

Diagnosis, Treatment and Prevention of Infectious Diseases in the City: New for 2016

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SINCE 1910

ANIMAL MEDICAL CENTER



Screening for Tick Borne Diseases - What about Non-Clinical Dogs? Should We??



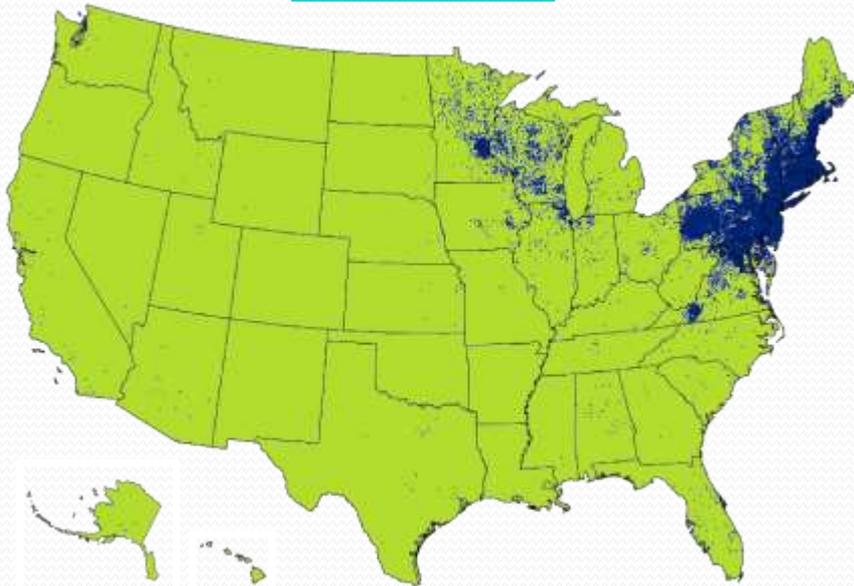
Goals of Screening

- To treat if justified
 - To prevent damage/disease
 - Lyme nephritis
- To serve as marker for infected ticks
 - Co-infections
 - Public health
 - Improve tick control
 - Vaccinate

INCREASING PREVALENCE OF LYME DISEASE

CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC) PREVALENCE MAP¹

HUMANS 2014

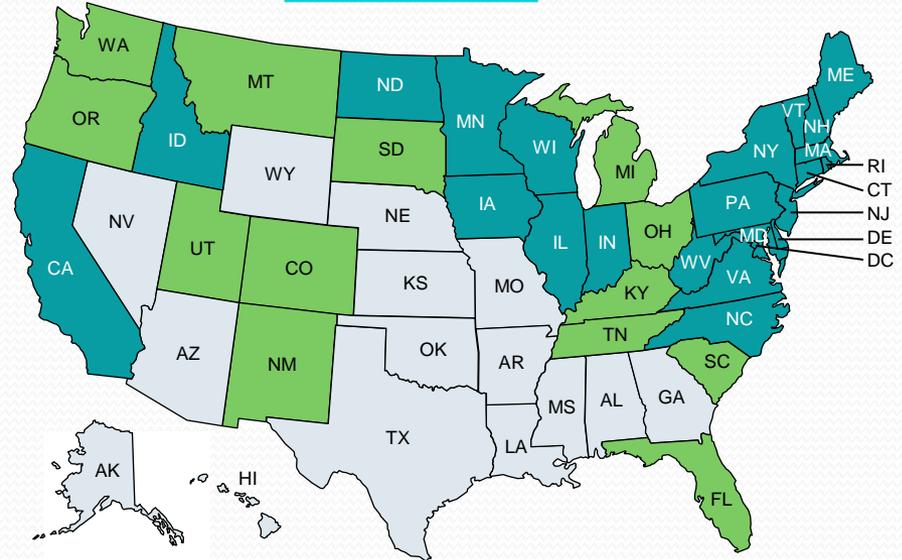


1 dot placed randomly within county of residence for each confirmed case

- 28% increase in human incidence rate between 2005 and 2013, with 30,000 cases reported annually²
- CDC believes actual cases to be closer to 329,000 per year²

COMPANION ANIMAL PARASITE COUNCIL (CAPC) PREVALENCE MAP³

CANINES 2015



□ LOW ■ MODERATE ■ HIGH

- 1 in 16 dogs tested were positive for Lyme disease in 2015³
- 166,000 cases per year³

More Than Just Lyme!

- **Lyme disease/Lyme borreliosis**

Borrelia burgdorferi

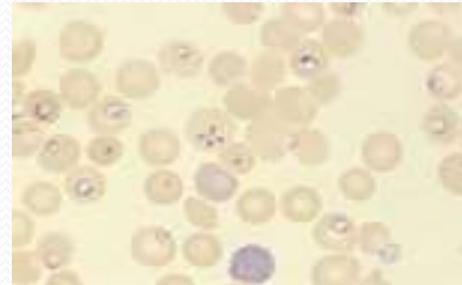


- **Ehrlichiosis/Anaplasmosis**

- *Ehrlichia canis, Ehrlichia ewingii, Ehrlichia chaffeensis*
- *Anaplasma phagocytophilum, Anaplasma platys*

- **Rocky Mountain spotted fever**

Rickettsia rickettsii



- **Babesiosis**

Babesia canis, Babesia gibsoni

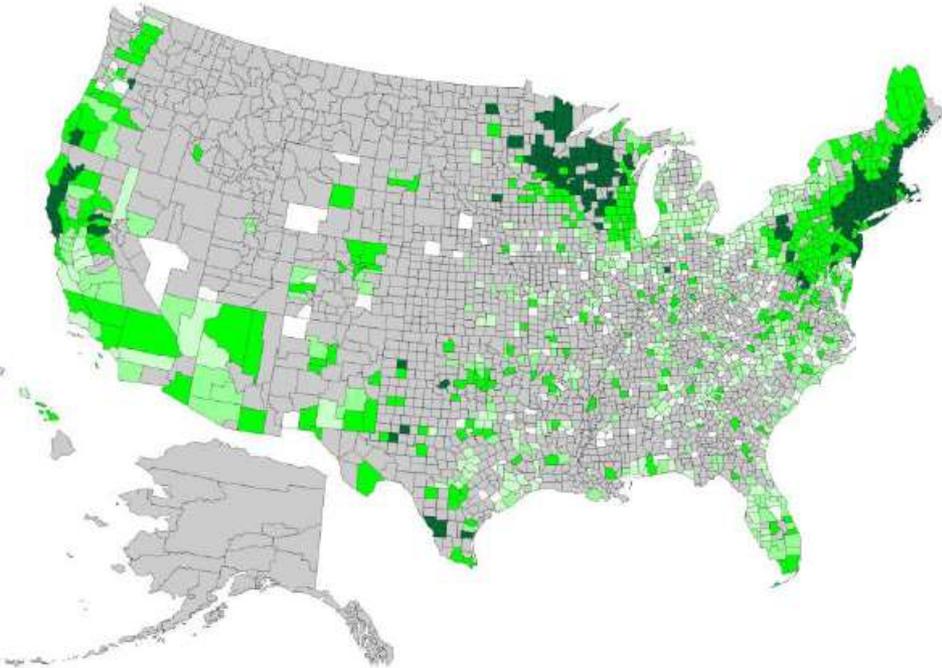
- **Bartonella**

- **Canine hepatozoonosis**

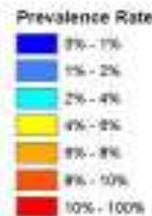
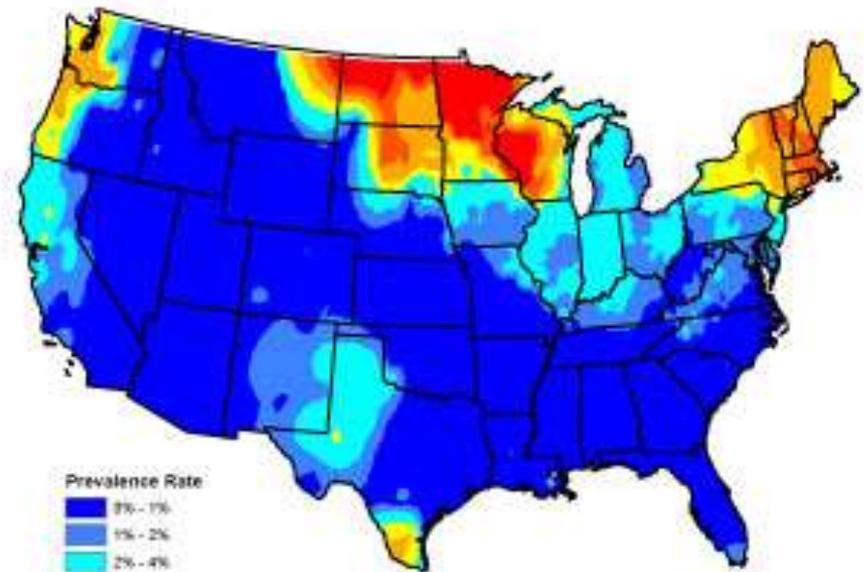
Hepatozoon americanum
Hepatozoon canis



