

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

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International Workshop on Alternative Methods for
Leptospira Vaccine Testing

Challenges for Leptospirosis Hamster model

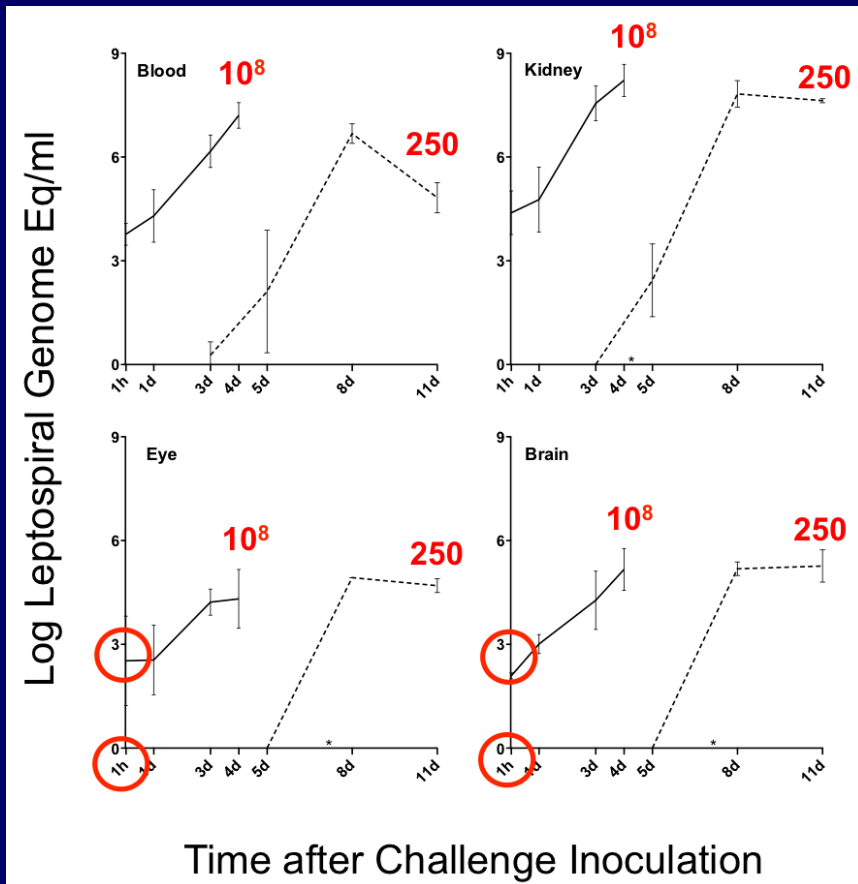
- Acute disease
 - ✓ Rapid dissemination
 - ✓ Death occurs within few hours
- Different serovars
 - ✓ Different outcomes
 - ✓ Different LD50s
- Infection route
 - ✓ Intraperitoneal doesn't occur in nature
 - ✓ Penetration - pathogenesis
- Death as endpoint
 - ✓ More efficient determinants for disease

