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

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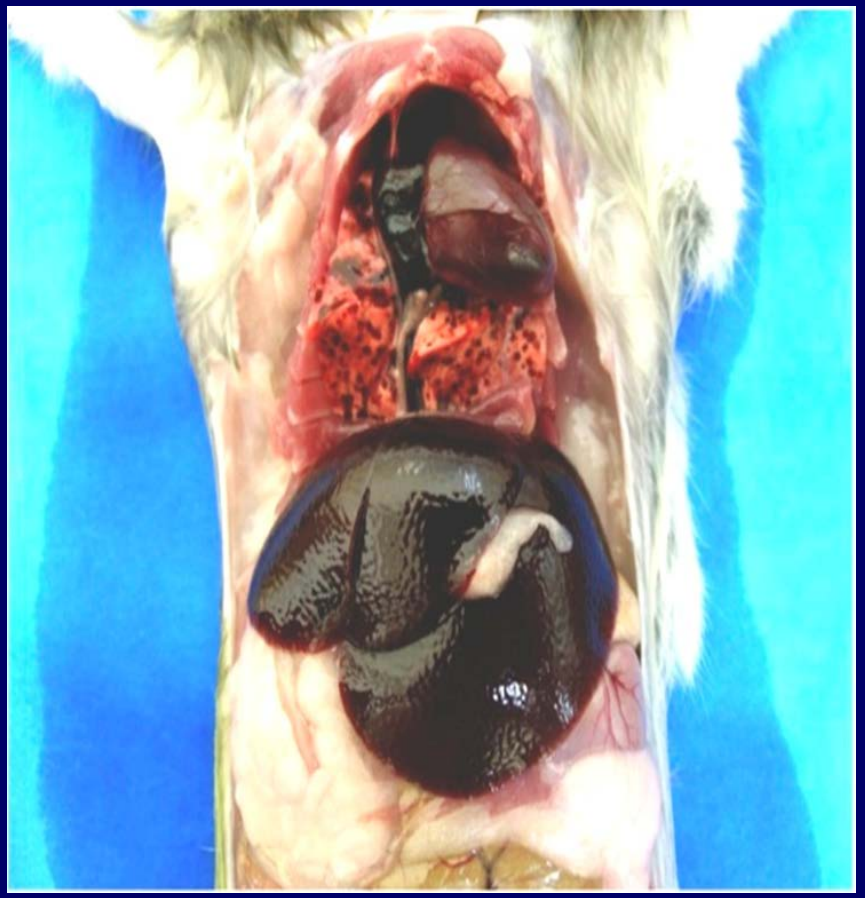
Challenges for Leptospirosis Hamster model

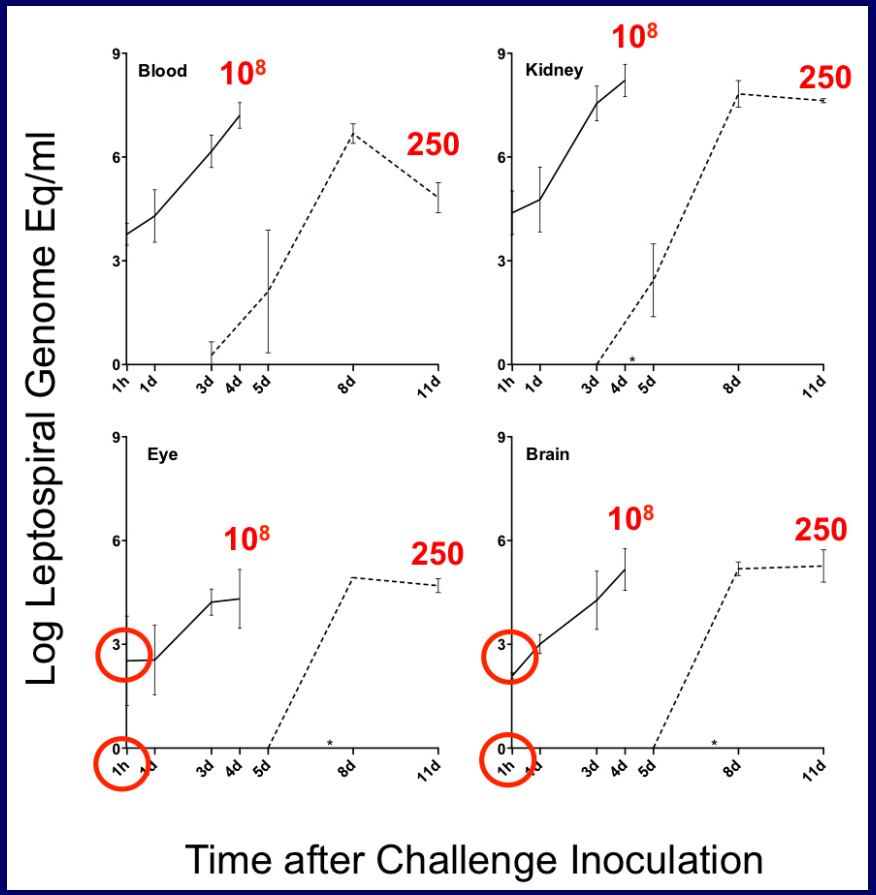
- Acute disease
  - ✓ Rapid dissemination
  - ✓ Death occurs within few hours
- Different serovars
  - ✓ Different outcomes
  - ✓ Different LD50s
- Infection route
  - ✓ Intraperitoneal doesn't occurs in nature
  - ✓ Penetration - pathogenesis
- Death as endpoint
  - ✓ More efficient determinants for disease

