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

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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

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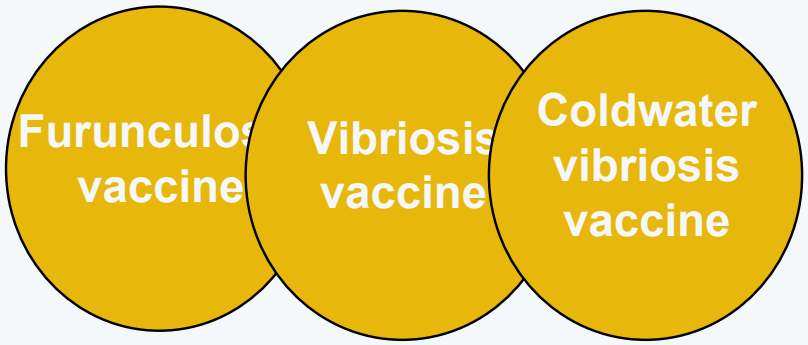
Regulatory framework
-Licensing documentation

- **European Monographs**
  - **Mandatory**
  - **Must be implemented for all <u>new and existing</u> 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

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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

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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

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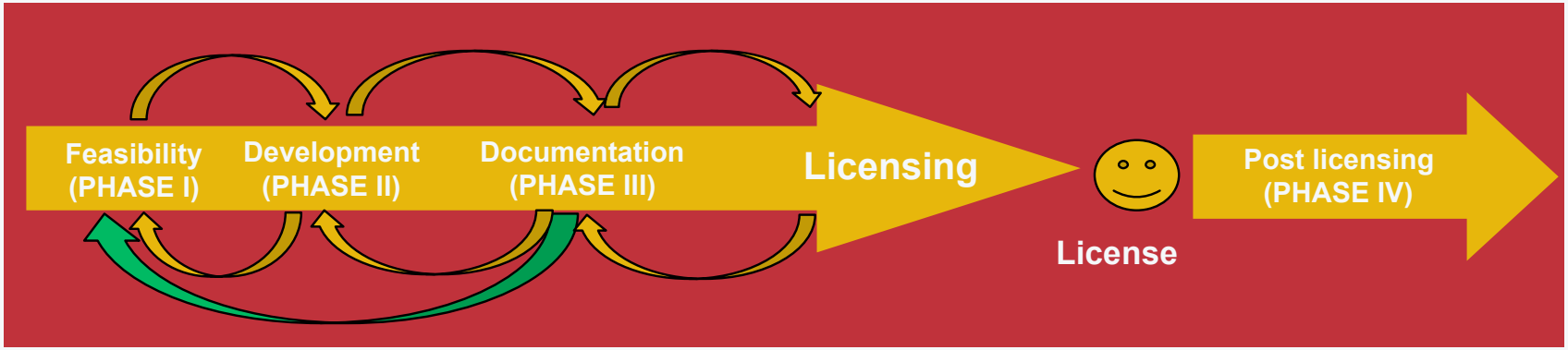
Documenting a new product

-From R&D to fish farmer



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

Waiting for response from authotities

Post marketing studies
- Field studies.
- Batch release (S&P)
- Stability testing (S&P)

The feasibility-development and documentation studies which include fish

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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.**

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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....)**

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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.

Test	Guideline	# fish / batch and antigen	# fish (total)	Observation
Efficacy	Ph. Eur.	100		21 days after the first specific death
Monovalent			800	
Hexavalent			4000	

Efficacy test is important and is performed once only.