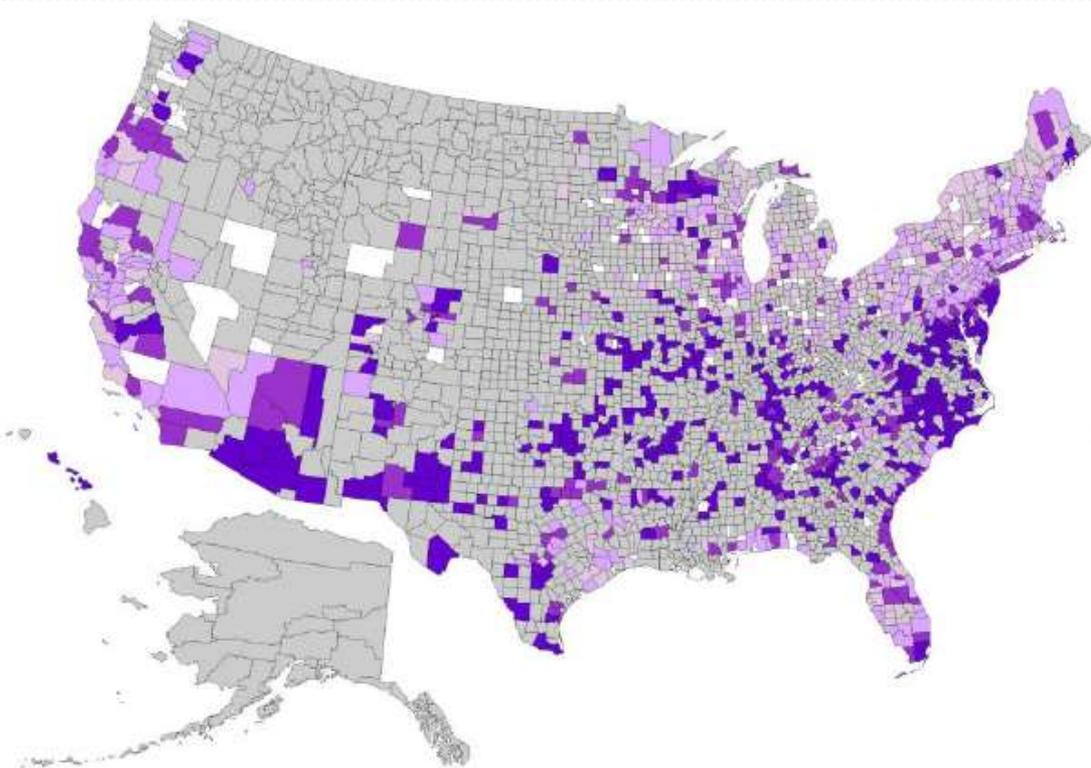
Antibodies to *Anaplasma* in Dogs



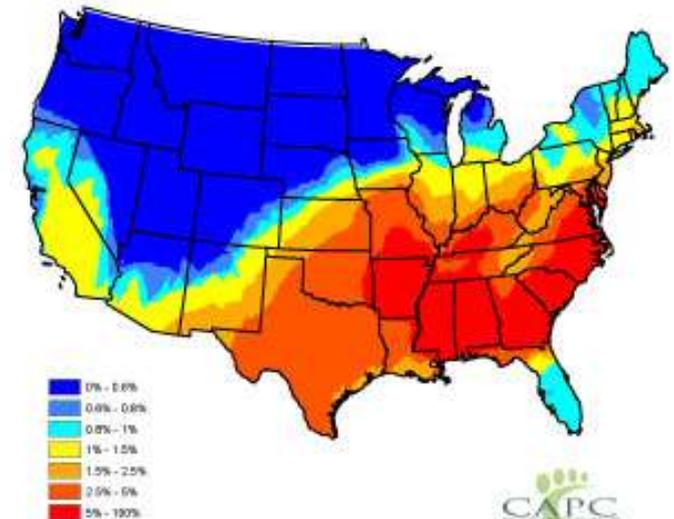
2014 Predicted Anaplasmosis Prevalence



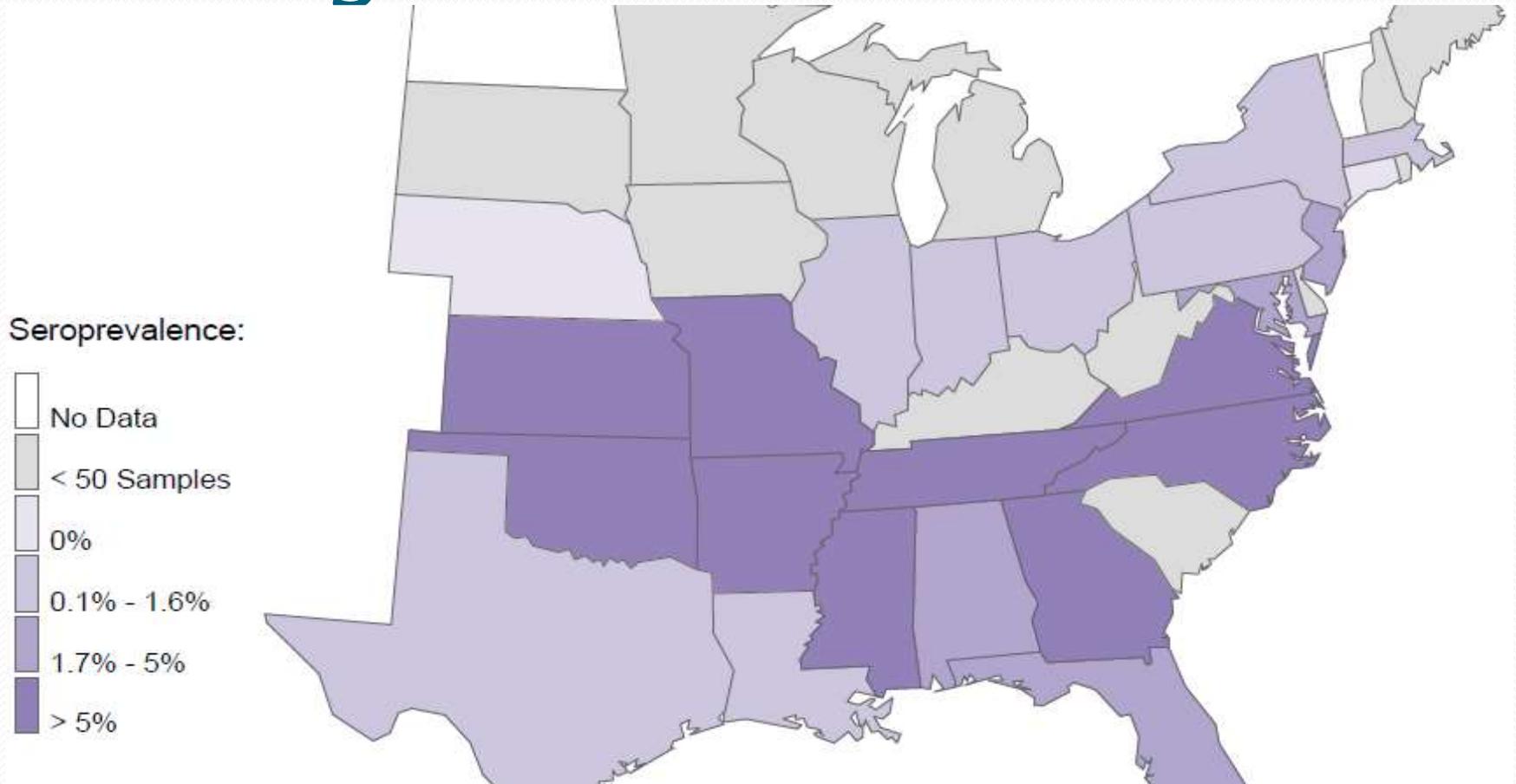
Antibodies to *Ehrlichia* spp., Dogs



2014 Predicted Ehrlichiosis Prevalence



E. ewingii



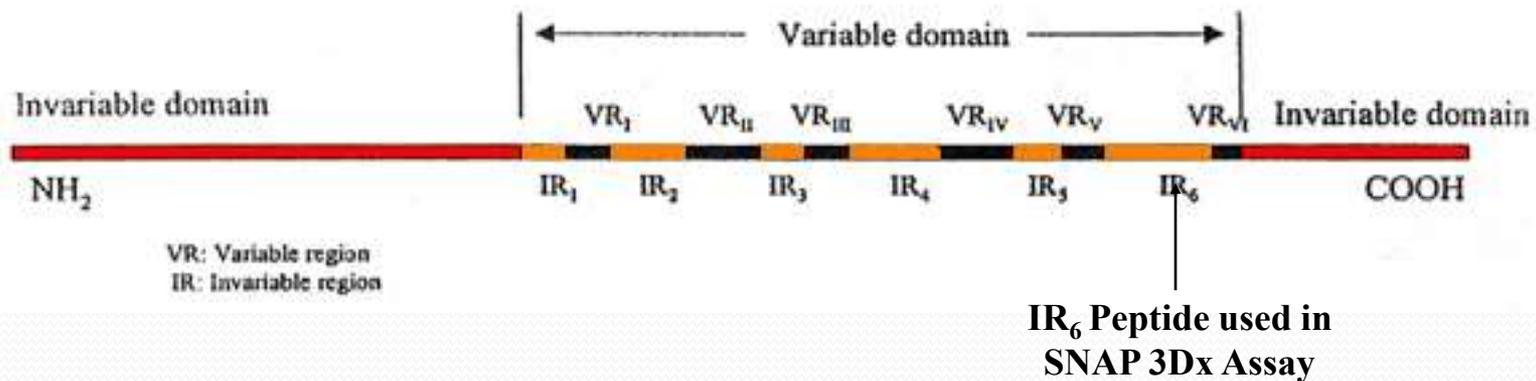
Canine Tick Borne Disease Testing



C6 Testing

VlsE Protein of *B. burgdorferi*

- 34 kDa surface lipoprotein
- IR₆ is one of six invariable regions within the central variable domain
- Six variable regions (VR_I - IR_{VI}) are interspersed

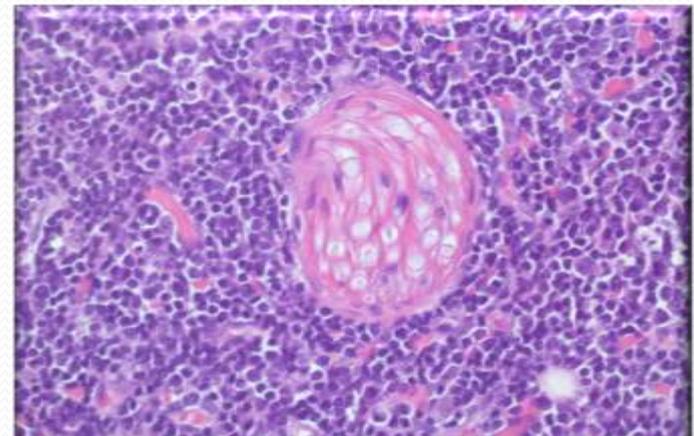
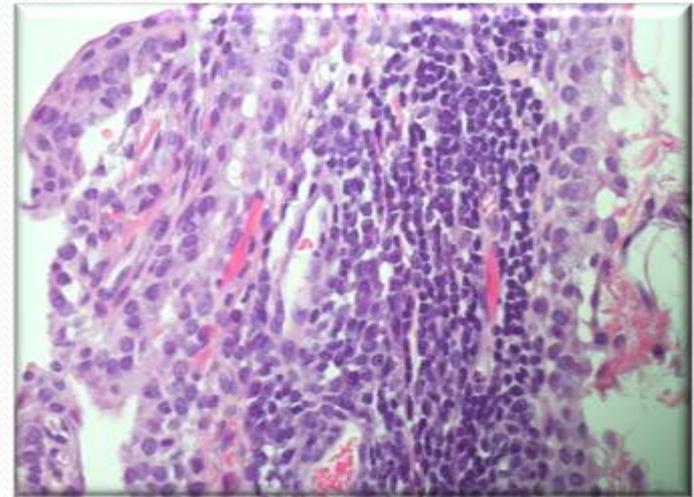