Hamster model of acute leptospirosis



In hamsters, leptospires rapidly disseminate, and death can occur within a few hours

Real Time PCR in Infected Hamsters



Status	N°	%
Survived	63	20.7
Euthanized	21	73.3
Deaths	220	72.4
Deaths within 8h	59	36.7
Deaths within 16h	161	73.3
TOTAL	304	100

- Over 68% of the animals that died were considered healthy on the last assessment

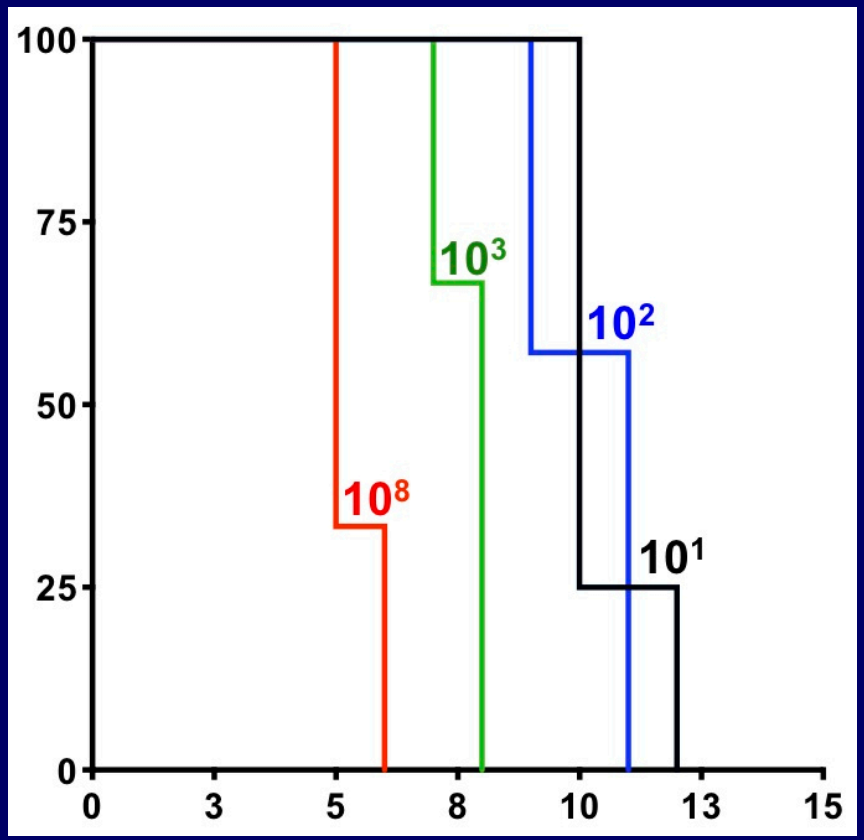
Within the same pathogenic specie (*L. interrogans*), different serovars and routes of infection can't be compared