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

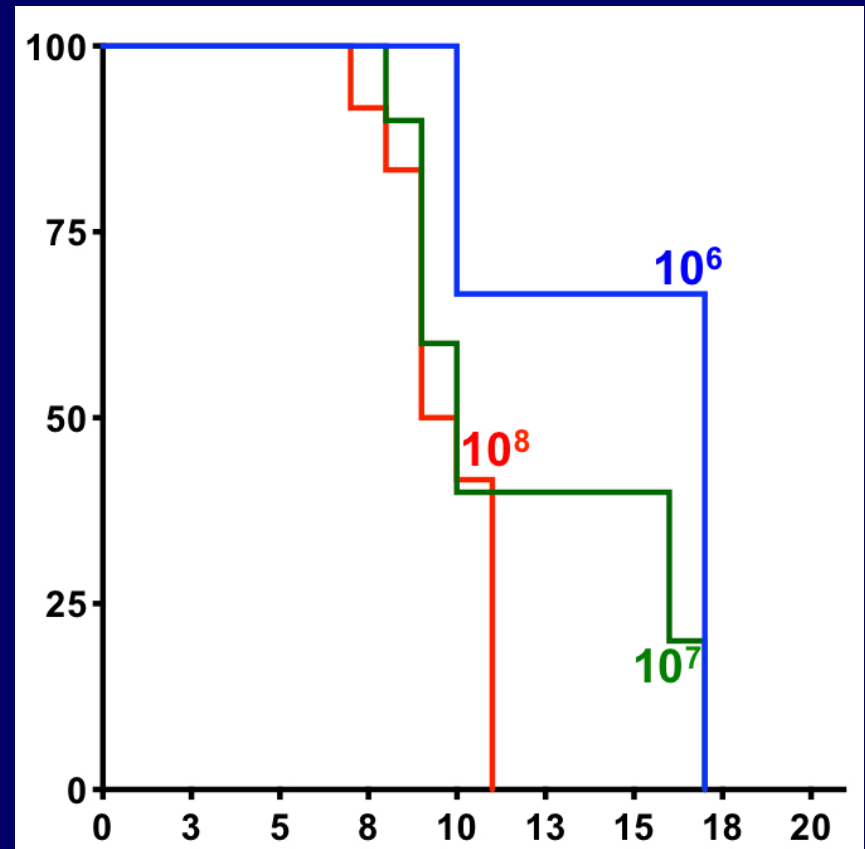
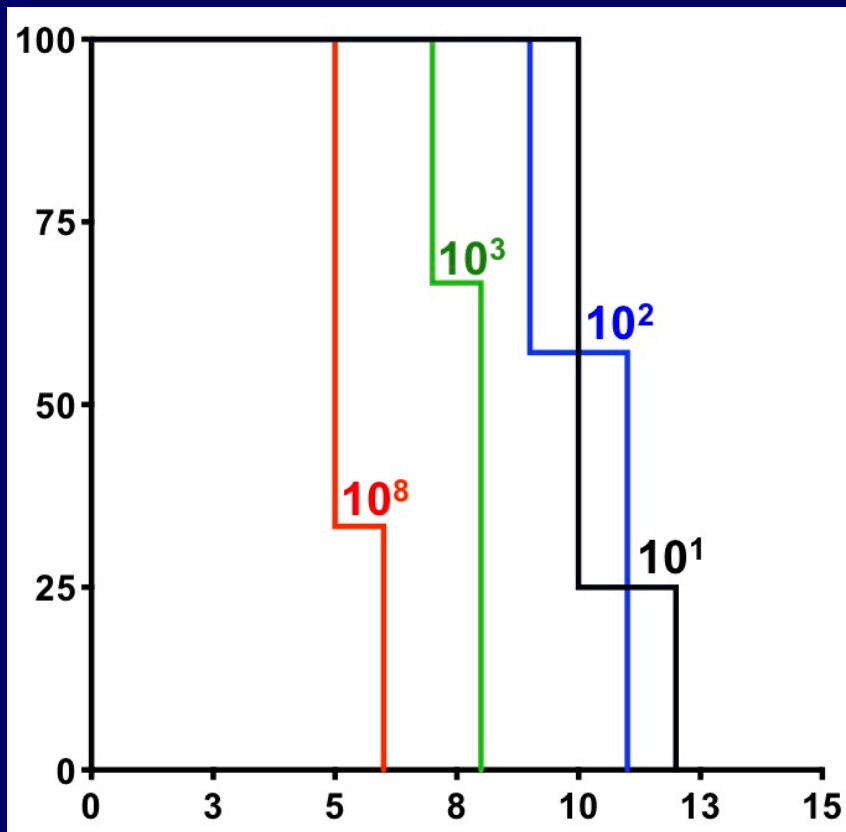
Serovar	Route	Inoculation dose leptospire	Median of days for death	Range of days for death
Manilae	IP	10^8	5	5
Manilae	IP	100	10	9 - 12
Manilae	Ocular	10^8	8.5	8 - 9
Copenhageni	IP	10^8	5	5 - 6
Copenhageni	IP	100	9	9
Copenhageni	Ocular	10^8	9	9

