

# Development of Livestock and Aquaculture Infectious Disease Models

The development of new disease challenge models is required where new pathogens (or pathogens of new significance), pathogen strains or serotypes emerge. It may also be necessary where pathogen strains have developed resistance to existing licensed products. In order to assess the efficacy of veterinary medicinal products (VMPs) against these pathogens, it is necessary to have validated disease models available for conducting tests under controlled conditions.

Validated experimental models of livestock and aquaculture infectious disease are important tools for the generation of efficacy data for candidate veterinary medicinal products (VMPs) or feed products / supplements. For efficacy data to be submitted for registration purposes in Europe, the US and Japan, pivotal efficacy studies must be conducted to the standards defined in the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products, Good Clinical Practice (VICH-GCP).

While it is widely acknowledged that the number and scale of *in vivo* assays should be reduced, replaced and or refined (the 3Rs) most of the dossiers submitted contain data from *in vivo* challenge model efficacy studies. It is unlikely that the requirements for animal challenge models will reduce markedly in the near future, since in most cases it is not possible to produce the same quality of efficacy data using *in vitro* assays. There is, however, a responsibility to ensure the *in vivo* models used to generate registration data are industry relevant, biologically valid and statistically robust.

Challenge model development can be complex and costly. It is generally undertaken by animal health companies, aquaculture companies, contract research organisations or academic groups. Prior to undertaking any development, it is necessary to have a clear idea of what will be required in the development, the resources required and the likely problems that may occur. In some cases, it is possible to review the scientific literature for similar models which can help to determine appropriate model design, however, such information is not always available and, even with well-established model designs, there can be considerable variability in clinical signs, pathology and other outputs for different isolates for the same pathogen species.

There are 5 main steps involved in the development of a new disease model:

1. Sourcing of pathogen isolates
2. Identification of challenge titre, volume and route
3. Identification of target species parameters (eg age range)
4. *In vitro* validation
5. *In vivo* validation

This article will review the critical success factors for each of these steps.

## Sourcing of Pathogen Isolates

Once the need for a disease model for a particular pathogen has been identified, it should also be considered whether

there is a particular serotype, subtype or toxin production profile of the pathogen that is of particular significance in the geographic region(s) where the VMP will be marketed. This will increase the likelihood that data generated from the use of the model will be acceptable to the regulatory authorities. With changing epidemiology of disease worldwide, this selection can prove challenging, but sufficient information is generally available within the literature and through contacts with industry to allow an informed choice to be made. With improvements in the speed and cost for DNA sequencing in recent years, the genotype of different isolates can now also be used to assist in determining the most appropriate strains to use, and identification of particular pathogenic gene sequences can aid in accurate selection of pathogen strains.

Generally, it is suggested that selection of isolates should be based on a number of factors, taking into account:

- Geographical location
- Where the isolate was sourced
- The year of isolation – (as often the regulators require the use of field isolates which are <5 years old for regulatory studies)
- The age of the animal – and, in fish, the development stage and production environment, from which it was sourced (selecting isolates from animals similar to the intended target for the model)
- The clinical history of the isolate (i.e. what clinical signs were observed in the host animal and what pathology was observed at postmortem, if applicable).

This can be particularly important where pathogens can cause a range of different clinical signs – offering a wide range of parameters where statistical significance can be evaluated.

Where possible, isolates should be selected from cases where a single isolate was recovered. This is important since with multifactorial infections it is difficult, if not impossible, to attribute clinical signs to a single pathogen. Secondly, opportunistic species may or may not be disease-causing under certain conditions.

An additional factor to be considered for bacterial pathogens is antimicrobial sensitivity. For bacterial challenge models to be used in antimicrobial efficacy studies, the sensitivity of the pathogen against the common veterinary antimicrobial families in use should be established. For some studies, it may be appropriate to use a bacterial challenge isolate with high or low minimum inhibitory concentration (MIC). This is increasingly important as the incidence of resistant bacterial populations grows. The use of challenge isolates with higher MICs against a particular antimicrobial product provides the option of monitoring field effectiveness of the product against increasingly resistant pathogens.

## Identification of Challenge Titre, Volume, and Route

Challenge models should be designed to produce clinical disease to a level that allows for a stringent test of the VMP but without being overly severe. Models producing very mild disease, or which are sub-clinical in nature, and models that are acute or severe, are unlikely to be wholly representative of the field situation and may result in an unrealistic assessment