Serovar	Route	Inoculation dose leptospires	Median of days for death	Range of days for death
Manilae	IP	10^8	5	5
Manilae	IP	100	10	9 - 12
Manilae	Ocular	10^8	8.5	8 - 9
Copenhageni	IP	10^8	5	5 - 6
Copenhageni	IP	100	9	9
Copenhageni	Ocular	10^8	9	9

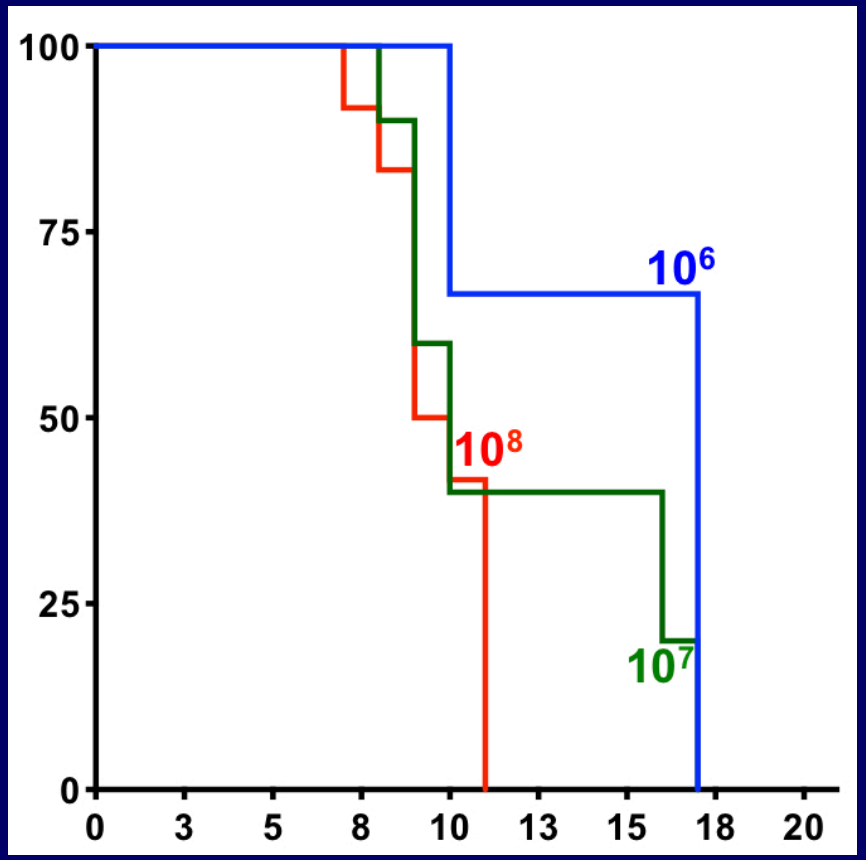
- Serovar Lai has a LD_{50} of $5x10^7$ leptospires

