had a specific booster program developed, based on:
  - Size
  - Condition of the fish
  - Timing of the immersion vaccination
  - The date of transfer to the farm relative to the disease risks and temperature profiles.

Resulting Benefits

- *No delay in harvest due to withdrawal times and*
- *A better grade and quality of fish being produced.*
- *The farm was able to avoid any significant losses for the duration of the production cycle.*

AquaVac ERM Immersion Vaccination

Fish are fully immersed in a diluted solution containing the vaccine for 30 seconds after which they are returned to the holding tanks. The methods involved are simple and easy to implement and will give good results providing that a few basic rules are adhered to. In brief the rules are:

1. Only vaccinate healthy fish.
2. Vaccinate the correct weight of fish, i.e. 100 kg per litre of undiluted vaccine.
3. Observe the 30 second immersion time.
4. Make sure that the conditions in the vaccine solution are appropriate for the fish. The solution should be well oxygenated, at the correct temperature and clean. The fish should not be stressed by the procedure.
5. The time for development of immunity will depend on the water temperature. 28 days at 12°C should be allowed for fish to develop full immunity before being exposed to infection.

I. Preparation of Fish for Immersion Vaccination

Prerequisites

Minimum size 2g; prefered size 5g.
Immune induction period 28 days at 12°C.
Minimum temperature 5°C,
prefered temperature 12°C.
Maximum of 100kg fish per litre of undiluted vaccine.

Time

The time to vaccinate will be determined by the expected time of first exposure to the disease (i.e. movement from hatchery to ongrowing site), size of the fish and water temperature.

The following information is provided as a guide to ensure optimum results from immersion vaccination using AquaVac ERM.

Condition and Health

In order to respond well to vaccination and to ensure that the vaccination procedures do not precipitate additional disease or husbandry problems, the condition and health of the fish must be good.

Immersion vaccines are taken up through the gills. Therefore, these must be in good condition.
Any signs or symptoms of gill disease or adverse environmental conditions must be treated and resolved prior to vaccination.

Starvation

As with any handling procedure, the fish should be starved prior to vaccination. This is both to reduce the level of stress associated with the procedure and also to avoid fouling of the vaccination solution, which results in a build up of toxic waste material and ammonia causing acute stress and damage to the fish.

II. Preparation of Equipment

Immersion Vaccination Equipment

1. Vaccination tank for vaccine solution (1 litre of vaccine and 9 litres of water) – designed to optimise the volume/surface area ratio.
2. Knotless net or strainer basket for de-watering fish.
3. Scales and weighing system.
4. Stop watch.
5. Vaccination record sheet.

Preparing the Vaccine Solution

1 litre AquaVac* ERM vaccine per 100kg fish to be vaccinated. If a greater weight of fish are to be vaccinated, more than 1 litre of vaccine can be used at a time e.g. 300kg fish = 3 litres vaccine + 27 litres water.

1. Shake vaccine well to ensure all of the antigen pellet is suspended.
2. Measure out 9 litres clean hatchery water per litre of vaccine to be diluted.
3. Add vaccine to water and mix well.
4. Oxygenate lightly to maintain good saturation. Avoid super saturation.

III. Vaccination

Immersion Vaccination Procedure

1. Place vaccination tank on scales and tare to zero.
2. Drain a netful of fish.
3. Immerse fish in the vaccine solution for 30 seconds
   Ensure that:
   - fish are not overcrowded
   - solution circulates around fish
   - fish are able to breathe freely.
4. Note weight of fish vaccinated.
5. Drain fish carefully maintaining the vaccine solution in the vaccination tank.
6. Place vaccinated fish in a separate holding tank.
7. Repeat process until the maximum allowable weight of fish has been vaccinated (100kg/litre of vaccine used).

Precaution during Process

1. Fish should not show any signs of distress during or after the vaccination procedure. If they do, stop vaccinating and resolve the problem.
2. Minimise the time out of water to reduce stress.
3. Ensure that no scale or skin damage is caused by the equipment or handling procedures.