- Serovar Lai has a LD₅₀ of 5×10^7 leptospire

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

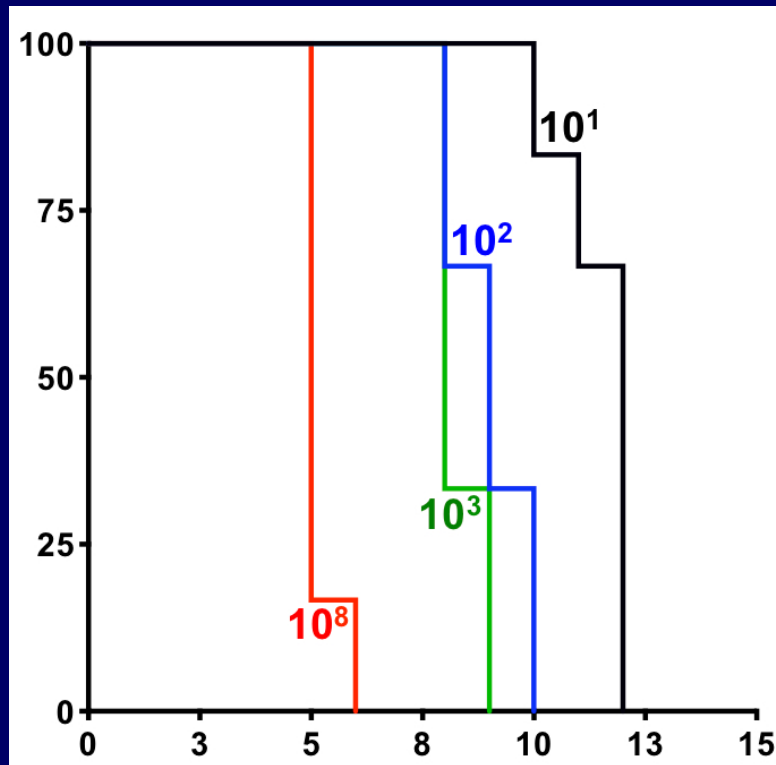
LD₅₀ Conjunctival



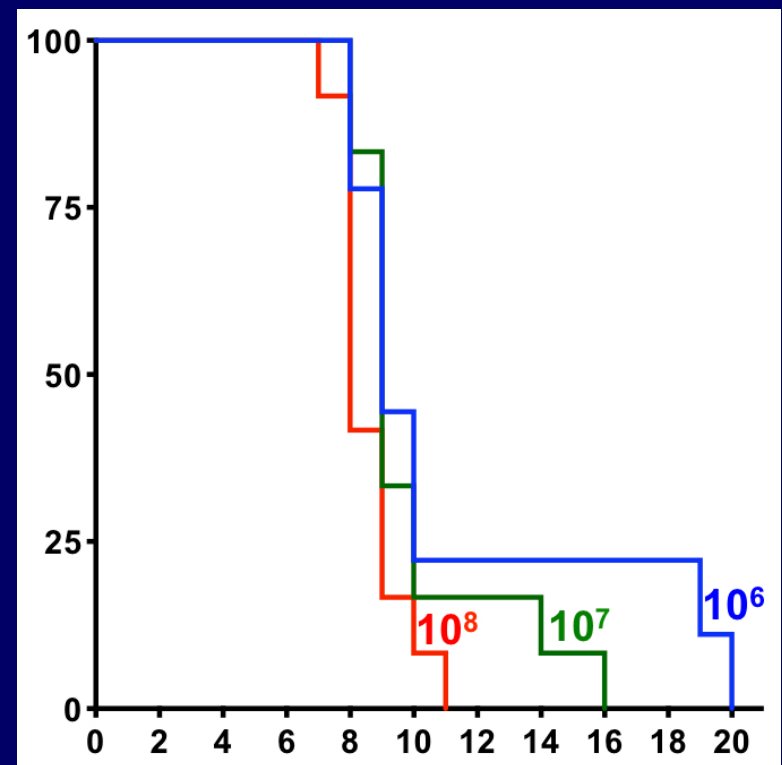
It's important to have a well established and standardized hamster model for each serovar, as for different routes of infection

