



Leptospirosis: Animal Health Perspectives

Ronald D. Schultz, Professor and Chair
Department of Pathobiological Sciences
School of Veterinary Medicine
University of Wisconsin-Madison

Animals Commonly Infected with Leptospira



Mice

Asymptomatic Rodent Carriers



Rats



Wildlife Species

Raccoons



Dogs



Livestock and Domestic Animals

Cattle

Swine



Opposum

Horses

Sheep/Goats





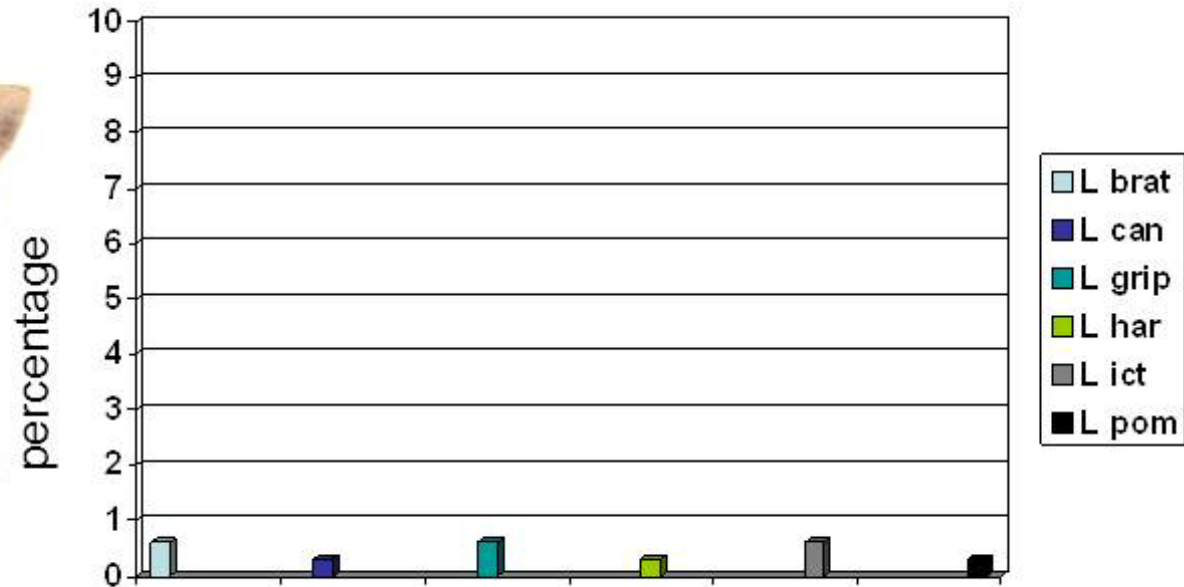
Serologic Prevalence of *Leptospira* Infection of Raccoons in Southeastern WI 1983-84 and 2008-09

Serologic Prevalence of Antibody (≥ 100)

<u>Serovars</u>	<u>1983-84 (n=98)</u>	<u>2008-09 (n=135)</u>
<u><i>L. canicola</i></u>	20/98=20%	23/135=17%
<u><i>L. grippotyphosa</i></u>	39/98=40%	58/135=43%
<u><i>L. pomona</i></u>	12/98=12%	11/135=8%

No Change over 25 year period!

Percent occurrence of antibody titers against *Lepto spp* in sub-population of 290 feral cats



Titers were seen in 3 of 290 cats tested

	L brat	L can	L grip	L har	L ict	L pom
cat762	1:6400	1:200	1:800	1:200	1:800	1:3200
cat808	1:200	neg	neg	neg	1:400	neg
cat966	neg	neg	1:300	neg	neg	neg

Note: Cats develop excellent antibody responses when vaccinated with Canine 4-Way Vaccine!

Canine Leptospira Vaccines and Diagnostics

E. Mukhtar, L.J. Larson, O. Okwumbua, R.D. Schultz

Department of Pathobiological Sciences, School of Veterinary
Medicine, University of Wisconsin-Madison and Wisconsin Veterinary
Diagnostic Laboratory



SCHOOL OF
VETERINARY MEDICINE
University of Wisconsin-Madison

Introduction

- *Leptospira* spp are water-borne, zoonotic spirochetes which have been shown to cause disease in many animal species, including the dog.
- Clinical signs of leptospirosis are often vague and non-specific.
- Older canine vaccines contain 2 serovars – *L. canicola* and *icterohemorrhagiae* (2 way). More recent vaccines have all added *L. pomona* and *grippityphosa* (4 way).
- The current “gold standard” for diagnosis of leptospirosis is the Microscopic Agglutination Test (MAT) which is based on the presence of agglutinating antibody in the serum.
- The recent suggestion that cases of canine leptospirosis are becoming more frequent may stem from misdiagnosis based on MAT and the current criterion for determining infection.