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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

Test	Guideline	# fish /batch	# fish (total)	Observation
Double dose safety	Ph Eur.	50	150 (v)+ 50(c)	21 days

The GLP-Safety test is relevant but does not disclose true local reaction profile

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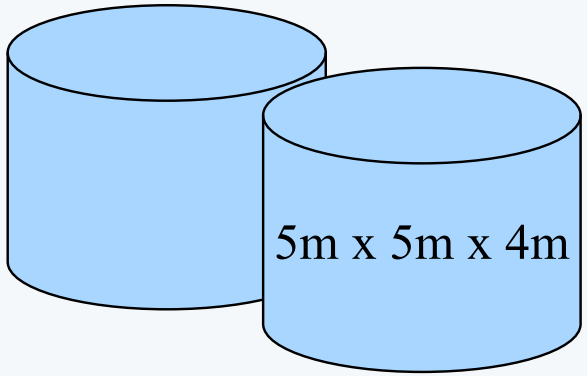
Research fish used during: -Field studies

Trial in mini cages



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 commersial consumption

The mini cage studies give good and reliable documentation

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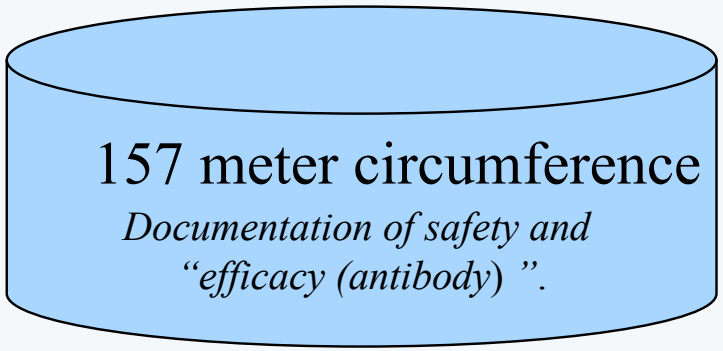
Fish used during: GPC-Field trials

Trial in production cages



Design

- Min 3 sites included in the trial
- One or two cages/vaccine per site
- 4-500.000fish 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?

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R-R-R

Replacement-Reduction-Refinement

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R-R-R;

Related to *Feasibility-Development-Documentation* studies.

- ***In vivo*** **tests are necessary tools in order to develop safe and efficacous vaccines. In my opinion these testet 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 stressfull situation of a certain magnitude.**
  - ✓ **High water quality should be available for the trial fish.**
  - ✓ **Automatic survailance systems should monitor the environment of the fish.**
  - ✓ **Clinical trail 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).**

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Cont.

- **Batch Safety**

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

  - ✓ **Dobbel dose batch safety could be elliminated and combined with the batch potency test (single injection). ”No” risk of dobbel injection during commersial operations.**

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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 respons .**
     - **Validated methods for detecting specific antibodies post vaccination ”may” have the potential as a alternative tool as a potency release test.**

     Question: Serological respons 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?

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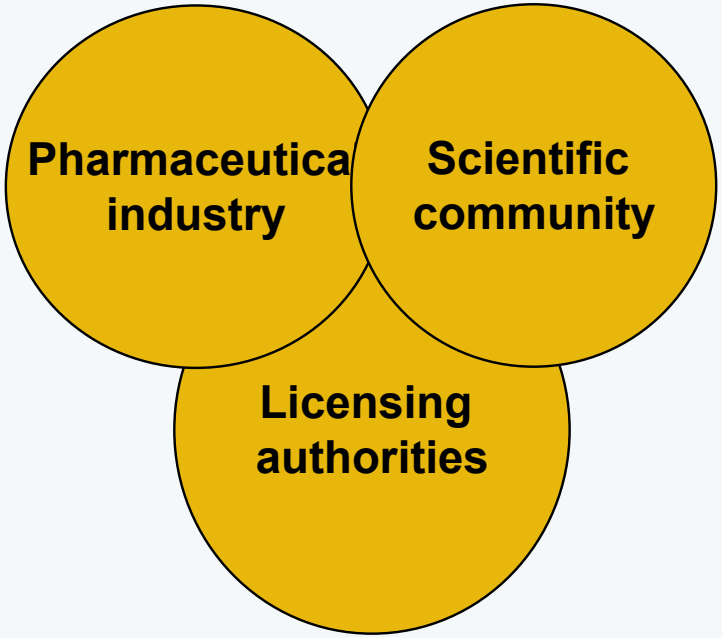
Future



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.**

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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?

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Thank you for your attention!

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