AccuPlex

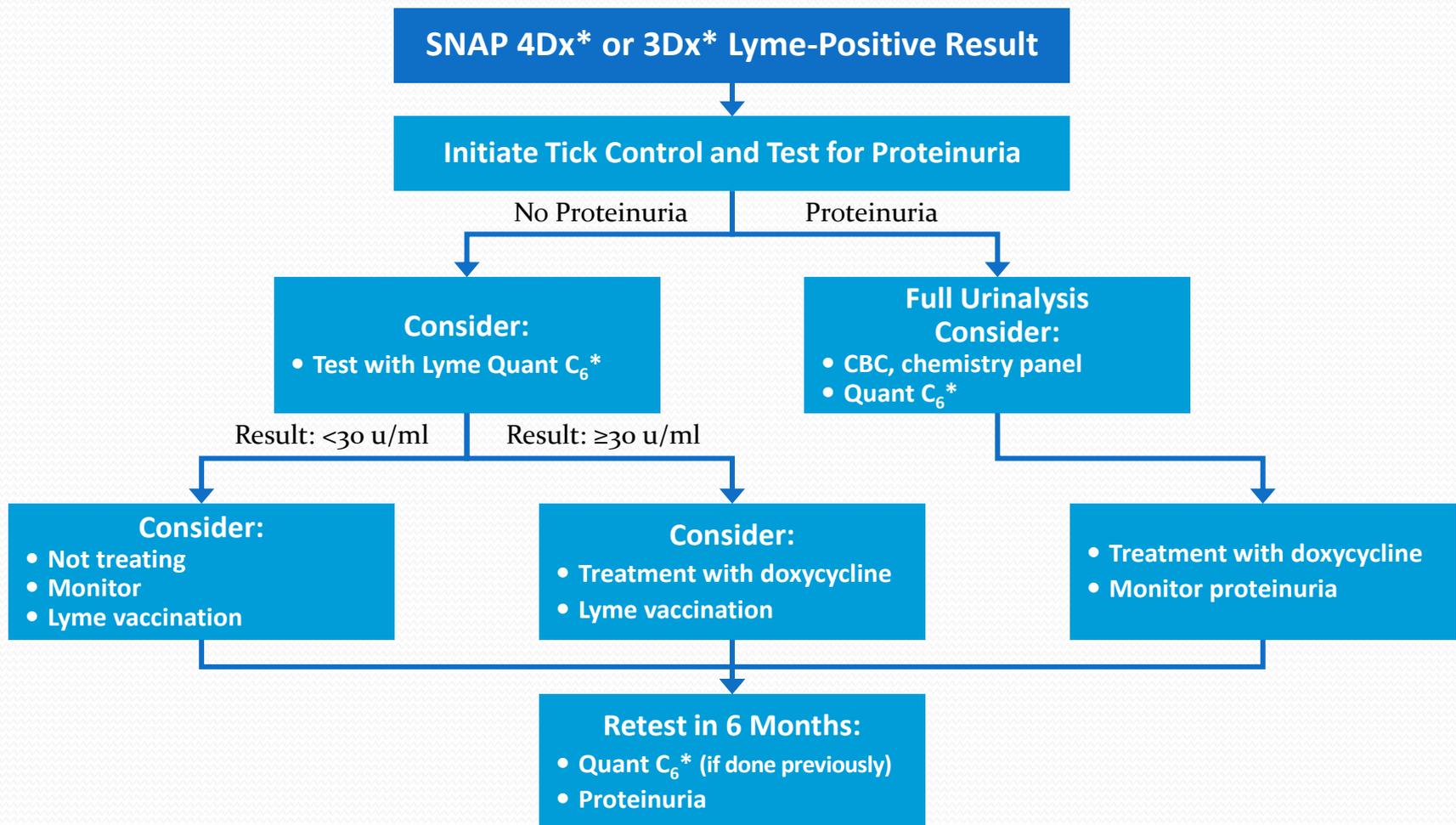
- Bio-CD
 - OspA
 - OspC
 - OspF
 - P31
 - SLP



“Non Clinical” Lyme Cases



Treatment – Reasonable Approach?



Treatment

- Antibiotic Tx usually results in rapid response
 - Doxycycline drug of choice because of co-infections – disadvantages
 - Oral, side effects
 - Recall!
 - Treat for at least 30 days, but still can not guarantee removal of the organism.
 - Convenia –
 - Effective – 2 doses 2 weeks apart
- Re-Tx reoccurrences.

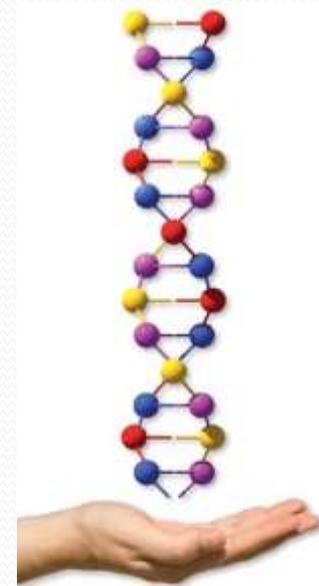
What About Sick Dogs??



Combining serology with PCR



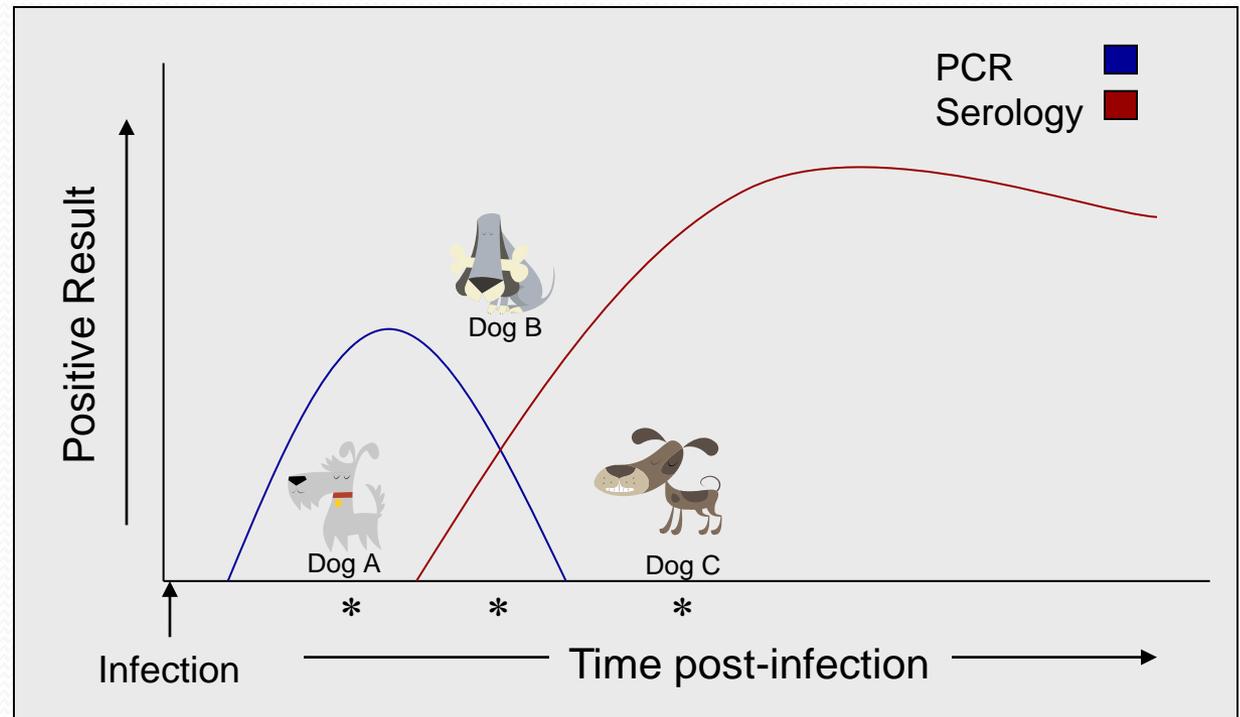
	Serology	Polymerase Chain Reaction (PCR)
Measures	Antibody response of host	Nucleic acid (DNA) from pathogen
Sensitivity	High	Variable, depending on pathogen
Specificity	Variable, depending on test	High
Benefit	Useful for screening	Identify suspected pathogens and confirm active infection
Limitations	Clinical signs may precede a measurable antibody response	A negative PCR result does not necessarily rule out infection



Lyme is an exception: Clinical signs of *Borrelia burgdorferi* infection (Lyme disease) occur post-seroconversion. Serology testing alone (SNAP 3Dx/4Dx or Lyme Quant C6) is the recommended diagnostic methodology. *B. burgdorferi* is rarely detected by PCR in the peripheral blood of infected animals.

The benefits of PCR and serology for sick patients

The amount of time between infection and the onset of clinical signs is typically unknown and will have an effect on diagnostic test results.



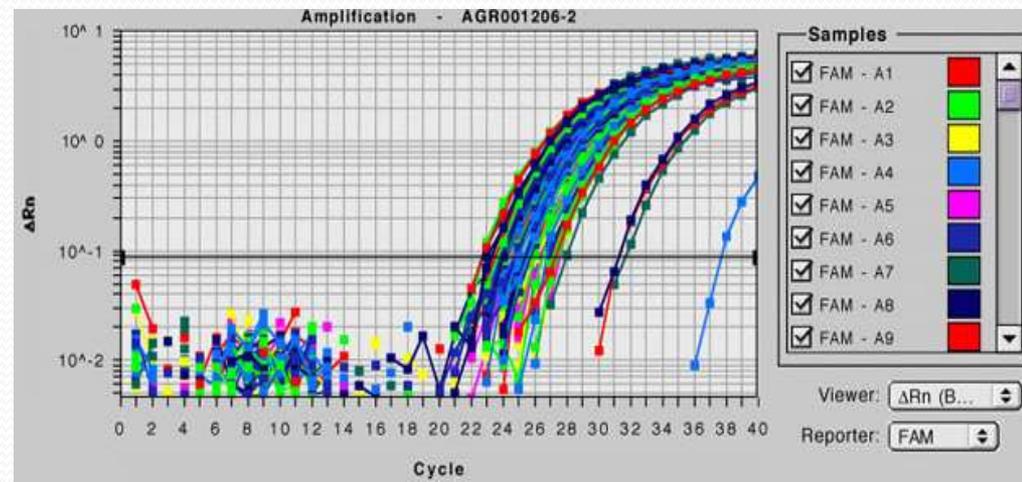
Using serology and PCR together improves your ability to make a complete and accurate diagnosis.

Conclusions

- **Good, Better, Best: Don't Miss Infectious Disease**
- **The only way to not miss infectious disease**
- **Screen and test Broadly!!!**

Diagnosing Leptospirosis

- Identifying the organism
 - Urine and blood cultures
 - Gold standard
 - Blood – only first few days, negative if abs
 - Urine after 4-10 days, negative if abs
 - Furosemide increases yield (dilute urine)
 - Difficult to culture
 - Rarely offered
 - Blood PCR
 - Urine PCR



Diagnosis

- Serology
 - MAT – Microscopic agglutination test
 - Standard serological test in companion animals
 - Serum mixed with serovar specific organisms
 - Highest dilution causing 50% agglutination of the organisms is reported as the titer
 - Highest titer the infecting serogroup???

