

SAFETY AND EFFICACY RESULTS AFTER VACCINATION WITH ALPHA MARINE® Vibject.

ALPHA MARINE® Vibject is an emulsion vaccine for injection against vibriosis in cod. It contains formaldehyde-inactivated cultures of *Vibrio anguillarum* serotype O1, *Vibrio anguillarum* serotype O2a and *Vibrio anguillarum* serotype O2b. The recommended dosage is 0.1 ml per fish weighing a minimum of 30 g at the time of vaccination. The vaccine reduces mortality and clinical signs caused by *Vibrio anguillarum* O1, O2a and O2b.

1. INTRODUCTION

Farming of Atlantic cod is a growing industry in Norway and it is expected that several bacterial and viral diseases may cause problems in the future. The disease causing pathogens may be well-known, adopted from other species, more virulent strains and/ or new pathogens.

Sustainable farming of cod includes the development of effective disease control measures. Today vibriosis causes large losses in farmed cod and disease outbreaks can be recorded from early stages of the lifecycle up to slaughter. Vaccines that protect against vibriosis throughout the whole production cycle of the fish are needed.

Oil adjuvanted vaccines are necessary for long term protection against diseases, but these vaccines induce adverse reactions in the form of adhesions and dark pigmentation in the abdominal cavity in vaccinated fish.

The major concern for PHARMAQ during the development of an oil based vibriosis vaccine for cod, was to secure the long term safety with regard to induction of adhesions and pigmentation after vaccination. In farmed cod, the liver is of economical value, therefore any damage of this organ due to vaccination must be prevented. To avoid damage to the liver investigations on the proper injection site has been performed, and implemented.

Oil-adjuvanted vaccines tested in preliminary studies seem to induce acceptable adverse reactions in vaccinated cod. The reactions in the abdominal cavity after vaccination also seem to differ between cod and salmon. In cod the lesions are less extensive and less firm than in salmonids. The principal characteristics in this species are that the vaccine is very quickly encapsulated in the abdominal cavity, the presence of adhesions between the organs and peritoneal wall are not common. The development of pigmentation on organs and in muscle, as observed in salmonids, is practically not observed.

One of the purposes of the field studies was to confirm the results on safety (adhesions and melanisation), observed in the preliminary safety laboratory studies with oil based vaccine. Side effects have been evaluated at different time points in each of the field studies using the Speilberg – scale developed for salmonids.

An overview of the results obtained in laboratory and field studies after vaccination with ALPHA MARINE Vibject is presented.

Seven field studies have been performed at commercial farms to evaluate the safety and efficacy of the vaccine. Some of the studies are still ongoing, but the preliminary data includes results up to 18 months post vaccination. The studies will continue until slaughter of the fish, minimum 24 months post vaccination.

2. SAFETY

2.1 *Results and conclusions from laboratory studies*

The safety of ALPHA MARINE Vibject for the target species cod (*Gadus morhua*) under controlled laboratory conditions is considered satisfactory administered at twice the recommended dose for fish with a minimum weight of 23 g at vaccination.

ALPHA MARINE Vibject is safe to use after administration of the recommended dose in cod of approximately the minimum recommended size maintained at semi commercial conditions.

The table below presents an overview of side effects observed at injection doses of 0.1ml and 0.2ml for batches of ALPHA MARINE Vibject in the laboratory trials.

Groups	Months post vaccination	Average adhesion score (0.1ml)	Average adhesion score (0.2ml)	Vaccine residues (0.1 ml)	Vaccine residues (0.2 ml)
Marin 02.04 FO-2	3	1.3	-	1.1	-
Marin 02.04 FO-2	6	1.3	-	0.1	-
Marin 02.04 FO-2	12	1.3	-	0.9	-
Marin 02.04 FO-2	1	-	1.6		1.2
Marin 07.05 FO-2	1	-	1.7	-	1.0
600001	1	-	1.6	-	0.9

The level of adverse effects caused by the product in standard laboratory test and at semi commercial condition is considered acceptable. The side effects observed should not decrease the growth / quality of the fish.

2.2 *Results and conclusions of field trials*

Side effects have been evaluated in each of the field studies based on the Speilberg – scale. The side effect evaluations have been done at different time points post vaccination and will continue throughout the production cycle.

The results obtained in field trials under commercial conditions confirm the results of safety (adhesions and melanisation) observed in the preliminary safety laboratory studies with ALPHA MARINE Vibject.

A summary with the results recorded at different time points post vaccination are presented below.

Summary of side effects recorded in all on-going field trials with ALPHA MARINE Vibject:

Trial	Batch	Months post vaccination	Average adhesion scores	Average melanin scores	Vaccine residues
Marin 08.05 FT	600001	5	1.8	0	1.0
Marin 03.05 FT	600001	2.5	1.8	0	1.0
Marin 03.06 FT	600002	4	1.3	0	0.5
	600002	7	1.3	0	0.1
Marin 04.06 FT	600002	2.5	1.2	0	0.9
	600002	5	1.5	0	0.4
Marin 01.06 FT	600001	5	1.0	0	0.1
	600001	10	1.3	0	0
Marin 05.06 FT	600002	3	1.2	0	0.1
	600002	5	1.1	0	0.1
Marin 05.04 FT	600001	4	1.5	0	0
	600001	13	1.6	0	0
	600001	18	1.3	0	0

The fish vaccinated with ALPHA MARINE Vibject has been examined for adverse effects in the form of adhesions and pigmentation in the abdominal cavity from 2.5 to 18 months post vaccination. The highest score recorded during the evaluations was 1.8 on the Speilberg scale.