AquaVac ERM immersion vaccine has a very wide safety margin and will not cause any damage to the fish. The handling procedures will cause harm if not carefully implemented.

Allow 21-28 days for immunity to build up following vaccination. Booster vaccinate using AquaVac ERM Oral 4-6 months after primary vaccination.

AquaVac ERM Oral Oral Vaccination

I. Planning Vaccination

AquaVac ERM Oral vaccine is administered to booster vaccinate rainbow trout that have been primary vaccinated using AquaVac ERM. Ideally, booster vaccination should be 4-6 months post primary vaccination. The exact timing will depend upon the size of fish, the season, the health and disease status of the farm and the production objectives for the particular batch of fish in operation.

II. Preparing the Fish for Vaccination

- Only vaccinate healthy fish.
- Ensure that all fish are feeding and that the size of feed pellet is appropriate for all fish in the population.
- Treat any suspect disease problem.
- Ensure that the holding tanks and water quality are adequate for the 15 days of vaccination and subsequent 21 days (at 12 °C) for full immune induction.
- Do not stress or move the fish during the vaccination or immune induction period.

III. Preparing the Vaccine Food

Vaccine should be mixed with feed at a minimum rate of 3% liquid to weight ratio. It may be necessary to add extra oil to the feed to achieve this.

Dosage and Administration

Dose rate: 0.1 ml per fish, i.e. 1 litre of vaccine treats 10,000 fish. The dose should be fed over 10 days according to the following protocol:

Day 1-5	0.01 ml per fish per day
Day 6-10	No vaccine feed
Day 11-15	0.01 ml per fish per day

1. Calculate the appropriate quantity of feed and vaccine for the population of fish. Prepare between 75-90% of the daily feed.
2. Weigh out quantity of feed. Place in mixer.
3. Mix.
4. Shake vaccine well before use.
5. Add the appropriate quantity of vaccine slowly while mixing.
6. Mix.
7. Allow mixed vaccine feed to settle and absorb all oil prior to bagging and using.
8. Store prepared vaccine feed in dry, dark place, (20-25°C).
9. Use within 19 days of preparation.

IV. Vaccination

Feeding

- Make sure feed is distributed in such a way that all fish in the population have access to it.
- Do not over-feed, ensure that all feed is consumed.
- Top up feeding with untreated feed if required. Do not over-feed.

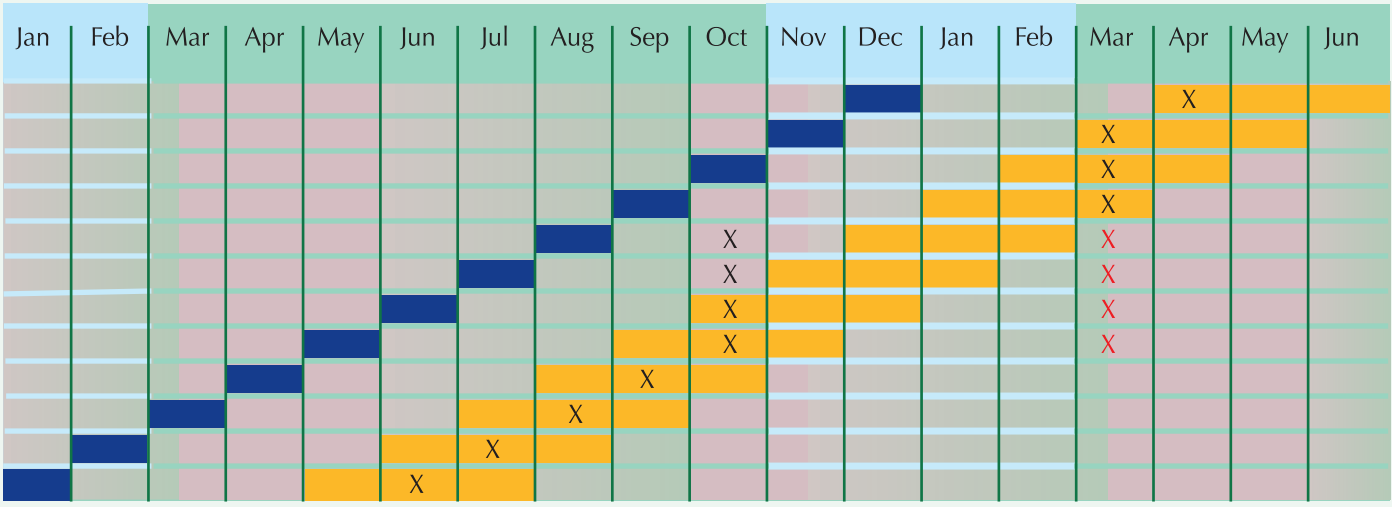
Total Protection Vaccination Strategy for the prevention of Enteric Redmouth Disease