Study Design

- Several trials were set up to determine if vaccination with either commercial 4-way, 2-way, or experimental monovalent *Leptospira* vaccines could induce MAT antibody titers above the criterion of ≥ 800 .
- One trial was set up to determine if Lyme vaccination would potentiate or abrogate Lepto vaccination.
- All dogs were housed in a *Leptospira*-free environment.
- Blood for MAT serology was drawn at various time points following vaccination.

**Percentage of Sixteen Vaccinated (2-Way Vaccine) Dogs Raised in a
Leptospira-free Facility with MAT Antibody Titer \geq 800****

SEROVAR	PERCENTAGE (HIGHEST TITER)
<i>L. bratislava</i>	13 % (800)
* <i>L. canicola</i>	0 %
<i>L. grippityphosa</i>	0 %
<i>L. hardjo</i>	0 %
* <i>L. icterohaemorrhagiae</i>	19 % (1600)
<i>L. pomona</i>	0 %
<i>L. autumnalis</i>	6% (800)

*Serovars in vaccine

**Single Titer of \geq 800 considered infected

Vaccinated annually for 4 yrs. prior to revaccination (serum collected 12 months after revaccination)

Outcome

1. One (1) would be considered *L. autumnalis* infected.
2. Two (2) would be considered *L. bratislava* infected.
3. Three (3) would be considered *L. icterohaemorrhagiae* infected.
4. None would be considered *L. canicola*, *L. grippotyphosa* or *L. hardjo* infected.

**Percentage of Forty Normal Vaccinated (4-Way Vaccine) Dogs Raised in
a *Leptospira*-free Facility with a MAT Antibody Titer of ≥ 800 ****

SEROVAR	PERCENTAGE (HIGHEST TITER)	
<i>L. bratislava</i>	10 %	(1600)
* <i>L. canicola</i>	30 %	(1600)
* <i>L. grippotyphosa</i>	10 %	(800)
<i>L. hardjo</i>	0 %	(<100)
* <i>L. icterohaemorrhagiae</i>	50 %	(6400)
* <i>L. pomona</i>	20 %	(6400)

*Serovars in vaccine

**Single Titer of ≥ 800 considered infected

Vaccinated at 12 & 16 wks then annually for 2 yrs. Revaccination at 2 yrs (serum collected 3 months after revaccination).

Outcome

Many of these 40 dogs would be considered positive (infected) for one or more *Leptospira* serovars.

1. Four (4) would be *L. bratislava* infected.
2. Twelve (12) would be *L. canicola* infected.
3. Four (4) would be considered *L. grippityphosa* infected.
4. None would be considered *L. hardjo* infected.
5. Twenty (20) *L. icterohaemorrhagiae* infected.
6. Eight (8) would be considered *L. pomona* infected.

**L. autumnalis* not tested

MAT Titer \geq 800 After 2 Doses Of a *Leptospira* Monovalent Vaccine

Group ID	Previous Vac.	Vac.	Bra	Can	Grip	Har	Ict	Pom	Aut
1	4 way vac.	Bra	800*				400	100	400
	4 way “	Bra	1600*				400		400
2	4 way “	Har					100		400
	4 way “	Har					100		200
3	4 way “	Pom	800*					800*	800*
	2 way “	Pom					100	400	800*
4	2 way “	Ict					800*		200
	2 way “	Ict					800*		400
5	2 way “	Grip							100
	2 way “	Grip			800*		100		200
6	2 way “	Aut	400				100		800*
	2 way “	Aut	800*				400	400	1600*
7	2 way “	Can	100	400	200		200		400
	2 way “	Can		100					200

Dogs Previously not Vaccinated then Vaccinated With 5 Doses of an Experimental Monovalent Vaccine

Animal (vaccine serovar)	Bra	Can	Grip	Har	Ict	Pom	Aut
M (Bra)	800*				800*		200
R (Can)	100	1600*	100		100		200
O (Grip)	800*		1600*		100		400
F (Har)	100			3200*	100		
B (Ict)	3200*				6400*		800*
Z (Pom)	800*				200	200	1600*
G (Aut)	200		6400*		100	800*	6400*

*Titer of ≥ 800 considered infected, thus these 7 dogs would be considered positive for infection with one or more serovars.

