International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Veterinary Vaccine Potency and Safety Testing

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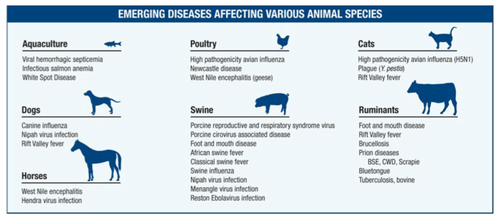
Introduction

- Vaccines improve animal and human health and welfare by preventing and controlling infectious diseases
- Veterinary vaccines play a major role in (**Figure 1**):
  - Protecting animal health and public health
  - Reducing animal suffering
  - Enabling efficient production of food animals to feed the burgeoning human population
  - Reducing the need for antibiotics to treat food and companion animals
- NICEATM and ICCVAM have identified vaccine potency and safety testing as one of the four highest priorities for reduction, refinement, and replacement of animal testing (ICCVAM 2008)
  - Priority based on the large numbers of animals and significant pain and distress that can occur for potency and safety testing of many human and veterinary vaccines
- NICEATM, ICCVAM, and their ICATM partners organized an international workshop held on September 14–16, 2010, to promote and advance the development and use of scientifically valid alternative methods for human and veterinary vaccine testing

Figure 1. Emerging Diseases Affecting Various Animal Species



Graphic provided by Dr. James Roth

Workshop Goals

- Review the state of the science of alternative methods that reduce, refine, and replace the use of animals in vaccine potency and safety testing, and discuss ways to promote their implementation
- Identify knowledge and data gaps that must be addressed through research, development, and validation efforts
- Identify and prioritize efforts needed to address these knowledge and data gaps

Workshop Organizing Committee: ICCVAM Interagency Biologics Working Group

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International Workshop Invited Experts

Figure Legend

Back Row: Paul Stickings, Ivo Claassen, Michael Schmitt, Richard Isbrucker, Warren Casey, Coenraad Hendriksen

Fourth Row: Janet Skerry, Dorothea Sesardic, Hajime Kojima

Third Row: Marlies Halder, Robin Levis, Johan Descamps, Geetha Srinivas, Karen Brown

Second Row: Juan Arciniega, Steven Rubin, Jeffrey Galvin

Front Row: Yoshinobu Horiuchi, Theresa Finn, Jodie Kulpa-Eddy, William Stokes, Richard McFarland

Not Shown: Hans Draayer, Glen Gifford, Richard Hill, James Keller, Suman Mukhopadhyay, James Roth, Anne Schuchat, Jinho Shin, Willie Vann, Daniela Verthelyi, Ralph Woodland

Workshop Sessions

Session 1: Overview of Public Health Needs and Regulatory Requirements for Vaccine Safety and Potency Testing

- Summarized public health needs for vaccines in the U.S., Europe, Asia, and developing countries, as well as regulatory requirements and rationale for determining potency and safety of vaccine products

Session 2: Replacement Methods for Vaccine Potency Testing: Current State of the Science and Knowledge Gaps

- Reviewed currently accepted replacement alternatives (i.e., antigen quantification), knowledge gaps associated with test methods not currently accepted, and areas that should be emphasized as targets for future development

Session 3: Animal Use for Vaccine Potency Testing: Refinement and Reduction Alternatives

- Provided an overview of alternative methods and approaches that could (1) refine current vaccine potency testing procedures to reduce or eliminate animal pain and distress associated with current vaccine potency testing procedures and/or (2) reduce the number of animals used for specific vaccine potency testing procedures

Session 3A: Refinement Alternatives: Using Serological Methods to Avoid Challenge Testing

Session 3B: Refinement Alternatives: Using Earlier Humane Endpoints to Avoid or Minimize Animal Pain and Distress in Vaccine Potency Challenge Testing

Session 3C: Reduction Alternatives: Strategies to Further Reduce Animal Numbers for Vaccine Potency Testing

Session 4: Vaccine Post-Licensing Safety Testing: Reduction, Refinement, and Replacement Methods and Strategies

- Focused on current regulatory requirements and rationale for post-licensing safety testing (e.g., general safety test, neurovirulence test, pyrogen test) for both human and veterinary vaccines

Poster Session: Fifteen poster presentations of ongoing research, development, and validation activities focused on reducing, refining, and replacing animal use for vaccine potency and safety testing

Detailed information on the workshop, including all presentations, can be obtained on the NICEATM-ICCVAM Web site at:
http://iccvam.niehs.nih.gov/meetings/BiologicsWksp-2010/BiologicsWksp.htm

Breakout Groups: Veterinary Vaccines

Objective

- Review the state of the science, knowledge gaps, and priority areas for future research, development, and validation to advance alternative methods for veterinary vaccine potency and safety testing

General Recommendations

Priority Vaccines

- Rabies
- Leptospira
- Clostridial
- Erysipelas
- Foreign animal disease vaccines
- Poultry vaccines
- Fish vaccines

Criteria for Prioritization

- Use of large numbers of animals per test
- The production of large numbers of serials annually
- Possibility of animal pain and distress during the challenge testing procedure
- Vaccines for which the functional protective antigen has been identified and characterized
- Vaccines for foreign animal diseases
- Zoonotic organisms that are also dangerous to humans
- Diseases that can be easily spread to wildlife populations

Achieving Broader Acceptance and Use of Alternative Methods Through:

