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

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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><strong>TOTAL</strong></td>
<td><strong>304</strong></td>
<td><strong>100</strong></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
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

**LD₅₀ Intraperitoneal**

```
100
75
50
25
0
```

**LD₅₀ Conjunctival**

```
100
75
50
25
0
```

![Graph showing LD₅₀ for Intraperitoneal and Conjunctival routes of infection](image-url)
There’s a need for better determinants of disease and/or death

- Weight loss
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**

| Time to death | Lethargic | | Sick | |
|---------------|-----------|------|------|
|               | #         | %    | #    | %    |
| 7-8h          | 14        | 24.12| 12   | 60.00|
| 17-18h        | 12        | 20.69| 6    | 30.00|
| 24h           | 24        | 41.38| 2    | 10.00|
| 30-33h        | 5         | 8.63 | -    | -    |
| 39-42h        | 3         | 5.18 | -    | -    |
| TOTAL         | 58        | -    | 20   | -    |
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
“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
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