AquaVac ERM
Immersion vaccination at a minimum of 2g.

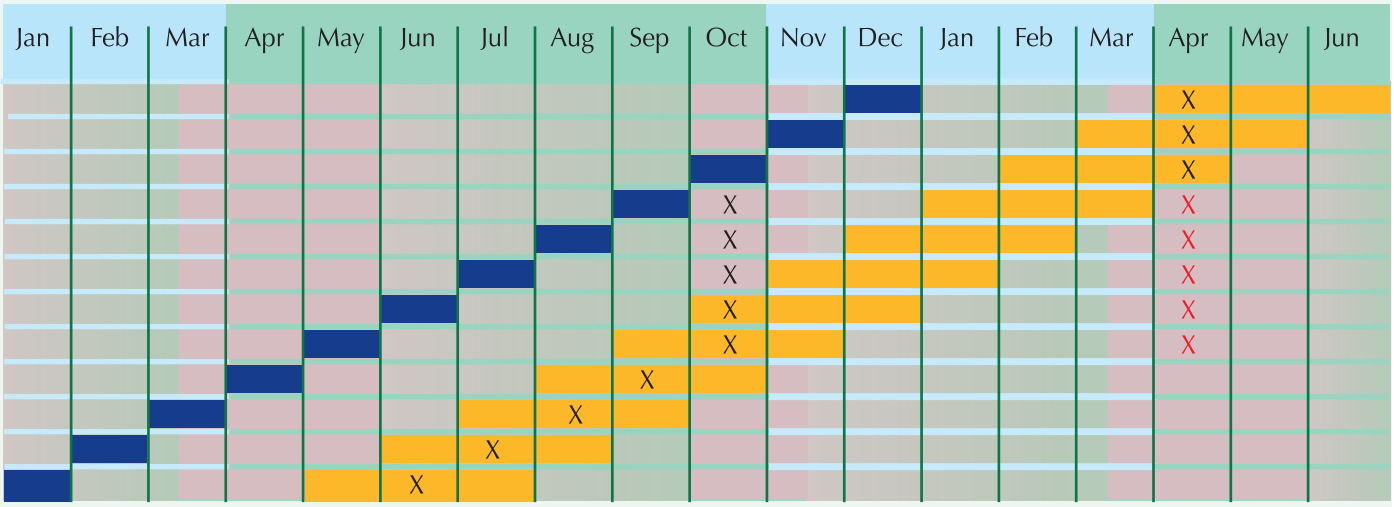
AquaVac Ergosan®
Complementary feed ensuring optimum nutritional status and good immune response.

AquaVac ERM Oral
Infeed oral booster used 3-6 months post immersion.

