CASE STUDY 4
Swine Erysipelas vaccine
In vitro ELISA assay to replace in vivo immunization-challenge test
Introduction

• History and background

• Situation & Methods in EU
  • Companies
  • Authorities

• Situation & Methods in US
Introduction

**Erysipelothrix rhusiopathiae**

- One of the oldest recognised diseases (isolated by Koch in 1876)
- Gram + bacteria
- Pathogenic for ≠ species
- Acute to chronic forms in pigs
Introduction

(sub)acute *Erysipelothrix rhusiopathiae* → Pathognomonic lesions
Erysipelothrix rhusiopathiae

• First attenuated vaccine by passages in rabbits in 1881-1882
• Art. on skin scarification test in swine 1944
  • Basis for the first reliable model for efficacy testing in domestic animals ≈ inactivated vaccine
History and background

Erysipelothrix rhusiopathiae

- Development of inactivated vaccines required QC $\rightarrow$ WHO $\rightarrow$ EP
  - Mice = highly susceptible $\Leftarrow \neq$ target species
  - Mice dilution challenge assay (1952)

- Paul Ehrlich Institute (Germany)
European Pharmacopoeia

POTENCY TEST ≠ immunogenicity

- Quantification of the vaccine’s
  - upper limit → Safety (III)
  - lower limit → Efficacy (IV - immunogenicity)

of the registration dossier

- Each produced batch of the vaccine
  (safety test abandoned in the EU (exceptions))
Potency and immunogenicity 2-2-1-1

• “the potency section establishes, by a well-controlled test, in experimental conditions the minimum acceptable vaccinating capacity for all vaccines within the scope of the definition which must be guaranteed throughout the period of validity.”
Potency and immunogenicity

- In pigs (10 vaccinates and 5 controls)
- Challenge with serotype 1 or 2 (EDQM)
- Observation
- ≥ 90% protection
- ≥ 80% controls symptoms
Potency and immunogenicity in pigs

+ Use of target species
+ Use of challenge pathogen

- Use of target species
- Use of challenge pathogen

- Time and cost
Batch potency test 2-4-2

• For most vaccines, the tests cited under Potency or Immunogenicity are not suitable for the routine testing of batches → alternatives (development)

• The acceptance criteria for the batch potency test are established by correlation with the test described under Potency.
Batch potency test 2-4-2

- Where a batch potency test is described in a monograph, this is given as an example of a test that is considered suitable, after establishment of correlation with the potency test; other test models can also be used.
Batch potency test 2-4-2

- Live vaccines → virus titre or bacterial count
- Inactivated vaccines → in-vitro methods recommended, provided consistency of production
  - according to well-controlled procedures with defined and monitored key parameters, in-process control tests and target formulation of the final product
Mice dilution challenge assay

- 3-4 dilutions of the vaccine + control group
- EDQM challenge strain serotype 1 & serotype 2
- Determine the cut-off
  - reliable ➔ historical & in-vivo
  - unreliable ➔ variability animals & production
- use of animals, animal suffering and time (1.5m)
Mice serology test

• Inject suitable dose (1/10\textsuperscript{th} pig-dose) to 10 mice
• bleed the animals under anaesthesia & pool the sera \(\Rightarrow\) determine the level of antibodies by a suitable immunochemical method
  o For example ELISA with erysipelas ELISA coating antigen BRP (EDQM).
Mice serology test

• Relative Potency: The antibody level is not significantly less than that obtained with a batch that has given satisfactory results in the test described under Potency.

→ Need for reference batches
Mice serology test

+ fewer animals

+ extensively validated (VICH GL 1&2)

+- time (1m)

- influence of adjuvant

- animal variability (mouse strains)

- still requires the use of animals (bleeding)
Alternatives

- SDS-PAGE
- Western blotting
  ➔ subunit vaccines or purified proteins
- Antigen quantification test
Supplemental Assay Method 613 for *In vitro Potency Testing* of *Erysipelothrix rhusiopathiae* Bacterins

**ELISA**

- 96-well plate
- coating with *monoclonal antibodies* against the 65kD protein (= protective antigen)
  - Article from 1991 describing the 66-64 kD antigen
- Min. 7 twofold dilutions of the test serial and the reference bacterin
**In vitro Potency Testing of Erysipelothrix rhusiopathiae Bacterins**

