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Committee for Medicinal Products for Veterinary Use (CVMP)

Guideline on the design of studies to evaluate the safety and efficacy of fish vaccines

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* This guideline replaces the Guideline on the design of studies to evaluate the safety and efficacy of fish vaccines (EMA/CVMP/IWP/314550/2010). The current revision consists of administrative changes made in order to align the guideline to Regulation (EU) 2019/6. The references to the legislation applicable and other scientific guidelines have also been updated. As no changes were made to the scientific content, no concept paper and no public consultation were deemed necessary.

Keywords	***Immunological veterinary medicinal products, veterinary vaccines, fish, finfish, safety, efficacy, study design***

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Executive summary

This document provides information on items to be considered in the design and conduct of studies to support the safety and efficacy of immunological veterinary medicinal products (IVMPs) in finfish.

The guideline outlines important items to take into account for both pre-clinical laboratory scale size studies and clinical (field) trials so that the studies are representative of the safety and efficacy of the vaccine when administered in accordance with its intended use (e.g. type of finfish to be used; water conditions, method of administration, use of control groups etc).

The guideline also outlines aspects to be considered in the determination of the duration of immunity for vaccines intended for use in finfish.

1. Introduction (background)

This document provides guidance in respect of the design of studies for the evaluation of the safety and efficacy of immunological veterinary medicinal products (IVMPs) for use in finfish. The procedures outlined should be considered for all submissions but may not be applicable for all IVMPs for use in aquaculture. If certain aspects are modified or omitted, justification should be provided.

In principle the results of all studies should be applicable irrespective of where they are carried out; however, the applicant should take into account the various conditions (e.g. climatic, disease situation, water temperature and salinity) as these may influence the outcome of the studies.

IVMPs for use in finfish meet the definition of 'limited market' in Article 4(29) of the Regulation (EU) 2019/6. Therefore, the guidance provided for IVMPs intended for limited markets could be considered.

2. Scope

The aim of this guideline is to provide guidance regarding the conduct of studies to demonstrate the target animal safety and efficacy for immunological veterinary medicinal products intended for use in farmed finfish.

3. Legal basis

This document should be read in conjunction with Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 of the December 2018 of veterinary medicinal products, and relevant European Pharmacopeia (Ph. Eur.) monographs and chapters (e.g. Ph. Eur. 0062, 5.2.6 and 5.2.7). Furthermore, relevant guidelines, particularly the Guideline on data requirements for applications for immunological veterinary medicinal products intended for limited markets submitted under Article 23 of Regulation (EU) 2019/6 (EMA/CVMP/59531/2020-Rev.1) and/or the Guideline on safety and efficacy data requirements for applications for immunological veterinary medicinal products intended for limited markets but not eligible for authorisation under Article 23 of Regulation (EU) 2019/6 (EMA/CVMP/IWP/224724/2022) should be considered.

All animal experiments should be conducted taking into account section I.1.7 of Annex II of Regulation (EU) 2019/6 and the 3Rs principles (replacement, reduction and refinement), notwithstanding the place of conduct of the experiments. Alternatives to in vivo test methods should be employed whenever possible.

4. General considerations for studies involving fish

The following animal welfare concerns should be taken into consideration when designing studies to test the safety and efficacy of IVMPs in finfish:

Personnel conducting the studies should be appropriately trained to detect behavioural changes / signs of illness in the participating fish.

The method used to identify vaccinated and controls fish should involve the least harmful technique.

The number of fish in the vaccinated and control groups should be sufficient to obtain statistically significant and clinically reliable results. However, for vaccination-challenge studies, the possibility of reducing the number of non-vaccinated control fish should be investigated as these fish will suffer disease and associated distress.

Mortality as an evaluation parameter in vaccination-challenge studies should be questioned whenever possible; humane endpoints are preferable. Moribund fish should be humanely slaughtered.

Relevant items to be considered in the design / performance of both safety and efficacy studies for fish vaccines are outlined below.

4.1. Fish to be used

The fish to be used must not have been vaccinated against any of the antigens in the vaccine and should be from a population shown to be free from specific antibodies against any of the vaccine antigens against which protection is claimed.

The range of species, ages, sizes, weights and physiological status (e.g. smoltification, sexual maturation) of fish used in the studies must be representative of those to which the vaccine will be administered in the field for commercial purposes. For pre-clinical studies, fish of the minimum recommended vaccination age or size should be used.

The origin/varying genetics of the experimental fish used is important to obtain valid reproducible results. Variation from those which will be encountered under commercial use conditions should be addressed. Fish used in studies should preferably be derived from stock which will be used commercially.

All finfish species shall be identified by their colloquial name followed in parenthesis by the Latin or Linnean description.

