Vaccine testing: How can we reduce fish numbers and/or avoid the use of fish?

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PHARMAQ AS
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Content of the presentation

• Regulatory framework
• From R&D to fish farmer
• The use of study animals during:
  — Development
  — Documentation
  — Field tests
• Study animals used for batch release
• Reduce, Refine and Replace
• Conclusion
Regulatory framework
- Licensing documentation

- **European Monographs**
  - Mandatory
  - Must be implemented for all *new and existing* products

- **Guidelines and Position papers**
  - Neither mandatory for the industry nor the authorities

- **Production and Control**
- **Safety**
- **Efficacy**

The framework sets the standard the industry
Regulatory framework
-Pharmacopoeia

- Evaluation of safety of veterinary vaccines (Ph. Eur. 5.2.6)
- Evaluation of efficacy of veterinary vaccines (Ph. Eur. 5.2.7)
- Furunculosis vaccine (inactivated, oil-adjuvanted, injectable) for salmonids (Ph. Eur. 1521)
- Vibriosis (Cold water) vaccine (Inactivated) for salmonids (Ph. Eur. 1580)
- Vibriosis vaccine (inactivated) for salmonids (Ph. Eur. 1581)

Mandatory for the industry
Regulatory framework
- Guidelines and Position Papers

- Guideline on good clinical practice (CVMP/VICH/595/98)
- Good Laboratory Practice
- The general requirement for the production and control of live and inactivated vaccines intended for fish (81/852/EEC)
- Data requirement for removing the target animal safety test for immunological veterinary medicinal products in EU (EMEA/CVMP/865/03/Final)

Guidelines may be deviated, when thoroughly justified
Documenting a new product
From R&D to fish farmer

The feasibility-development and documentation studies which include fish

Lab studies
- Virulence test
- Challenge mod (IP/Imm/Cohab)
- Potency

Lab/field studies
- Field studies
- Onset of imm
- DOI
- Safety
- Potency
- Stability
- Dose-finding

Lab/field trials
- Field trials (GCP)
- Safety (GLP)
- Efficacy
- Stability

License
Waiting for response from authorities

Post marketing studies
- Field studies.
- Batch release (S&P)
- Stability testing (S&P)
Commonly used methods
-In clinical vaccine studies

• Administration of vaccines (Imm; I.P or Oral).
• Anaesthesia (Metacain, Benzokain,)
  ✓ Always used prior to invasive procedures
• Blood-sampling from *vena caudalis*.
• Marking of fish by; removal of adipose fin, fluorescent dye, implant or tattooing (Alcian blue).
• Challenge of vaccinated fish with pathogens (I.P;Imm;Cohab).
• Euthanized prior to sampling.
Clinical development and documentation studies

- Studies must be relevant, using sufficient numbers of animals to obtain true differences between groups
  - Statistical design and methods should be used in order to optimise the study design. Statistical differences may not be of clinical relevance.

- Tests and methods employed should be validated (high specificity; repeatability and reproducible).

- Clinical laboratory and field studies should mimic the situation in field (this is a challenge....)
Research fish used during: Documentation of efficacy – lab.

- Documentation of three batches of final product.
- Show consistency between batches.
- One dose of vaccine injected.
- Fish marked for identification.
- Challenge I.P at 6-8 weeks post vaccination (relevant method?).
- Control mortality ≥ 60%.
- Mortality observed until 21 days after the first specific death of fish.

<table>
<thead>
<tr>
<th>Test</th>
<th>Guideline</th>
<th># fish / batch and antigen</th>
<th># fish (total)</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy</td>
<td>Ph. Eur.</td>
<td>100</td>
<td>800</td>
<td>21 days after the first specific death</td>
</tr>
<tr>
<td>Monovalent</td>
<td></td>
<td></td>
<td>4000</td>
<td></td>
</tr>
<tr>
<td>Hexavalent</td>
<td></td>
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</tbody>
</table>

Efficacy test is important and is performed once only.
Research fish used during: Documentation of safety - lab. (GLP)

• Secure that the product is safe to use (toxicity test)
• Documentation of 3 batches
• Fish blood sampled prior to vaccination (doc of seronegativity)
• Marking by fin clipping
• Injected double dose of vaccine and observed for 21 days