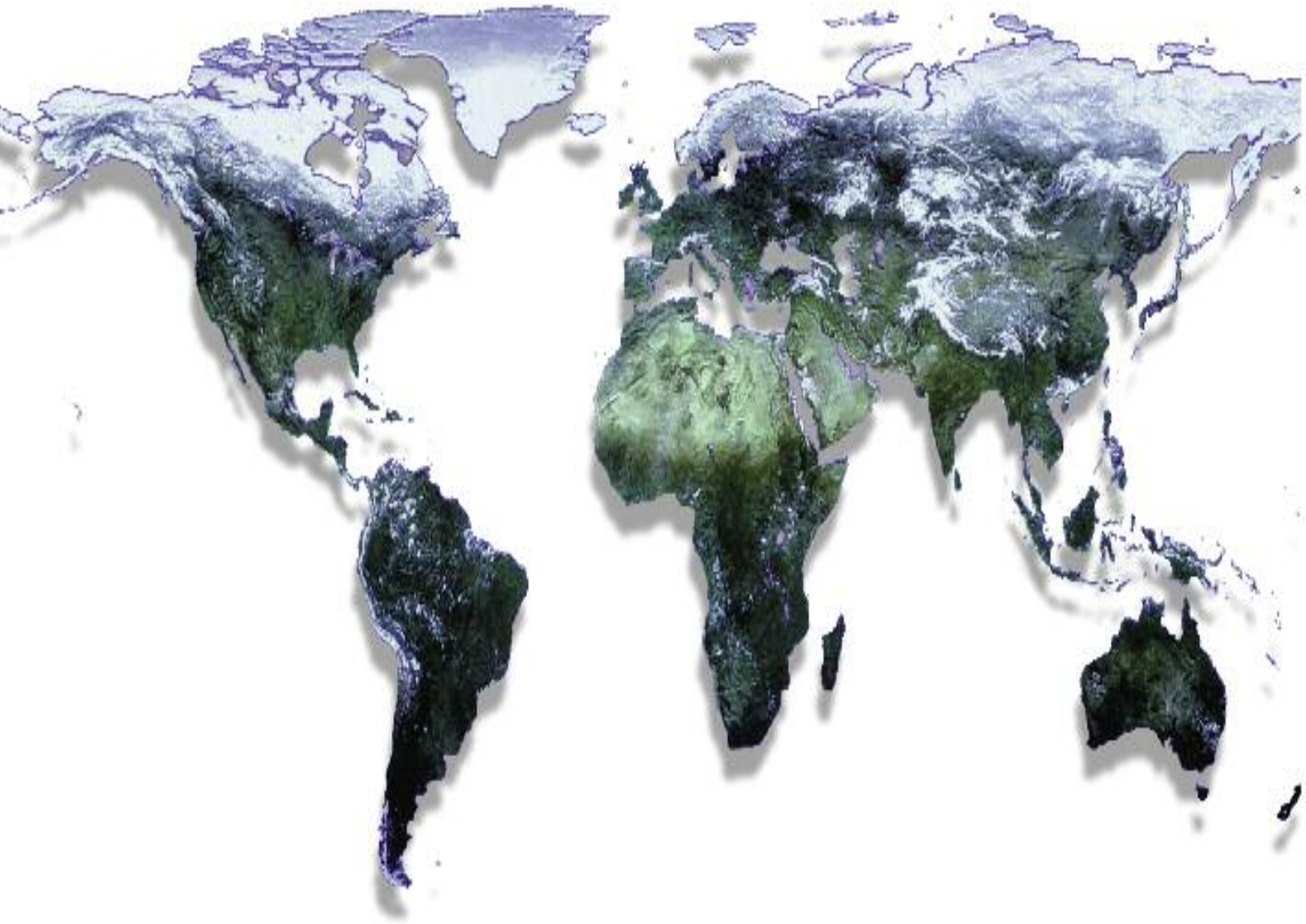
Warm Water Farm



Cold Water Farm



5g Immersion vaccination
Basic recommended for Oral Boost (4-6 Months Post Immersion)
X Actual time for Oral Boost
X Time of Oral re-Boost if required
Periods when ERM is at its peak
Water temperature is too low to make Oral Boost practical (< 6°C)



As with all pharmaceutical products please follow the label directions and advice provided by your veterinarian

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