

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

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

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# Potential for Hamster Potency Test Refinements

- **What humane endpoints have been studied in *Leptospira* infections and which appear to be of potential benefit?**
- **Are there opportunities to use analgesics post challenge?**
- **Are humane endpoints written into any *Leptospira* vaccine potency testing guidelines?**
- **What funding opportunities are available for development of humane endpoints?**

# What humane endpoints have been studied in *Leptospira* infections?

## Clinical Signs

- **Weight Loss**
- **Temperature drop**
- **Appearance indicative of death**

# What humane endpoints have been studied in *Leptospira* infections?

## ➤ **Weight Loss – No Consensus**

- **Coutinho, et al - Weighed day of challenge and daily thereafter – Euthanized at 10% weight loss – vaccinates gained weight, controls all lost weight and were euthanized\***
- **Reed – Weight loss was not a determinant for death\*\***
- **Both -- Concern with handling of infected hamsters to place them on a balance\*\***

•Coutinho, et al. 2011. A LigA Three-Domain Region Protects Hamsters from Lethal Infection by *Leptospira interrogans*. [www.plosntds.org](http://www.plosntds.org)

\*\* Nick Reed presentation –

[http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=2&ved=0CGgQFjAB&url=http%3A%2F%2Fwww.humane-endpoints.org%2Fimages%2FReed.pdf&ei=DoMZUNirN4zVigKXv4GQCQ&usg=AFQjCNHaXwKaKQDBzvfjCrbdtTks\\_TD8lQ&sig2=TF5mg\\_eUwZYk1Qf1S8Tu9w](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=2&ved=0CGgQFjAB&url=http%3A%2F%2Fwww.humane-endpoints.org%2Fimages%2FReed.pdf&ei=DoMZUNirN4zVigKXv4GQCQ&usg=AFQjCNHaXwKaKQDBzvfjCrbdtTks_TD8lQ&sig2=TF5mg_eUwZYk1Qf1S8Tu9w)

# What humane endpoints have been studied in *Leptospira* infections?

## ➤ Temperature Drop

- **Reed\* and Personal Experience– Temperature drop was not predictive of death**
  - Used hand-held devices that caused major variability
  - As devices improve (more accurate implants or hand-held devices this may be an option)
  - If hamsters have to be handled, safety is an issue
- **Wunder – Rectal Temperature not predictive of death**
- **Personal Experience**
  - Animals often huddle together and increase body temperature
  - Activity of animals affects body temperature
  - Environmental temperature affects body temperature

\* Nick Reed presentation –

[http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=2&ved=0CGgQFjAB&url=http%3A%2F%2Fwww.humane-endpoints.org%2Fimages%2FReed.pdf&ei=DoMZUNirN4zVigKXv4GQCQ&usg=AFQjCNHaXwKaKQDBzvfjCrbdtTks\\_TD8IQ&sig2=TF5mg\\_eUwZYk1Qf1S8Tu9w](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=2&ved=0CGgQFjAB&url=http%3A%2F%2Fwww.humane-endpoints.org%2Fimages%2FReed.pdf&ei=DoMZUNirN4zVigKXv4GQCQ&usg=AFQjCNHaXwKaKQDBzvfjCrbdtTks_TD8IQ&sig2=TF5mg_eUwZYk1Qf1S8Tu9w)

# What humane endpoints have been studied in *Leptospira* infections?

More promising humane endpoint is appearance of clinical signs

Healthy Hamster on left

Sick hamster on right



\* Nick Reed presentation – Animal Health and Veterinary Laboratories Agencies --

[http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=2&ved=0CGgQFjAB&url=http%3A%2F%2Fwww.humane-endpoints.org%2Fimages%2Freed.pdf&ei=DoMZUNirN4zVigKXv4GQCQ&usg=AFQjCNHaXwKaKQDBzvfjCrbdtTks\\_TD8lQ&sig2=T F5mg\\_eUwZYk1Qf1S8Tu9w](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=2&ved=0CGgQFjAB&url=http%3A%2F%2Fwww.humane-endpoints.org%2Fimages%2Freed.pdf&ei=DoMZUNirN4zVigKXv4GQCQ&usg=AFQjCNHaXwKaKQDBzvfjCrbdtTks_TD8lQ&sig2=T F5mg_eUwZYk1Qf1S8Tu9w)

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<http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=2&ved=0CGgQFjAB&url=http%3A%2F%2Fwww.humane>

[endpoints.org%2Fimages%2FReed.pdf&ei=DoMZUNirN4zVigKXv4GQCQ&usg=AFQjCNHaXwKaKQDBzvfjCrbdtTks\\_TD8lQ&sig2=TF5mg\\_eUwZYk1Qf1S8Tu9w](http://www.humane-endpoints.org%2Fimages%2FReed.pdf&ei=DoMZUNirN4zVigKXv4GQCQ&usg=AFQjCNHaXwKaKQDBzvfjCrbdtTks_TD8lQ&sig2=TF5mg_eUwZYk1Qf1S8Tu9w)