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



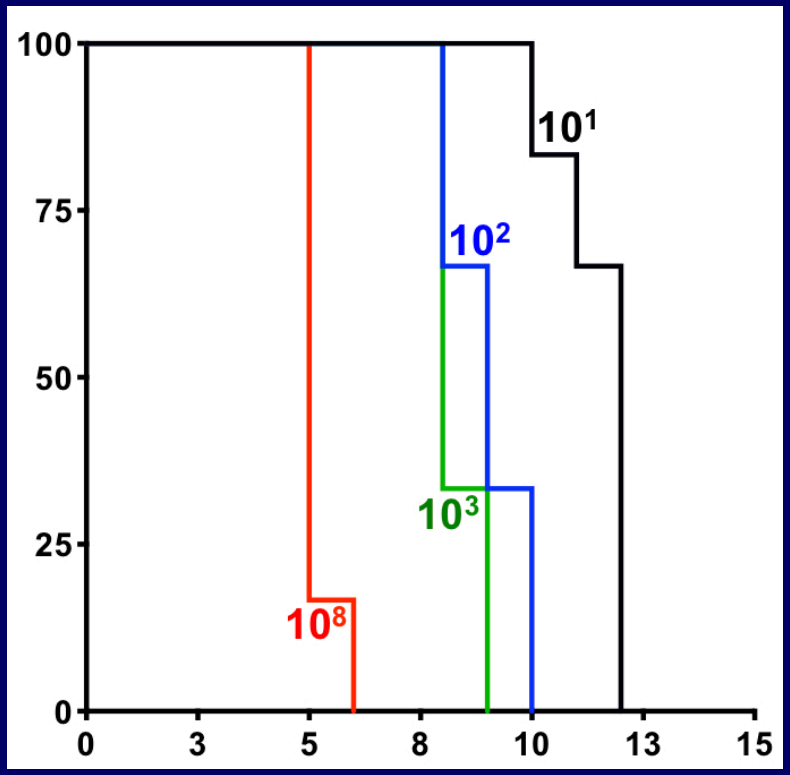
LD_{50} Conjunctival



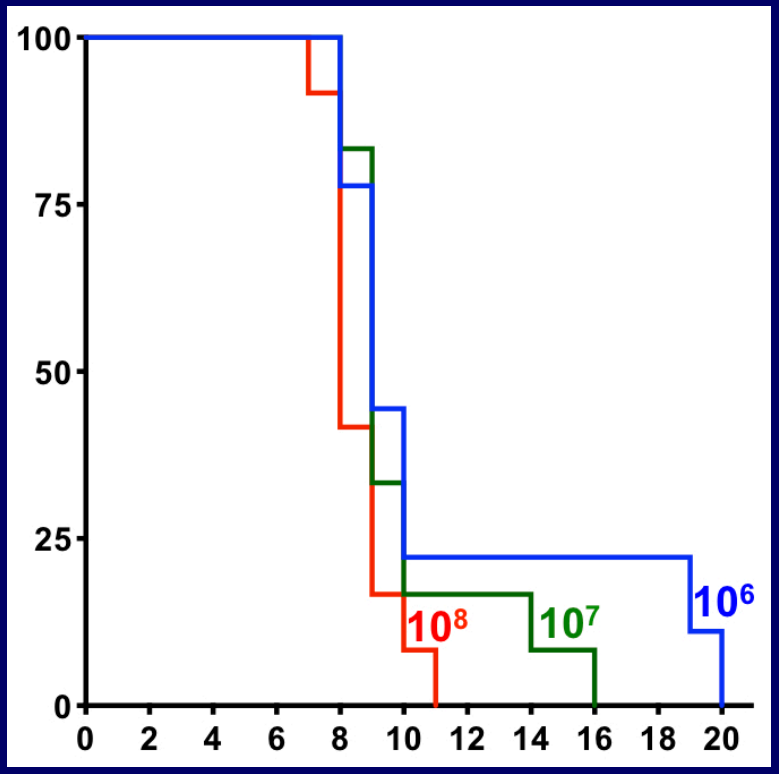
It’s important to have a well established and standardized hamster model for each serovar, as for different routes of infection