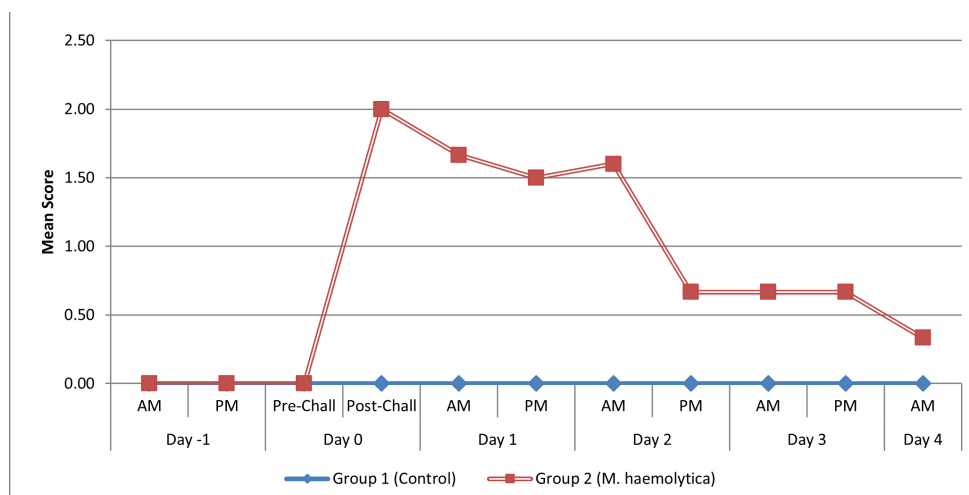


Figure 1

of the product efficacy. Models should be designed to produce a moderate level of clinical disease where appropriate, with clear clinical signs that are consistent and reproducible and with significant differences in the measured parameters between challenge and control animals. Large differences between control and challenged animals and low variability of clinical signs in the challenged group, means that a smaller number of animals are likely to be required to give statistical significance to the study. This is ethically more acceptable and also reduces development costs.

An example of the clinical signs that can be generated during challenge model development is shown in Figure 1. This data shows the clear difference in mean group respiratory scores for calves challenged with a respiratory bacteria and a control (unchallenged) group.

Other considerations include the route of administration of the challenge (e.g. intravenous, intranasal, subcutaneous, oral, intra-tracheal or in fish, intra-peritoneal injection, immersion in a suspension of pathogen or co-habitation with infected animals, or topical application e.g. of a pathogen suspension directly onto the gills), the volume of the challenge to be administered, the challenge concentration, and also the number of occasions and/or period over which the challenge has to be administered. The route of administration should mimic the route of entry of the pathogen in the field where possible. For example, the administration of respiratory bacteria by intravenous injection is unlikely to produce field-type symptoms, whereas the use of an intranasal delivery method is likely to produce disease more consistent with field infections. The volume of the challenge is important as in some cases, due to specific pathogen characteristics, it is not possible to use a small volume of challenge material. For example, the respiratory pathogen *Mannheimia haemolytica* challenge can require a large volume to be used, which is made up of a diluted neat broth culture. *M. haemolytica* produces endotoxins which, when concentrated, will produce acute endotoxic reactions resulting in rapid death of animals. The use of a large, diluted, volume of challenge material dilutes the concentration of the endotoxin and negates, or at least reduces, the toxic effect. The challenge titre is very important as this normally determines the resulting clinical signs. Too low a titre and no disease will occur; too high and the disease may be too acute and therefore, ineffective as a model. Finally, the number of doses of challenge and their frequency should be determined. Some challenges work well with only a single challenge occasion; others require multiple doses over a period of time. For example, some *Mycoplasma bovis* challenge models are most successful when a low volume / high titre is administered daily over a number of days.

Another example is that of a sea lice challenge model for farmed Atlantic salmon which uses seawater adapted 'post-smolt' salmon. Sea lice copepodid larvae are introduced into a tank containing fish, attaching and developing into the more harmful, mobile stages. The number of copepodids must be carefully controlled to ensure that the number of mobile lice is sufficient to enable valid statistical comparisons between treated and untreated groups, whilst avoiding damaging levels of parasite infestation. This is achieved by use of copepodids from a standard source and age, and careful control of environmental conditions, particularly water temperature and light, which influence settlement success. With careful control of the challenge two or more cohorts can be established on one group of fish which provides efficiencies in animal numbers and costs without causing undue harm to the experimental animals. For VMPs with preventative action, fish may be treated and then challenged, adult lice manually removed at sampling, and fish allowed to recover and challenged again at a later date in order to assess the duration of efficacy. For therapeutic products, fish may be infected with two cohorts of sea lice at an interval of several weeks in order to be able to measure effects on different parasite stages in a single study.

These factors need to be carefully considered and it is not uncommon during new model development for a range of different routes, volumes and concentrations to be tested. Literature searches can provide clues to possible challenge procedures for different species, however, with strain-to-strain variability the model that works in one situation may not be suitable for a situation with a different isolate of the same species.

#### Identification of Target Species Parameters

Following pathogen selection, the next stage in the development of a model is to consider the nature of the VMPs that will be tested with the model to ensure that the age range, development stage, production environment, gender, breed or reproductive status of the target species reflects the planned use of the product. Should it be developed in weaning age animals to support antimicrobial product development, or is the model to be used for vaccine development, where older animals will be the target?