- Broader access to information
- Harmonization and development of the testing requirements for individual protective antigens and their development
- Increased interaction/communication between regulatory agencies, research institutions, and vaccine manufacturers worldwide through workshops, scientific meetings, and conferences
- Harmonization of requirements, methods, and specifications
- Investigations into the impact of adjuvants on alternative *in vitro* assays
- Quality assurance and availability of necessary reagents

Veterinary Vaccine Potency Testing: Replacement, Refinement, and Reduction Methods

Replacement Methods for Veterinary Vaccine Potency Testing

State of the Science

- Potency of certain vaccines can now be determined in the final vaccine product by *in vitro* antigen quantification
  - Avian Newcastle disease, canine leptospirosis (nonadjuvanted, inactivated), and feline leukemia
- These assays are typically based on binding of key protective antigens to specific antibodies in an *in vitro* immunoassay

Priority Research Needs and Recommendations

- Identify, purify, and characterize vaccine protective antigens in veterinary vaccines
- Develop separation methodologies to extract the protective antigen from adjuvants to avoid adjuvant interference in subsequent antigen quantification assays
- Encourage early and frequent interactions with regulators

Refinement Methods for Veterinary Vaccine Potency Testing

1. Humane Endpoints

State of the Science

- Moribund euthanasia, not death, can now be used as an endpoint for all vaccine challenge studies
- Earlier humane endpoints have been approved for rabies and swine erysipelas vaccine challenge testing

Priority Research Needs and Recommendations

- Identify earlier humane endpoints for vaccines requiring challenge testing
  - Systematically collect and evaluate all clinical signs and other objective parameters
    - Investigate objective quantitative endpoints, e.g., body temperature changes, body weight
- Collect data and identify clinical endpoints for control groups
  - If a required percentage of controls have reached the specified endpoint, then all controls might be euthanized
- Focus on vaccines for which animals take a longer period of time to develop disease (e.g., leptospira)
- Collect and apply data (clinical observations/measurements) from premarketing efficacy tests
- Monitor animals at least twice daily for moribund condition or evidence of an established humane endpoint
  - Develop innovative methods to observe animals
- Share information between manufacturers and regulators to support change to earlier humane endpoints

2. Serological Methods

State of the Science

- In many cases the lethal challenge test has been replaced by a serological assay
  - Either *in vitro* titration of protective antibodies from serum or *in vitro* toxin neutralization using cell cultures or immunoassays
- Examples include *Clostridium novyi/perfringens/septicum/tetani, Leptospira hardjo*, and rabies

Priority Research Needs and Recommendations

- Identify and understand the antibodies involved in protective immunity
- For rabies, convene focused working group of both human and veterinary researchers
- Convert *in vivo* toxin neutralization tests to ELISA or other cell-based methods for appropriate clostridial vaccines
- Research new methods to assess functionality of antibodies or other immune responses
- Develop and validate assays and reagents to measure antibodies

3. Reduction Methods for Veterinary Vaccine Potency Testing

State of the Science

- A single-dilution test is available that represents a strategy to reduce the number of animals used in the challenge test for rabies vaccine

Priority Research Needs and Recommendations

- Systematically identify causes of excessive variation and repeat testing
- Investigate ways to reduce or eliminate the sources of variation and causes of inconclusive test results
- Conduct a retrospective review of archival data to determine if the minimum number of animals (including control animals) may be reduced while maintaining the necessary statistical power for current tests
- Incorporate flexibility into the regulatory process so that the reduction of animals can be applied on a case-by-case basis, particularly for minor-use situations

Post-Licensing Veterinary Vaccine Safety Testing: Replacement, Refinement, and Reduction Methods

State of the Science

- Alternative methods are currently available for vaccine safety testing of avian live virus vaccines
- In other cases safety assessment has been incorporated into the potency challenge test:
  - Canine distemper
  - Mink enteritis
  - Bovine virus
  - Canine parainfluenza

Priority Research Needs and Recommendations

- Assess the need for the general safety test and determine if waiving this test could be implemented globally
- Continue investigating cell culture and PCR techniques as promising approaches to replace *in vivo* tests for extraneous agents in poultry vaccines
- Determine if the *in vivo* veterinary rabies inactivation test could be replaced with cell culture techniques already in use in the European Union for the testing of virus inactivation for human rabies virus
- Investigate how to develop, validate, and implement safety testing using various tests in a consistency approach

Conclusions

- This was the first international workshop in the U.S. to bring together stakeholders from both the human and veterinary vaccine communities to discuss opportunities to reduce, refine, and replace animal use for potency and safety testing
- The workshop reviewed the state of the science for existing alternative methods and approaches that could be implemented immediately to provide for animal reduction, refinement, and replacement for vaccine potency and safety testing
  - Alternative methods have been incorporated into the potency and safety testing of several human and veterinary vaccines
- The workshop identified knowledge and data gaps, as well as research, development, and validation activities needed to address these gaps and to advance alternative methods for vaccine potency and safety testing
  - Advances in science and technology that can and should be applied to these efforts were highlighted and identified as priorities for future initiatives
- The workshop emphasized the value and role of international cooperation, collaboration, and harmonization in advancing alternative methods for vaccine potency and safety testing
  - Increased international cooperation is essential to maximize the impact of new methods and to accelerate their implementation globally
- Implementation of the workshop recommendations is expected to advance new methods for vaccine testing that will benefit animal welfare and ensure continued protection of human and animal health

References

ICCVAM. 2008. The NICEATM-ICCVAM Five-Year Plan (2008-2012): A Plan to Advance Alternative Test Methods of High Scientific Quality to Protect and Advance the Health of People, Animals, and the Environment. Available at: http://iccvam.niehs.nih.gov/docs/5yearplan.htm.

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