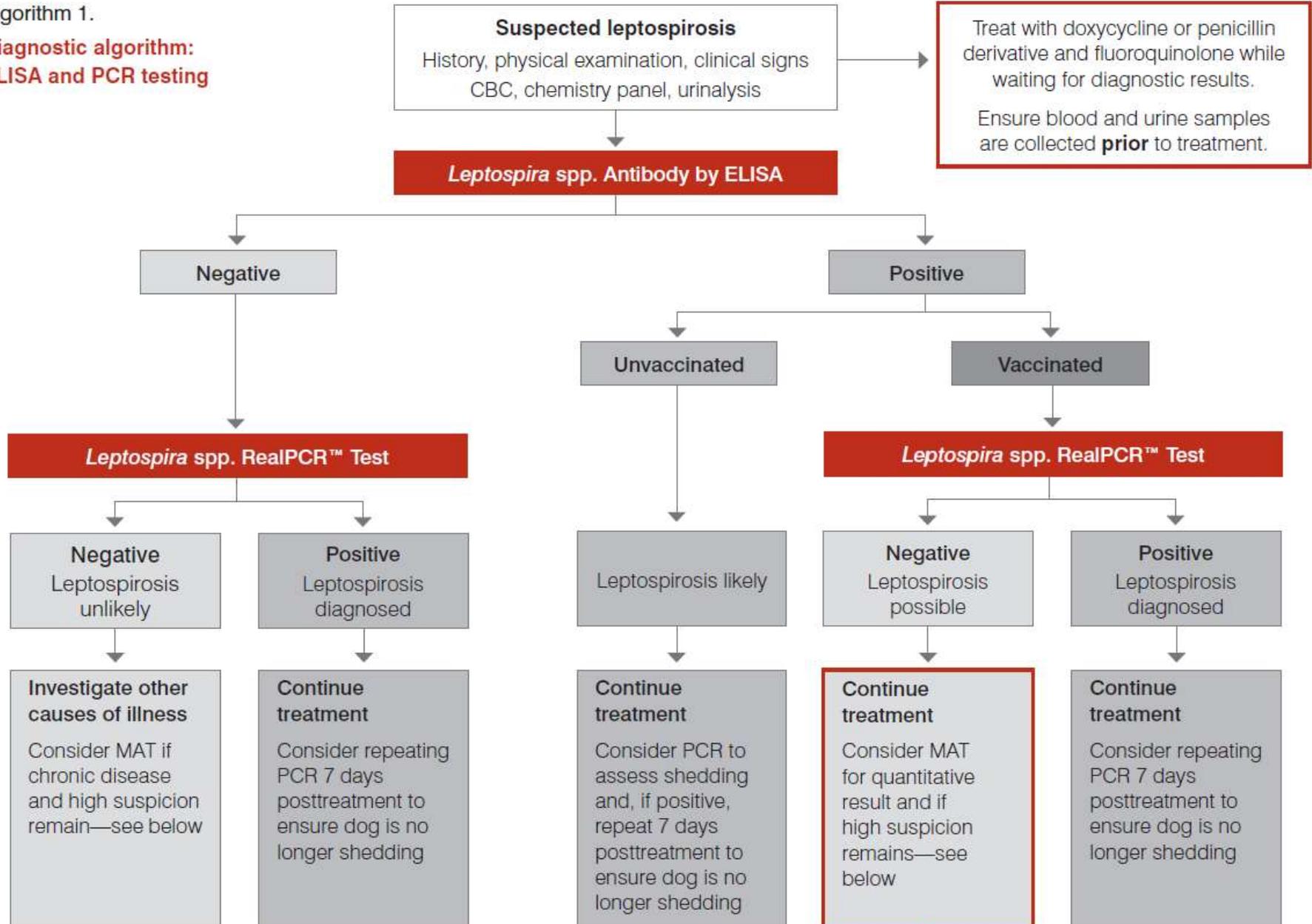
New Lepto ELISA

- Single peptide
- Non-serovar specific
- Rapid turn-around
- Still picks up vaccinal abs

- As good/better than MAT
- Repeat if neg early on
- Combine with PCR?

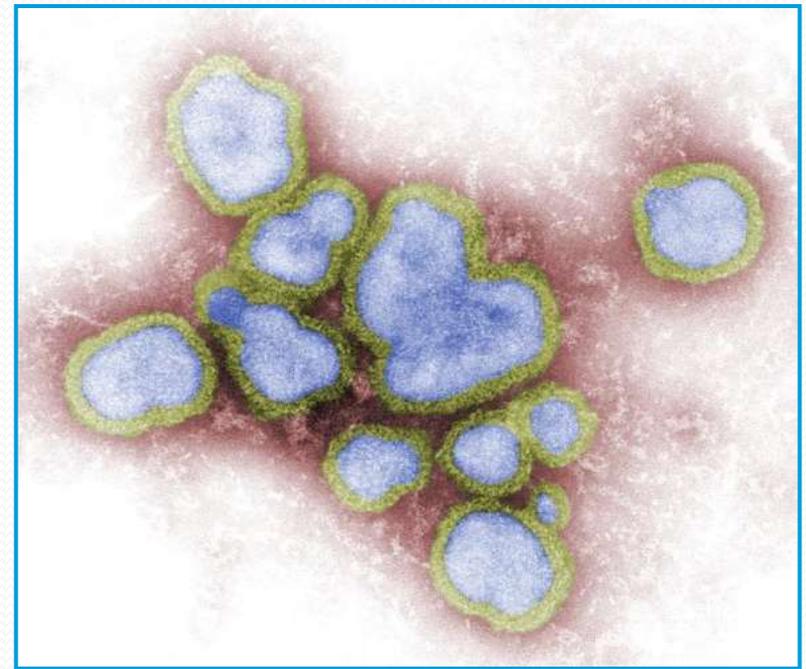
Algorithm 1.

**Diagnostic algorithm:
ELISA and PCR testing**



Canine Influenza Virus

- Influenza A
 - Family Orthomyxoviridae
- Classified into subtypes
 - Hemagglutinin
 - 16 antigens (H1 to H16)
 - Neuraminidase
 - 9 antigens (N1 to N9)
 - Typically species specific



This digitally-colored negative-stained transmission electron micrograph (TEM) depicts a number of influenza A virions.
Source: CDC Public Health Image Library

Canine Influenza Virus Emergence

Subtype H3N8

- Jumped directly from horses to dogs, discovered in 2004
 - Likely a one-time event
- Developed ability to spread from dog-to-dog



Canine Influenza Virus Emergence

Subtype H3N2

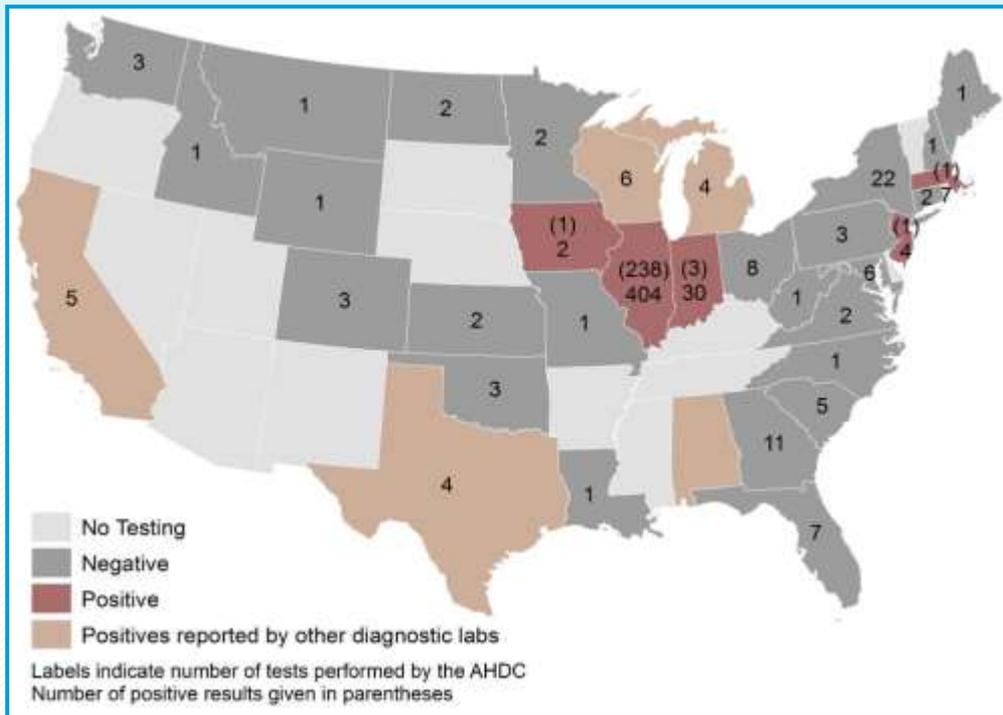
- Avian origin virus
- Been in dogs in Asia since at least 2007



Emerging Disease Monitoring

Canine Influenza Virus, Testing Summary March 13 – May 6, 2015

CIV Testing and Results



California (5/0), Colorado (3/0), Connecticut (2/0), Florida (7/0), Georgia (11/0), Idaho (1/0), Illinois (166/238), Indiana (27/3), Iowa (1/1), Kansas (2/0), Louisiana (1/0), Maine (1/0), Maryland (6/0), Massachusetts (6/1), Michigan (4/0), Minnesota (2/0), Missouri (1/0), Montana (1/0), New Hampshire (1/0), New Jersey (3/0), New York (22/1), North Carolina (1/0), North Dakota (2/0), Ohio (8/0), Oklahoma (3/0), Pennsylvania (3/0), South Carolina (5/0), Texas (4/0), Virginia (2/0), Washington (3/0), West Virginia (1/0), Wisconsin (6/0), Wyoming (1/0), State Unknown (43/2)

¹Unconfirmed report in Idexx Press Release (5/7/15)

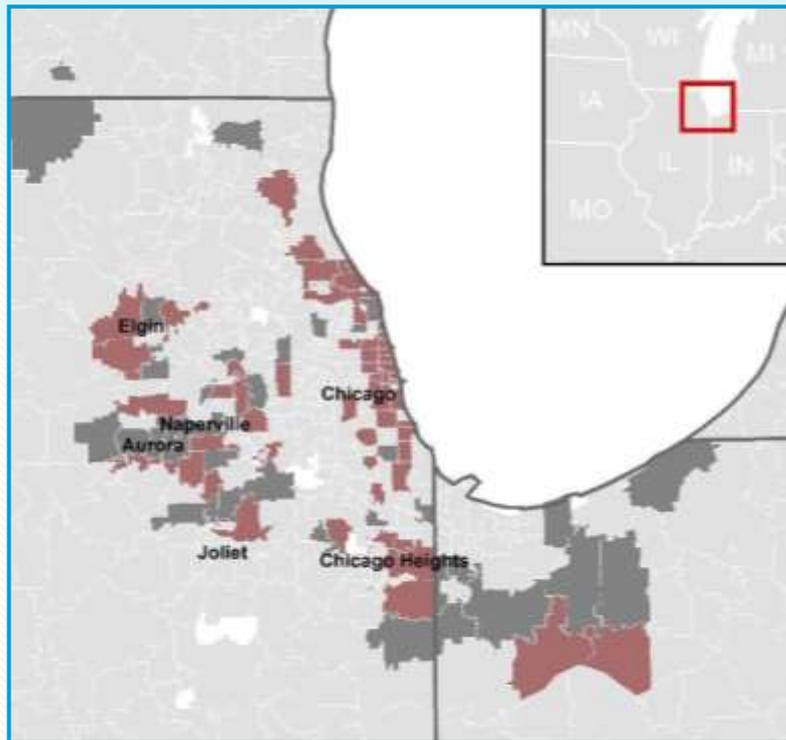
Source: Cornell University Animal Health Diagnostic Center. https://ahdc.vet.cornell.edu/docs/CIV_Monitoring_2015_05_o8.pdf.