An example of this would be *Streptococcus suis*, which can cause lameness, septicemia and/or meningitis in piglets where the models for testing vaccines or therapeutic products are generally quite different with regard to challenge volume, titre and route of administration. On the other hand, for pig respiratory bacterial disease models, it is possible in many

cases to set up one model with a wider range of possible uses, such as challenge in weaning age (four-week-old) piglets for use with therapeutic VMPs or with 10–12-week-old animals for vaccine studies. The only differences in these models are likely to be either a higher volume or higher concentration of the challenge material.

In farmed Atlantic salmon, vaccines tend to be administered to juvenile fish prior to transfer to seawater, whereas therapeutics for control of several economically important diseases are administered to larger fish in the seawater phase. Ideally, the model should be able to service a number of different requirements (e.g. both preventative and therapeutic VMPs), but this may require multiple models to be developed with the same pathogen.

### **In Vitro Validation of Pathogen Isolates**

Once isolates are sourced, the growth in the laboratory must be validated to determine the optimal conditions required to produce the pre-determined challenge titre and determine the reproducibility of the culture conditions. This is necessary as most challenge models use a fresh challenge culture prepared on the day of challenge, and in these situations it is generally not possible to confirm the titre accurately prior to use, therefore, there must be considerable confidence in the growth conditions and challenge titre produced.

Retrospective confirmation is possible following titration of the material, however, the results will take at least 24 hours to become available and the challenge will have already been administered before the titre is known. It is necessary to show that in the laboratory an isolate can be produced to the required titre, within the required timeframe, on a number of occasions, before it can be confirmed as suitable for use. For all isolates, large seed stocks should be prepared and maintained to ensure that testing is always carried out from the same basic stock material, and stability checks should be carried out before any use of the isolates in challenge efficacy work to ensure viability is retained during the freezing / storage process. All seed stocks must be confirmed as free from contamination and tested to confirm that they are pure before use, since the presence of other organisms may result in unexpected clinical disease or increased severity of disease.

However, *in vitro* culture is not possible for all pathogens and alternative approaches may be required. For example, *in vitro* culture conditions for replication of Piscine Myocarditis Virus (which is responsible for Cardiac Myopathy Syndrome in farmed Atlantic salmon), have not been successfully established and in this case, the challenge model uses a pool of tissue homogenate prepared from heart, spleen or kidney reservoir tissue from affected fish. The material is screened for other pathogens and purified by filtration and centrifugation. QPCR is used to confirm consistency of virus load and viability is confirmed by assessment of cytopathic effect in cell culture.

### **In Vivo Validation**

Once the laboratory validation is complete, isolates should be selected for *in vivo* model development. Generally, isolates are selected based on meeting different criteria relating to growth level and growth rate, however, successful growth in the laboratory does not necessarily mean that the isolates will be successful at producing clinical disease. While isolates are selected from clinical cases, it is not possible to ensure that they are capable of causing clinical disease prior to use. Unknown factors may be present in the environment of the source farms where clinical cases were observed, which allowed previously benign organisms to become pathogenic and may not be present or reproducible in research facilities.

These unknown factors could be related to stress from high stocking densities, poor quality feed or previous disease outbreaks, as well as other factors. The high animal care standards in disease research facilities means that these stressors are normally reduced in comparison to some commercial farms.

Selected isolates should be administered to the target species using the recommended route, volume and titre. During validation, animals must be observed at routine intervals to track the progress of any disease and to monitor the welfare of the animals. This information is vital in determining appropriate parameters of disease for use in regulatory efficacy studies, as well as allowing data to be collected for use in determining ethical endpoints for veterinary intervention. Many *in vivo* model development studies will include a range of different routes, volumes and concentrations, however, it is generally recommended that only one parameter is assessed at any one time. For example, for a bovine respiratory model, the concentration of the challenge may be assessed at three different titres but the route and volume should remain constant for all. *In vivo* studies should include sufficient numbers of animals to allow identification of variability of the model, but ethical use of animals will limit the maximum number used. In general, groups of between five and ten animals are sufficient for this purpose, although this can be disease and species dependent. A control group is normally also included to provide baseline species data for comparison.

It would also be expected as a minimum that a repeat of the model following the optimised procedures is carried out. This allows identification of any variability and, only once this has been assessed and confirmed to be of an appropriate level, can the model be considered as fully validated.

### **Summary**

Experimental disease challenge models are essential for the generation of efficacy data for VMPs for regulatory submission. Model development is a complex and time-consuming process incorporating biological, financial and logistical factors. Companies developing VMPs or novel feed products must take these factors into consideration when deciding between in-house development and potential outsourcing to a contract research organisation.



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