- *Leptospira interrogans* serovar Manilae

LD₅₀ Intraperitoneal



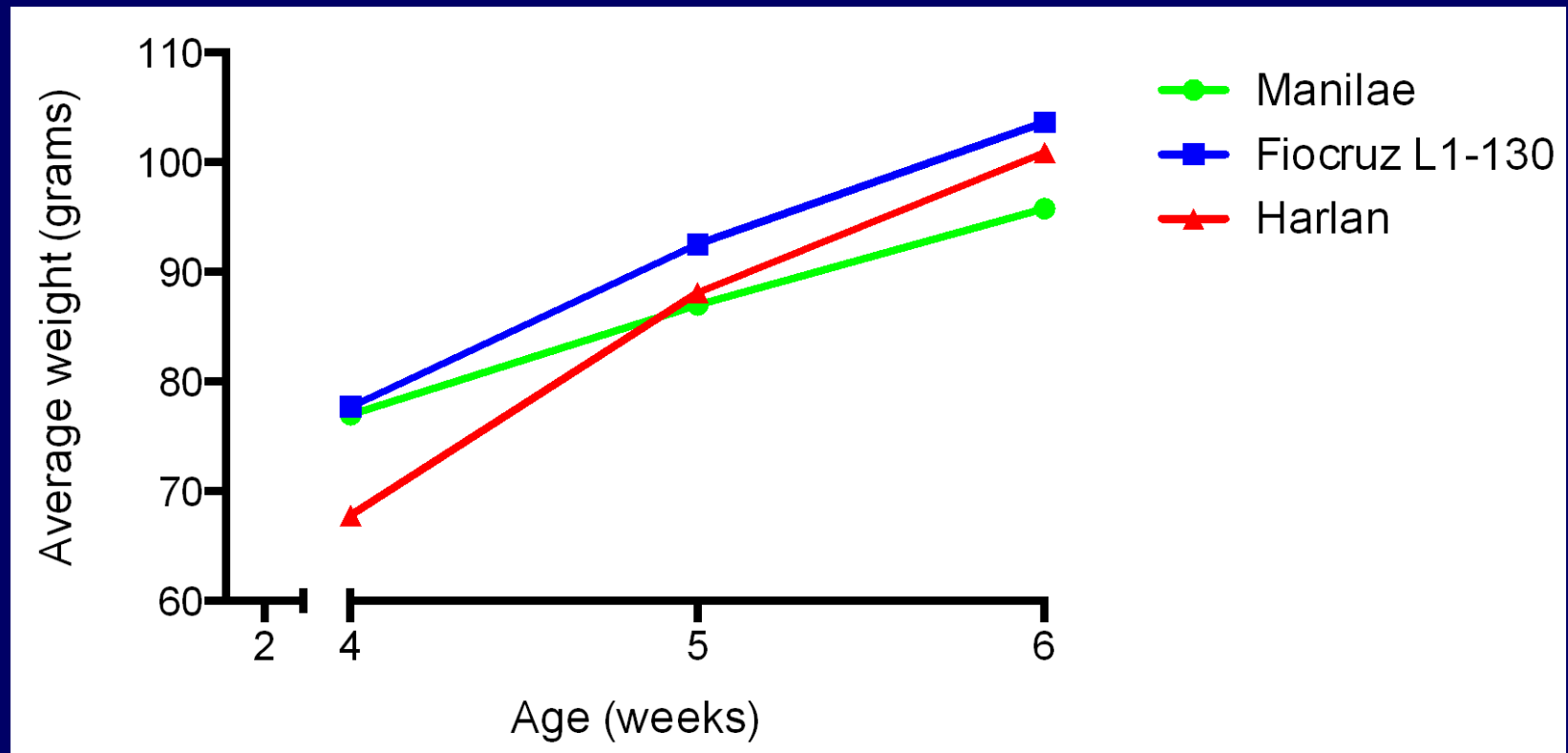
LD₅₀ Conjunctival



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	-

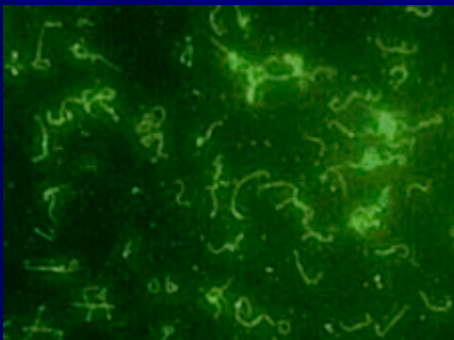
Future experiments at Yale to avoid the high numbers of deaths without evident symptoms