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<tbody>
<tr>
<td>Double dose safety</td>
<td>Ph Eur.</td>
<td>50</td>
<td>150 (v)+ 50(c)</td>
<td>21 days</td>
</tr>
</tbody>
</table>

The GLP-Safety test is relevant but does not disclose true local reaction profile.
Research fish used during:
- Field studies

**Design**
- Two replicate cages
- 1000 – 3000 fish per cage
- 6 groups per cage
- Groups are marked and mixed
- Two premises ran in parallel

**Advantages**
- Frequent sampling
- Pilot vaccines may be tested
- Eliminate cage variation
- May be exposed to natural challenge
- Use a limited number of fish

**Disadvantages**
- Outbreak of disease rarely occurs
- Does not equal production cages
- Growth not optimal
- Fish not for commercial consumption

The mini cage studies give good and reliable documentation.

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Fish used during: GPC-Field trials

Trial in production cages

157 meter circumference

Documentation of safety and "efficacy (antibody)".

Design
• Min 3 sites included in the trial
• One or two cages/vaccine per site
• 4-500,000 fish per cage
• Test and control (positive) product in separate cages

Advantages
• Production conditions
• Self experience for farmer
• May be exposed to natural challenge
• Fish used for human consumption

Disadvantages
• Outbreak of disease rarely occurs
• Replicates more difficult
• Difficult to do proper sampling
• Lot of vaccine necessary
• Approx 2 mill fish needed per site

Are fish vaccinated with licensed vaccines (control), under standard conditions research animals?
R-R-R-R
Replacement-Reduction-Refinement
R-R-R; Related to *Feasibility-Development-Documentation* studies.

- *In vivo* tests are necessary tools in order to develop safe and efficacious vaccines. In my opinion these tests will not (on a short term perspective) be possible to replace by *in vitro* tests, but:
  - Optimising study design could reduce the number of fish included in each *in vivo* study.
  - All equipment at the trial facility should be optimised for conducting fish trials.
  - Anaesthetic should be used prior to all stressful situations of a certain magnitude.
  - High water quality should be available for the trial fish.
  - Automatic surveillance systems should monitor the environment of the fish.
  - Clinical trial staff needs to be trained in order to handle the trial fish.
  - The least invasive (but relevant) vaccination/challenge/marking methods should be employed.
  - Hard endpoints should be identified (i.e. mortality vs. morbidity).
• Batch Safety

✓ Applying consistency approach by removing batch safety test (EMEA/CVMP/865/03/final) after approval of 10 consecutive production batches (applicable for fully licensed products only?).

✓ Dobbel dose batch safety could be eliminated and combined with the batch potency test (single injection). ”No” risk of dobbel injection during commercial operations.
Cont.

• Batch Potency
  ✓ Replace *in vivo* challenge test with:
  
  1. *In vitro* methods
     • Antigen quantification assay (ie ELISA, quantitative immuneblot etc.) could replace most *in vivo* procedures (i.e batch release, stability of vaccine/bulk antigen).

  **Question:** Is this approach possible with multivalent vaccines?

  
  2. Serological antigen/antibody responses
     • Validated methods for detecting specific antibodies post vaccination ”may” have the potential as an alternative tool as a potency release test.

  **Question:** Serological responses in fish may vary a lot also within family groups. Is fish the right target animal or would an alternative like chicken/rat be a better target animal for a serological test?
Future

Within 5 years
• Eliminate batch safety tests after 10 approved batches

Within 5-10 years
• Replaced batch potency by *In vitro* tests.

Refine the definition of research animal:
  - Discriminate between fish animals that suffer (i.e. challenge) and animals that are handled by standard procedures used in the industry.
Conclusion

• *In vivo* vaccination-challenge studies are necessary tools in order to develop new vaccines that are safe and efficacious for the fish.

• The greatest potential of replacing *in vivo* test by *in vitro* assay is related to batch release and quality control of final product.

• The definition of study animals should be considered and clarified.
  ✓ Should there be distinction between laboratory and commercial animals used in *in vivo* research studies?
  ✓ Are fish vaccinated with autogenous vaccines research animals?
Thank you for your attention!

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