Evaluations performed under semi –intensive production condition and under commercial conditions show that side effects in cod after vaccination with the oil based vaccine (ALPHA MARINE Vibject) are less extensive and firm than observed with oil based vaccine in salmonids.

The main characteristics of the side effect reactions are:

- Adhesions are recorded mainly between the intestines, pyloric caeca and the stomach.
- The vaccine is very quickly encapsulated in the abdominal cavity.
- The presence of adhesions between the organs and the abdominal cavity wall are not common, unless the injection of the vaccine is not performed at the recommended injection site.
- The development of pigmentation on organs and in muscle, as observed in salmonids, is practically none existent in cod.
- Visible oil based vaccine residues are significantly reduced from 3 weeks to 12 weeks post vaccination. It is not common to find vaccine residues at later time point evaluations.

= In salmonids an average score lower than 2.0 – 2.5 at time point eight weeks after sea transfer, is regarded as acceptable. Individual scores of 4 or higher are

regarded unacceptable and will decrease the growth and/or the quality of the fish. These levels of side effect in cod have never been observed.

The field studies are not finished and the last evaluation will be performed at slaughter approximately 24 months post vaccination. Our experience in salmonids shows that adverse effects can increase approximately up to 6 - 9 months post vaccination. After 6-9 months adverse effects will decrease or stay at the same level. We therefore presume that a similar development of the adverse reactions will be seen in cod as well.

The side effects observed up to 18 months post vaccination indicate that the use of oil based vaccines in cod should not represent a risk to commercial cod production.

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3. EFFICACY

3.1 Results and conclusions of laboratory trials

The efficacy of ALPHA MARINE Vibject has been tested in laboratory studies using three batches of vaccine.

The laboratory studies demonstrate that vaccination with ALPHA MARINE Vibject reduces the mortality caused by *V. anguillarum* O1. After challenge with the pathogen 6 weeks post vaccination a RPS₆₀ value between 98% to 100% was obtained for the three batches.

The challenge with *V. anguillarum* O2a did not reach a control mortality of 60% for any of the batches, therefore no RPS₆₀ values could be calculated. The challenge with *V. anguillarum* O2a did however pass the test criteria of 40% mortality, maximum 21 days after the first specific mortality for all three batches. The RPS_{END} for these batches varied between 98% and 100%.

In cod *V. anguillarum* O2b is the major cause of vibriosis, but *V. anguillarum* O2a and O1 do also cause outbreaks. The low mortality recorded in control group after challenge with *V. anguillarum* O2a and O1 indicates a lower susceptibility of the fish used in challenges to these pathogens.

The studies show that vaccination with ALPHA MARINE Vibject reduces the mortality caused by *V. anguillarum* O2b for all three batches. After challenge with this pathogen 6 weeks post vaccination, a RPS₆₀ value higher than 98% was obtained.

The onset of immunity occurs no later than 500 degree days post vaccination for *Vibrio anguillarum* serotype O1, serotype O2a and serotype O2b.

The duration of protection against vibriosis can not be tested for ALPHA MARINE Vibject in controlled laboratory studies, because of difficulty in maintaining cod under laboratory conditions and because the vibrio challenge model is only developed for small fish. The duration of protection must therefore be evaluated after use under field conditions.

3.2 Results and conclusions of field trials

Vibriosis represents a problem in farmed cod and is the major reason for the use of antibiotics in this industry.

Cod is exposed to vibriosis already from hatching. PHARMAQ does therefore recommend a vaccination administration where a combination of immersion vaccination at 1 gram and 5 gram sizes and injection vaccination at later stages is used to protect the fish throughout the life cycle.

Based on experience with the water based vaccine ALPHA MARINE Vibrio it is evident that new formulations that induce stronger immunity and longer duration of protection is needed in order to protect cod against disease throughout the life cycle. With ALPHA MARINE Vibject, PHARMAQ has developed an emulsion for injection vaccine that has shown to give improved protection relatively to ALPHA MARINE Vibrio.

The preliminary data from five field trials are presented. The data includes results from 5 to 18 months post vaccination. In the field trials approximately 530 000 cod have been vaccinated with ALPHA MARINE Vibject under commercial conditions in different geographical regions of Norway. All trials are still ongoing.

Efficacy has been evaluated based on the mortality caused by vibriosis and the difference in mortality recorded between the test vaccine ALPHA MARINE Vibject and the control group ALPHA MARINE Vibrio.

A summary with results recorded at the different observation periods post vaccination is presented below.