- 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

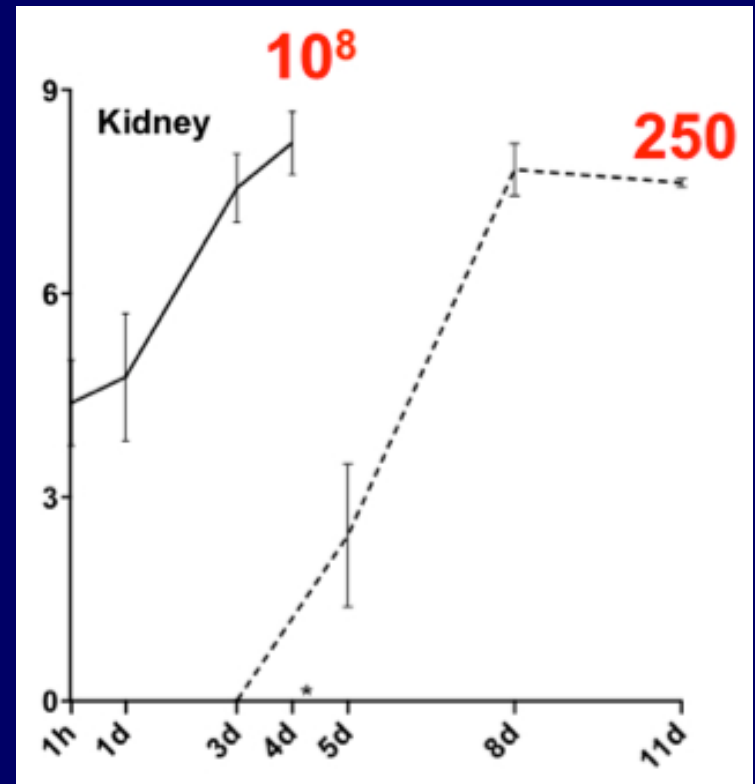
“New” possible outcomes other than death

- 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

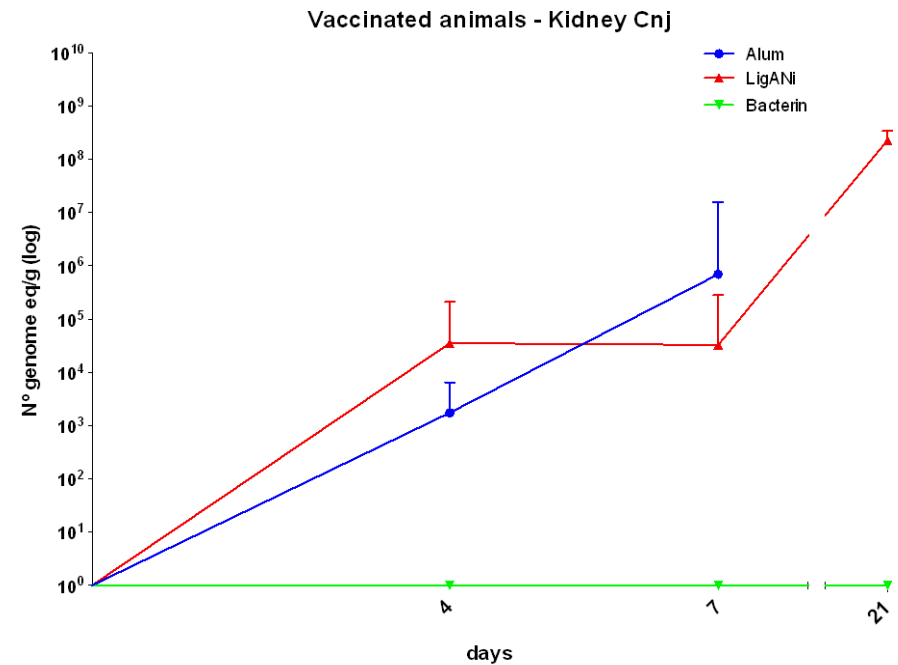
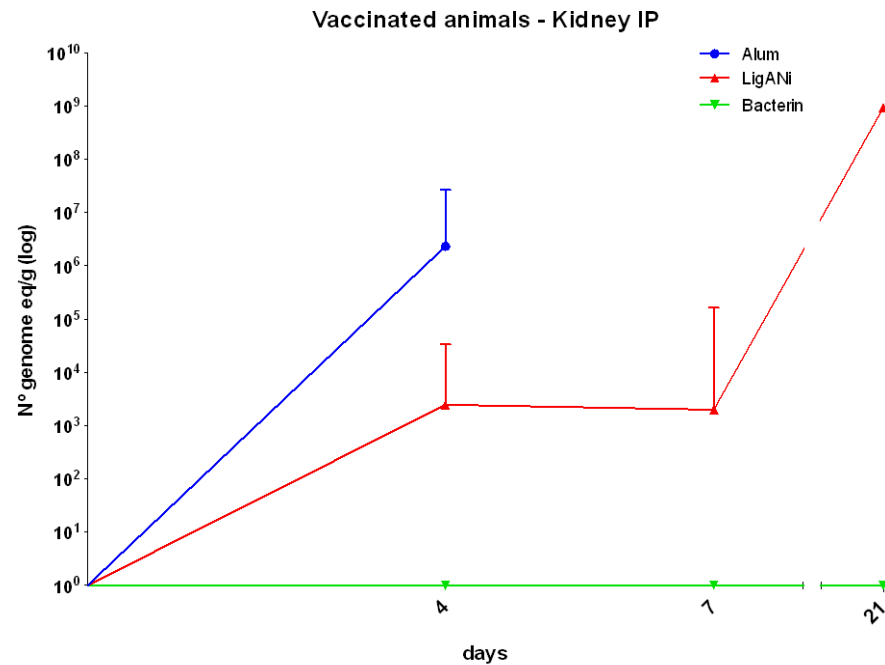