The allocation of fish to vaccinated and control groups should be done randomly, using an appropriate method. The numbers of fish per group should be justified and the sample size in each group should be sufficient to allow the results to be statistically significant and clinically reliable.

If vaccinated and control fish are not housed in the same tank, to overcome tank effects which may be experienced between groups of fish which are kept under identical conditions but in different tanks, measures should be taken to overcome such effects e.g. a minimum of two tanks could be used for each of the vaccinated and control groups. Effects of stocking density on the parameters to be determined should be taken into consideration.

4.2. Water conditions

Water quality parameters such as temperature and salinity (e.g. freshwater versus seawater) used in each pre-clinical study during vaccination should be relevant to the environment under which the vaccine will be used for commercial purposes. For challenge model development consideration can also

be given to possible changes in the water conditions/environment which fish may be exposed to during the life cycle (e.g. changes associated with transfer to sea etc).

Water quality parameters such as temperature and salinity should be documented in each study report.

The different climatic conditions and water temperatures within the Community should be considered, when relevant for the fish species/disease in question. Some studies may need to be performed both at high and low temperature for the relevant fish species/disease distribution. Similarly, some studies may need to be performed following the main production practices for the species (e.g. S0 and S1 stocking for salmon).

The chosen conditions should be justified by the applicant for each study.

To account for the fish being poikilothermic animals and taking into account the fact that immunity in fish is temperature dependant and that the frequency and intensity of injection site reactions increases with higher water temperatures, comparative data from safety and efficacy pre-clinical studies involving fish should be based on "degree-days".

4.3. Vaccine to be administered

The vaccine formulation to be administered in the safety and efficacy studies should be the final formulation proposed for marketing. Data from studies performed with a formulation(s) which differs to the proposed final commercial formulation can only be used as supportive information unless the applicant can justify that the differences have no impact on the safety or efficacy profile of the vaccine. The vaccine dose (i.e. dose volume and amount) and administration method(s) employed in the safety and efficacy studies must represent those proposed for commercial use of the vaccine.

4.4. Study reports

The applicant is encouraged to standardise study protocols and study reports as far as possible to facilitate the comparison of study results and the possible extrapolation between species.

Each pre-clinical study or clinical trial and the conditions under which they are performed should be described in detail.

Separate reports on all studies, whether favourable or not, should be provided. All adverse events should be reported. An explanation of non-specific mortalities and comments on any physical or behavioural abnormalities should be provided.

5. Pre-clinical studies

Requirements for pre-clinical safety and efficacy studies for veterinary vaccines are outlined in Regulation (EU) 2019/6, and in particular in section IIIb.3 and section IIIb.4 of Annex II thereof, and relevant Ph. Eur. texts (e.g. Ph. Eur. 0062, 5.2.6 and 5.2.7).

Additional items to be considered in the design and performance of pre-clinical safety and efficacy studies for fish other than those referred to in the above-mentioned documents are outlined in the following sections of this guideline.

5.1. Safety studies

The safety should be determined for all the proposed target species, unless otherwise justified.

Studies performed in one species of fish may be considered relevant for the evaluation of safety in a second species of fish, provided that the recommended conditions for use of the vaccine in both species are similar e.g. similar fish size/water temperature and quality etc at the time of vaccination. In such a case there should be supportive data from studies in the second species. It may for example be considered unnecessary to carry out pre-clinical safety studies in trout if such studies have been carried out on other species (e.g. salmon), and if clinical trials in trout are available.

The use of a negative control group may be useful in the evaluation of the safety of certain vaccines. For example, for parenteral vaccines inclusion of a mock-vaccinated group (e.g. saline) in the study should be considered to distinguish between reactions attributable to the vaccine itself and reactions associated with the injection process e.g. the anaesthetic used during vaccination and other manipulations.

In all tests, the vaccine and control should be administered in the same manner and control fish should be handled identically to vaccinated fish.

Details of the type of control group used should be clearly documented and the applicant should justify the contents of the control "vaccine".

To assess the acute safety characteristics of the vaccine, the fish should be monitored daily for mortality/morbidity over a minimum of a 14-day period (or as recommended in the relevant Ph. Eur. monograph for specific vaccines) taking into account the optimal water temperature for the target species. At the end of the monitoring period, when appropriate, the fish should be slaughtered humanely and examined for systemic and/or local reactions macroscopically and/or microscopically as appropriate.

For parenteral vaccines, post-mortem examination should include investigation of the occurrence of effects such as pigmentation (e.g. melanisation) and adhesions. (e.g. measured using the Speilberg score – refer to Annex 1of this guideline for details).

The nature and frequency of all adverse events should be monitored and recorded.