Outcome

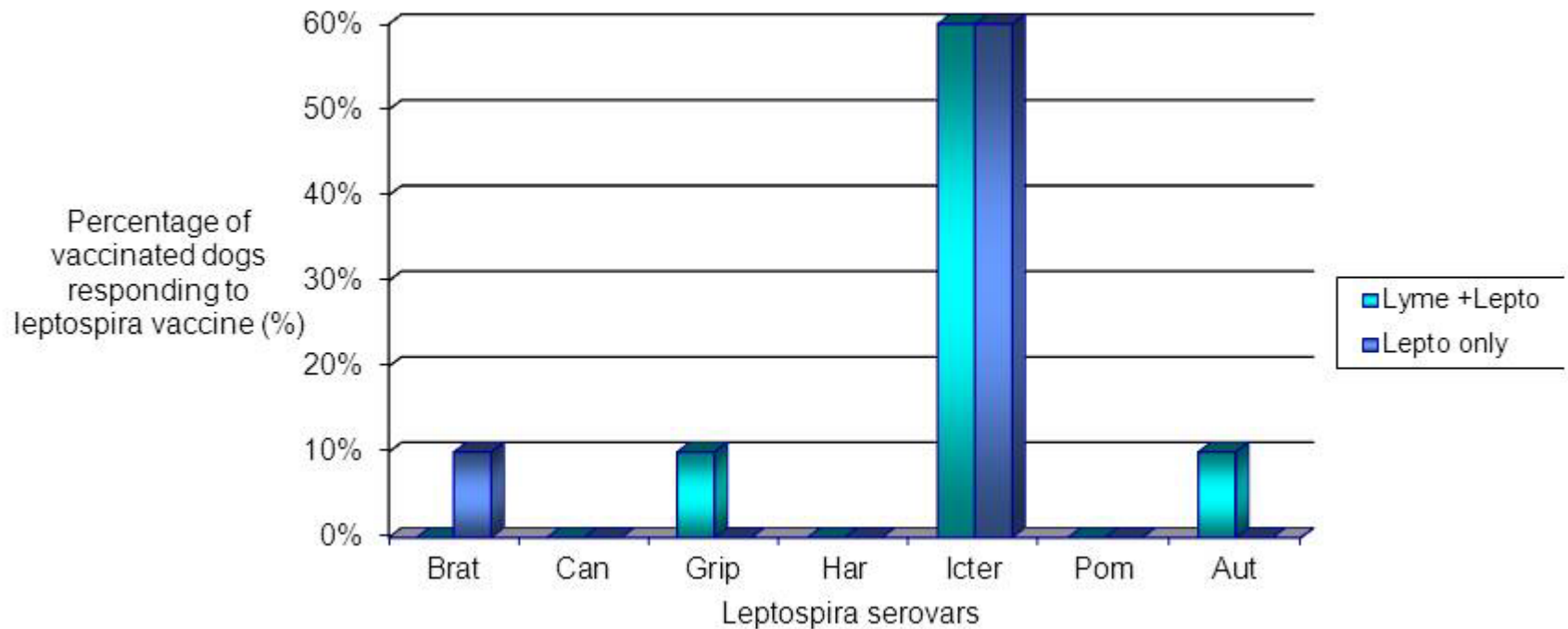
- This study shows considerable cross-reactivity on the MAT occurs among certain serovars, even when naïve dogs are vaccinated with monovalent serovars.
- Cross reactivity seemed to occur most frequently with the *L autumnalis* and *bratislava* serovars.
- Cross reactive MAT titers were sometimes seen at and above the criterion for infection.