Accessed 5/14/15

Emerging Disease Monitoring

Canine Influenza Virus, Testing Summary March 13 – May 6, 2015

Chicago Area Testing



Source: Cornell University Animal Health Diagnostic Center. https://ahdc.vet.cornell.edu/docs/CIV_Monitoring_2015_05_08.pdf
Accessed 5/14/15

CIV Is Part of CIRDC Complex

- Most commonly identified CIRDC pathogens
 - *Mycoplasma cynos*
 - *B. bronchiseptica*
 - Canine parainfluenza virus (CPiV)
 - Canine respiratory coronavirus (CRCoV)
- Current Chicago outbreak
 - Canine influenza virus (CIV) – New strain closely related to H₃N₂ Asian influenza A strains
 - Canine-specific influenza A H₃N₈ PCR tests run by commercial veterinary labs might not detect this new strain
- Usually multiple agents act sequentially or synergistically to cause disease

Datz, C. *Compendium on Continuing Education for the Practicing Veterinarian*. 2003; 25(12): 896 – 901 & 902-914.

Ford, R.B. Kirks. *Current Veterinary Therapy XIV*. Saunders-Elsevier, St. Louis, 2009: 646-649.

Chalker, V.J. *Clinical and Diagnostic Laboratory Immunology*. 2003; 10(3): 352 – 356.

Keil, D.J. *JAVMA*. 1998; 212(2): 200 – 207.

Ford, R.B. In: *Infectious Diseases of the Dog and Cat*. 3rd Ed. 2006: 54-61.

• Co-morbid infections likely to be more severe

CIRD Transmission

- Most outbreaks result from dog-to-dog contact
- Most common in high-stress, high-density environments
 - Pet shops, boarding and grooming facilities, commercial kennels, sporting events, daycare, animal shelters and veterinary hospitals
 - Oronasal contact with aerosolized respiratory secretions
 - Fomite spread also important, depending on the pathogen
 - Clinical signs usually develop 1–3 days post-exposure (up to 10 days)
 - Pathogen shedding varies with infectious agent
 - Viral agents commonly shed for 3–13 days post-infection
 - CIV - Viral shedding ceases after ~7 days for H3N8 and potentially longer for H3N2
 - Canine distemper virus (CDV), canine adenovirus 1 (CAV-1), *B. bronchiseptica* and *Mycoplasma* can be shed by recovered dogs for a few weeks to months

Datz, C. *Compendium on Continuing Education for the Practicing Veterinarian*. 2003; 25(12): 898 - 901 & 902-914.

Ford, R.B. *Kirks Current Clinical Veterinary Medicine*. 2003; 13(1): 240-241.

Chalker, V.J. *Clinical and Diagnostic Laboratory Immunology*. 2003; 10(3): 352 - 356.

Keil, D.J. *JAVMA*. 1998; 212(2): 200 - 207.

Ford, R.B. In: *Infectious Diseases of the Dog and Cat*. 3rd Ed. 2006: 54-61.

Clinical Signs of CIRDC

Rapid Clinical Course Means Clinical Diagnosis Most Common

Typical Presentation

- Paroxysmal coughing
- Elicitable tracheal cough
- Laryngitis – Hoarse/high pitched 'honking'
- Rhinitis
- Retching, hacking cough, gagging
- Otherwise unremarkable physical examination
- Duration of clinical signs is typically 1–2 weeks

Severe Presentation

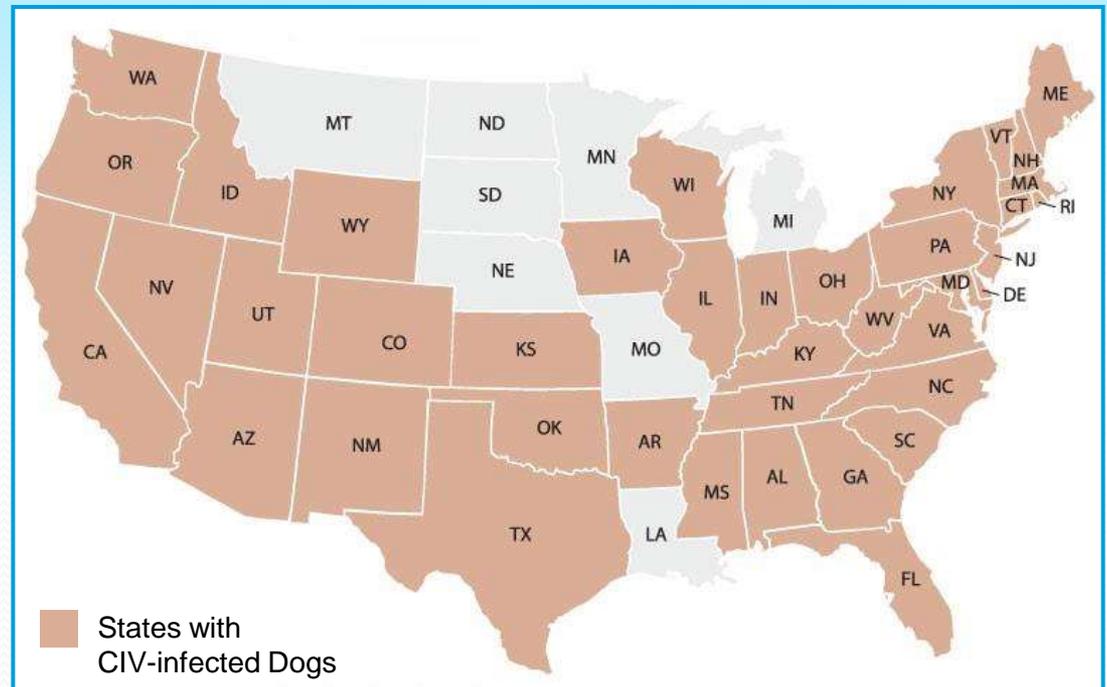
- More likely in puppies and unvaccinated dogs
- Signs of typical presentation **plus**
- **Fever, lethargy, anorexia**
- **Dyspnea, clinical signs of lower respiratory tract infection**
- **Prolonged clinical course**

From the Racetrack to the Backyard

CIV in the Pet Dog Population

- Regular CIV activity in NE states, CO, Las Vegas, CA
- Morbidity rate can be 60–80%
- Single-agent infection mortality rate very low
- 80–90% cases – Mild URT signs
- 10–20% cases – Severe LRT signs, co-infections

Canine Influenza H3N8 Now Documented in 40 States



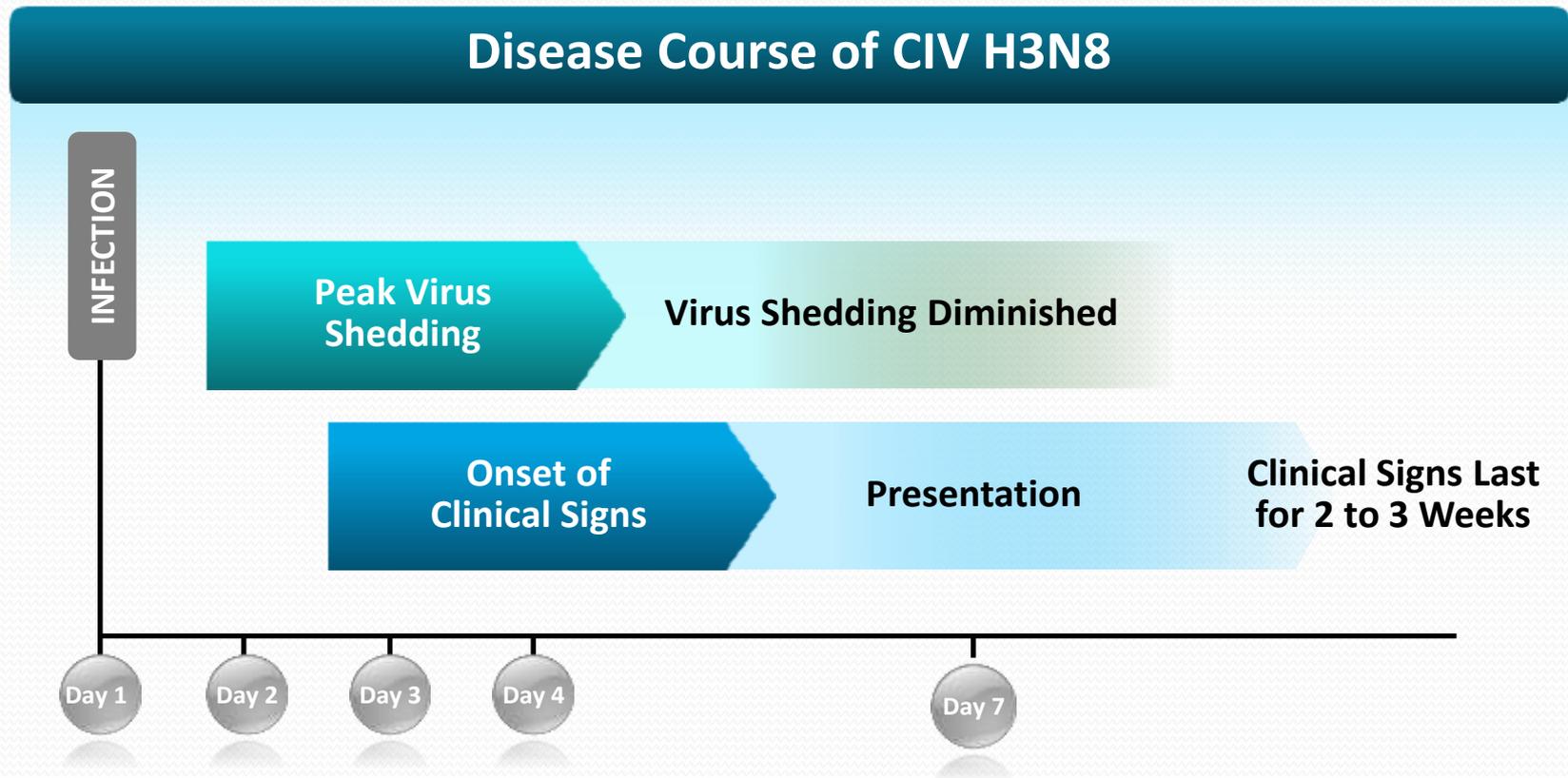
Map Source: Syndromic surveillance data of Cynda Crawford, DVM, PhD, University of Florida; Edward Dubovi, PhD, Cornell University; Sanjay Kapil, DVM, PhD, ACVIM, Oklahoma State University; Rhode Island State Veterinarian's office; and IDEXX Laboratories. April 2013.