Summary of efficacy field trials ongoing with ALPHA MARINE Vibject (test vaccine) and ALPHA MARINE Vibrio (control vaccine)

Trial	Observation period post vaccination	Disease outbreak (s)	Cum. Mortality (%) during observation period	Cum. mortality (%) during observation period	Treatment
			ALPHA MARINE Vibject	ALPHA MARINE Vibrio	
Marin 03.06 FT	7	Atypical furunculosis	1.2	6.7	yes
Marin 04.06 FT	5	Vibriosis	20	6	yes
Marin 05.06 FT	5	Vibriosis	1	2.5	no
Marin 01.06 FT	10	Atypical furunculosis	3	5	no
Marin 05.04 FT	18	Vibriosis	12	24	no

Vibriosis has been observed in 4 of the five farms. The presence of disease has in most of the cases been related to rise in temperature in the sea water above 14 °C. In most of the cases vibriosis has been recorded as a sub clinical condition and

vibrio has been isolated in dead fish from mortality sock during veterinary routine inspection.

The picture of disease has varied from case to case, from sub acute to acute vibriosis outbreaks. In two of the farms it was necessary to treat the fish with antibiotics, but in one of them the treatment was against atypical furunculosis.

The most serious mortality in the group vaccinated with ALPHA MARINE Vibject, was recorded in study Marin Injection 04.06 FT. In this study, vibriosis were recorded before and shortly (few days) after the fish was vaccinated. The recommended immunisation time of 500 degree days had not been followed and a new outbreak of vibriosis was recorded after transfer of the fish to the sea cages. The uncertainty of the health condition of this fish makes it difficult to evaluate the efficacy of ALPHA MARINE Vibject.

Repeated incidents of vibriosis indicate that the fish is not well protected against the pathogen. This could be explained by a suppressed ability to respond to vaccination due to outbreaks of vibriosis before vaccination. Rise in temperature and transportation, which represent a stress situation for the fish could also increase the incidences of disease outbreaks.

In study Marin Injection 05.06 FT, a low mortality was recorded up to 5 months post vaccination even vibriosis was observed. The mortality was low for both vaccines, but higher for the water based vaccine. It is most possible that, at 5 months post vaccination, a water based vaccine still provide appropriate protection against vibriosis.

Relatively low mortality was recorded up to 10 months post vaccination in study Marin 01.06 FT. Sub clinical vibriosis was diagnosed from a few occasional dead fish, but no clinical outbreak of vibriosis was recorded in any of the vaccinated groups. An outbreak of atypical furunculosis was observed. Lower mortality was recorded in the observation period for the ALPHA MARINE Vibject vaccinated group.

The study performed up to 18 months post vaccination (Marin 05.04 FT), show a distinct difference in mortality between vaccines in the complete observation period. The mortality caused by vibriosis in the ALPHA MARINE Vibject group was strongly reduced compared to the water based vaccine ALPHA MARINE Vibrio. The results indicate that ALPHA MARINE Vibject may induce a longer duration of protection, than ALPHA MARINE Vibrio.

In the summer of 2006 very high temperatures in the sea were recorded. In addition saithe located at the same site as the test cod, were affected by vibriosis. Even though vibrio was observed in the test cod, no treatment was necessary.

In two cases where vibriosis was observed, *V. anguillarum* O2 b was verified as the cause of dead. In outbreaks over the past years, vibriosis have also been recorded in cod during the grow out period and the disease has been identified as *V. anguillarum* O2a.

In some cases bacteria isolated from outbreaks have shown negative results to the test anti *V. anguillarum* O1, O2, O3 and O4.

Further examinations from the *V. anguillarum* O2a outbreaks classify these bacteria as atypical *V. anguillarum* O2a. PHARMAQ is currently working to clarify the cross protection between different *Vibrio anguillarum* O2a strains in cod.

The results presented in these field trials are preliminary and the trials are still ongoing. The data that will be collected the second summer in sea will be very important in order to conclude on duration of protection of the oil based vaccine ALPHA MARINE Vibject compared to the duration of protection of the water based vaccine ALPHA MARINE Vibrio.

PHARMAQ considers ALPHA MARINE Vibject, as a first generation emulsion for injection vaccine for cod. The experience with ALPHA MARINE Vibject and further development of the antigen composition of vaccines for farmed cod will be important to support a sustainable industry.

4. OVERALL CONCLUSION

- ALPHA MARINE Vibject does not excite any abnormal behaviour or toxic reactions in cod vaccinated at >30 grams.
- The side-effects recorded up to 18 months post vaccination with ALPHA MARINE Vibject are low and at an acceptable level.
- ALPHA MARINE Vibject administered at a dose of 0.1 ml/fish is safe to use in cod.
- The laboratory studies showed that vaccination with ALPHA JECT Vibject reduces the mortality caused by *V. anguillarum* O1, O2a and O2b, after challenge with the pathogen 6 weeks post vaccination
- The preliminary results indicate that ALPHA MARINE Vibject reduces mortality caused by vibriosis under commercial field conditions.

January 2007