qPCR Kidney – high and low dose



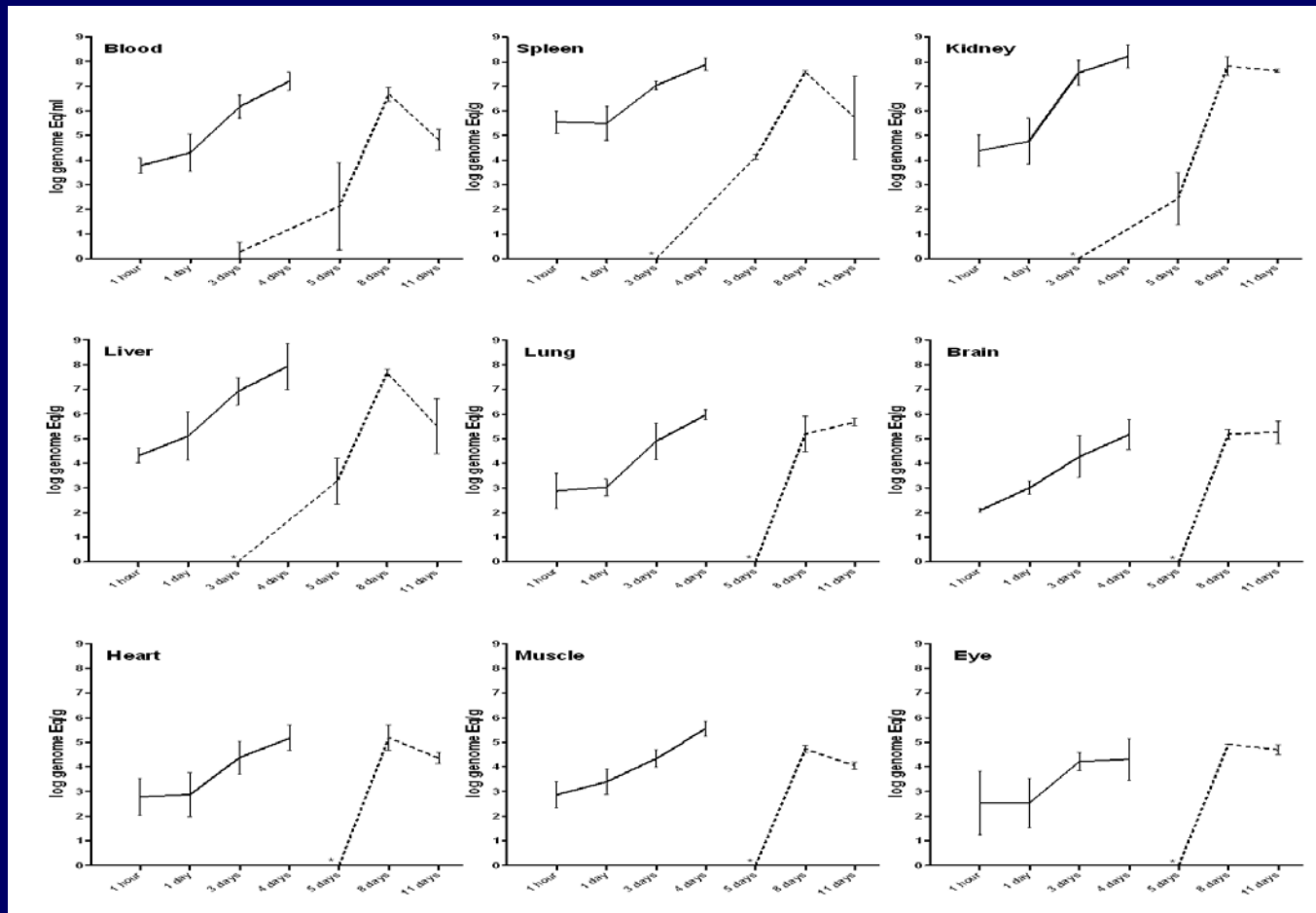
“New” possible outcomes other than death

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



“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

Aknowledges

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