It is important to take into account the possible adverse effects of vaccine administration on development over the life span of the target fish species. This is particularly important in the case of parenteral vaccines as adhesions may have a negative effect on spawning, and adhesions/pigmentation may result in rejection or down-grading of fish at slaughter.

On this basis, the safety studies should be capable of allowing a prediction to be made of the safety profile over the average life span of the fish species. For example, weight gain over the life span (for food producing fish) and for parenteral vaccines the percentage of fish downgraded on quality grounds due to adhesions/pigmentation at slaughter time etc are important aspects to be considered.

It may be more appropriate to evaluate the long-term safety effects of vaccine administration over the life span in clinical trials as discussed in Section 6 below.

5.2. Efficacy studies

The efficacy studies should be designed to support the use of the recommended vaccine dose and administration schedule (including the recommended re-vaccination, if applicable) in providing optimum protection against the claimed indications.

The parameters evaluated should be appropriate to the proposed indications (including onset and duration of immunity claims (if relevant to the study design)) and should be pre-determined prior to conducting the studies.

Every study should be designed to allow for appropriate statistical evaluation. A sample size analysis should be presented. The statistical evaluation methods should be justified.

The challenge model used in each study must be justified by the investigator and the relevance to the natural disease situation should be discussed. Items to be considered include: the relevance of the challenge organism(s) to the disease(s) against which protection is claimed, the method of administration of the challenge organism (e.g. cohabitant, injection etc), the water temperature, the timings of (i) the challenge and (ii) the recording of the evaluation parameters. In the case of claims for protection against mortality, it is important that the evaluation period is of sufficient duration to reveal the total development of the mortality curve, both in control and vaccinated animals, as vaccination may delay the onset of mortality. In efficacy studies where mortality is expected, the use of validated humane endpoints should be considered.
Data are required to support each proposed indication for all target species for which efficacy is claimed. Where justified, challenge studies can be replaced by an alternative method based on antibody response when a suitable correlation with efficacy has been demonstrated.

The studies should include a control group, and the applicant should justify the choice of the control group (mock-vaccinated or non-vaccinated) used.

Data from well controlled pre-clinical studies are preferred wherever relevant models are available, and clinical trials should serve to confirm the findings from the controlled studies. However, it is recognised that for some disease situations in fish, no or only poor challenge models exist. In such situations, with appropriate justification, more emphasis may be placed on clinical trials conducted under conditions which reflect the disease situation in the field as discussed in Section 6 below.

6. Clinical trials

The scope of clinical trials is to ensure that the vaccine is efficacious and safe in the diversified conditions for aquaculture found in Member States for the relevant fish species. The clinical trials are to be performed in established commercial farms. Controlled clinical semi-field studies performed in large scale research facilities may also be relevant, if justified. A satisfactory number of sites with conditions representative for the normal in-use conditions should be used. The applicant should justify the number of sites. The clinical trials should be performed in accordance with GCP as far as possible.

The number and suitability of the sites selected for the clinical studies should be justified by the applicant. These should be geographically well distributed to optimise the possibility of diversified environmental conditions, disease situation and management practices. Each site should have several pens or tanks with fish of the relevant size/age and physiological condition (e.g. smoltification, sexual maturation) for the proposed use of the vaccine. At least two of the pens or tanks, and preferably several pairs of pens/tanks should be used in the study per vaccinated and control group. The farmer should preferably be experienced in keeping detailed records on all important factors concerning the farm and its fish. Records on the source of fish and the disease history in different pens or tanks must be kept. Previous medication, use of chemicals and vaccines should be known. Daily records of outbreaks of disease, mortality and medication are required, as well as known and stable management practice concerning for example hygiene, feeding, handling and use of feed additives and chemicals.

Both a vaccinated and control group should be used. The allocation of the groups should be done randomly, using an appropriate method. The prevalence of disease, daily mortality, clinical symptoms and other relevant parameters should be comparable in the vaccinated and control group. The type of control group used (i.e. mock-vaccinated, non-vaccinated or positive control) should be justified. If a positive control (e.g. a comparator vaccine) is used, consideration should be given to maintaining a (small) group of non-vaccinated fish in a separate test pen to serve as indicators of exposure to

disease(s) at farm level. Once the relevant infection has been diagnosed in the controls they can be slaughtered humanely.

For clinical studies involving multivalent vaccines where one or more new antigens have been added to a previously approved vaccine, the control vaccine should ideally contain the same antigens as the test vaccine except for the new antigen(s).

Clinical trials in commercial fish farms should preferably be performed in farms known to be subject to spontaneous outbreaks of the disease(s) against which protection is claimed. Studies should thus be conducted at the time of year and under conditions relevant to the occurrence of a "natural challenge". The method of identification and confirmation of the presence of the causal agent(s) for the natural challenge in each group is an important factor for field studies involving fish. The method used must be relevant to the disease situation and should be recorded for a representative number of fish in each group. Justification should be provided for the diagnostic method(s) and representative number of fish used with consideration being given to guidance on diagnostic methods from official disease control laboratories.