# What humane endpoints have been studied in *Leptospira* infections?

More promising humane endpoint is appearance of clinical signs

**Blood on nose and paws – also in urine**



•\* Nick Reed presentation – Animal Health and Veterinary Laboratories Agencies --  
[http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=2&ved=0CGgQFjAB&url=http%3A%2F%2Fwww.humane-endpoints.org%2Fimages%2Freed.pdf&ei=DoMZUNirN4zVigKXv4GQCQ&usg=AFQjCNHaXwKaKQDBzvfjCrbdtTks\\_TD8IQ&sig2=T F5mg\\_eUwZYk1Qf1S8Tu9w](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=2&ved=0CGgQFjAB&url=http%3A%2F%2Fwww.humane-endpoints.org%2Fimages%2Freed.pdf&ei=DoMZUNirN4zVigKXv4GQCQ&usg=AFQjCNHaXwKaKQDBzvfjCrbdtTks_TD8IQ&sig2=T F5mg_eUwZYk1Qf1S8Tu9w)

# What humane endpoints have been studied in *Leptospira* infections?

More promising is appearance of clinical signs

Clinical Signs	Action
Normal	None
Arched back Slightly rough coat	Observe again in 2 hours If signs persist, observe again in 2 hours, etc.
Dull sunken eyes, Moderately rough coat, Unstable on feet Subdued but responds on stimulus Disinterested in surroundings	Observe again in 2 hours, etc. <b>If at end of day, euthanize</b>
Prostate, Nasal bleeding, Blood in urine, permanently closed eyes	Euthanize

# Implementing Use of Early Humane Endpoints

- **Data sources indicate most death occurs between day 7 and 10 days post challenge (Critical Phase) for all serovars\***
- **Need to define “Critical Phase” for each serovar and each facility**
  - Define based on a retrospective evaluation of data
  - Define by collecting data from on-going hamster potency testing program documenting the clinical signs of hamsters post challenge
- **More frequent observations should occur during the Critical Phase**

\* USDA/CVB,. Wunder,. Reed, AHI companies,

# Implementing Use of Early Humane Endpoints

- **Challenge so that clinical signs should occur during the daytime observations**
- **Reverse Lighting – Reed\***
  - **Hamsters are nocturnal so they are more active during darkness**
  - **Leave lights off during the daytime and have lights on during the night.**
  - **Provides a greater opportunity to determine true lethargy and clinical signs during the daytime observations**
- **Use of white bedding material to detect bleeding**
- **Training of observers to detect clinical signs**

\* Nick Reed presentation –

[http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=2&ved=0CGgQFjAB&url=http%3A%2F%2Fwww.humane-endpoints.org%2Fimages%2FReed.pdf&ei=DoMZUNirN4zVigKXv4GQCQ&usg=AFQjCNHaXwKaKQDBzvfjCrbdtTks\\_TD8lQ&sig2=TF5mg\\_eUwZYk1Qf1S8Tu9w](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=2&ved=0CGgQFjAB&url=http%3A%2F%2Fwww.humane-endpoints.org%2Fimages%2FReed.pdf&ei=DoMZUNirN4zVigKXv4GQCQ&usg=AFQjCNHaXwKaKQDBzvfjCrbdtTks_TD8lQ&sig2=TF5mg_eUwZYk1Qf1S8Tu9w)

# Potential use of analgesics during critical phase of *Leptospira* challenges

Drug	Hamster	Mice	Rat	Guinea Pig
Ketoprofen	5 mg/Kg SC, SID	5 mg/Kg SC, SID	5 mg/Kg SC, SID	5 mg/Kg SC, SID
Meperidine	4-8 mg/Kg SC or IM every 3 hours	2-5 mg/Kg SC or IM every 4 hours	4-8 mg/Kg SC or IM every 3 hours	N/A
Butorphenol	5 mg/Kg SC every 4 hrs	2 mg/Kg SC every 4 hours	5 mg/Kg SC every 4 hrs	5 mg/Kg SC every 4 hrs
Buprenorphine	0.5 mg/Kg SC every 8 hours	0.05-0.1 mg/Kg SC every 12 hours	0.1-0.5 mg/Kg SC every 12 hours	0.05 mg/Kg SC every 12 hours

Table from [http://vetmed.duhs.duke.edu/guidelines\\_for\\_rodent\\_analgesia.htm](http://vetmed.duhs.duke.edu/guidelines_for_rodent_analgesia.htm)

- **Buprenorphine added to water effectively relieved pain and distress in mice challenged with *Toxoplasma gondii* without interference in the death outcome.**