- Leptospira interrogans serovar Manilae

LD_{50} Intraperitoneal



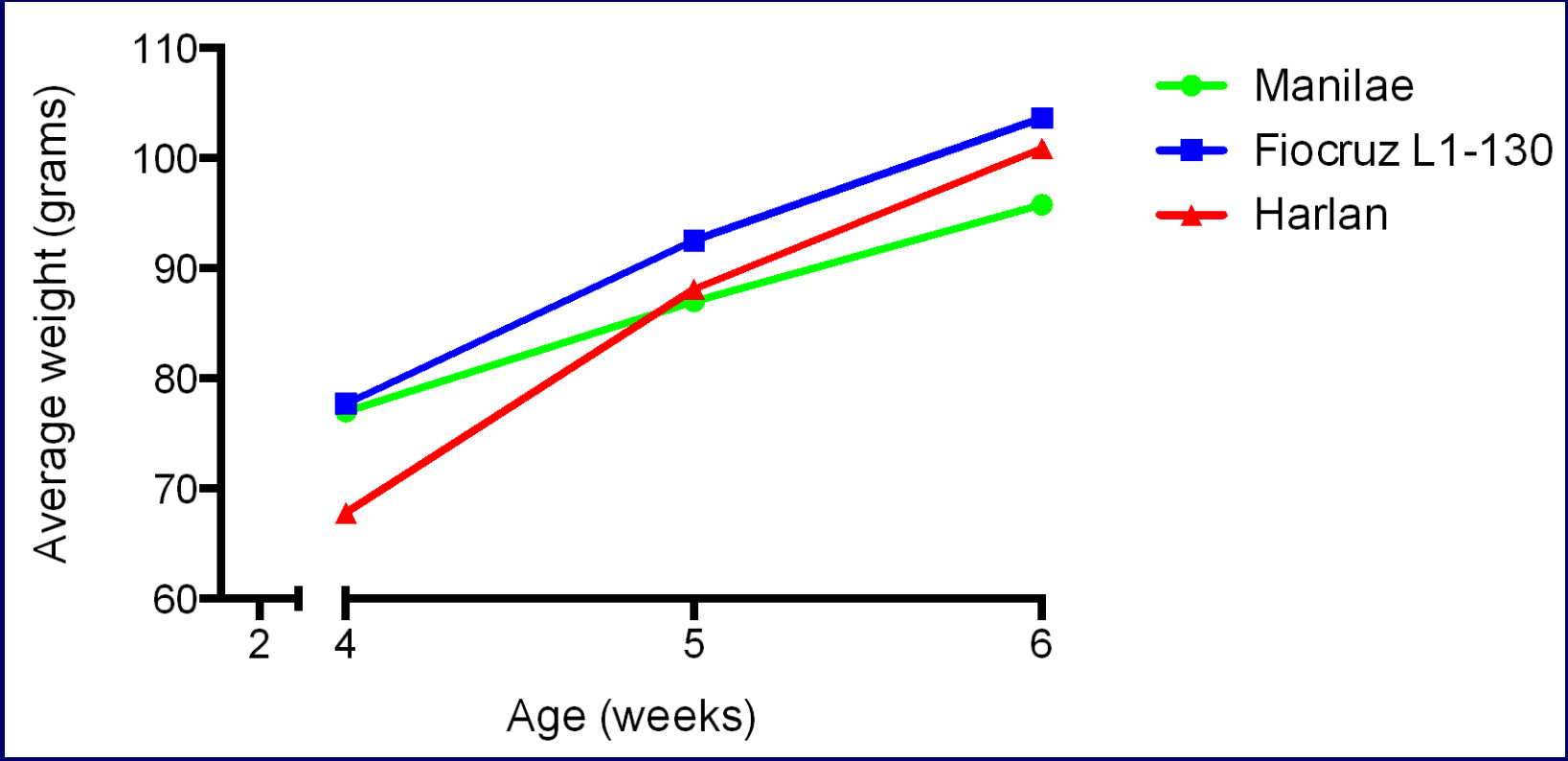
LD_{50} Conjunctival



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

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**	**-**

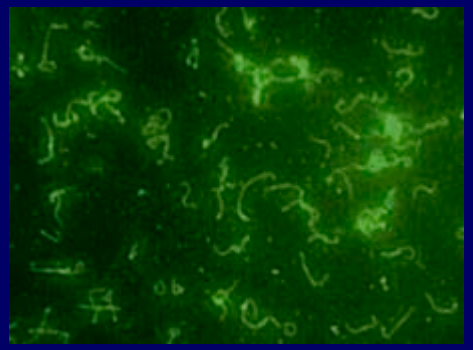
Future experiments at Yale to avoid the high numbers of deaths without evident symptoms