Map permission provided by Dr. C. Crawford, University of Florida. <https://ahdc.vet.cornell.edu/news/civ.cfm> .

Accessed 2/17/15

CIV – H3N8 Peak Viral Shedding Precedes Onset of Clinical Signs

CIV H3N8 Has a Shorter Incubation Period Than Other Causes of CIRDC



Dubovi, E.J. *Vet Clin Nth America Small Anim.* 2008; 38: 827-835.

American Veterinary Medical Association. <https://www.avma.org/KB/Resources/Backgrounders/Pages/Canine-Influenza-Backgrounder.aspx>. Accessed January 31, 2015.

Crawford, C. . <http://www.sheltermedicine.com/node/32>. Accessed March 31, 2015.

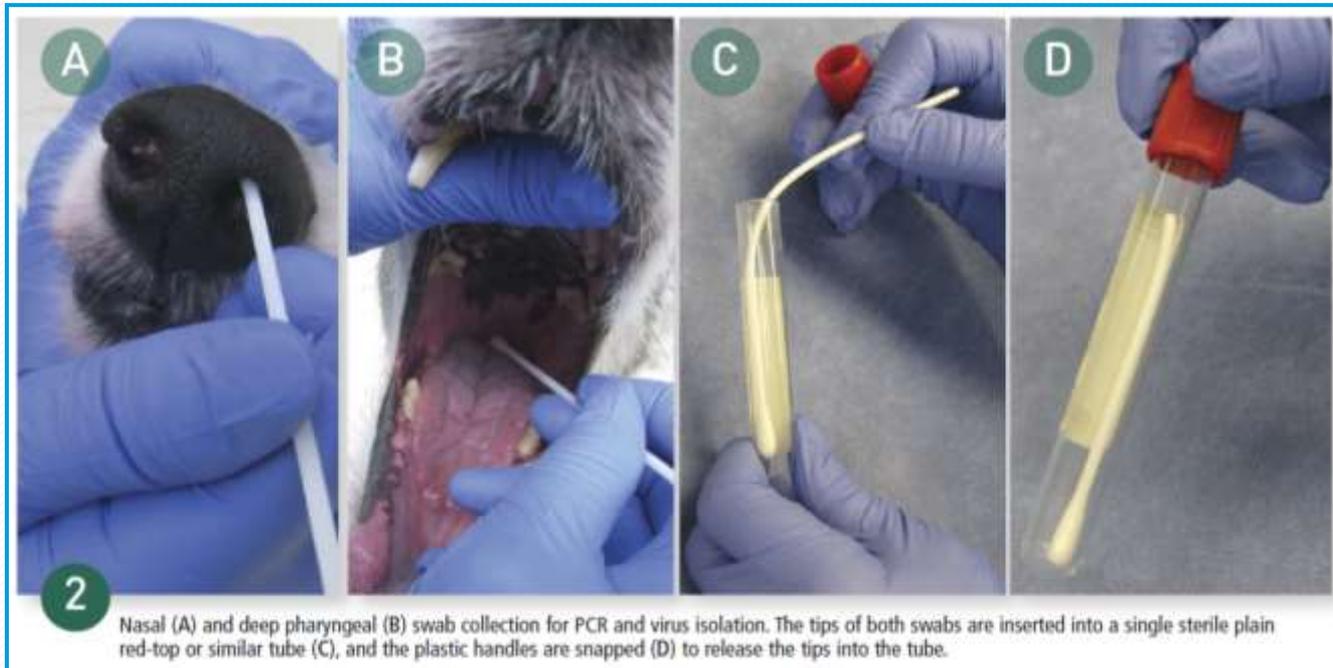
CIRD – Confirmation of Etiologic Agents

Upper Respiratory Signs

- Deep nasal/oropharyngeal swabs (nasal preferred)

Lower Respiratory Signs

- Tracheal wash



CIRD Diagnostics – PCR

- Commercially available panels
 - *Bordetella bronchiseptica*, CAV₂, CDV, canine herpesvirus, canine influenza virus (H₃N₈, H₃N₂), CPiV, canine pneumovirus, CRCoV, H₁N₁ pandemic influenza virus, *Mycoplasma cynos*, *Streptococcus equi* subsp. zooepidemicus
- Nasal swabs twice as successful as nasopharyngeal swabs
- Don't use transport medium or wooden-stemmed swabs
- False negatives
 - Specimen taken after viral shedding, especially CIV
 - Poor sampling technique, sample quality and handling
- False positives
 - Shedding only occurs after vaccination with modified live vaccines up to 3 weeks
- Post vaccination CDV can be differentiated from natural disease using RT-PCR

Dubovi, E. et. al. *Vet Clinic Sm An Pract.* Canine Influenza.38 (2008) 827–835.

- 1–3 day turnaround

CIRD Diagnostics – Serology & Culture

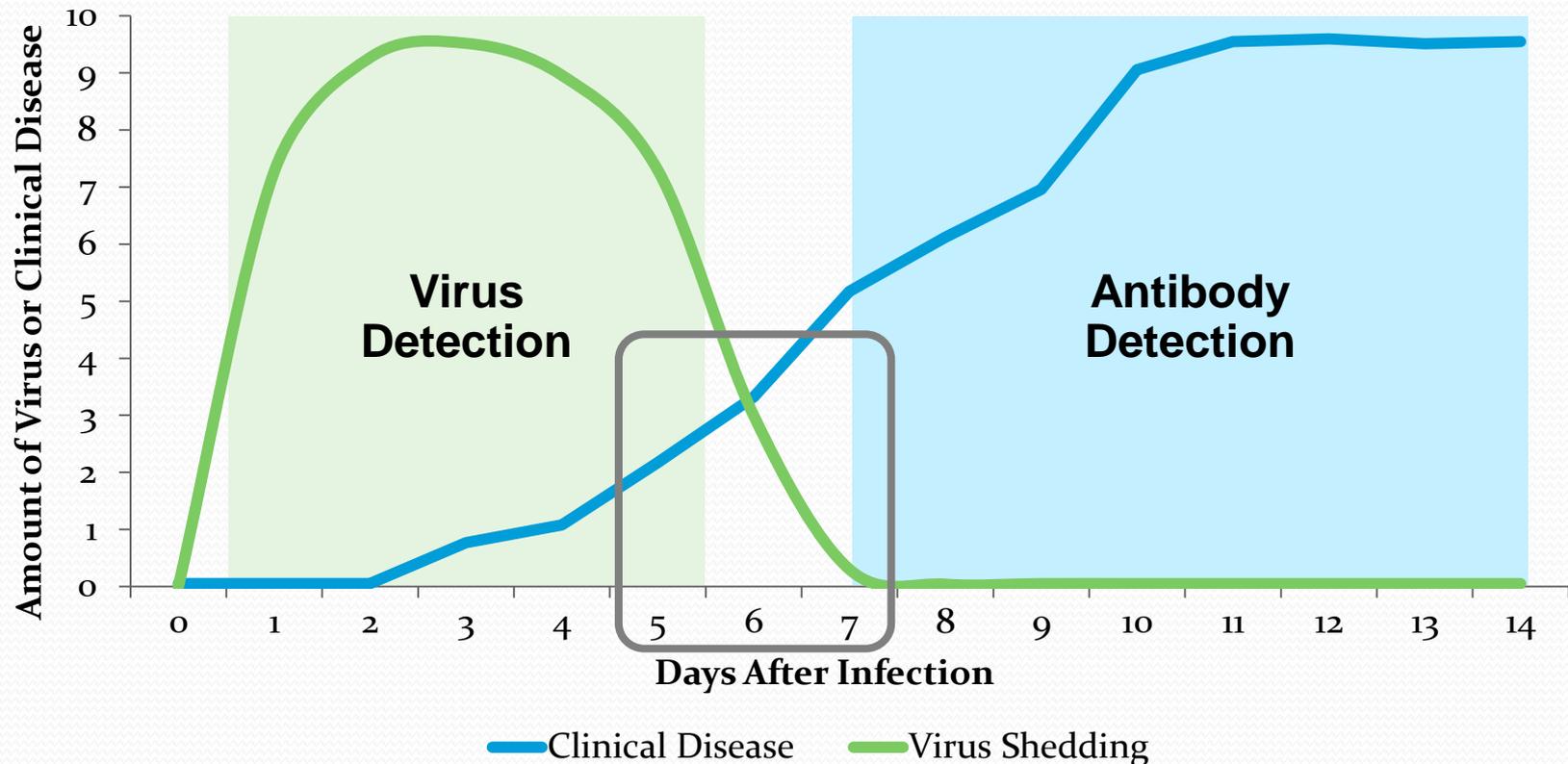
CIV – Hemagglutination Inhibition (HI) Testing for Anti-CIV Antibodies

- Paired antibody titer to demonstrate rising convalescent antibody titer 2–3 weeks later
- Vaccination can produce low antibody titer – 1:16 – 1:64
- Active infection antibody titer – 1:512 – 1:2048
- History important
 - Usually low seroprevalence and low vaccination rate in USA → Moderate antibody titer + appropriate history could be significant
 - Antibody titer starting to rise 10 days after infection
 - Prolonged elevated antibody titer after infection in rare cases

CIRD – Bacterial Culture and Sensitivity

- *Bordetella, Mycoplasma, Streptococcus*
- Also *E. coli, Klebsiella, Pasteurella, Enterobacter*
- Might yield multiple opportunistic isolates rather than primary pathogen