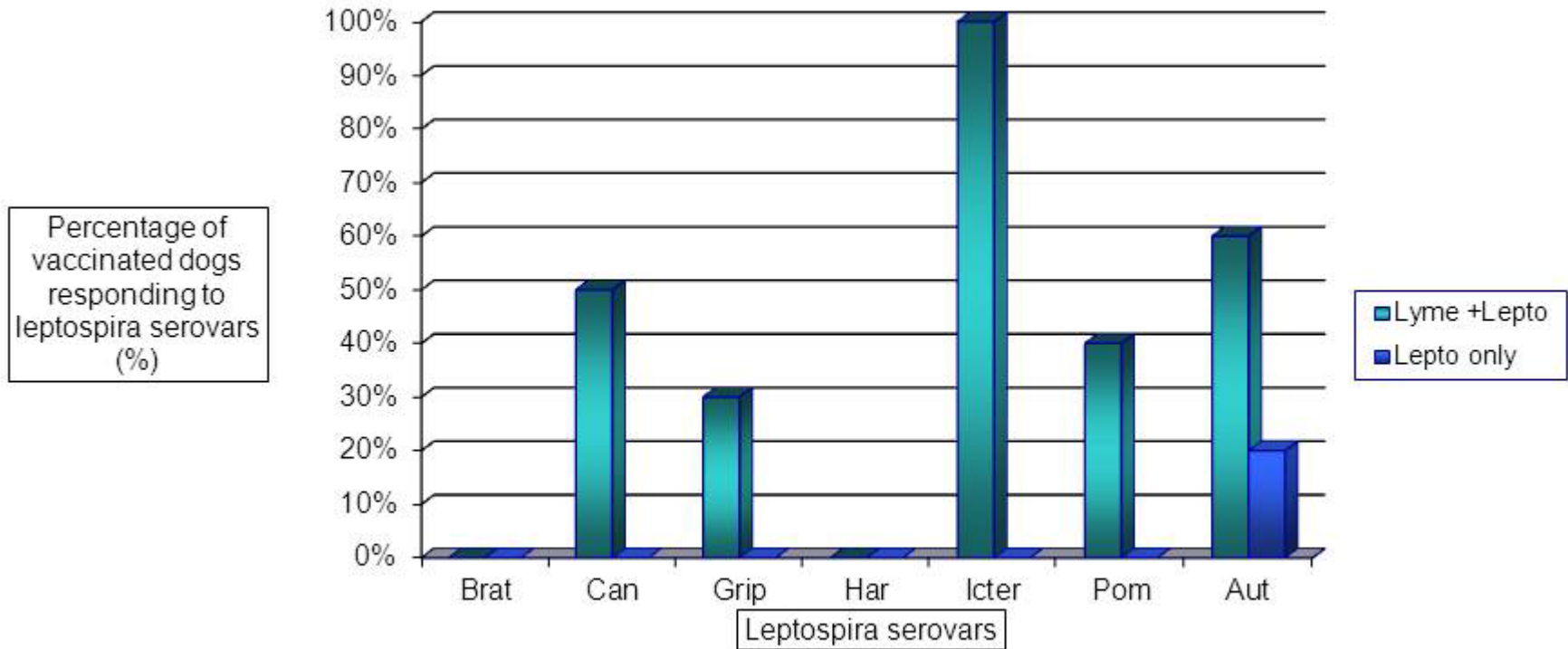
Questions

- Does pre-vaccination with Lyme vaccine influence the MAT response to individual serovars or the peak of the response?
- Could Lyme vaccine prime the animal for a Lepto response?
- Does Lyme vaccination increase titer levels to Lepto serovars?
- Does Lyme vaccination increase the likelihood of cross-reaction to Lepto serovars?
- Study Design: one group of dogs was vaccinated first with Lyme and then 1 week later with Leptospira; the second group was given Leptospira only. Sera were tested for antibody via MAT at 5 and 8 weeks post Leptospira vaccination.

Percentage of dogs responding with MAT titer ≥ 100 to four-way *Leptospira* vaccine five weeks later



Percentage of dogs responding with MAT titer ≥ 100 to four-way *Leptospira* vaccine eight weeks later

