Lyme Questions

• 1. Diagnosis
  – Can we diagnose Lyme infection and Lyme disease?
  – Clinical signs
  – What test should we actually use

• 2. Prevention
  – Can we prevent Lyme Infection?
  – Environmental Control?
  – Tick Control?
  – To vaccinate or not to vaccinate?

Questions

3. What about non-clinical dogs?
   • Should we screen, what should we do with positives?
   • Should we treat?

4. Lyme nephritis
   • What is it? Diagnosis?
   • Treatment?

The Organism

• *Borrelia burgdorferi* are helical shaped bacteria about 10-25µm long.

• Related to Leptospira

[Link to CDC website](www.cdc.gov)
The Tick – Deer Ticks

- Northeast – Ixodes scapularis (dammini)
- Northwest – Ixodes pacificus

Positive Test Map

How important is Lyme?
Human Cases 2002 & 2004

www.cdc.gov

The Tick –
Is Co-infection Important?

Aph tracks closely with Lyme
(Aph data from R&D Labs serosurvey, n=37K+).
Can We Diagnose Canine Lyme Disease??

Human Clinical Signs

Frequency of Clinical Findings Among 31,120 Lyme Disease Patients, 2001-2002

- 60% erythema migrans
- 33% arthritis
- 8% Bell's palsy
- 3% radiculopathy
- 1% meningitis
- 1% encephalitis
- 1% deafness

*These totals may not be 100% because some patients may have more than one clinical finding. Data reported in MMWR vol. 50, No. 17, pg 301.

www.cdc.gov

Clinical Signs

www.Lyme.org
Lyme: Clinical Signs

- Dogs – 10% show clinical signs
  - Onset 2 – 5 months post-infection
  - Skin lesion uncommon in dogs
  - Arthritis (often multiple joints but usually only lame in one leg at a time). Lymphadenopathy.
  - Lethargy, fever – resolve after ~3 days
  - Chronic recurrent disease?
  - Glomerulonephropathy?
  - Rare Dz: cardiac, neurologic.

Diagnosis: controversies

- Not all infected animals show clinical signs (as many as 80% seropositive with only 5% - 10% showing signs in some areas).
- DDx: based on wide criteria – not just one test.
- Do dogs with clinical disease due to lyme invariably have antibody titer?
- How should we make the diagnosis?

Lyme: Diagnostics

- Isolation and IFA:
  - Difficult due to small numbers of organisms and culture system is complex.
  - Use skin near tick attachment for best results (for PCR and culture).
### Diagnosis - Serology

- **Elisa**
  - Very sensitive
  - Whole cell antigens
  - **Does not differentiate** antibodies induced by vaccination from antibodies induced by natural infection

### Diagnosis - Serology

- **Western blot**
  - Human gold standard
  - Differentiates
    - Vaccinational
    - Exposure
    - Exposure + vaccinational

### Diagnosis - Serology

- **C6 SNAP**
  - Elisa
  - **C₆** protein
  - Only positive with natural exposure
Diagnosis Serology

- Quantitative C6 assay
  - Elisa
  - C6 protein
  - Only be positive with natural exposure
  - Does titer correlate with antigen load? With chance of clinical disease?

Classic 2 Tier Approach vs. 3Dx in Healthy Young Labrador and Golden Retrievers
Goldstein ACVIM 2005

3Dx Results n=268
- 62% negative
- 9% low positive
- 6% medium positive
- 23% high positive

50/268 dogs were Lyme positive on the 3Dx

Western Blot Results (exposure) n=259
- 55/259 dogs were positive for Lyme exposure on the Western blot

Cross Tabulation of Lyme Results: Western Blot (exposure) vs. 3Dx

<table>
<thead>
<tr>
<th></th>
<th>Western Negative</th>
<th>Western Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>3Dx</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>200</td>
<td>14</td>
<td>214</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>41</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>204</td>
<td>55</td>
<td>259</td>
</tr>
</tbody>
</table>

agreement = 93%, K = 0.78, p<0.0001
What about Non-Clinical Dogs? Should We Screen?

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

Circulating Immune Complexes In Canine Lyme Disease
Results

- Sera from over 150 dogs have been evaluated
  - 96 Non-clinical
  - 34 Clinical
    - Veterinarians assessment of Lameness, fever, lethargy

Immune Complexes Pre and Post Treatment – Non Clinical Dogs

![Graph showing immune complex scores](image)

- Pre-treatment: 400, Post-treatment: 200
- p < 0.001
- p = 0.345

Treatment – 2 Tier Approach?

![Diagram showing 2-tier approach](image)
Treatment

- Antibiotic Tx usually results in rapid response
- Doxycycline may be better than amoxicillin
  - Co-infections?
- Treat for at least 30 days, but still can not guarantee removal of the organism.
- Re-Tx reoccurrences.