CIV H3N8 – Peak Viral Shedding Precedes Onset of Clinical Signs

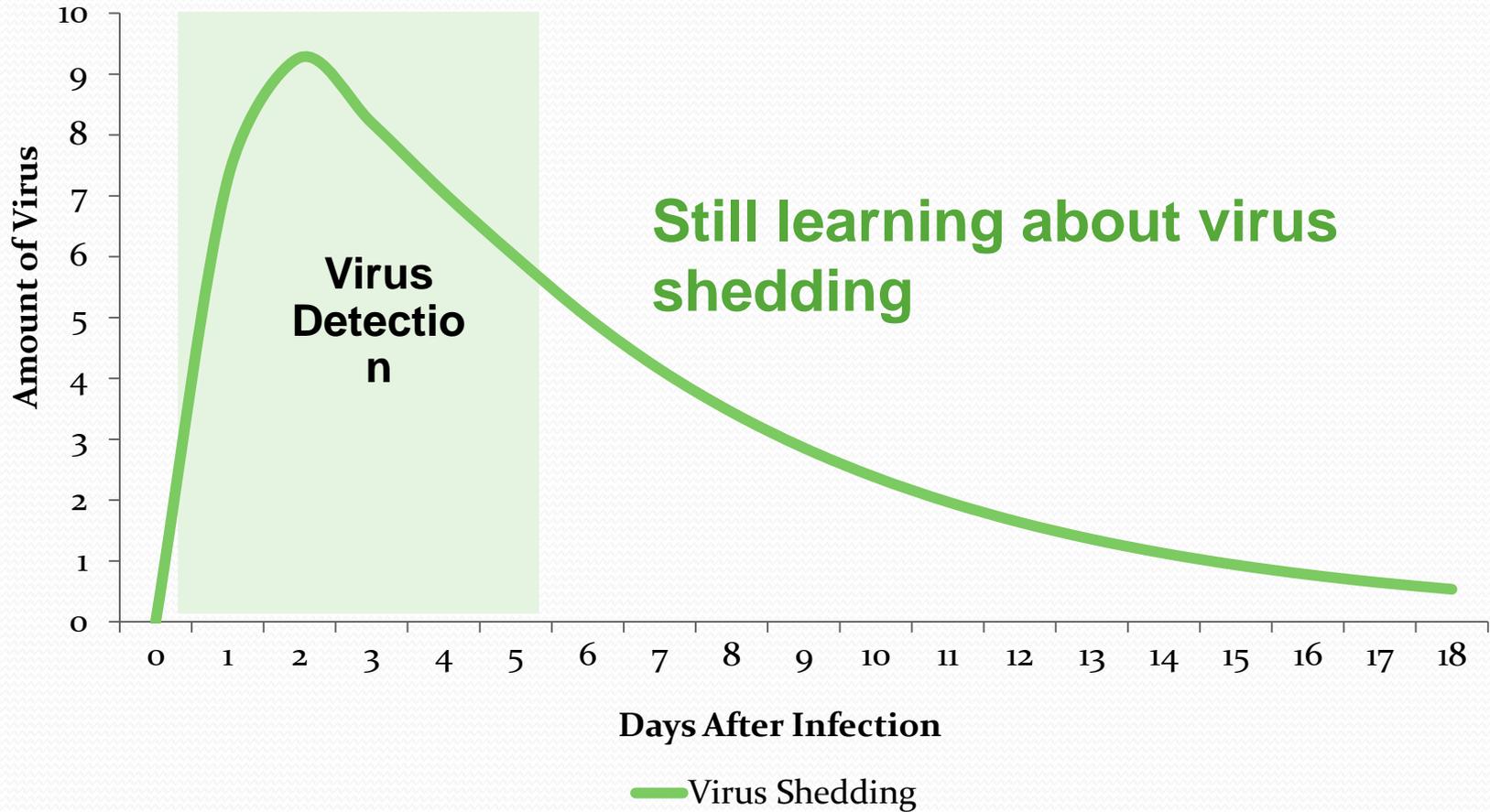


Dubovi, E.J. *Vet Clin Nth America Small Anim.* 2008; 38: 827-835.

American Veterinary Medical Association. <https://www.avma.org/KB/Resources/Backgrounders/Pages/Canine-Influenza-Backgrounder.aspx>. Accessed October 31, 2013.

Crawford, C. <http://www.sheltermedicine.com/node/32>. Accessed March 31, 2015.

CIV H3N2 – Shedding May Occur for Longer Duration



How Can We Prepare Outside of Chicago?

Education

- Know the travel history
- Avoid travel to endemic areas
- Quarantine dogs coming in from endemic areas

Vaccination!!!!

- Use H₃N₈ and H₃N₂
- Vaccinate for other components of CIRDC

Be Diligent

- TISC – Test and Isolate All Suspected Cases

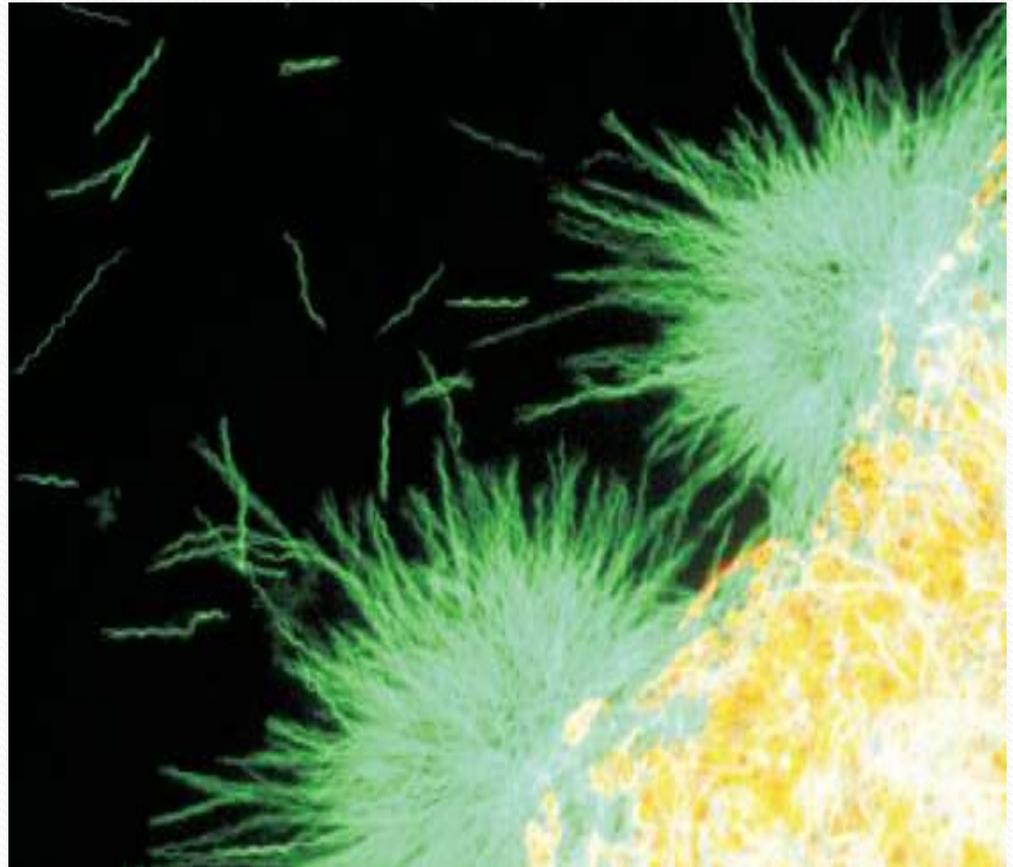
Lyme Prevention

- How does prevention work?
- Package
 - Tick removal
 - Tick control
 - **Vaccination**

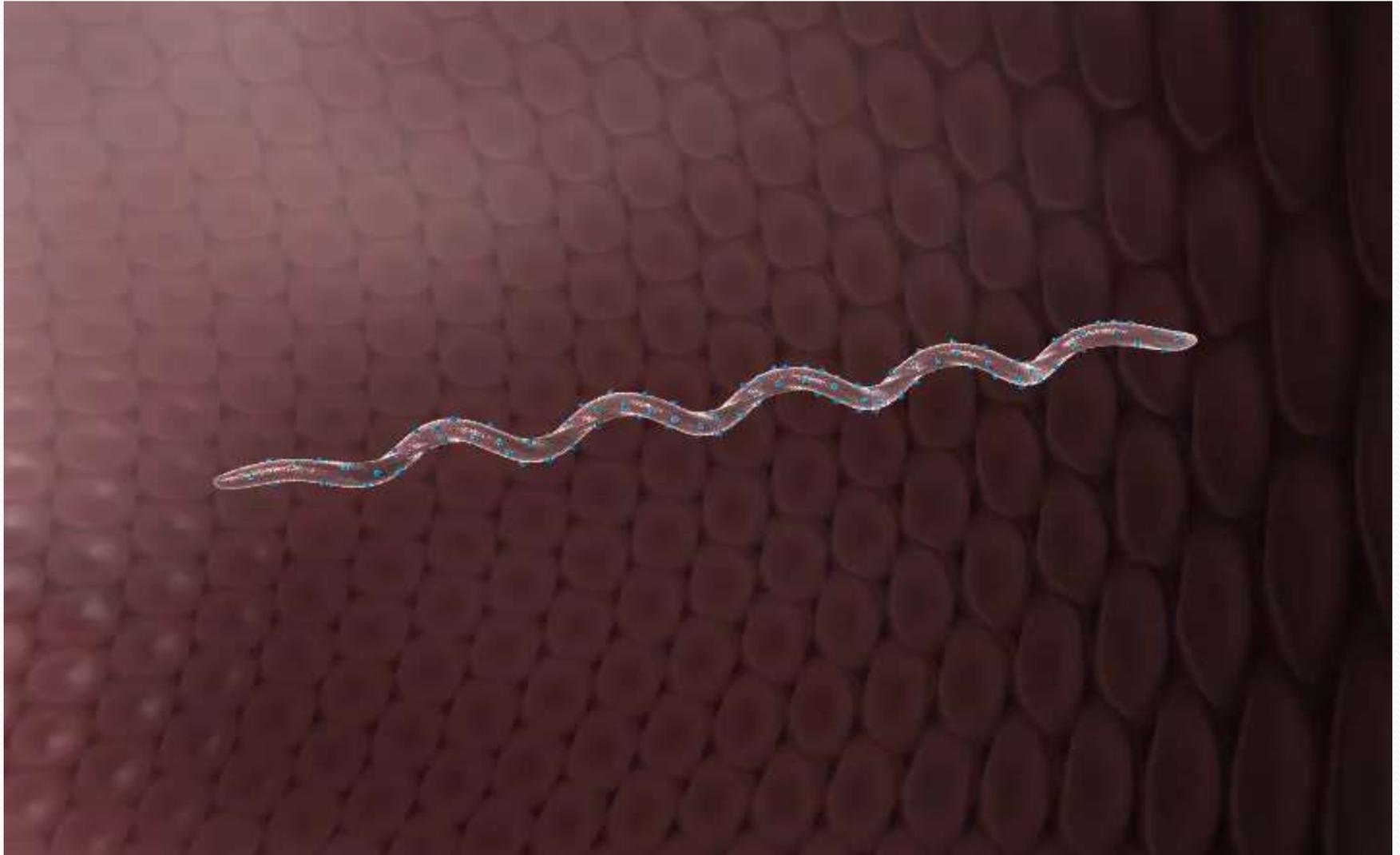


Why Is That?

