Opportunities and Strategies to Further Refine Animal Use for *Leptospira* Vaccine Potency Testing

Elsio Wunder DVM, PhD
Associate Research Scientist

Yale School of Public Health
Epidemiology of Microbial Diseases

Oswaldo Cruz Foundation (Fiocruz)
Brazilian Ministry of Health
Salvador, Brazil

09/21/2012
International Workshop on Alternative Methods for *Leptospira* Vaccine Testing
Challenges for Leptospirosis Hamster model

- Acute disease
  - Rapid dissemination
  - Death occurs within few hours
- Different serovars
  - Different outcomes
  - Different LD50s
- Infection route
  - Intraperitoneal doesn’t occur in nature
  - Penetration - pathogenesis
- Death as endpoint
  - More efficient determinants for disease
In hamsters, leptospires rapidly disseminate, and death can occur within a few hours.

Real Time PCR in Infected Hamsters

<table>
<thead>
<tr>
<th>Status</th>
<th>N°</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survived</td>
<td>63</td>
<td>20.7</td>
</tr>
<tr>
<td>Euthanized</td>
<td>21</td>
<td>73.3</td>
</tr>
<tr>
<td>Deaths</td>
<td>220</td>
<td>72.4</td>
</tr>
<tr>
<td>Deaths within 8h</td>
<td>59</td>
<td>36.7</td>
</tr>
<tr>
<td>Deaths within 16h</td>
<td>161</td>
<td>73.3</td>
</tr>
<tr>
<td>TOTAL</td>
<td>304</td>
<td>100</td>
</tr>
</tbody>
</table>

- Over 68% of the animals that died were considered healthy on the last assessment.
Within the same pathogenic specie (*L. interrogans*), different serovars and routes of infection can’t be compared

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Route</th>
<th>Inoculation dose leptospires</th>
<th>Median of days for death</th>
<th>Range of days for death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manilae</td>
<td>IP</td>
<td>$10^8$</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Manilae</td>
<td>IP</td>
<td>100</td>
<td>10</td>
<td>9 - 12</td>
</tr>
<tr>
<td>Manilae</td>
<td>Ocular</td>
<td>$10^8$</td>
<td>8.5</td>
<td>8 - 9</td>
</tr>
<tr>
<td>Copenhageni</td>
<td>IP</td>
<td>$10^8$</td>
<td>5</td>
<td>5 - 6</td>
</tr>
<tr>
<td>Copenhageni</td>
<td>IP</td>
<td>100</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Copenhageni</td>
<td>Ocular</td>
<td>$10^8$</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

- Serovar Lai has a LD$_{50}$ of $5 \times 10^7$ leptospires
It’s important to have a well established and standardized hamster model for each serovar, as for different routes of infection.

- *Leptospira interrogans* serovar Copenhageni

![Graph showing LD50 Intraperitoneal and LD50 Conjunctival dosages.](image-url)
It’s important to have a well established and standardized hamster model for each serovar, as for different routes of infection

- *Leptospira interrogans* serovar Manilae

**LD$_{50}$ Intraperitoneal**

**LD$_{50}$ Conjunctival**
There’s a need for better determinants of disease and/or death

- Weight loss

Average weight curve comparing animals infected with Leptospira and the standard for the breed
Alternative determinants of disease and/or death tested so far, are not good enough

• Age
  ✓ Previous results didn’t show differences;
  ✓ Coutinho et al. (2011 PLoS NTD, 5:12, e1422): animals were infected with 11-12 weeks of age = 10% weight loss;
  ✓ At Yale: infection with 3 weeks of age

• Rectal temperature
  ✓ Previous experiments showed no patterns to estimate disease and/or death

• General Clinical Signs - Appearance
  ✓ Lethargy: how to define specific parameters to consider an animals as lethargic?
  ✓ Sick animals: which symptoms should be considered?
Alternative determinants of disease and/or death tested so far, are not good enough

- General Clinical Signs - Appearance

<table>
<thead>
<tr>
<th>Time to death</th>
<th>Lethargic</th>
<th></th>
<th>Sick</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>%</td>
<td>#</td>
<td>%</td>
</tr>
<tr>
<td>7-8h</td>
<td>14</td>
<td>24.12</td>
<td>12</td>
<td>60.00</td>
</tr>
<tr>
<td>17-18h</td>
<td>12</td>
<td>20.69</td>
<td>6</td>
<td>30.00</td>
</tr>
<tr>
<td>24h</td>
<td>24</td>
<td>41.38</td>
<td>2</td>
<td>10.00</td>
</tr>
<tr>
<td>30-33h</td>
<td>5</td>
<td>8.63</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>39-42h</td>
<td>3</td>
<td>5.18</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TOTAL</td>
<td>58</td>
<td>-</td>
<td>20</td>
<td>-</td>
</tr>
</tbody>
</table>
Future experiments at Yale to avoid the high numbers of deaths without evident symptoms

• Simplified daily follow-up of the animals (flowchart);
  ✓ Animals will be checked twice a day during light cycle with interval between 8-10h;

• Check for any abnormal scurry and/or movement of the animals based on tactile stimuli:
  ✓ Normal: moving more than 30cm in the cage;
  ✓ Euthanasia: any animal with diminished movement (unable to move more than 30cm);

• Immediately euthanasia of symptomatic animals:
  ✓ Seizures, dyspnea, bleeding, or ruffled fur
“New” possible outcomes other than death

- Sterilizing immunity?
  - ✓ Bovine/Swine (“herd”) vaccines
  - ✓ Humans and Canine
  - ✓ Public Health point of view
  - ✓ Quantitative Real Time PCR
  - ✓ Touch Prep analysis – IFA

qPCR Kidney – high and low dose

- IFA with α-LipL32
- Kidney touch prep
“New” possible outcomes other than death

- Dissemination
  - ✓ Quantitative Real Time PCR
  - ✓ Proof-of-concept: death is correlated with burden of agent in tissues

![Graphs showing the dissemination of agents in vaccinated animals' kidneys IP and CnJ.](image)
“New” possible outcomes other than death

- Dissemination
  - Proof-of-concept: death is correlated with burden of agent in tissues
Goals for a improved hamster model for leptospirosis

• Well standardized animal model, considering location, animal, agent, dose of infection and route of infection

• Well established timeframe and expected symptoms and/or death, taking in account the different doses of infection, and also the route of infection used

• Better and more quantifiable description of parameters to identify symptomatic animals

• Complete and efficient record spreadsheet to collect information of animal monitoring

• Identification and evaluation of new surrogates for determination of infection
Acknowledges

Fundação Oswaldo Cruz
Mitermayer Reis

Yale School of Public Health
Albert Ko
Elsio Wunder
Vimla Bisht

Yale Animal Resources Center (YARC)
Rachel Ardito
General staff

Yale Institutional Animal Care and Use Committee (IACUC)
Patricia Preisig
Heidi Voegeli
Claudia Swanson
Steven Wilson

Support

IACUC 2011-11424
CNPq/Brazil
NIAID U01AI088752
NIAID R01 AI052473
FIC D43 TW00919