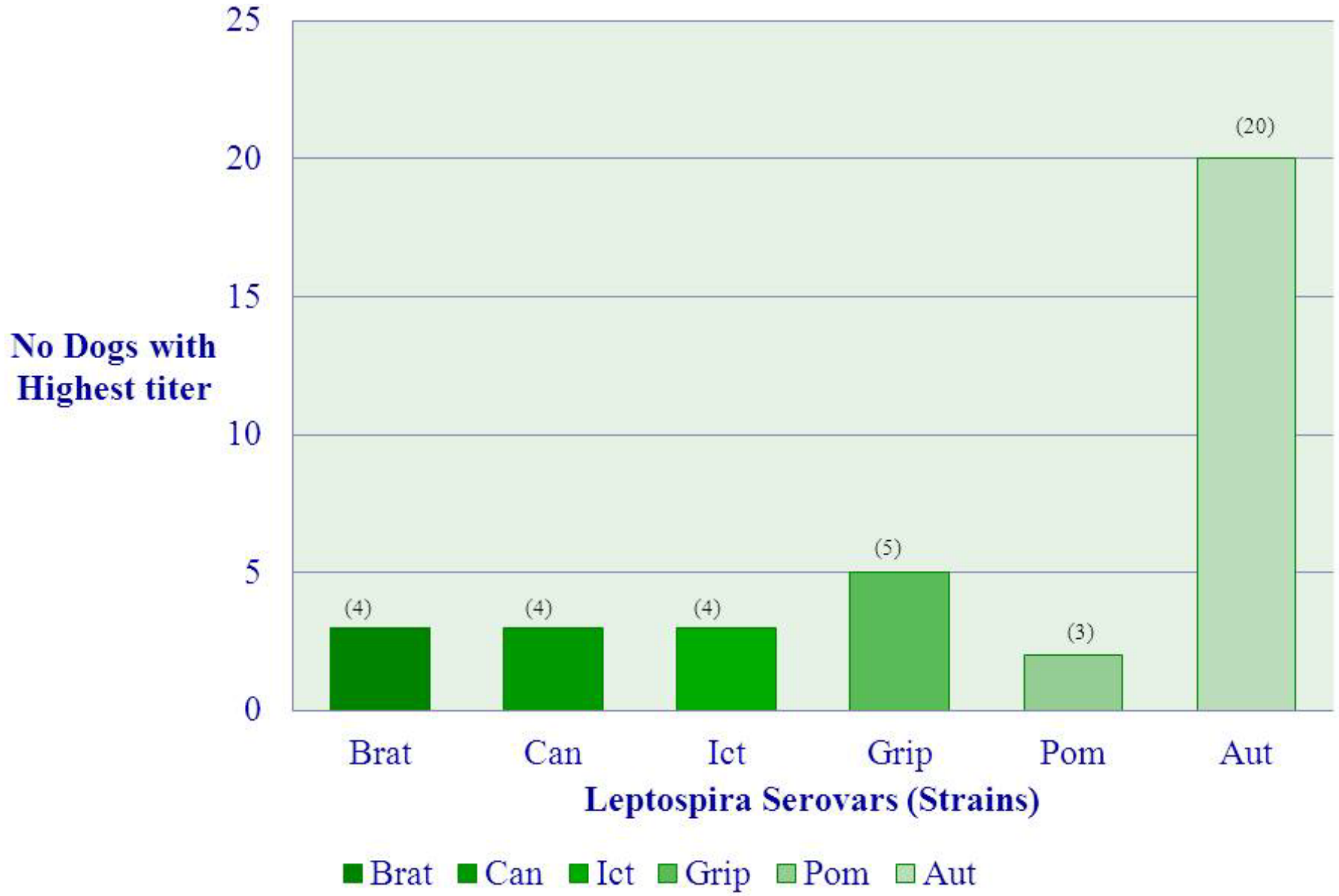


Outcome

- In dogs initially vaccinated with Lyme vaccine (2 doses) and then vaccinated with *Leptospira* vaccine, a more rapid and higher response to more serovars in the *Leptospira* vaccine was observed.
- Lyme vaccination may increase frequency of cross-reactive antibody responses. However, both groups cross-reacted with *L autumnalis*.

Which Leptospira Serovar Has the Highest Titer in a Dog?

N=40



Canine Leptospira Vaccines:



Immunologic Memory for IgG can persist for 1 to 2 yrs. in the dog.

Immunologic Memory for IgE can persist for 4 years or longer.

Therefore:

An adverse (Type I hypersensitivity) reaction can occur in genetically predisposed dogs for much longer time than protective immunity is present.

How Can We Demonstrate Protective Immunity to Leptospira Vaccines Without Performing Animal Challenge Studies?

Demonstrate Experimentally that the serum “Leptocidal Test” is positive!*

R.C. Johnson and L.H. Muschel developed a serum Antileptospiral Assay and published it in J. Bact. 91: 1403-1409, 1966!

R. F. Bey and R.C. Johnson demonstrated that there was a direct correlation between protective antibodies in the hamster protective test and antibodies reactive in the Antileptospiral Assay.

Note: There appears to be a significant correlation for protection with Lyme Vaccines in the dog and the Borreliacidal Assay!

*Validation of the Leptocidal Test would be required using Dog Challenge studies!



The new criteria we propose using for diagnosis of canine leptospiral disease are:

- 1. MAT titers to only serovars *L. canicola*, *L. grippotyphosa*, *L. icterohaemorrhagiae*, and/or *L. pomona* should be used for the diagnosis of canine leptospirosis.**
- 2. With a single sample, a titer should be equal to or greater than 1600 for one or more of these 4 serovars.**
- 3. When acute and convalescent samples are available, the increase in titer to one or more of the 4 serovars should be an 8 fold or greater increase.**
- 4. An acute titer of 1600 or greater would constitute a positive sample, even if the convalescent sample did not show an 8 fold increase.**