- Antigen shifting
- Outer surface antigens (Osps)
 - OspA
 - OspC
- Bacteria hiding



The A and C's of Outer Surface Protein Production



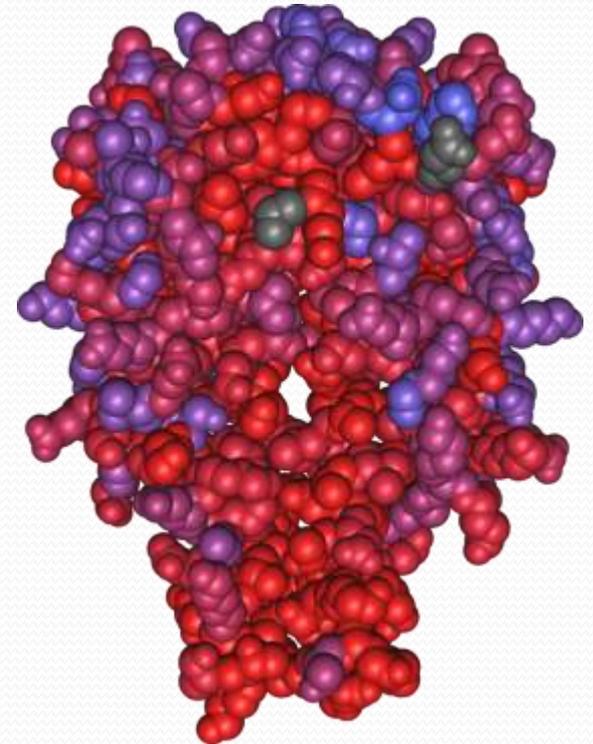
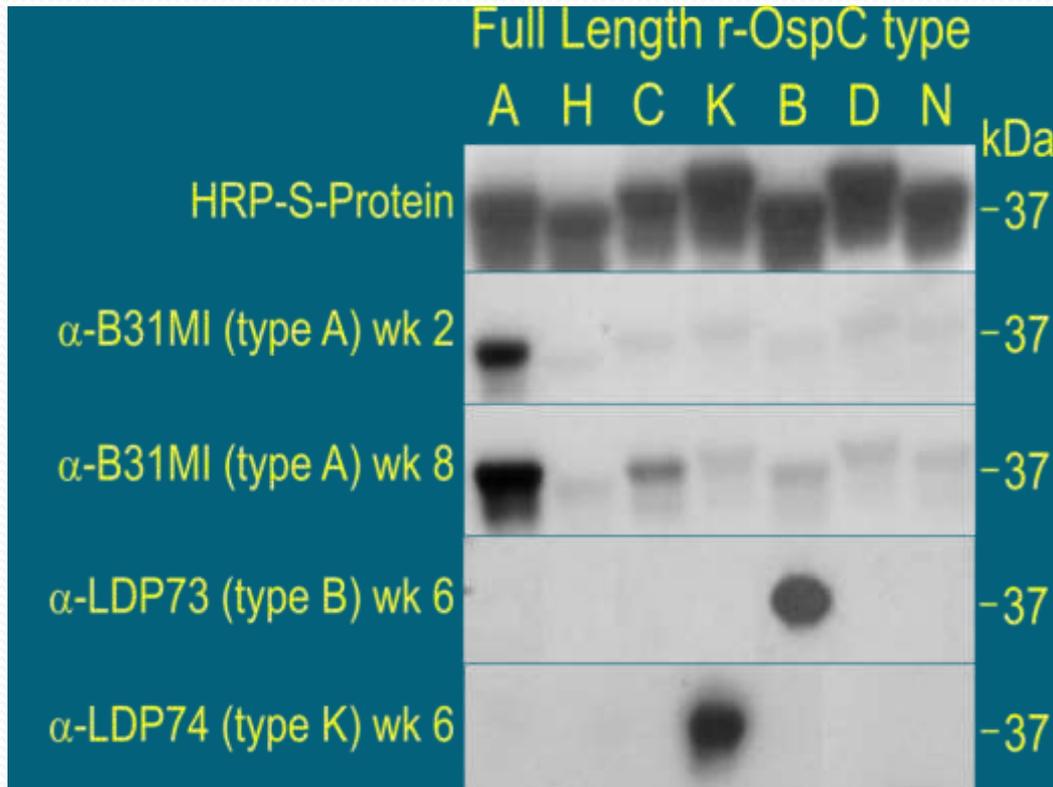
Other Lyme Vaccines

- Recombinant OspA or whole cell vaccine are really both providing OspA
 - Recombinant – just OspA
 - Whole-cell (bacterin) - OspA and other antigens (OspC)
- In most cases the immune system cannot clear the infection completely and cannot effectively prevent re-infection (dependent on Ab titers and antigens being displayed by the bacteria)
- Antibiotics help active disease but often do not clear the infection

Chimeritopes: A New Vaccine Direction

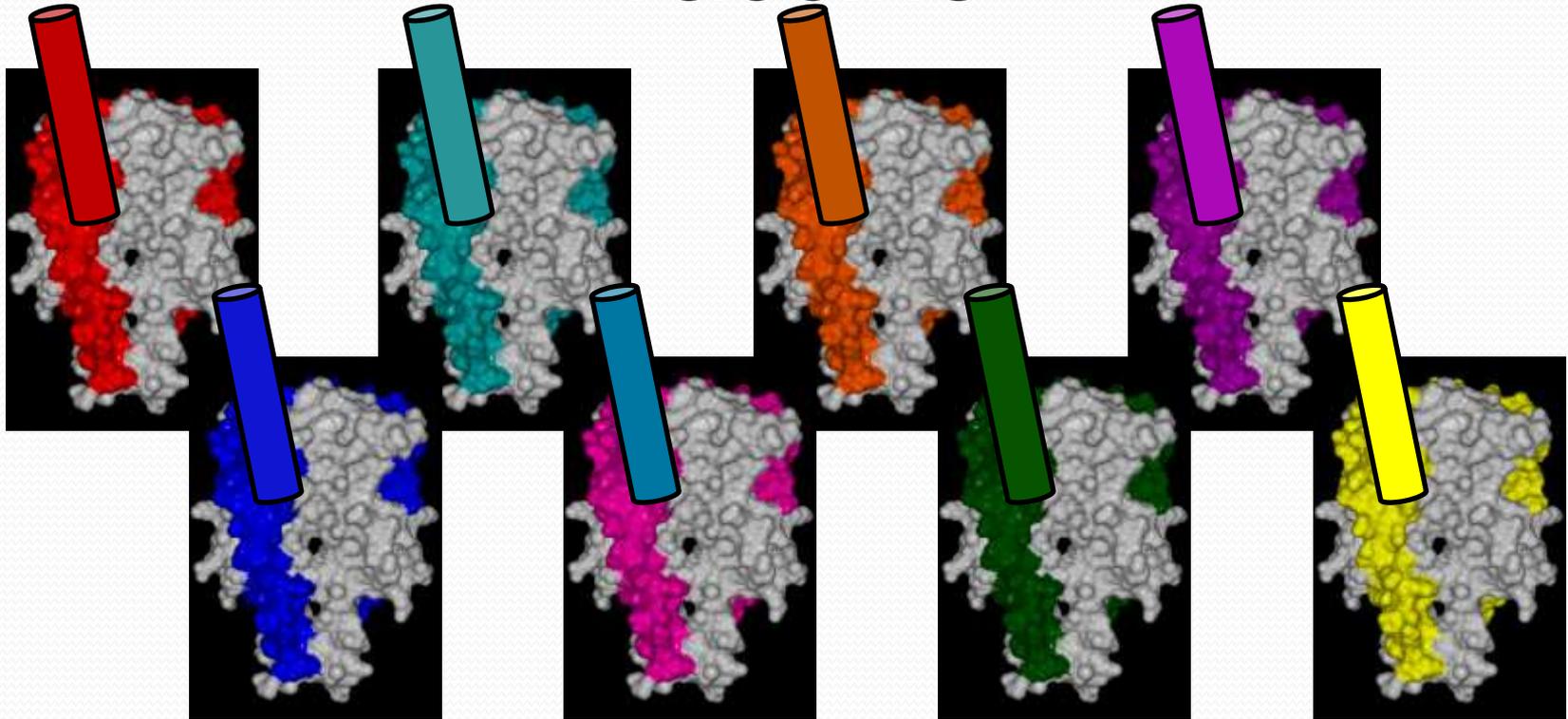
- Chimeritope - a novel protein consisting of short, well defined, protective-linear epitopes derived from one or more proteins or protein variants from one or more pathogens
- Advantages:
 - Eliminate regions that are non-productive or elicit adverse events
 - Linear epitopes vs conformational epitopes
 - Protect against diverse pathogens
 - Synergistic mechanisms of protection

OspC Ab Response During Infection is Type-Specific



> 30 OspC types exist in nature but only a small subset cause invasive infections in canines

Polyvalent Chimeric Epitope Vaccine



OspC-Chv2/OspA: Protective Efficacy in Canines

% of Sites with Lesions Consistent with LD

Control	– 55
OspC-Chv1	– 25
OspC-Chv2	– 17
OspA	– 19
OspC-Chv2/OspA	0%

OspC-Chv2 - minimal joint involvement

OspA – high joint involvement: 6/8 dogs

OspC-Chv2/OspA - prevented LD lesion formation, dissemination and seroconversion in all dogs

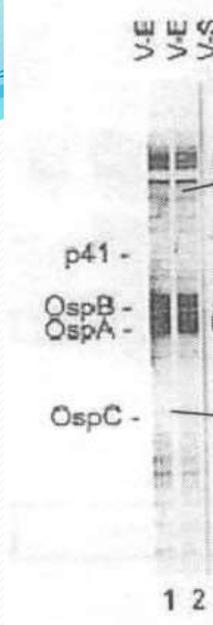
Treatment (Vaccine)	Lesion by location	# Dogs
Not vaccinated –infection control	Skin, 1 joint	1
	Skin, 4 joints	1
	Skin, 6 joints	2
	Skin, 7 joints	1
	Skin, 9 joints	1
	Skin, 10 joints	1
OspC-Chv1	Skin only	2
	Skin, 3 joints	1
	Skin, 4 joints	1
	Skin, 6 joints	1
	4 joints	1
	No lesions	2
OspC-Chv2	Skin only	5
	Skin, 1 joint	1
	Skin, 6 joints	1
	No lesions	1
OspC-Chv2 + OspA	No lesions	8
OspA alone	Skin, 2 joints	1
	Skin, 3 joints	1
	1 joint	1
	2 joints	1
	3 joints	1
	4 joints	1
	No lesions	2

Chimeritope Technology: Next Generation Vaccines



So up until now...

- Recombinant or whole cell vaccine are really both providing OspA
 - Recombinant – just OspA
 - Whole-cell: Osp A and other antigens including Osp C – Significance?
- The dog's immune system cannot clear the infection completely in most cases. Likely to not be able to effectively prevent re-infection from a tick, dependent on titers and antigens being displayed by the bacteria.
- Antibiotics help active disease but often are unable to clear the organism.



But now with crLyme

- Still Osp A
- Chimeric Osp C
 - Promote Osp C antibodies that for the first time will actually
 - Recognize the borrelia in the mid gut at the end of the blood meal
 - Recognize the borrelia in the dog!!!
 - The ones that escaped the Osp A barrier
 - Just like a real vaccine

What does that all mean?

- Although we still need the full package
 - Coinfection
 - Decrease chance of Lyme infection
- But - No longer is this a numbers game!
- We are no longer at the mercy of the waning OspA titer and unknown numbers of ticks getting through the tick control barrier.
- We actually for the first time have a vaccine that will work in the dog!!!

Recommendations for Lyme Prevention

- Still use vaccines as part of a Lyme prevention package
- Test every Lyme positive dog for proteinuria and if pos then treat
- Vaccinate clinical dogs? No
- Vaccinate negative dogs – Yes!
- Vaccinating positive dogs – Yes! if risk of reinfection

Questions???

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