- 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

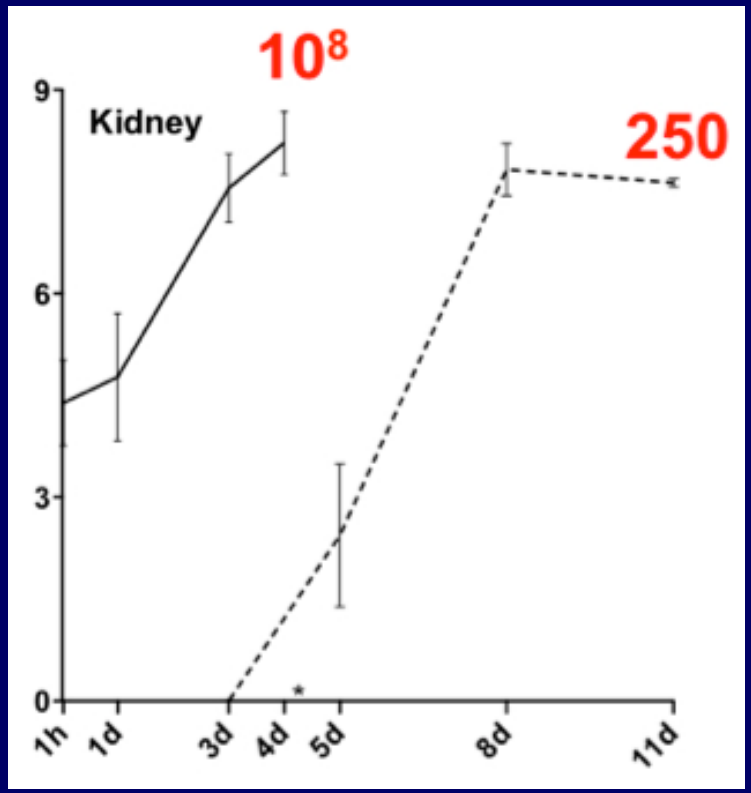
“New” possible outcomes other than death