Current Protocol Prospective Clinical Trial - Protocol

- Monitor dogs at risk for proteinuria/microalbuminuria
- Test all PLN or GN cases especially in endemic areas for Lyme
- If positive (SNAP or Western blot) and proteinuric treat:
  - Non specific therapy to reduce proteinuria
  - Long term doxycycline
- Be aggressive about biopsying
  - Add immunosuppression if GN
    - Imuran-Leukeran

How Can We Prevent All This??
Should We Vaccinate??

• Considerations:
  – How common is the pathogen?
  – How common is the disease?
  – How severe is the disease?
  – How treatable is the disease?
  – How good is the natural immunity?
  – How expensive, reliable, safe is the vaccine?
  – Is there a zoonotic potential?

Common?

• Considerations:
  – How common is the pathogen?
    • Common
  – How common is the disease?
    • Not that common but 10% of ~20-30% is a lot!
  – How severe is the disease?
    • Usually not that severe
      – Lyme nephritis?
      – Recurrent disease?
How Treatable is the Disease?

- What do we know?
  - Some dogs do not completely get rid of the bacteria with:
    - Their immune response
    - Antibiotic therapy
  - Status of Borrelia burgdorferi infection after Antibiotic Treatment and the Effects of Corticosteroids: an Experimental Study. Straubinger et al J Infect Dis 2000

Why Is That??

- Antigen shifting and hiding
  - Outer surface antigens
    - Ospa
    - OspC
  - Bacteria hiding

In the Tick

- All Ospa
- No OspC
In Culture

- Traditional strains:
  - All OspA
  - No OspC
- Nobivac®
  - OspA
  - OspC

In the dog

- No OspA
- Initially OspC

So – What happens in the dog?

- 1. If no clinical disease or after clinical disease
  - Bacterial load is low but not cleared!!
  - Down regulation and shift of OspC
  - Hiding from in the immune system and antibiotics in membranes, cartilage etc.?!
  - Antibody titers drop despite persistence of organism?

- No OspA
- Very little and different OspC
Conclusions

- 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.

What does that mean?

- How do the vaccines work? In the tick!
- Should we vaccinate in endemic areas along with tick control?
  - Vaccinate negative dogs - yes
  - Vaccinate positive dogs - yes
    - Not protected from re-infection
    - Treat first?

Lyme nephritis???
Lyme Nephritis?


Lyme Nephritis – So What Do We Know?

- A unique lesion in the kidneys of dogs with a devastating glomerular-tubular disease is described
- Relatively good circumstantial evidence for a Lyme connection
- Many anecdotal reports of a similar clinical syndrome in Labs and Goldens in Lyme endemic areas

Lyme Nephritis – So What Don’t We Know?

- Is it really Lyme or something else? Is Lyme the whole story?
- What does an alive dog look like?
- What is the real time frame of the disease?
- What does a mild case look like?
- Can some of them resolve?
- Is there a vaccine issue?
THE SEARCH FOR INTACT BORRELIA BURGDORFERI BACTERIA IN KIDNEYS FROM DOGS SUSPECTED OF SUFFERING FROM “LYME NEPHRITIS”

TAHutton, RE Goldstein, BJ Njaa, DZ Atwater, YF Chang and KW Simpson

Lyme PCR Testing

“Lyme Fish” Study

Positive control

Affected renal tissue
Summary and Conclusions

- Using sensitive molecular techniques (PCR and FISH), we found limited evidence of the presence of intact *B. burgdorferi* or any other bacteria in the renal tissue of dogs with suspected “Lyme nephritis”.

- The inconsistent presence of any organism, including *B. burgdorferi*, suggests that bacterial invasion of renal tissue is an unlikely pathogenesis for this disease, and that immune complex disease may be more likely.

Conclusion: Lyme is not Lepto

Do dogs with Lyme nephritis have renal deposition of immune complexes?
Yes – They Do!

Renal Immune Complexes!!

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- Be aggressive about biopsy:
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    * Imuran-Leukeran
Thank You

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2. After completing the evaluation, you will automatically be linked to the Continuing Education Certificate. The CE certificate can only be accessed after the evaluation is completed.
3. Download the CE Certificate (in pdf format) to your computer and print enough copies for those persons viewing the webcast with you.

Your input is very important! We take feedback seriously in order to provide you with the highest-quality experience possible.

If you have any questions about completing the evaluation or accessing your CE certificate, please email us at webconference@aahanet.org or call 800/252-2242.

Questions to the Speaker

Please email your questions to webconference@aahanet.org by Thursday, October 1, 2009.

Dr. Goldstein will provide written responses to all of the questions and they will be posted on AAHA's website by Tuesday, October 13, 2009.

AAHA gratefully acknowledges the following for their sponsorship of this Web Conference: