Validation of Livestock Infectious Disease Models – Critical Success Factors

PEER REVIEWED

Introduction
Validated experimental models of livestock infectious disease are important tools for the generation of efficacy data for candidate veterinary medicinal products (VMPs). 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 Harmonization 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.

In this article, the procedures involved in the production of new challenge models and critical success factors are reviewed.

Development of New Models
The development of new disease challenge models is required where new pathogens (or pathogens of new significance), new pathogen strains or serotypes emerge, and may also be necessary where pathogen strains have developed resistance to existing licensed products. In order to assess the efficacy of VMPs against these pathogens, it is necessary to have validated disease models available for conducting tests under controlled conditions. Challenge model development can be complex and costly and it is generally undertaken by animal health 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, inter-isolate variation can result in considerable differences between models for the same pathogen species.

There are a number of key factors which should be carefully considered before developing a challenge model. These include selection of a challenge pathogen, biological factors (complexity of development, availability of pathogen isolates), financial factors and logistical factors (resources available for development, facilities available to conduct the development).

Selection of Pathogen Species
The pathogen species selected for model development must be representative of the species and strain causing disease in the country or region where the VMP is planned to be registered for use. Where possible, the development of models using pathogens with clinical significance in several geographic regions, and which produce discernible and reproducible clinical disease, is desirable.

Once 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 region 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 to allow an informed choice to be made. With improvements in the speed and cost for gene sequencing in recent years, the genotype of different isolates can now also be used to assist in determining the most appropriate strains to use.

Identification of Target Species Age Range
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, gender, breed or reproductive status of the target species reflects the planned use of the product. For example, 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 have to be tested? 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. An example of this would be *Mycoplasma bovis*, which can cause respiratory disease in cattle, 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 ten-twelve-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.

Sourcing of Pathogen Isolates
Once the type of model has been determined, it is then necessary to source a range of possible isolates of the selected pathogen which can be further investigated. Generally it is suggested that selection of isolates should be

based on a number of factors which will take into account the 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 from which it was sourced (select isolates from animals of a similar age range to the intended target for the model) and the clinical history of the isolate (i.e. what clinical signs were observed in the host animal and what pathology was observed at post mortem, 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 dual infections it is difficult, if not impossible, to attribute clinical signs to a single pathogen. Secondary opportunistic species may or may not be disease-causing.

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 currently becoming increasingly important as the incidence of resistant bacterial populations becomes greater. 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 which are very acute or severe, are unlikely to be wholly representative of the field situation and may result in an unrealistic assessment 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, with obvious differences between challenge animals and controls. A large difference between control and challenged animals and low variability of clinical signs in the challenged group means that a smaller number of animals is 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 entry of the challenge (e.g. intravenous, intranasal, subcutaneous, oral, intra-tracheal), the volume of the challenge to be administered, the challenge concentration, and also the number of occasions over which the challenge has to be administered. The route of entry should mimic the route of entry of the pathogen in the field. 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 for three days.

All of 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.

Figure 1: Group Mean Respiration Scores
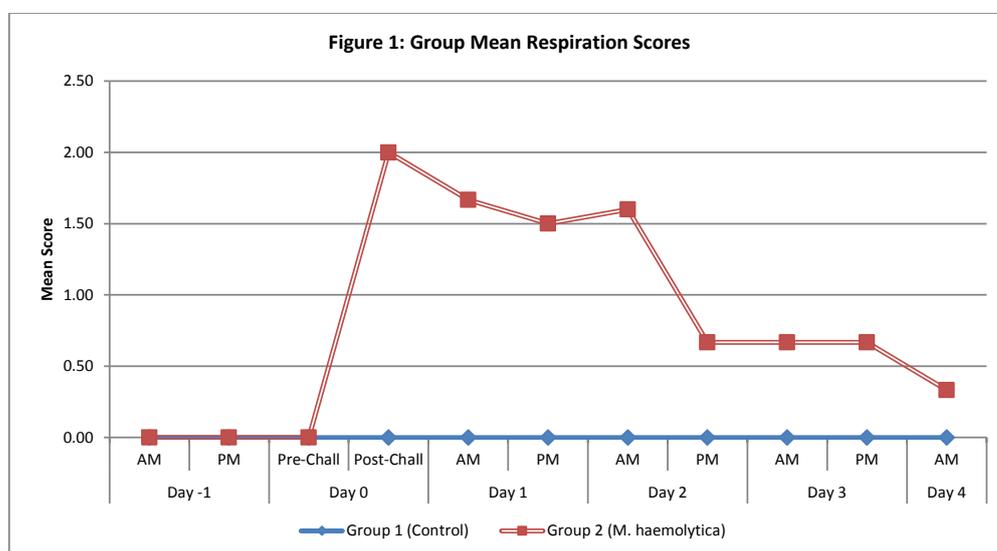
Literature searches can provide clues to possible challenge procedures for different species, however with strain-to-strain variability, the model that works in one situation may not be suitable for a situation with a different isolate of the same species.

In vitro Validation of Pathogen Isolates

Once isolates are sourced, the growth in the laboratory must be validated to determine the optimal growth conditions required to produce the pre-determined challenge titre and to 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. All seed stocks must be confirmed as free from contamination and tested to confirm that they are monocultures before use, since the presence of other organisms may result in unexpected clinical disease or increased severity of disease.

Selection of Pathogen Isolates for Animal Phase

Once the laboratory validation is completed, 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 definitely 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 which 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 animal 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-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. Animal health companies must take these factors into consideration when deciding between in-house development and potential outsourcing to a contract research organisation.

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