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

IFA with α-LipL32
Kidney touch prep

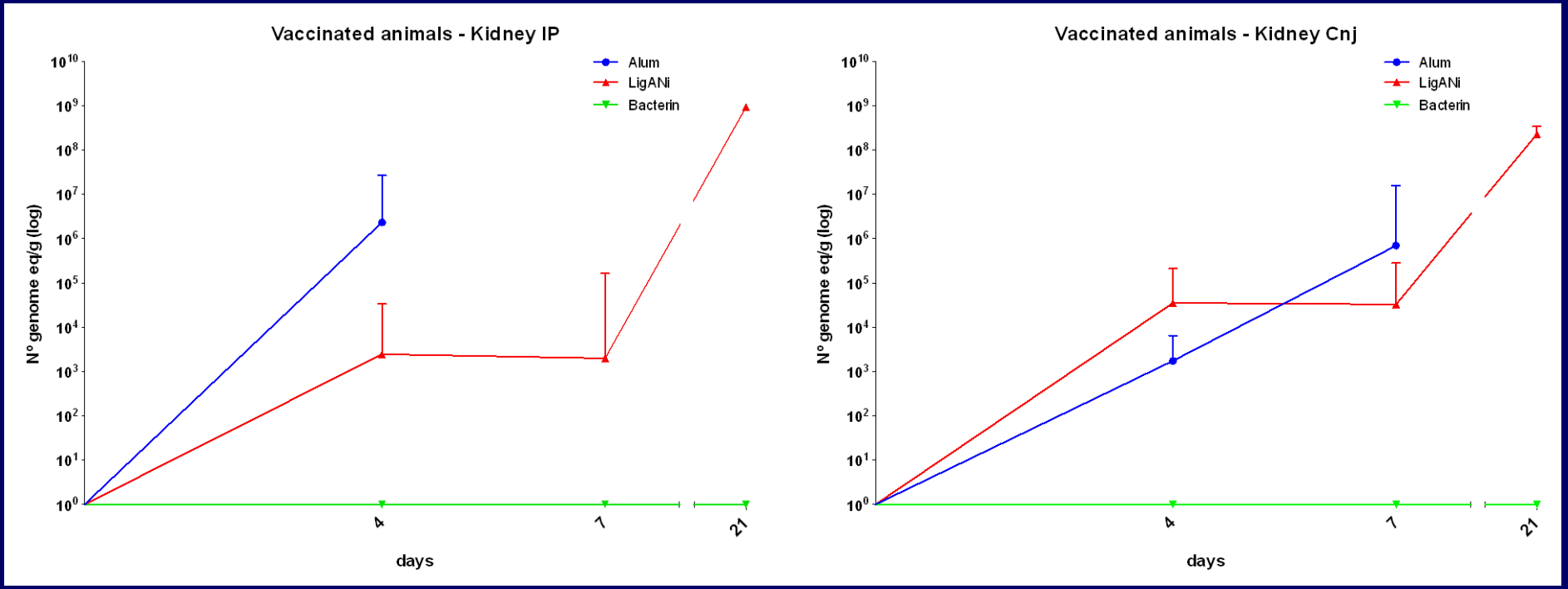


qPCR Kidney – high and low dose



“New” possible outcomes other than death

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



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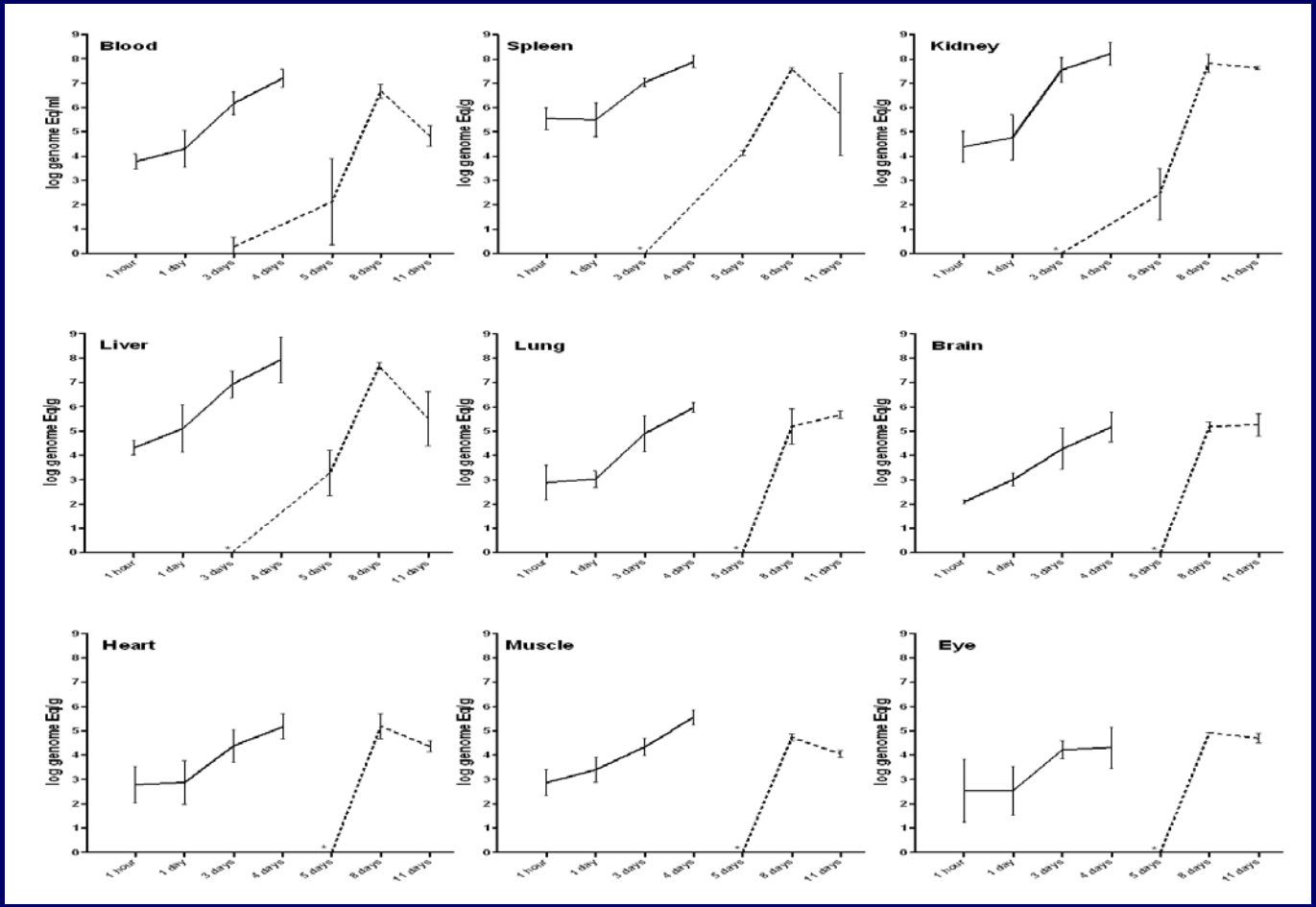

Goals for a improved hamster model for leptospirosis

- 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

Aknowledges

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