Information from studies where a natural challenge was not detected should be provided along with a discussion of the relevance of the parameters chosen as endpoints for the proposed claims. A full evaluation of the safety data from these studies should be provided.

Unless otherwise justified, data from both pre-clinical studies and full-scale clinical trials will be required. Where appropriate the applicant should justify the lack of relevant data.

Omission of clinical trials and submission of pre-clinical studies may be accepted if adequately justified by the investigator. For example, in case of a second species closely related to a first species for which the product is fully documented and where recognised models to establish vaccine efficacy (e.g. challenge or antibody response) exist, pre-clinical studies may be sufficient to document efficacy also in the second species.

In situations where clinical trials are not expected to be of value for assessment of efficacy due to absence / low occurrence of natural challenge and efficacy data are only available from pre-clinical studies, consideration should be given to including additional efficacy endpoints in the pre-clinical studies, and/or waterborne challenge in addition to challenge via injection in order to mimic the field conditions. In cases where pre-clinical studies fully support the claims made in the summary of product characteristics, efficacy trials carried out in the field are not required. However, the omission of clinical trials for this reason still has to be duly justified.

7. Duration of immunity (DOI) claims

It is important that DOI claims are supported by reliable data. The following aspects should be considered:

Studies conducted under field-like conditions where groups of fish are taken from the holding tank / cage / pen etc at different intervals and subjected to challenge infection or evaluation of a specific antibody response (where a suitable correlation with efficacy has been established) are useful in evaluating the DOI. For these studies, the relevance of the holding conditions used (e.g. freshwater vs seawater; water temperature / quality etc) to the conditions which will be encountered when the vaccine is used naturally in the field should be considered when proposing a DOI for the vaccine.

Clinical trials used to determine DOI should involve monitoring for the occurrence of disease / causal agent(s) at the participating site(s) on a regular basis to ensure that the disease is detected as soon as possible after the outbreak. Large time periods between monitoring points could result in detection at a

much later time than the actual outbreak of the disease. In addition, it is possible that natural exposure to the pathogen/s may boost immunity.

Ideally, the DOI claims will be based on a combination of the results from challenge studies conducted under semi-field conditions and disease monitoring data and serological testing results from the clinical trials.

In general, for the DOI claims proposed for the product information it is not necessary to refer to the design and the conditions used in the studies unless for specified reasons (e.g. different production methods exist for the target species, DOI has not been determined for all target species or different DOI for different target species).

If a reliable DOI cannot be determined from the challenge / field studies, this should be stated in the product information.

Definitions

For the purpose of this guideline, the following definitions apply:

Finfish: A term used to separate true fish from shellfish, crayfish, jellyfish etc. All the species of fish mentioned in this guideline are examples of true finfish.

Degree days: The amount of degree days is determined by multiplying the water temperature each day with number of days. For example, 10 days with 5° C equal 50 degree days.

Parenteral administration: the vaccine is administered by injection.

Immersion administration: vaccine is administered by dipping or bathing the fish in an immersion bath/tank. Spray vaccination is a form of immersion vaccination.

Oral administration: vaccine is administered via the feed.

Annex 1

Speilberg scoring system (Midtlyng *et al*. 1996).

Score	Visual appearance of abdominal cavity	Severity of lesion
0	No visual lesions	None
1	Very slight adhesions most frequently localised close to the injection site. Unlikely to be noticed by laymen during evisceration	No or minor opacity of peritoneum after evisceration
2	Minor adhesions, which may connect colon, spleen or caudal pyloric caeca to the abdominal wall. May be noticed by laymen during evisceration.	Only opacity of peritoneum remaining after manually disconnecting the adhesions.
3	Moderate adhesions including more cranial parts of the abdominal cavity, partly involving pyloric caeca, the liver or ventricle, connecting them to the abdominal wall. May be noticed by laymen during evisceration.	Minor visible lesions after evisceration, which may be removed manually.
4	Major adhesions with granuloma, extensively interconnecting internal organs, which thereby appear as one unit. Likely to be noticed by laymen during evisceration.	Moderate lesions, which may be hard to remove manually.
5	Extensive lesions affecting nearly every internal organ in the abdominal cavity. In large areas, the peritoneum is thickened and opaque, and the fillet may carry focal, prominent and/or heavily pigmented lesions or granulomas.	Leaving visible damage to the carcass after evisceration and removal of lesions.
6	Even more pronounced than 5, often with considerable amounts of melanin. Viscera cannot be removed without damage to fillet integrity.	Leaving major damage to the carcass.