Lindsey, et al. 2005. J. Parasitology 91 (6), pp 1488-1490

- **CVB – Buprenorphine works during Erysipelas challenges**

# Potential use of analgesics during death phase of *Leptospira* challenges

Administration of analgesics to hamsters via drinking water during the expected death phase

Normal Daily Water Consumption (healthy animal)	30 mL/day
Dose of Buprenorphine in drinking water	0.5 mg/Kg per animal/day

**Complication: when hamsters become sick, they drink less water**

**However, with Critical Phase being short (<12 hours) this may work**

**Some indication that Buprenorphine interferes with endpoint for *Leptospira* – need further studies**

# Potential use of analgesics during death phase of *Leptospira* challenges

## ➤ Buprenorphine can be administered via Jello

- Must be introduced to Jello to get them used to eating it – once they try it they like it
- Prepare Jello according to package for “jigglers” with the drug dose mixed into the water. Pour into ice cube trays to solidify into cubes the right size.
- Give one cube BID

# Are humane endpoints written into any *Leptospira* vaccine potency testing guidelines?

## ➤ USDA – Notice 12-12 (Draft Memo 465)

- Issued May 25, 2012
- All codified potency tests (except Rabies) that use live virus, bacteria or a bacterial toxin and which are expected to be lethal should be modified (via the Outline of Production) to include the following wording
  - *Moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power should be humanely euthanized and considered as death as outlined in CFR 117.4. Observations must be made with sufficient frequency to ensure suffering is minimized.*
- In the case of rabies:
  - *Animals exhibiting paresis,, signs of paralysis, and/or convulsions must be humanely euthanized and considered as deaths as outlined in 9CFR 117.4.*

# Are humane endpoints written into any *Leptospira* vaccine potency testing guidelines?

## ➤ EU – Directive 86/609/EEC

- Experiments “shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practically available”.
- When choosing test methods “those which use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm.....should be selected”.
- Currently being replaced by Directive 2010/63/EU – demands that methods to reduce, refine or replace animals are applied (see recitals 11 ff, Art 23, Annex V, Art 47).

## ➤ EU Applicants must pay an additional fee if an alternative test is submitted for replacement of an accepted test – Major disincentive

- Halder -- Germany (PEI) has waived this fee for submission of alternative non-animal test submission

# Are humane endpoints written into any *Leptospira* vaccine potency testing guidelines?

➤ EU – Monograph 62 -- The general monograph on "Vaccines for veterinary use" states the following:

## ***“Animal tests***

*In accordance with the provisions of the European convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes, **tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm.** The criteria for judging tests in monographs must be applied in the light of this. For example, if it is indicated that an animal is considered to be positive, infected, etc **when typical clinical signs occur then as soon as it is clear that the result will not be affected, the animal in question shall be either humanely killed or given suitable treatment to prevent unnecessary suffering.** In accordance with the General Notices alternative methods may be used to demonstrate compliance with the monograph and the use of such tests is particularly encouraged when this leads to replacement or reduction of animals use or reduction of suffering.”*

# Are humane endpoints written into any *Leptospira* vaccine potency testing guidelines?

## ➤ ICATM – The International Cooperation on Alternative Test Methods

- U.S. (NICEATM-ICCVAM), Europe (ECVAM) Canada (Health Canada), Japan (JaCVAM), Korea (KoCVAM)
  - Memorandum of Cooperation signed 2009
  - Voluntary collaboration to support the 3Rs
  - Support harmonization of alternative test methods through cooperative validation and acceptance
  - Support development of humane endpoints where replacement is not possible

# What funding opportunities are available for development of tests utilizing the 3Rs, including evaluation of humane endpoints?

- Center for Alternatives to Animal Testing (CAAT)
- Animal Welfare Information Center (AWIC) Bulletin
- American College of Laboratory Animal Medicine (ACLAM) Foundation
- American Society of Laboratory Animal Practitioners (ASLAP) Foundation
- The National Center for Research Resources (NCRR)
- National Science Foundation Animal Behavior Grants
- National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs, UK)
- Fund for the Replacement of Animals in Medical Research (FRAME, UK)
- Medical Advances without Animals (MAWA, Australia)

A photograph of a desert landscape. In the foreground, a paved road with white lane markings leads towards the horizon. The middle ground features several large, flat-topped mesas and buttes, characteristic of a desert environment. The sky is filled with heavy, grey, and white clouds, creating a dramatic atmosphere. The overall scene is captured in a wide-angle shot, emphasizing the vastness of the landscape.

**THANK YOU FOR YOUR ATTENTION**

**If you cannot replace the hamster potency test with the *in vitro* test, at least work to apply early humane endpoints AND, where possible, Use analgesics when conducting any animal testing.**