Reducing Hamster Usage In *Leptospira* Vaccine Potency Testing

Carol Clarke, DVM, DACLAM  
Research Program Manager  
Animal and Plant Health Inspection Service-Animal Care (APHIS-AC)
Live Animal Test

• **Virus-Serum-Toxin Act**
  – Enforced by APHIS-Center for Veterinary Biologics CVB
  – *Leptospira* bacterin potency testing is mandated under 9 CFR §113.101- 113.104

• Syrian Hamsters are very sensitive to *Leptospira*
  – Used as part of the standard regulatory test for *Leptospira* vaccine potency
Creation of Challenge Inoculum For Potency Testing

Back-titration hamsters:

• *In vivo* passaging: 4 groups of 5 hamsters injected with serial dilutions of a serogroup to estimate $LD_{50}$
  – Symptoms: Hemorrhage (nasal and urine), and death.
    • 5% display neurologic ataxia as a result of hepatic failure.
    • 33% of the animals display no symptoms before becoming moribund.
**9 CFR Live Animal Potency Tests for Leptospira Vaccines**

**Overview**

- **Vaccinate**
  - Wait 14 - 18 days
- **Challenge w/Leptospira**
  - Observe 14 days
- **Count survivors**

**Animal Numbers**

<table>
<thead>
<tr>
<th>Vaccinates</th>
<th>Challenge Controls</th>
<th>Concurrent Challenge (LD_{50} Titration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>
14 Days Post-Challenge

- A valid test requires 80% mortality in controls
- The vaccine test batch is deemed potent if 80% of the vaccinates survive
Hamster Numbers Add Up!

- Example: A canine vaccine vial with 3 serogroups
  - 20 Back-titration hamsters x 3 serogroups = 60
  - 20 hamsters for live animal test x 3 serogroups = 60
    Total= 120 hamsters

- Now consider the hundreds of vials needed for batch testing
Our Goal

• Reduce hamster usage in potency testing through initiatives begun by the APHIS-CVB:
  – **2013**: Exemption to use *in vitro* ELISA test developed by APHIS-CVB instead of standard test
  – **2015**: Exemption by CVB to eliminate back-titration hamsters for *L. canicola* & *L. icterohaemorrhagiae*
  – **2017**: Exemption by CVB to eliminate back-titration hamsters for *L. pomona* & *L. grippotyphosa*
Alternative: ELISA Test
Replacement of the Live Animal

\textit{In vitro} ELISA antigen quantification methods have been developed and validated by CVB for serogroups:

- \textit{L. pomona}
- \textit{L. canicola}
- \textit{L. icterohaemorrhagiae}
- \textit{L. grippotyphosa}
Enzyme-linked Immunosorbent Assay (ELISA)
2013: Guidance to use the ELISA test in Leptospira bacterin potency testing in hamsters.
- Provides information on alternative *in vitro* potency assays
- Provides information on reagents available from CVB
Supplemental Assay Methods (SAM)

- Protocols for *in vitro* potency assays using the ELISA as compared to a suitable non-expired reference
  - SAM 624: *L. interrogans* serogroup Pomona
  - SAM 625: *L. interrogans* serogroup Canicola
  - SAM 626: *L. kirschneri* serogroup Grippotyphosa
  - SAM 627: *L. interrogans* serogroup Icterohaemorrhagiae
Exemption requirements

• Firms are to obtain an exemption from CVB before using the ELISA.
  – Use of ELISA is optional

• Firms must validate the potency assays for their products and submit data to CVB for acceptance
Alternative: Elimination of Back-Titration Hamsters
Reduction in Live Animals

• **2015 APHIS-CVB Notice # 15-13:**
  – Provided biologic licensees, permitees and applicants the option to remove back-titration hamsters from *in vivo* potency tests for Leptospira serogroups icterohaemorrhagiae and canicola
  
  – CVB would allow an exemption from the titration requirement in vaccination-challenge potency assays for these serogroups

• *Removal of the back-titration hamsters could reduce animal use by 50% when potency testing these two fractions*
Alternative: Elimination of Back-Titration Hamsters
Reduction in Live Animals

• **2017 APHIS-CVB Notice # 17-06:**
  – Provided biologic licensees, permittees and applicants the option to remove back-titration hamsters from *in vivo* potency tests for Leptospira serogroups pomona and grippotyphosa

  – CVB would allow an exemption from the titration requirement in vaccination-challenge potency assays for these serogroups

• *Removal of the back-titration hamsters could reduce animal use by 50% when potency testing these two fractions*
USDA Team Effort

National Agricultural Library-
Animal Welfare Information Center (NAL-AWIC)

– Will inform the public about the exemptions

– Mandated under the Animal Welfare Act to provide information on improved methods of animal experimentation,
  • includes methods which could reduce or replace animal use or refine a study to minimize pain and distress to animals (3Rs alternatives)
Animal Welfare Information Center

The Animal Welfare Information Center (AWIC) is mandated by the Animal Welfare Act (AWA) to provide information for improved animal care and use in research, testing, and teaching.

News

EPA Launches Voluntary Pilot Program to Reduce Animal Testing

Wednesday, January 04, 2017

DHHS. NIH. NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).
USDA Team Effort

APHIS- Animal Care

– Will track impact of exemptions using the Annual Report submitted by manufacturers of a *Leptospira* biological

– The facilities are listed in the Product Code Book (on-line)
#7- Hamsters

Category E:
- Pain unrelieved
- Explanation required
Hamster Usage Under Category E

Number of Hamsters

<table>
<thead>
<tr>
<th>Year</th>
<th>Usage for Potency Testing</th>
<th>Total Usage Under Category E</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>35000</td>
<td>45000</td>
</tr>
<tr>
<td>2014</td>
<td>30000</td>
<td>40000</td>
</tr>
<tr>
<td>2015</td>
<td>26000</td>
<td>33000</td>
</tr>
<tr>
<td>2016</td>
<td>25000</td>
<td>32000</td>
</tr>
</tbody>
</table>
Challenges

• USDA cannot require companies to use the alternatives

• More work needs to be done
  – CVB exploring the use of cryopreserved *Leptospira* stock to eliminate maintenance of virulent challenge through continuous transfer in live animals
Conclusion

• The USDA continuously works towards reducing hamster usage in *Leptospira* vaccine potency testing by:
  
  – Providing approved exemptions to the standard live animal test i.e. ELISA test and elimination of back-titration hamsters
  – Providing Information on available alternatives
  – Monitoring impact of exemptions via the Annual Reports
  – Researching use of cryopreserved *Leptospira* strains
Acknowledgements

• **Center of Veterinary Biologics (CVB)**
  – Dr. Donna Malloy
  – Dr. Larry Ludemann
  – Dr. Angela Walker

• **Animal Care (AC)**
  – Emil Ewing

• **Animal Welfare Information Center (AWIC)**
  – Kristina Adams
  – D’Anna Jensen
THANK YOU