**ELISA**

- Polyclonal rabbit anti-65kD protein
- Antirabbit peroxidase conjugate
  - read + determine RP (≥ 1)

- Monoclonal Antibodies
  - Method of Preparation: Ascites fluid was collected from BALB/c mice injected with hybridoma ERHU1-B60-91. Ascites fluid was pooled, sterilized, aliquoted, and frozen
US – SAM 613

In vitro Potency Testing of Erysipelothrix rhusiopathiae Bacterins

+ measuring directly (no immunisation step)
+ quick
+ no use of animals

- additional method development
- measuring quantity
  ≠ measuring biological activity
  ≠ stability indicating profile
US – SAM 613

Published in 2009 and replaced

• SAM 601 Potency Testing of E. Antiserum in Mice
• SAM 605 Potency Testing of E. Bacterins in Swine
• SAM 606 Potency Testing of E. vaccines in Swine
Potency Testing of E. Bacterins in Mice

- 9CFR, part 113.119
- Vaccination
  - 80 mice (20/dilution) per serial to be tested
  - 80 mice for the reference bacterin
  - 30 mice to determine the LD$_{50}$ of the challenge inoculum (>100 LD$_{50}$/0.2ml dose)
Potency Testing of E. Bacterins in Mice

- Challenge with inoculum
- Record deaths (humane endpoints?)
- Calculate \( PD_{50} \) of reference & serial
- \( PD_{50_{\text{serial}}} / PD_{50_{\text{reference}}} = \text{Relative Potency} \geq 0.6 \)
Regulatory Background

Legislation – **New Registration**

- latest science?

- Vaccination / challenge model often
  - cheaper
  - quicker
  - Easier → more certainty
Regulatory Background

Legislation - Existing registrations

→ Existing registered potency test = golden standard

→ Change to new method
  → comparing with the registered potency test
  → often additional tests (ex. measuring of adjuvant)

→ Cost & duration validation regulatory

→ Incentives? → easier
MAHs & Methods in the EU

Inact. serovar 2 / adjuvant / preservatives

• MSD: Porcilis Ery (RP) / (Colisorb)
• Merial: Ruvax (RP)
• Zoetis: Suvaxyn (RP)
  → changed potency test in 2005
• Hipra: Eryseng (Ag) / Parvosuis / (Hiprasuis)
MAHs & Methods in the EU

Eryseng

- Authorised in the EU in July 2014

- Batch release potency test = antigen quantification
  - Correlation ELISA – antigen content ≠ batches (discrimination)
  - Correlation ELISA – protection in the target species
  - Correlation ELISA – stability indicating profile
  - Company reference standards
Methods in the EU

Batch release/OCABR vaccines

- Part of the shortlist
- Testing prior to release to the market
- Germany, Belgium and Hungary

- additional time and animals
Thoughts / discussion points

• > 130y experience with disease & vaccination → what with other vaccines?
• Can the US direct in-vitro method (SAM 613) be implemented in the EU (i.e. EU Pharmacopoeia)? Or vice-versa?
  • What hurdles exist on the authority side?
  • What hurdles exist on the industry side?
• Can a vaccine manufacturer in the EU refer to the US method as a “suitable validated alternative” (as mentioned in the EU monograph)?
Thoughts / discussion points

What is acceptable risk?

- What are the major hurdles to implement a method that has been validated elsewhere in the world? How can they be overcome?

- Instead of extensive validation before implementation of a new method, would it be possible to replace part of that validation data by post-implementation monitoring?
Thoughts / discussion points

Few monographs → increase - harmonisation

- 1st to market → use validation work for registration dossier to determine the “standard” method

+ MAH = “own” method is reference (sharing)

+ other MAH = not necessary to develop other method → validation/implementation (advantageous for smaller companies)
options

1. Choice/preference of method
   → for example antigen quantification
2. Choice of protective antigen
   → for example antigen quantification
3. Reference standards:
   → monoclonal Abs specific for vaccines/manufacturer

(Obligation of) golden standard method?
Thanks to Pierre Grognét (CODA/CERVA)

Thank you for your attention

Questions?

Discussion?